Anatomical variations of the thoracolumbar nerves with reference to transverse abdominal plane (TAP) block

by

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Declaration of original work

I, Bianca Smit, hereby declare that the dissertation entitled,

Anatomical variations of the thoracolumbar nerves with reference to transverse abdominal plane (TAP) block,

is my original work and has not been submitted to any other tertiary institute for degree or award purposes other than the University of Pretoria for the degree in M.Sc. Anatomy.

B Smit 31-10-2015

Date
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Summary

Introduction

The transverse abdominal plane (TAP), formed between the transversus abdominis muscle (TAM) and the internal oblique muscle (IOM), contains the thoracolumbar nerve plexus. The plexus is anaesthetised through a blind or ultrasound-guided TAP block, mainly used for post-operative pain management. Ultrasounds are not always readily available in the public sector, creating a need to improve the blind TAP block. The L1 nerve and its terminal branches, also running in the TAP, can be blocked with a TAP block or separately. By studying the anatomy of the nerve plexus, the TAP block and the iliohypogastric and ilioinguinal blocks could be improved. This study aimed to determine the course and branching patterns of the thoracolumbar nerve plexus, as well as the branching of the L1 nerve in the posterior abdominal wall before entering the TAP.

Methods

Bilateral dissections were done on 54 embalmed cadavers to examine the TAP by noting the number of nerves at the mid-axillary line (MAL) and at the linea semilunaris. The needle tip position, as well as the general branching patterns were evaluated. For the L1 dissections, the root contributions and branching patterns were evaluated before entering the TAP. Abdominal ultrasounds were taken bilaterally on 43 volunteers to measure depth, individual muscle layer thickness, and subcutaneous fat thickness on a line at the injection point and at the IOM and TAM tendon junction. Differences between sides and the effect of BMI categories were analysed.

Results

The average number of nerves from the MAL to the linea semilunaris increased by one nerve. The needle was in the correct plane in only 7.6% of cases, with the needle going too deep in 79.3% of cases. The “pop” method used in the blind TAP block ensures the needle tip is not too superficial, but it is easy to go deeper than required. A nerve was pierced in 6.5% of cases, while the mean distance between the needle
tip and the closest nerve (4.56 mm – 6.83 mm) indicated the needle tip is generally close enough to the nerves to provide anaesthesia without nerve damage. Nerve interactions observed includes branching or not, merging or not, or any combination thereof. Various variations were seen for the root contributions of the L1 nerve, affecting the innervation of the anterolateral abdominal wall. The fourth lumbar artery accompanied the L1 or its terminal branches to enter the TAP in 40.7% of cases. The IOM and TAM tendon junction appears as a hyperechoic dot on an ultrasound, which can be used as an additional landmark. Measurements revealed the needle should be advanced at least 2 cm and 3 cm in healthy and overweight BMI individuals respectively.

Conclusions

Notable differences were seen between the current study and studies using a different population, indicating the anatomy of the TAP block is specific to population. The anatomy surrounding the TAP showed significance for a South African population. By analysing the anatomy, this study adds ways to improve the blind and ultrasound-guided TAP blocks.

Keywords: transverse abdominal plane (TAP); TAP block; L1 nerve; iliohypogastric nerve; ilioinguinal nerve; ultrasound anatomy
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List of Abbreviations

TAP Transverse abdominal plane
IOM Internal oblique muscle
TAM Transversus abdominis muscle
BMI Body mass index
MAL Mid-axillary line
EOM External oblique muscle
ASIS Anterior superior iliac spine
CT Computed tomography
CIs Confidence intervals
Chapter 1: Introduction

1.1 Background

The transverse abdominal plane (TAP) is formed between the internal oblique muscle (IOM) and the transversus abdominis muscle (TAM). Here the thoracolumbar nerves, T7-L1, run to form a nerve plexus (Standring, 2008). As these nerves innervate the anterolateral abdomen (Standring, 2008), their position in a fascial plane makes for a logical option for regional anaesthesia. As such, a landmark technique TAP block was introduced in 2001 (Rafi, 2001) with the first ultrasound-guided modification following a few years later (Hebbard et al., 2007). The TAP block is used in several surgical procedures such as abdominal wall reconstructive flaps and lumbotomy (Rozen et al., 2008; Van Der Graaf et al., 2011), but is mainly used in post-operative pain management (McDonnell et al., 2008; Sharkey et al., 2013).

The exact anatomy of the thoracolumbar nerves once they enter the TAP is variable, causing problems for both the blind TAP block and the ultrasound guided technique (Rozen et al., 2008). The amount of fat present on the patient in the injection area creates a problem in both the TAP block techniques. In the blind block, an increase in abdominal fat reduces the ease of finding the correct landmarks required as well as finding the correct depth for the needle point (Rafi, 2001). In the ultrasound guided technique, the abdominal fat impairs the ideal angle at which the ultrasound transducer should be placed, thereby increasing the difficulty for an efficient block (Toshniwal and Soskin, 2012). Another possible problem for the TAP block is the absence of the triangle of Petit in about 17.5% of patients. This triangle is used as an important landmark for both the blind and ultrasound guided methods (Sharkey et al., 2013; Walter et al., 2008). By analysing the course and variations of the thoracolumbar nerves as well as the depth of the TAP, and then analysing the data, especially with regards to weight, the efficacy of the TAP block can potentially be improved.

