Feasibility, accuracy and safety of a percutaneous fine-needle biopsy technique to obtain qualitative muscle samples of the lumbar multifidus and erector spinae muscle in persons with low back pain

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FEASIBILITY, ACCURACY AND SAFETY OF A PERCUTANEOUS FINE NEEDLE BIOPSY TECHNIQUE TO OBTAIN QUALITATIVE MUSCLE SAMPLES OF THE LUMBAR MULTIFIDUS AND ERECTOR SPINAE MUSCLE IN PERSONS WITH LOW BACK PAIN

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ABSTRACT

The lumbar muscular system, in particular the lumbar multifidus muscle (LM) and the erector spinae muscle (ES), plays an important role in stabilizing and mobilizing the lumbar spine. Based on the topography, the lumbar paraspinal muscles can be classified into local and global muscles. The LM is part of the local system, while the ES is part of the global system. Therefore, it is interesting to investigate the muscle fibre type composition in both muscles. There is accumulating evidence that non-specific chronic low back pain is associated with lumbar muscle dysfunction. To further elucidate this lumbar paraspinal muscle dysfunction, it is important to understand the structural characteristics of individual muscle fibres of the LM and ES. Muscle fibre type composition can be investigated in muscle tissue samples. So far, muscle samples are taken by using invasive procedures that are not very well tolerated. The aim of this article is to evaluate the feasibility, accuracy and safety of a percutaneous fine needle biopsy technique to obtain muscle samples from the LM and ES in persons with NSCLBP and to evaluate the feasibility of performing immunofluorescence analysis of myosin heavy chain isoforms expression to investigate muscle fibre type composition. Preliminary investigations in cadavers were performed to determine the optimal vertebral level and puncture site to obtain muscle samples of the LM and ES through a single skin puncture. In 15 persons with NSCLBP, muscle samples of the LM and ES were taken under local anaesthesia with the percutaneous fine needle biopsy technique, preceded by determination of the puncture site with ultrasonography. Muscle fibre type composition was investigated using immunofluorescence analysis of myosin heavy chain expression. The subjects reported little or no pain and were willing to repeat the procedure. The obtained muscle tissue contained transverse-sectioned muscle fibres in which muscle fibre contractile characteristics of the paraspinal muscles could be evaluated with immunofluorescence analysis of the myosin heavy chains. We can conclude that percutaneous microbiopsy appears to be feasible, accurate and safe to obtain muscle tissue from the paraspinal muscles. The use of ultrasonography to determine the puncture site is necessary to ensure biopsy of the correct muscles and to ensure safety of the procedure.

KEYWORDS

Lumbar paraspinal muscles, muscle biopsy, muscle fibre characteristics, muscle fibre typing, muscle morphology, muscle architecture

INTRODUCTION

Low back pain (LBP) is one of the most disabling musculoskeletal disorders(Airaksinen et al., 2006) with a lifetime prevalence of 84% (Balague et al., 2012). In 23% of all persons with LBP, the cause remains unknown and the diagnosis of non-specific chronic low back pain (NSCLBP) is indicated (Balague et al., 2012, Airaksinen et al., 2006). The unknown cause impedes the effective treatment and prevention (Wong et al., 2014).

The lumbar muscular system, in particular the lumbar multifidus muscle (LM) and the erector spinae muscle (ES), plays an important role in stabilizing the lumbar spine (Goel et al., 1993). The multifidus muscle consists of different fasciculi, which lie at the sides of the spinous processes of the vertebrae, from the sacrum to the axis. The multifidus muscle can be divided in a thoracic and lumbar part. The LM is more superficial, thick and consists of five bands, each originating from a lumbar spinous process (L1-L5). These band spread caudolaterally to attach distally onto the mammillary processes, the sacrum and/or the ilium (Rosatelli et al., 2008, Macintosh et al., 1986, Hansen et al., 2006). The lumbar ES appears to be one muscle mass lying lateral to the multifidus muscle and extending from the thoracic vertebrae to the ilium and sacrum. However, dissection of this common muscle mass reveals that it consists of two muscles, longissimus thoracis and iliocostalis lumborum, each with thoracic and lumbar fascicles. In the lumbar region, the longissimus thoracis pars lumborum attaches cranially to accessory processes and the medial part of the transverse processes of the lumbar vertebrae, while the iliocostalis lumborum pars lumborum attaches cranially to the lateral parts of the transverse processes (Bustami, 1986, Bogduk, 1980, Macintosh et al., 1986, Daggfeldt et al., 2000). Although these lumbar fibres appear to be one muscle mass, they are separated by the lumbar intermuscular aponeurosis. It is unclear whether the lumbar parts attach directly to the iliac crest (Bogduk, 1980, Macintosh et al., 1986) or that they have a caudal attachment to the ES aponeurosis (Bustami, 1986, Daggfeldt et al., 2000).