The main branches of L1, the iliohypogastric and ilioinguinal nerves, are also plagued by the problems of weight as they run in the TAP (Standring, 2008). Needle positioning on L1 blocks could be improved by investigating the precise location where the L1 nerve divides and where it passes through the transversus abdominis muscle.
1.2 Problem statement

Although the thoracolumbar nerves form a plexus, the exact branching patterns are not fully described. Often in undergraduate degrees the nerves are only mentioned but never seen. Any physician attempting the TAP block needs to be aware of the typical and variant anatomy of the TAP and the nerves running within it. The effect that an increase in body weight has on the difficulty in performing both the TAP block, blind or ultrasound guided, and the L1 block, has not been fully explored. The current L1 block techniques require an injection into the TAP as well as the plane between the external oblique and internal oblique muscles. This technique therefore requires a greater amount of anaesthetic and runs the risk of additional nerves, such as the femoral nerve, obturator nerve, and the lateral femoral cutaneous nerves being blocked unintentionally. These problems have led to research questions pertinent to the current study.

1.3 Research questions

- Can the current blind TAP block be improved by providing more information on the underlying anatomy and more specific landmark positions?
- Is there a more suitable anatomical position for needle placement more posteriorly than the current insertion for L1 blind blocks so local anaesthetic is injected only into the TAP?
- How are the course, variations and nerve depth of the thoracolumbar nerves influenced by Body Mass Index (BMI), if at all?

1.4 Aims and objectives

1.4.1 Aims

This study was divided into three aims. The first aim was to determine the course and branching patterns of the thoracolumbar nerve plexus (T7-L1) in the TAP after emerging from the costal margins, by using landmarks such as the mid-axillary line (MAL), as well as the costal margins, iliac crest, and surface of the skin. This study
also aimed to determine the position where the L1 nerve divides into its two terminal branches and the general position where these branches enter the TAP. Additionally, this study aimed to investigate the relationship between BMI and TAP plexus positioning so as to improve TAP block accuracy.

1.4.2 Specific objectives

The objectives of the study were as follows:

- To determine the course of the thoracolumbar nerves (T7-T12, L1) in the transverse abdominal plane by cadaver dissection.
- To determine the position where the L1 nerve splits into its two main branches with cadaver specimens.
- To determine the position where the iliohypogastric and ilioinguinal nerves enter the TAP after piercing the TAM by measuring the nerves at constant intervals in relation to specific landmarks.
- To determine the depth of the TAP through ultrasound in live volunteers of a healthy and overweight BMI.
Chapter 2: Literature review

In the following section the typical anatomy of the TAP is discussed before elaborating on the clinical importance of the current study. This includes background on peripheral nerve blocks, ultrasonography, the TAP block, and blocks of the L1 nerve terminal branches.

2.1 Typical anatomy of the transverse abdominal plane

The TAP is formed between the IOM and the TAM (Figure 2.1). A neurovascular plexus is formed in the plane and contains the terminal branches the inferior six intercostal nerves (T6-T11), the subcostal nerve (T12), and the first lumbar nerve (L1) (Rozen et al., 2008).

![Figure 2.1: Lateral view of the TAP nerve plexus superficial to the transverse abdominal muscle (Toronto Western Hospital, 2008).](image)

As T7-T11 branches proceed anteriorly within the TAP, they eventually exit the plane to enter the rectus sheath posterior to the rectus abdominis muscle, although T12-L1 branches have also been documented within the rectus sheath (Rozen et al., 2008). Together these thoracolumbar nerves innervate the lateral abdominal wall, the anterior...
abdominal wall (including the rectus abdominis muscle), and the inguinal region (Figure 2.2) (Sharkey et al., 2013; Standring, 2008; Sviggum et al., 2012). It is important to note that even though the neurovascular plexus runs in the plane, it has been found to be predominantly deep to the fascial layer inside the TAP (Rozen et al., 2008).

![Figure 2.2: Innervation of the thoracoabdominal nerves (T7-L1). The shaded area (T10-L1) indicates the area anesthetised in the typical posterior TAP block (Mukhtar, 2009).](image)

The TAP can be easily accessed through the triangle of Petit, formed by the iliac crest inferiorly, the posterior border of the external oblique muscle (EOM) anteriorly, and the anterior border of the latissimus dorsi muscle posteriorly (Figure 2.3). The IOM forms the floor of this triangle (Standring, 2008). Two anatomically important structures giving blood supply to the region, runs in relation to the TAP plexus, the deep inferior epigastric artery and the deep circumflex iliac artery (Sharkey et al., 2013).
Figure 2.3: The formation of the triangle of Petit, an important landmark for the TAP block.

The L1 nerve differs from the rest of the nerves involved in the TAP nerve plexus as it originates from the lumbar plexus. The nerve typically has two terminal branches, the iliohypogastric and the ilioinguinal nerves. This bifurcation normally occurs before the nerves emerge at the lateral border of psoas major muscle. After this emergence, the iliohypogastric and ilioinguinal nerves pierce the TAM to enter the TAP where they can remain for a variable distance before exiting the TAP by piercing the IOM (Figure 2.4).
Typically, the iliohypogastric nerve pierces the TAM in the region superior to the iliac crest. The exact location where this event takes place is disputed (Klaassen et al., 2011; Reinpold et al., 2015; Sviggum et al., 2012). The course of the ilioinguinal nerve is better described and most sources agree that the ilioinguinal nerve would pierce the muscle specifically superior to the anterior region of the iliac crest (Standring, 2008).

### 2.2 Clinical importance

#### 2.2.1 Peripheral nerve blocks

##### 2.2.1.1 History of peripheral nerve blocks

Peripheral nerve blocks allow for the administration of local anaesthesia to a specific region (Longnecker et al., 2012). The first documented cases of injection of an anaesthetic agent as a peripheral blockade was in the mid to late 19th century (Hadzic, 2007; Miller et al., 2014). Cocaine as a local anaesthetic started out with Sigmund Freud passing a sample along to a colleague who soon discovered its use for eye
surgeries in the form of a suspension. Although this was a breakthrough at the time, it did not involve injection of the substance. A few years later, another ophthalmologist, N.J. Hepburn, gave himself subcutaneous injections to test the efficacy of the drug (Hadzic, 2007).

In the 1880’s, researchers W.S. Halsted and R.J. Hall injected cocaine into sites including the ulnar and musculocutaneous nerves in the upper limb, and into the infraorbital and supratrochlear nerves in the face (Miller et al., 2014). Injection of a local anaesthetic has moved away from cocaine since then and now aids in reducing the amount of anaesthetic needed while lowering the risks and complications associated with general anaesthesia, such as nerve damage, brain damage, or perioperative death due to respiratory or cardiac complications (Butterworth et al., 2013; Longnecker et al., 2012).

2.2.1.2 Common nerve blocks

Multiple nerve blocks have been developed according to need. Common nerve blocks used today can be divided according to body region. Popular upper limb nerve blocks include the interscalene block, supraclavicular and infraclavicular blocks, axillary block, and several terminal nerve blocks (Butterworth et al., 2013; Miller et al., 2014). These blocks are generally easily accessible with landmark techniques or simple ultrasound modifications and deliver all the advantages of regional anaesthesia over general anaesthesia.

Lower limb nerve blocks target the nerves from the lumbar and sacral nerve plexuses. The femoral nerve can be targeted by more than one block. The 3-in-one block targets the femoral nerve, the lateral femoral cutaneous nerve, as well as the obturator nerve in one technique, whereas the femoral nerve block targets the femoral nerve only. Other popular lower limb nerve blocks include the parasacral and sciatic nerve blocks (Butterworth et al., 2013; Miller et al., 2014).

For trunk blocks, intercostal blocks and paravertebral blocks are regularly done (Butterworth et al., 2013; Miller et al., 2014). The TAP block is generally used for postoperative pain management following abdominal procedures (McDonnell et al.,
The TAP block procedures are described later on.

2.2.1.3 Advantages and disadvantages of nerve blocks

Nerve blocks can be administered as a single injection, or as a continuous peripheral nerve block (also called perineural local anaesthetic infusion). The latter is used in the case of chronic pain management or in opioid tolerance (Butterworth et al., 2013).

The first nerve blocks were all blind blocks based on anatomical landmarks to determine the needle insertion point and then depending on “clicks and pops” to establish the correct depth of the needle (Figure 2.5) (Longnecker et al., 2012). Several modifications have been introduced for needle localisation, the first of which is nerve stimulation. This technique uses electrical stimulation of a nerve to determine if the needle has been placed in the correct position. Nerve stimulation does not provide a method of visualising the nerve, but it can be combined with some of the later techniques such as ultrasound-guidance (Butterworth et al., 2013; Miller et al., 2014). Possible complications for nerve blocks (as regional anaesthesia techniques) include intravascular injections, infection, and block failures (Sviggum et al., 2012).

In an attempt to reduce noted failure rates of blind nerve blocks, visualisation techniques such as ultrasound-guidance, fluoroscopy, and computed tomography (CT) scans have been added to different procedures (Miller et al., 2014; Soneji and Peng, 2013). Ultrasound-guidance is by far the most widely used visualisation technique and can be combined with nerve stimulation if deemed necessary (Butterworth et al., 2013; Soneji and Peng, 2013).
2.2.2 Ultrasonography

2.2.2.1 General mechanism of ultrasonography

Ultrasonography uses sound waves that are above human audibility (greater than 20 kHz) to generate an image of tissues. Because these sound waves have a shorter wave length, it is easier to visualise smaller objects due to less diffraction of the waves (Giancoli, 2008; Miller et al., 2014). For medical imaging, the waves are typically 1-20 MHz (Miller et al., 2014). A transducer receives an electrical impulse which it converts into and sends out as a sound wave using a pulse-echo technique. As the sound wave comes into contact with the body’s different tissues, parts of the pulse is reflected back to the transducer while the main pulse continues. The depth is calculated by the time it takes for the reflected sound waves to return to the transducer. An image is formed based on the reflected sound waves (Giancoli, 2008). Structures that reflect more sound waves, such as bone, appear whiter on the image (hyperechoic), while structures that allow more sound waves to pass through, such as blood, appear darker (hypoechoic) (Butterworth et al., 2013).
The transducer type used will influence the image generated as a linear array transducer will produce a higher quality image for the same specifications as a curvilinear transducer. However, the curvilinear transducer provides a wider view (Figure 2.6) (Miller et al., 2014). Furthermore, higher frequency transducers will produce a higher quality image, but lack penetration power and therefore is used more for superficial structures (Butterworth et al., 2013).