There is accumulating evidence that NSCLBP is associated with lumbar muscle dysfunction (Chan et al., 2012, Danneels et al., 2000, Hides et al., 2008, Wallwork et al., 2009, Kamaz et al., 2007, Kjaer et al.,

2007). Macroscopically, this muscle dysfunction is characterized by a decrease in anatomical muscle cross sectional area (CSA) of the entire paraspinal muscles and an increase in fat content, although somewhat inconsistent results have been reported on this aspect (Chan et al., 2012, Danneels et al., 2000, Hides et al., 2008, Wallwork et al., 2009, Kamaz et al., 2007, Kjaer et al., 2007, Paalanne et al., 2011, Crossman et al., 2004, Mannion et al., 1997). Some studies suggest significant differences in morphology of the paraspinal muscles between persons with LBP and healthy controls (Chan et al., 2012, Kamaz et al., 2007, Danneels et al., 2000, Hides et al., 2008, Wallwork et al., 2009, Kjaer et al., 2007), while others suggest no differences (Paalanne et al., 2011, Crossman et al., 2004, Mannion et al., 1997). Therefore, knowledge of the structural characteristics of individual muscle fibres of the lumbar paraspinal muscles in persons with NSCLBP is essential to clarify the role of lumbar muscle dysfunction.

Human skeletal muscles consist of different fibre types, that have different morphological and functional characteristics. In humans, these muscle fibres are classified into three groups based on their contractile speed, myosin heavy chain (MHC) expression and metabolic capacity: type I slow twitch oxidative fibres (type I), type II fast twitch oxidative/glycolytic fibres (type IIa) and type II fast twitch glycolytic fibres (type IIx). Mobilizing muscles contain large numbers of rapid contracting muscle fibres (type II), while stabilizing muscles may be more dominated by fatigue-resistant muscle fibres (type I) (Widmaier et al., 2014, Schiaffino and Reggiani, 1996, Caiozzo, 2002). Consequently, it might be expected that the back muscles would predominantly consist of type I fibres, because these muscles need to ensure upright body positioning during much of the day. However, the back muscles also play an important role in the spinal movement. Bergmark (Bergmark, 1989) stated there are two muscular systems that are involved in the stability and mobility of the lumbar spine. This classification is based on the topography of the muscles. The local muscles have an origin and insertion at the vertebrae and span only one or a few intervertebral joints. These local muscles are short and firm muscles and control the structural integrity of the lumbar spine. The global muscles have long lever arms since they connect the pelvis with the thorax. Therefore global muscles can produce great forces to change the position

of the thorax in relation to the pelvis. The LM is part of the local system, while the ES is part of the global system (Bergmark, 1989). Based on this classification, the ES and LM might have different fibre type compositions. However, previous studies were not able to find significant differences in muscle fibre type composition between ES and LM (Rantanen et al., 1994, Jorgensen et al., 1993, Thorstensson and Carlson, 1987).

Muscle fibre type composition can be investigated in muscle tissue obtained during surgery (open muscle biopsy) or by a percutaneous muscle biopsy (semi-open muscle biopsy). The technique, described by Bergström (Bergstrom, 1962), is considered to be the golden standard to obtain human muscle samples. This semi-open muscle biopsy technique is suitable for biochemical, histochemical and histomorphometric analyses, with similar results to muscle samples obtained during surgery (Hennessey et al., 1997). Another semi-open muscle biopsy technique is the percutaneous conchotome muscle biopsy in which a Tilley-Henckel punch forceps is used (Dietrichson et al., 1987). The latter has previously been used to obtain muscle samples from the ES in persons with LBP and in healthy persons (Mannion et al., 2000). However, in both semi-open biopsy techniques, an incision of 5-8mm through the skin and the underlying thoracolumbar fascia (TLF) is necessary, which makes it an invasive procedure that can be painful. Moreover, it is not well accepted by patients because of the relatively large needle size (Hayot et al., 2005, Tarnopolsky et al., 2011). A recent development in percutaneous skeletal muscle biopsies is the use of a fine needle biopsy technique, also termed microbiopsy (Hayot et al., 2005, Magistris et al., 1998, Welker et al., 2000, Cote et al., 1992). This alternative for the semi-open muscle biopsy is already used in several medical areas, such as oncology and neurology. Hayot et al. stated that the microbiopsy was very well tolerated, since subjects reported little or no pain and would all prefer the microbiopsy instead of the semi-open biopsy technique by Bergström (Hayot et al., 2005). Moreover, the muscle samples obtained by microbiopsies are of good quality and provide similar results compared to the golden standard, the Bergström technique (Hayot et al., 2005). Traditionally, muscle fibre type composition has been identified by determining myosin ATPase activity based on differential responses to various pH levels (Brooke and