![Figure 2.6: Ultrasound transducer types. A: Linear array transducer. B: Curvilinear transducer (Hadzic and NYSORA, 2012).](image)

### 2.2.2.2 Ultrasonography in regional anaesthesia

Ultrasound-guidance of peripheral nerve blocks was first introduced with the supraclavicular block by P. La Grange in 1978 (Hadzic and NYSORA, 2012; Miller et al., 2014). Ultrasound imaging ultimately allows for visualisation of adjacent structures and spaces which permits injection of local anaesthetic without damaging the nerve or any significant surrounding structures. Viewing peripheral nerves on an ultrasound involves looking at the fascicles covering the nerve, but differentiating nerves from other structures requires a trained eye. It is important to keep in mind the typical anatomy as well as known anatomical variations. It can also be challenging to
differentiate between a tendon and a nerve without high resolution ultrasound imaging (Miller et al., 2014).

There are several advantages to using ultrasound guidance. It allows for real time visualisation of needle placement leading to a decreased risk in damaging the nerve or other associated structures, and it aids in delivering local anaesthesia despite unexpected anatomical variations (Soneji and Peng, 2013). It also visualises the spread of the local anaesthesia in real time, allowing for modification of needle placement if needed (Longnecker et al., 2012; Wells, 2010). Visualisation of the local anaesthetic further allows for modification of the volume, leading to a decrease in injected volume when compared to blind nerve blocks (Butterworth et al., 2013). A decrease in anaesthetic volume is associated with a reduced risk for toxicity (Longnecker et al., 2012). Ultrasound is also more affordable than other imaging modalities and is portable and non-invasive (Soneji and Peng, 2013; Wells, 2010).

As with any technique or procedure, there are also limitations to ultrasound guidance. Structures deep to or obscured by bone cannot be visualised as bone has a high absorption coefficient (Miller et al., 2014; Soneji and Peng, 2013). Visualisation of deeper structures can require a curvilinear probe with a lower frequency, which will reduce the quality of the image produced. Ultrasonography is also very dependent on the user with structures such as peripheral nerves being difficult to identify and it being challenging to keep the needle in the correct plane related to the transducer. Failure to keep the typical anatomy as well as known anatomical variations in mind can also lead to misunderstanding of the image (Soneji and Peng, 2013). In developing countries such as South Africa, an ultrasound machine is not always readily available in the public sector. When a machine is available, there is no guarantee in the quality. This elicits the need to improve the existing blind blocks.

2.2.3 TAP block

The TAP block, first described in 2001 (Rafi, 2001) and subsequently modified for ultrasound-guidance (Hebbard et al., 2007), has undergone a great deal of investigation and has various clinical uses due to its relative simplicity and use in post-operative pain management (McDonnell et al., 2008; Sharkey et al., 2013; Wheble et al., 2015; Zhong et al., 2014). Both the landmark method and the first ultrasound
approach use the triangle of Petit, also known as the inferior lumbar triangle, as an important reference point (Hebbard et al., 2007; Rafi, 2001), with the landmark relying on fascial “pops” felt when a blunt needle is used (Rafi, 2001; Sharkey et al., 2013; Young et al., 2012). The landmark TAP block requires inserting the needle into the superior aspect of the triangle of Petit, while the ultrasound-guided TAP block uses the triangle as a landmark for the initial probe placement (Figure 2.7).

Figure 2.7: TAP block needle insertions. Left: The landmark TAP block. Right: The ultrasound-guided TAP block (Lissauer et al., 2014). Key: CM = costal margin; EO = external oblique muscle; IC = iliac crest; LD = latissimus dorsi muscle; MAL = mid-axillary line.

Previous studies have reported an absence of the triangle of Petit in 17.5% of hemi-abdomens, and this problem is often compounded in overweight and obese individuals (Sharkey et al., 2013; Walter et al., 2008). Some studies have reported the “double pop” technique is effective for accurate needle placement (McDonnell et al., 2007; Young et al., 2012), however others report the “pops” required for the landmark method is often not distinctly felt (Young et al., 2012). The ultrasound methods have shown great promise in the clinical setting, but it is important to keep the anatomy of the region in mind to further increase the efficacy of the procedure.

The ultrasound-guided TAP block has already been shown to be very useful and versatile in its uses with very few reported complications (Hebbard et al., 2007). The biggest difference with the ultrasound modification is the angle of the needle. In the
original “blind” method, the needle is perpendicular to the skin, and in so doing the plane, whereas with the ultrasound, the needle is inserted from the anterior into and along the plane. This allows the needle to be visualised longitudinally as well as reducing the risk of inserting the needle into the peritoneal cavity (Abrahams et al., 2010; Hebbard et al., 2007).

To date, there have been two reported cases of liver damage following TAP blocks. One of which was a blind block, while the other used the ultrasound-guided technique (Farooq and Carey, 2008; Lancaster and Chadwick, 2010). Although the ultrasound guidance has many advantages, it has been shown to have a reduced coverage when compared to the blind technique which has a complete coverage of T9 to L1 between the costal margin and iliac crest (Figure 2.2). This reduction has led to several modifications of the original method, each with a different anaesthetic coverage (Abdallah et al., 2012; Lissauer et al., 2014). Confusingly, many of these modifications that use essentially the same method, have different names according to authors (Lissauer et al., 2014).

The large amount of subcutaneous adipose tissue in obese individuals makes ultrasound-guided block difficult. This adipose layer, located between the skin and the EOM, impedes identification of the correct layers for needle placement (Standring, 2008; Toshniwal and Soskin, 2012). The increased depth also complicates the needle-beam angle, as it becomes more difficult to have an angle greater than 55 degrees. It has been recommended that an obese patient should lie in a semilateral position instead of the standard supine position, allowing for easier real-time ultrasound imaging as the subcutaneous fat is displaced more medially, thus leading to less adipose tissue in the path of the beam (Toshniwal and Soskin, 2012).

The most common indications for TAP blocks are for post-operative pain management and it plays an integral part in lowering opioid utilisation (McDonnell et al., 2008; Sharkey et al., 2013). The TAP block is also considered a feasible substitute in cases where an epidural in contra-indicated (Findlay et al., 2012; Hebbard et al., 2007). As previous studies on the injectate spread suggest that the block is volume dependent (Sviggum et al., 2012), the use of ultrasound reduces the volume needed, as discussed above. Other procedures where the thoracolumbar nerves are blocked include abdominal wall reconstructive flaps (Rozen et al., 2008) and lumbotomy (Van
Der Graaf et al., 2011), although the block does not seem to improve post operative pain scores following inguinal hernia repair (Petersen et al., 2013). As the ilioinguinal nerve, a terminal branch of the L1 nerve, travels in the inguinal canal (Standring, 2008), this is addressed by an L1 or an ilioinguinal/iliohypogastric block.

### 2.2.4 Blocking of L1 branches

Blocks of the terminal branches of L1 are commonly used in ambulatory procedures below the umbilicus, including herniorrhaphy surgery and gynaecological surgery. Much like the TAP block, blocks of the L1 branches aid in post-operative pain management and decrease opioid consumption (Sviggum et al., 2012). Although there is a use for blind ilioinguinal and iliohypogastric nerve blocks, there are relatively high failure rates (10%-40%) associated with the blind block which requires large injection volumes as 86% of the local anaesthetic is administered into muscle (Finnerty et al., 2010; Soneji and Peng, 2013; Sviggum et al., 2012). Damage to L1 and its terminal branches can cause decreased sensation, as well as groin pain (Tagliafico et al., 2014). The ultrasound guided versions of these blocks have been used in an attempt to improve the failure rate.

A recent study had confirmed that it is possible to properly visualise the terminal branches of L1 and the related structures with ultrasound, not just the plane where the nerve are supposed to lie, but it requires high-resolution ultrasound (Tagliafico et al., 2014). The exact position where the L1 branches exit the TAP to enter the plane between the IOM and EOM is variable (Tagliafico et al., 2014).

The guidelines for the blind block require needle insertion 2 cm medial and superior to the anterior superior iliac spine (ASIS) and then injecting into both the plane between the EOM and the IOM, and into the TAP between the IOM and TAM to counteract the variability in the nerves’ positions (Sviggum et al., 2012). Injecting this far anteriorly into both spaces could potentially be improved by inserting the needle more posteriorly and only injecting into the TAP. With the normal positioning of the probe in the ultrasound-guided technique, the L1 branches can be seen either in the TAP or between the IOM and EOM. It is often suggested that the nerves be found posterior to the ASIS, where they should be in the TAP, and then follow the nerves more anteriorly with the ultrasound probe (Sviggum et al., 2012).
Chapter 3: Materials and Methods

This study consisted of two samples: a cadaver aspect, using the abdomens of 54 embalmed cadavers, and an ultrasound aspect, using abdominal scans from 43 live volunteers. Furthermore, the cadaver aspect was divided into the TAP component and the L1 nerves component, while the ultrasound aspect only analysed the TAP aspects of this study. As all measurements for both aspects were done bilaterally, if no significant difference was seen between the left and right sides, the data was pooled together to increase the sample size. All cadavers were handled in accordance with the National Health Act, nr. 61 of 2003. All live volunteers for the ultrasound gave written informed consent. All personal information is being kept confidential and anonymous.

3.1 Materials

3.1.1 Cadaver aspect

Fifty four embalmed cadavers, obtained from the University of Pretoria and housed at the Department of Anatomy (Prinshof campus), were used in this study. In total, 95 needles were inserted into cadavers. Needle insertion of cadavers was done as with the conventional TAP injection methods (section 3.2.1.1. Nerve plexus in the TAP) after embalming and before dissection, and were used to determine the course and branching of nerves. It was noted if the needle was in the correct plane, whether it pierced any branches of the TAP plexus, and if not, how close the needle came to the closest nerve.