Kaiser, 1970, Guth and Samaha, 1970). Immunofluorescence analysis is a more sensitive method for the investigation of the muscle fibre type composition. It allows the evaluation of multiple myosin heavy chain (MHC) isoforms on a single cross-section. Moreover, pure fibres (single MHC isoform) as well as so-called hybrid fibres (co-expressing more than a single MHC isoform) could be clearly identified (Bloemberg and Quadrilatero, 2012). The amount and the CSA of the different individual muscle fibres could be measured in muscles samples that contain sufficient transverse-sectioned muscle fibres. This transverse sectioning is important for the correct measurement of the CSA, since oblique sectioning will overestimate the CSA of the muscle fibre. In single cryo-sections at least 150 muscle fibres should be accessible to achieve a representative sample of the entire muscle (Ceglia et al., 2013).

To our knowledge, the ES is the only lumbar paraspinal muscle that has been sampled using muscle microbiopsy in order to investigate differences in muscle fibre characteristics between persons with LBP and healthy persons. The main aim of the current study was to evaluate the feasibility, accuracy and safety of a microbiopsy technique in obtaining muscle samples of the LM and the ES through a single skin puncture. The second aim of the study was to evaluate the quality of the muscle samples and the feasibility of performing immunofluorescence analysis of the different MHC isoforms on the obtained muscle samples to investigate muscle fibre type composition.

METHODS

Part 1: Preliminary investigations to ensure accurate biopsy of LM and ES

Cadaveric study

The puncture procedure of the LM and ES and identification of the optimal puncture site were investigated in three cadavers (layer-by-layer and cross-sectional dissection). Two cadavers were used to observe and identify the relevant layer-by-layer anatomy in order to determine optimal vertebral level for taking muscle samples from the LM and ES. Two cadavers were used to determine the optimal puncture site in between the LM and ES, as well as the optimal puncture depth and angle for both muscles by cross-sectional dissection at the vertebral level L4 on one side of the body.

Microbiopsy under guidance of ultrasound

In one subject with LBP, the biopsy procedure, as described in detail in part 2, was performed while guided by ultrasound imaging to visualize the biopsy needle tip, to ensure that muscle tissue was sampled in the specific region of interest.

Part 2: Feasibility and safety of the biopsy protocol in persons with NSCLBP

Subjects

Fifteen persons with NSCLBP were recruited by local advertisement and included in the study after provision of their informed consent. Inclusion criteria were (1) medically diagnosed with non-specific chronic low back pain, (2) >18 years old, and (3) able to understand Dutch (spoken and written). The study was approved by the Ethical Committee of UHasselt (BE) and Jessa Hospital Hasselt (BE).

Microbiopsy device and needle

The microbiopsy was performed with an automated biopsy device, the Magnum Biopsy System (MG1522; Bard). The authors used 16-gauge core disposable biopsy needles (Magnum Needle,

MN1610; Bard; length 100mm, length of the sample notch 22mm) and a 15-gauge coaxial needle (Magnum Needle, C1610B, Bard; length 7cm).

Microbiopsy procedure

The successive steps of the microbiopsy procedure are illustrated in Figure 1. Participants were placed in a prone position with a small amount of lumbar flexion. To determine the correct location of the puncture site, all patients underwent an ultrasound examination (Philips iU22 Ultrasound machine with Philips C5-3 MHz Curved Array Transducer) prior to biopsy sampling. The distance between skin and TLF is essential to ensure a correct and safe biopsy depth and to avoid puncture of spinal nerve branches and vertebral bone. The puncture site was determined by the point of separation between the LM and ES at the level of the spinous process of vertebra L4. The vertebral level L4 was chosen since the muscle mass of the LM was too small at the level of vertebra L3 to obtain a muscle sample without risking damage to surrounding non-muscular tissue and at the level of vertebra L5, there is interaction with os sacrum. The distance between the spinous process and the puncture site, as well as the depth of the TLF were measured on ultrasound and the sample location was marked by the sonographer. The puncture site was confirmed by clinical palpation. To be consistent, all biopsies were taken at the right side of the vertebral column since all patients reported bilateral pain.

This ultrasound-guided determination of the puncture site was performed prior to biopsy sampling to establish the necessary landmarks in every subject. After this ultrasonography, the biopsies were performed without ultrasound guidance. In all subjects, the skin and the region subcutaneously was anaesthetized with 5ml of xylocaine 1% after skin application of povidone-iodine (Fig 1a). A small incision of approximately 2mm was made through the skin at the entry site of the coaxial biopsy needle (Fig 1b). The coaxial needle was perpendicularly inserted through the incision piercing the TLF (Fig 1c). This coaxial needle was used to provide a clear path through which the biopsy needle can be inserted to obtain muscle biopsies from two different muscles (LM and ES) without the need for repeated skin punctures. After inserting the biopsy needle into the biopsy device, it was inserted through the coaxial