Results from the left and right sides were grouped together where no statistical difference was found between sides. The sample was divided into two weight groups based on BMI. The weight groupings, consisted of 40 healthy weight individuals (80 hemi-abdomens, 18-25 kg/m$^2$) and 14 overweight individuals (28 hemi-abdomens, 25-35 kg/m$^2$). The height and weight of each cadaver was determined post-mortem at the University of Pretoria before embalming took place. The heights and weights were used to calculate BMI with the standard formula for area density, mass (in kg) divided by height squared (m$^2$), to give a value measured in kg/m$^2$. Normally a healthy BMI
falls between 18-25 kg/m² (Boron and Boulpaep, 2008). The exact BMI between overweight and obese is under debate by clinicians, with 30 kg/m² being the most widely acceptable cut-off for overweight. For practicality, the upper limit for overweight in this study was taken as 35 kg/m². The mean BMI of cadavers falling under healthy was 21.29 kg/m² and a BMI of 28.81 kg/m² for overweight cadavers.

### 3.1.2 Ultrasound aspect

Forty three live volunteers, divided into the same BMI groups as the cadavers, were used in the ultrasound study. Ultrasounds were taken bilaterally on 21 volunteers of a healthy BMI (7 males and 14 females), and 22 volunteers of an overweight BMI (12 males and 10 females). Volunteers were only used in the ultrasound aspect of this study and were recruited from the University of Pretoria. The mean BMI of the volunteers in the normal BMI group was 22.40 kg/m², while the mean for the overweight BMI volunteers was 29.18 kg/m².

### 3.1.3 Inclusion and exclusion criteria

Both cadavers and volunteers for the ultrasound aspect of the study had to adhere to the following inclusion and exclusion criteria:

- All individuals must be 18 years or older.

- Any scars, trauma, or previous surgical procedures in the inguinal area were grounds for exclusion from the study as this could have disturbed the natural course of the nerves.

- The BMI values of all individuals must fall between 18 and 35 kg/m².
3.2 Methods

3.2.1 Cadaver aspect

3.2.1.1 Nerve plexus in the TAP (T7 - T12, L1)

The technique for a standard blind TAP block (Rafi, 2001) was used to insert a needle bilaterally into the abdomen of the cadavers. The triangle of Petit was first palpated and the needle was then inserted into this triangle until two fascial pops were felt. The first pop indicates the piercing of the external oblique fascia (covering the IOM) and the second pop indicates the piercing of the internal oblique fascia (Young et al., 2012). The needle was only inserted to the same depth as in a clinical setting, to ensure accuracy of findings and to avoid puncturing any abdominal organs. The needle was temporarily fixed to the skin using superglue. The skin was then carefully removed, but leaving behind a square centimetre of skin around the needle, to expose the lateral abdominal area between the costal margin and the iliac crest. Hereafter, the EOM and the IOM were reflected to expose the TAP with the neurovascular structures inside the fascial layer. The thoracolumbar nerves were carefully exposed in the TAP fascial layer without moving the needle and without disturbing the natural course of the nerves (Figure 3.1).

Figure 3.1: Photo of TAP dissection from lateral view. The TAP was exposed between the costal margin and the iliac crest, leaving a one square centimetre piece of skin and underlying muscle layers surrounding the needle. The ASIS was indicated with a coloured pin and a scale was placed in frame.
Photos were taken from an anterior and a lateral view with a scale in frame for digital evaluation. These computer analyses, done after all dissections had been completed, enabled the precise calculation of the course of each nerve and its branches running inside the TAP. The most prominent point of the ASIS was marked by a coloured pin. At this stage, the number of nerves visible on the lateral aspect of the dissection (in the MAL), as well as at the linea semilunaris, was noted. After this, the square centimetre piece of skin surrounding the needle, and the muscle layers deep to it, was carefully dissected to determine the depth of the needle tip by noting which muscle layers had been pierced. In the event where the needle pierced the TAM, the distance from the needle to the closest nerve in the TAP was measured digitally from detailed photographs with a scale in frame (Figure 3.2).

Figure 3.2: Closest measurement from position where needle pierced TAM to the closest nerve (line a).

All digital measurements from photographs were done after calibration using Adobe Photoshop CS6 extended, version 13.0.1 x32 (Adobe Inc.). The course of the nerves running in the nerve plexus was analysed from photographs according to the criteria shown in the flow chart below (Figure 3.3). This takes into account the interactions between individual nerves. Interactions include nerve branching, mergers of individual nerves or their branches, either with the same nerve or other nerves and branches, as well as the anatomical position of the mergers.
3.2.1.2 Ilioinguinal and iliohypogastric nerves (L1)

Once the abdominal cavity had been opened, L1 and its terminal branches, the ilioinguinal and iliohypogastric nerves, were exposed in the posterior abdominal wall through careful dissection. The nerves were marked with coloured pins. Photos were taken from an anterior view with a scale present in frame. The roots of the nerves were then exposed, marked with pins, and photos taken again. The images were digitally analysed, using the same software as for the TAP component, to determine the root values of L1, the general positions where L1 divided into the two branches, and where these branches pierced the TAM to enter the TAP. Accidental anatomical findings were noted.
3.2.2 Ultrasound aspect

Written informed consent was obtained from each volunteer prior to participating in the study. Volunteers were asked to lie in a lateral recumbent position while an ultrasound image was obtained using the standard posterior approach (Hebbard et al., 2007). A SonoSite Edge Ultrasound system was employed with a low frequency, curvilinear transducer (SonoSite C60x/5-2 MHz 2013-03; SonoSite Inc., Bothell, WA, USA). The three layers of the abdominal muscles (EOM, IOM, and TAM) were first identified in the ultrasound image before the transducer was moved to the position necessary to perform an ultrasound-guided TAP block.