needle. The biopsy needle was directed laterally at an angle of 35° relative to the initial perpendicular position (Fig 1d). A muscle sample of the ES was obtained by activating the trigger button. After withdrawing the biopsy needle, it was removed from the biopsy device and a new biopsy needle was inserted. The coaxial needle was maintained and brought back to its initial position to obtain a muscle sample from the LM. The procedure was repeated, only the biopsy needle was directed medially at an angle of 15-20° relative to the initial perpendicular position (a maximal angle that might be used without risk of contacting the articular process was predetermined by the sonographer) (Fig 1e). A muscle sample of the LM was obtained by activating the trigger button. When necessary, the biopsy procedure was repeated in order to obtain sufficient muscle tissue. After withdrawing the biopsy needle, the coaxial needle was removed, sterile strips and sterile gauze were applied on the puncture wound. Firm pressure was applied at the biopsy site for approximately 5 minutes to avoid intramuscular bleeding.

The muscle samples were removed from the biopsy needle with a sterile needle and placed and oriented on a piece of cork prior to be embedded (Fig 1f). The samples were covered with Optimum Cutting Temperature-compound (TissueTek) and immediately frozen in isopentane, precooled in liquid nitrogen. Frozen samples were stored at -80°C until further analysis.

Clinical tolerance

Immediately after the biopsy procedure and one day later, the subjects were asked to evaluate the pain associated with the biopsy procedure using a modified Borg 0-10 scale: 0 indicated no pain at all and 10 indicated unbearable pain(Borg, 1982). Pain during admission of local anaesthesia was not evaluated. A follow-up of the patients was performed to check for infections.

Part 3: Assessment of the muscle sample quality

Orientation of the muscle fibres

Serial transverse sections (10µm) were cut with a microtome and stained with a haematoxylin-eosin

(HE) staining to check the orientation of the muscle fibres. The amount of transverse-sectioned fibres per muscle sample was counted.

Muscle fibre type composition

Immunofluorescent staining was performed to analyse MHC expression. Primary antibodies, specific to laminin (ab11575, Abcam), MHC I (BA-F8), MHC IIa (SC-71) and MHC IIx (6H1) (Development Studies Hybridoma Bank, Iowa City, IA, USA) were used. The sections were counterstained with appropriately conjugated secondary antibodies (Alexa Fluor 532, Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 555, Life Technologies) to visualize the different muscle fibres and their endomysium (Bloemberg and Quadrilatero, 2012). Stained samples were viewed with a fluorescent microscope (Leica EL6000, Leica).

RESULTS

Part 1: Preliminary investigations to ensure accurate biopsy of LM and ES

Cadaveric study

The layer-by-layer anatomy of the lumbar paraspinal muscles was investigated in two cadavers (Fig 2). A cross-sectional investigation of the paraspinal muscles at the level of vertebra L4, showed a clear separation between the ES and LM. When inserting the biopsy needle at this point of separation, a muscle sample from both the ES and LM could be obtained by a single skin puncture (Fig 3a). Specifically, by directing the biopsy needle laterally at an angle of 30-35° relative to the perpendicular position, a muscle sample of the ES could be obtained (Fig 3b). A muscle sample of the LM could be obtained specifically by directing the needle medially at an angle of 15-20° relative to the perpendicular position (Fig 3c). These angles ensured that ES and LM could be obtained without the risk of damaging spinal nerve branches and vertebral bone. Because laterally there are no other tissues in the close proximity, the biopsy angle for the ES could be greater.

Microbiopsy under ultrasonography guidance

The biopsy procedure was performed following the protocol described in the methods section under ultrasonography guidance. The tip of the biopsy needle was visualized with ultrasound while taking a biopsy of ES (Fig 4a) and LM (Fig 4b) to ensure the correct muscle was biopsied.

Phase 2: Feasibility and safety of the biopsy protocol in persons with NSCLBP

Patient characteristics

Seven of the patients were male and eight female. Further demographic and clinical data are shown in table 1.

Ultrasound-guided determination of the puncture site

The targeted needle insertion site was determined with ultrasound imaging as the point of separation between the LM and ES(Fig 5). The distance between spinous process of vertebrae L4 and the puncture site was 30.1 ± 3.3 mm. The depth of the TLF, which is defined as the distance between the skin and the TLF, was 8.3± 2.7 mm. The maximal angle at which the biopsy needle could be directed medially without risking damage the surrounding non-muscular tissue was 35.8 ± 1.8° (Fig 5).

Clinical tolerance

The biopsy procedure was performed without any problems or complications. All participants reported little or no pain associated with the microbiopsy with a mean pain score of 0.97 (ranging from 0-2) out of 10. The day after the microbiopsy, some participants experienced a slight feeling of swelling or tenderness at the puncture site, most likely compatible with a small hematoma. The mean pain score was 0.62 (ranging from 0-2) out of 10. All participants declared to be willing to repeat the test in the future. No infections or other complications were reported.

Part 3: Assessment of the muscle sample quality

Orientation of the muscle fibres

The muscle tissue of the LM and ES obtained by microbiopsy sampling contained transverse-sectioned muscle fibres in 29 out of 30 biopsies, which makes them appropriate for immunofluorescence analysis of MHC expression. The mean number of transverse-sectioned fibres was 286 ± 217 for the ES and 210 ± 160 for the LM. The amount of transverse-sectioned muscle fibres progressively increased in consecutive biopsy samples during our study. The mean number of transverse-sectioned fibres improved from 153 in the muscle samples of the first five patients to 352 in the muscle samples of the last five patients.

Muscle fibre type composition

It was feasible to perform a multicolor immunofluorescence staining procedure to visualize different muscle fibre types. The pure muscle fibres type I, type IIa and type IIx could be identified as well as the hybrid muscle fibres containing two types of myosin heavy chains (i.e., type I/IIa, IIax) (Fig 6).

DISCUSSION

The results of the present study demonstrate that the percutaneous microbiopsy technique is a feasible, accurate and safe tool to obtain human muscle tissue samples in vivo from the ES and LM in persons with NSCLBP. The most commonly used techniques to obtain muscle samples, the Bergström muscle biopsy and the conchotome muscle biopsy, are both semi-open biopsy techniques in which an incision of 5-8mm through the skin and underlying TLF is necessary, making it invasive procedures that can be very painful (Hayot et al., 2005, Tarnopolsky et al., 2011). Both approaches have the advantage of providing abundant muscle tissue for further analysis. However, their major disadvantage is the limited patient acceptance and relatively high pain scores. A percutaneous microbiopsy technique can be used to reduce the needle size and improve patient acceptance and tolerance.

Muscle fibre type characteristics play an important role in the functional capacity of a muscle. Beside ensuring the upright position of humans, back muscles also have an important role in the movement of the spine. To meet these functions, different back muscles may play different roles. Therefore, it might be interesting to investigate the muscle fibre type characteristics of the ES, a torque-producing muscle and the LM, a stabilizing muscle (Panjabi, 1992, Bergmark, 1989). Until now, only few studies have described the muscle fibre type characteristics in patients with NSCLBP by using muscle biopsy techniques (Mannion et al., 2000, Mannion et al., 1997, Crossman et al., 2004). These studies used the conchotome technique to obtain muscle tissue from the ES in healthy subjects and persons with NSCLBP. However, due to the invasiveness of the procedure, only the ES was biopsied in these studies. In healthy subjects, the ES and the LM were biopsies by using the Bergström technique (Jorgensen et al., 1993) and a small biopsy needle (Thorstensson and Carlson, 1987). However, both techniques required the need to incise the skin and the fascia, which increases the invasiveness of the procedure. In the present study, muscle tissue from the ES and LM was obtained. The position of the sample location and the use of a coaxial needle make it possible to obtain muscle tissue from both muscles through a single skin puncture, without incision of the fascia. Moreover, the percutaneous microbiopsy or fine needle biopsy technique comprise a fast procedure which has good patient acceptance and

tolerance with a pain level similar to a peripheral venous blood sampling (Tobina et al., 2009). In the present study, the microbiopsy was very well tolerated by all subjects, since they reported little or no pain, ranging from 0-2 on a VAS scale for pain (10 reflecting unbearable pain). All participant were willing to undergo a second procedure if necessary . These results confirm the findings of Hayot et al. who demonstrated that microbiopsy sampling was less painful than biopsy sampling using the Bergström needle, and that all participant unanimously prefered the microbiopsy technique (Hayot et al., 2005). Moreover, in the present study late-occurring discomfort/pain was also scored very low, ranging from 0-2 on a VAS scale for pain. In the present study, the combination of percutaneous microbiopsy sampling with ultrasonography prior to the biopsy sampling appeared well suited to examine small human skeletal muscles or deeper muscles without compromising safety or sample reliability. Therefore, percutaneous microbiopsy sampling seems a viable alternative to obtain muscle tissue from paraspinal muscles especially in patients with low back pain.

The cadaveric study has shown that the ideal puncture site is located at the separation between ES and MF. However, interindividual anatomical variations must be taken into account. Therefore, it is necessary to determine the septum between the ES and LM, and thus the puncture site, with ultrasonography prior to the biopsy procedure. Besides measuring the distance between the spinous process and the puncture site, the distance between the skin and the fascia and the angle to obtain a muscle sample of the LM were also measured in every participant. These measurement were performed to avoid the risk of contacting the articular process and to ensure biopsy of the correct muscle, which was confirmed earlier by the ultrasonography-guided biopsy.