Once the TAP had been identified, several measurements were done at two sites visible on the image (Figure 3.4). The first site was at the tendon junctions of the IOM and TAM, and the second site was the position where an ultrasound-guided TAP block is typically administered, as judged by a consultant anaesthesiologist. Measurements were performed as follows (Figure 3.4): Distance between skin and injection point or the depth of TAP (line a); Distance between skin and tendon junction of IOM and TAM (line b); Distance between sites (line c); Subcutaneous fat thickness (line d); EOM thickness (line e); IOM thickness (line f). All digital measurements from ultrasounds were done using Adobe Photoshop CS6 extended, version 13.0.1 x32 (Adobe Inc.) after calibration. This data was analysed and compared between the separate BMI groups.

Figure 3.4: Ultrasound measurements taken at the typical injection point in the TAP, and the tendon junction of the TAM and the IOM. Key: SF = Subcutaneous fat; EOM = External oblique muscle; IOM = Internal oblique muscle; TAM = Transversus abdominis muscle.
3.3 Statistical analysis

Descriptive statistics in the form of means, medians, ranges, quartiles, minimums, maximums, standard deviation, variance skewness and 95% confidence intervals (CIs) were calculated for measurements taken using Microsoft® Office Excel 2013. All other statistical analyses were done using IBM SPSS Statistics, version 20.0 (IBM Corp). Personal information taken included the age, sex, height, weight, and BMI for both cadavers and live volunteers. Handedness was also noted in volunteers.

Statistically significant differences in data between sides, as well as between healthy and overweight BMI categories, were determined according to data type. Left and right sides were treated as dependent groups as they were from the same individual, whereas the differences between healthy and overweight BMI groupings were taken as independent groups because they were done on different individuals. With left and right sides, continuous data was analysed by a paired samples t-test, ordinal data by a Wilcoxon signed rank test, and categorical data by a McNemar test. With healthy and overweight BMI groupings, continuous data was analysed by an independent samples t-test, ordinal data by a Mann-Whitney U test, and categorical data by a chi-squared test. A default threshold of 5% was used to evaluate significance, with \( p < 0.05 \) being considered as significant. For both the cadaver and ultrasound aspect, intra- and inter-rater statistics were performed after repeating the photos and digital analyses on a sample of ten abdomen halves.

3.4 Ethical considerations

Cadavers used in this study were obtained from the Department of Anatomy, University of Pretoria. All cadaveric material was handled in accordance with the National Health Act, nr. 61 of 2003. This study also made use of volunteers from the University of Pretoria. All volunteers fit the inclusion criteria required for the study. The researcher obtained permission from the head of the School of Medicine, Prof Lindeque, as well as the Dean of Students, Dr Madiba.

Informed consent was obtained from all participants (Appendix A). Ethical clearance was obtained on 14/05/2014 from the University of Pretoria’s Faculty of Health
Sciences Research Ethics Committee under protocol number 73/2014. Approval was also obtained from the University of Pretoria's Faculty of Health Sciences MSc committee on 26/03/2014.

Information regarding the volunteers’ and cadavers’ sex, age, height and weight, as well as handedness in volunteers only, was noted (Appendices B1 and B2). No other personal information was obtained. All information is being kept confidential and anonymous. Each volunteering participant received a total of R50.00 as travel compensation.
Chapter 4: Results

4.1 Cadaver aspect

4.1.1 TAP component

By increasing the knowledge of the anatomy of the TAP, the efficacy of the blind TAP block could be increased. In order to analyse the anatomy, the number of nerves observed, needle position and branching pattern of the nerve plexus was described.

4.1.1.1 Comparisons between sides and BMI groupings

Three different statistical analyses were performed between left and right and between healthy and overweight, depending on the data type. Categorical data included whether the needle pierced each of the three muscle layers and if the needle pierced a nerve. This data was analysed for differences between sides by a McNemar test, and between BMI groups by a chi-square test of independence. Ordinal data was the number of nerves at the MAL and at the linea semilunaris. The difference between sides was analysed by a Wilcoxon signed ranks test, and between weight groups a Mann-Whitney U test was used. The only measurement giving continuous data was the distance between the needle and the closest nerve. For the continuous data, a paired $t$-test was used to compare between sides, and an independent samples $t$-test was done between weight groups. In terms of all of the data for the TAP aspect, no significant difference was seen between sides, allowing for the pooling of data to increase sample size. No statistically significant difference was seen between BMI categories.

4.1.1.2 Number of nerves

To aid in the general description of the TAP nerve plexus, the number of nerves present at the MAL as well as at the linea semilunaris was noted. The mean number of nerves at the MAL was 4.40 (median 4 nerves, SD 1.3 nerves), while the mean at the linea semilunaris was 5.43 (median 5 nerves, SD 1.4 nerves). The minimum and
maximum number of nerves seen at both sites were two and nine nerves, respectively. The frequencies in the number of nerves at each site is shown below (Figure 4.1).