The amount of muscle tissue obtained by a percutaneous muscle microbiopsy is less compared to the semi-open biopsy techniques. In the present study, obtained muscle samples contained transverse-sectioned muscle fibres in 29 out of 30 muscle samples, on which immunohistochemical fibre type analysis can be performed. This finding is in line with Hayot et al. who showed that a microbiopsy provides muscle samples of excellent quality that are comparable to the muscle samples obtained by the golden standard, the Bergström technique (Hayot et al., 2005). To determine the yield, only

transverse-sectioned fibres were taken into account because only these can be used for measurements of myofibre CSA. The mean number of transverse-sectioned fibres was 286 ± 217 for the ES and 210 ± 160 for the LM, which is more than reported previously using comparable micro-needle sampling techniques (Hayot et al., 2005, Scheel et al., 2013, Shalabi et al., 2002, Townsend et al., 2016). Furthermore, there was a substantial improvement in the tissue quality over the course of the study as the authors gained progressively more experience in the biopsy technique and the transfer and orientation of the muscle sample.

The immunofluorescence analyses were performed on muscle samples containing transverse-sectioned muscle fibres. To our best knowledge there are no earlier reports on muscle biopsy sampling from the ES and LM in persons with NSCLBP, and further no previous study has used immunofluorescence analysis of MHC isoforms to examine muscle fibre characteristics with this sampling technique. The multicolour immunofluorescence analysis used in the present study has several advantages compared to other methods (e.g. ATPase staining). In particular, this technique is more reliable as it is easier to identify all pure and hybrid fibres on a single cross-section (Bloemberg and Quadrilatero, 2012). Our results show that it is feasible to perform immunofluorescence analysis of the different MHC isoforms on the muscle biopsy samples obtained using a microbiopsy method. Future research should be conducted to compare myofibre CSA and MHC composition in paraspinal muscles of persons with NSCLBP versus healthy persons. This muscle fibre typing may be important to evaluate the functional capacity of different back muscles. Future research should first focus on establishing reference data for muscle fibre type composition and muscle fibre characteristics in ES and LM. Previous studies were not able to find differences in muscle fibre type composition between ES and LM (Rantanen et al., 1994, Jorgensen et al., 1993, Thorstensson and Carlson, 1987). However, these studies might have some methodological problems. Rantanen et al. used cadavers in which rigor mortis can distort the results (Rantanen et al., 1994), while the other studies used a biopsy technique without using ultrasonography to ensure biopsy of the correct muscle (Jorgensen et al., 1993, Thorstensson and Carlson, 1987). Future research is necessary to assess morphological changes in

myofibre CSA that might occur in these muscles of persons with NSCLBP. This information likely will be helpful to clarify the role of the paraspinal muscles in low back pain and to develop more effective treatment and prevention programs in this patient population.

CONCLUSIONS

In conclusion, percutaneous microbiopsy sampling techniques can be used to obtain muscle tissue from the ES and LM in patients with NSCLBP. To ensure biopsy of the correct muscle and to ensure the safety of the procedure, the ideal puncture site was determined by using ultrasonography prior to biopsy. The use of ultrasonography in every participant is essential to reveal anatomical variations between individuals. Since, the ideal puncture site is marked during the ultrasonography, the biopsy sampling can be performed without ultrasound guidance. The present report demonstrates the feasibility, accuracy and safety of the percutaneous fine needle technique for obtaining biopsies of paraspinal muscles that provide transverse-sectioned images of high morphological quality for optimal immunofluorescence analysis of MHC based muscle fibre composition. The present protocol appears to be safe and useable for muscle research of the lumbar musculature in healthy subjects and patients with low back pain. Since lumbar paraspinal muscles may play a role in NSCLBP, this technique can be used to elucidate potential myocellular mechanisms related to lumbar paraspinal muscle dysfunction.

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AUTHOR CONTRIBUTIONS

BOE, AT, JV, FV, LB and AA contributed in the conception of the study. FV, SS and AA performed the cadaveric study. JV and AA contributed in the recruitment of the patients. LB performed the ultrasound examination. FV, AA and JV contributed in executing the muscle biopsies. SS and AA performed the immunohistochemical analysis. JV, AT, SS, FVA and AA analyzed and interpreted the data. AA was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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TABLES

Table 1. Patient characteristics

Characteristics	**Mean**	**Standard Deviation**
Age (years)	45.60	8.81
Height (cm)	173.96	8.39
Weight (kg)	75.09	12.62
Duration of symptoms (years)	9.57	10.07

FIGURE LEGENDS

Figure 1. Successive steps of the microbiopsy procedure. a. local anaesthesia 5ml of xylocaine 1%, b. incision through the skin, c. insertion of the coaxial needle, d. biopsy of the erector spinae muscle by directing the biopsy needle laterally, e. biopsy of the lumbar multifidus muscle by directing the biopsy needle medially, f. transferring and orienting the muscle sample on a piece of cork.

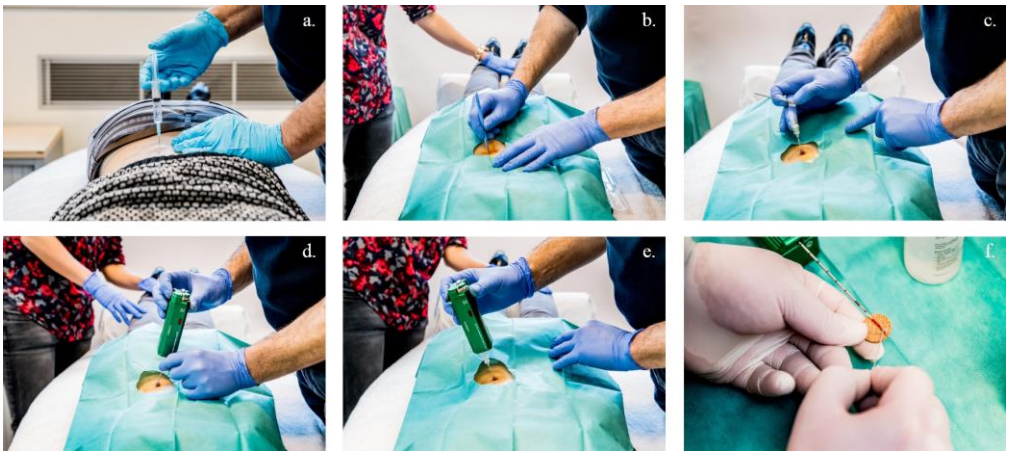

Figure 2. Layer-by-layer dissection of the back muscles. a. After removal of the skin, subcutaneous fat and the fascia, the superficial muscles of the back were exposed: trapezius muscle (TR), latissimus dorsi muscle (LD), serratus posterior inferior muscle (SPI) and erector spinae muscle (ES). Also the lumbar component of the thoracolumbar fascia (TLF) was exposed. b. focus on the lumbar part of the back muscles. c. reflection of the SPI and TLF exposed the complete ES group. d. a small incision in ES showed the lumbar multifidus (LM).

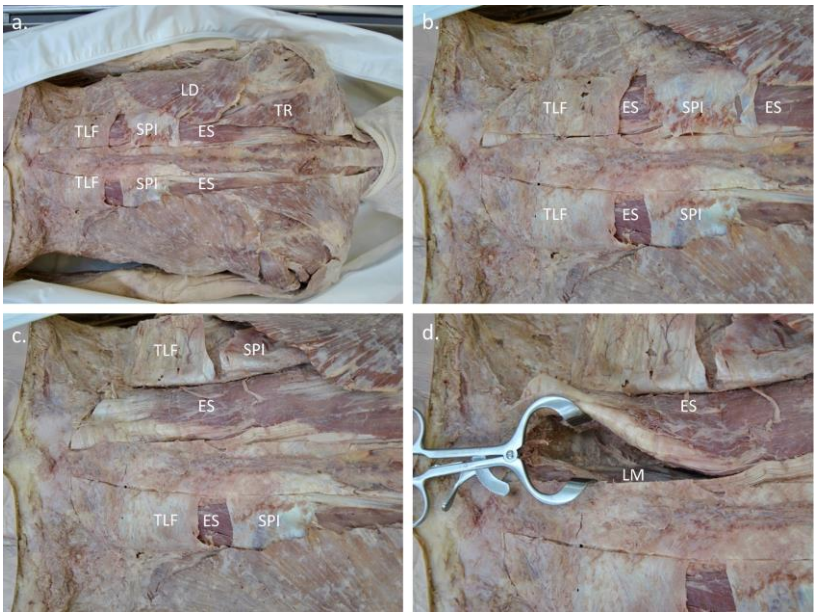

Figure 3. Determination of the puncture site in a cadaver. a. The coaxial needle was placed at the separation between ES and LM. b. The biopsy needle was directed laterally at an angle of 30-35° relative to the initial perpendicular position to obtain a muscle sample from ES. c. The biopsy needle was directed medially at an angle of 15-20° relative to the initial perpendicular position to obtain a muscle sample from LM.

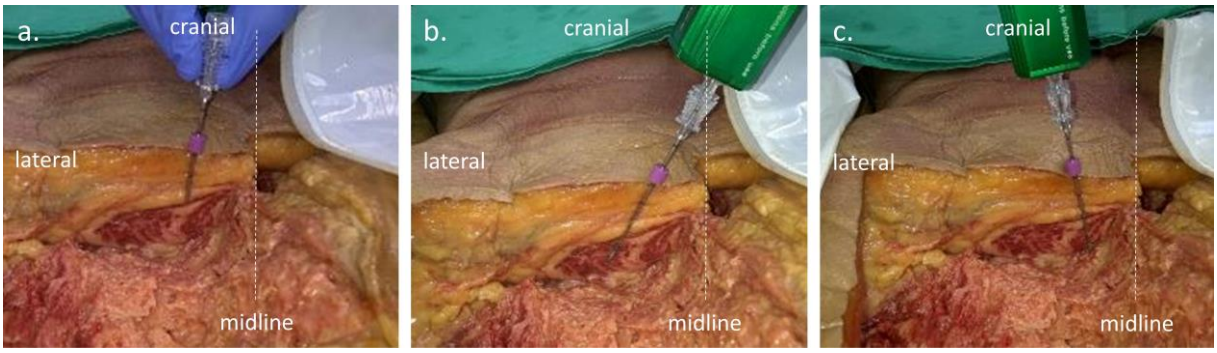

Figure 4. Ultrasound-guided percutaneous microbiopsy. The tip (yellow arrow) and direction (red arrow) of the biopsy needle was visualized when taking a muscle sample of erector spinae muscle (ES) in figure a and of the lumbar multifidus muscle (LM) in figure b.

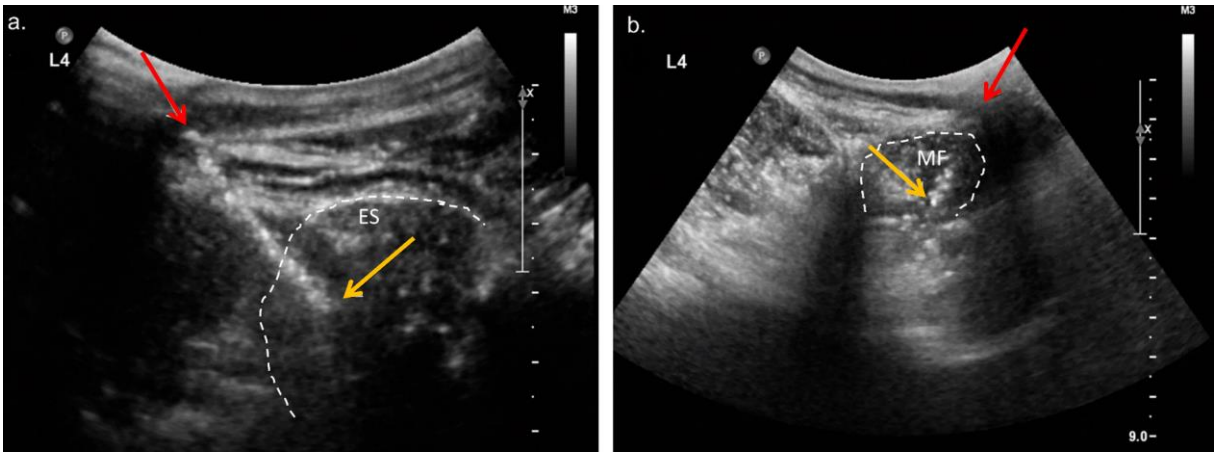

Figure 5. Ultrasound-guided determination of the puncture site. The distance (+) between spinous process (sp) and puncture site (ps), the depth of the fascia (*) and the maximal angle (arrow) to obtain a muscle sample of lumbar multifidus muscle (LM) was determined by a sonographer using ultrasound.

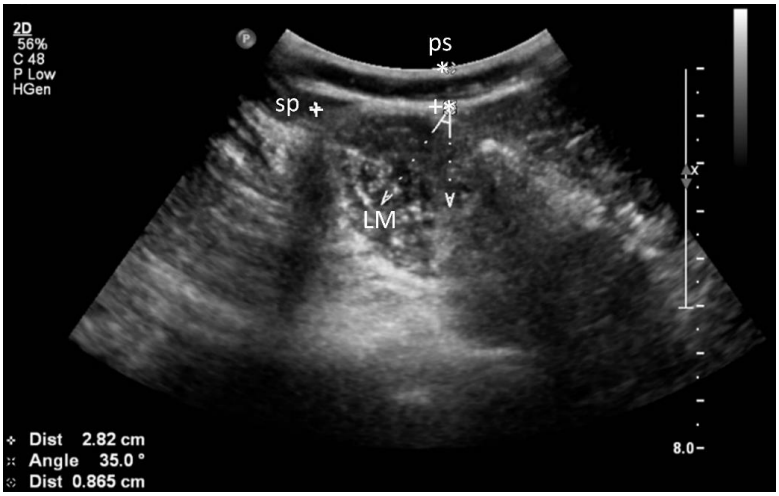

Figure 6. Immunofluorescence image of LM. Muscle cross-sections are incubated with primary antibody against a. MHCI, b. MHCIIa, c. MHCIIx and laminin. d. muscle cross-section which visualize all different fiber types. A hybrid fibre (IIax) is indicated with an arrow.