![Figure 4.1: Frequency of the number of nerves present at the MAL and the linea semilunaris.](image)

A significant difference was observed between the number of nerves present at the MAL and the linea semilunaris (Wilcoxon signed ranks test: $Z = -5.778$, $p < 0.05$). More nerves were found at the linea semilunaris than at the MAL. Despite this difference, samples with a larger number of nerves at the MAL tended to have a larger number of nerves at the linea semilunaris, as shown with the Spearman’s rank correlation coefficient. A moderate positive correlation was observed, indicating a significant relationship between the two variables (Spearman’s rank correlation coefficient: $\rho(85) = 0.515$, $p < 0.01$).

### 4.1.1.3 Needle position

In terms of the needle tip position, out of 92 analysed needles, the needle was in the correct plane in only 7.6% ($n = 7$). For the remaining cases the needle pierced the TAM in 79.3% ($n = 73$), the EOM in 9.8% ($n = 9$), and did not reach the EOM in 3.3% ($n = 3$). The needle depth was compared with BMI category by means of a chi-square
test. No significant relationship was found ($\chi^2(3) = 0.491, p > 0.05$). Needle depth and BMI category seemed to be independent of one another.

The needle pierced a thoracolumbar nerve in the TAP in 6.5% of cases ($n = 6$). Of the cases where the needle pierced the TAM without piercing a nerve, the mean distance of the needle to the closest nerve was 5.70 mm (median = 4.40 mm, SD 4.62 mm). The true mean is between 4.56 mm and 6.83 mm with 95% confidence. During dissection, it was noted that the nerves of the TAP plexus were consistently deep to the fascial layer of the plane, with the blood vessels more often superficial than deep. Despite the blood vessels being more superficial than the nerves, no damaged blood vessels were noted. Intra-rater and inter-rater statistics showed significant strong correlations for all cadaver measurements.

### 4.1.1.4 Branching pattern

The branching pattern of the nerve plexus observed in the current study showed several patterns of nerve interaction before reaching the linea semilunaris. A single nerve can continue from the MAL to the linea semilunaris without branching or interacting with any other nerves. One nerve can merge with an adjacent nerve, without branching, before the newly merged nerve reaches the linea semilunaris. One nerve can divide with one branch merging with an adjacent nerve before or at the linea semilunaris while the main branch continues without interacting with any other nerves. Two adjacent nerves can divide into two with the adjacent branches coming together at the linea semilunaris. Different possibilities encountered for nerve branching and interaction are shown in below (Figure 4.2). It was often noted that the nerves seen closest to the needle, gave off multiple branches.
Figure 4.2: Observed branching patterns for the TAP plexus. Top: Two adjacent nerves divide into two branches (Br), only for the two adjacent branches to merge (BrBrM), effectively increasing the number of nerves at the linea semilunaris. Bottom: A nerve merges with the branch of an adjacent nerve (BrNM), only to merge again with a nerve from the other side (NNM). Key: ASIS = Anterior superior iliac spine; Br = Branching; BrBrM = Merger of two branches; BrNM = Merger of an unbranched nerve and a branch of a nerve; NNM = Merger of two unbranched nerves.
4.1.2  L1 component

4.1.2.1  Root contributions

Concerning root contribution, a significant difference was seen between left and right sides ($\chi^2(9) = 29.193, p = 0.001$). Overall, a root contribution from L1 only was seen in 71% of cases ($n=41$). When breaking this down into sides, L1 was the only root contribution on the left in 60% of cases ($n=17$), and on the right in 80% of cases ($n=24$). A noticeable difference between sides was seen in the frequency of both a T12 and an L1 root contribution, with left having 32% (9 cases), and right having only 3% (1 case). For L1 and L2 contributions, there were no cases on the left and 4 cases (13%) on the right side. No cases were seen with a root contribution of L2 only. These results are shown in Table 4.1 below.

<table>
<thead>
<tr>
<th></th>
<th>Frequency % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall (n=58)</td>
</tr>
<tr>
<td>L1 only</td>
<td>70.7 (41)</td>
</tr>
<tr>
<td>T12 only</td>
<td>3.5 (2)</td>
</tr>
<tr>
<td>T12 and L1</td>
<td>17.2 (10)</td>
</tr>
<tr>
<td>L1, L2</td>
<td>6.9 (4)</td>
</tr>
<tr>
<td>T12, L1, L2</td>
<td>1.7 (1)</td>
</tr>
</tbody>
</table>

Left and right sides for each of the variables after origin of the root of the nerve was compared using McNemar’s test (Table 4.2). None of these showed a significant difference between sides, allowing the grouping of data for an increase in sample size.
Table 4.2: Frequencies for L1 variables overall and between sides.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency %</th>
<th>P-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall (n/total)</td>
<td>Left (n/s)</td>
<td>Right (n/s)</td>
</tr>
<tr>
<td>L1 divides</td>
<td>67.2 (39/58)</td>
<td>71.4 (20/28)</td>
<td>63.3 (19/30)</td>
</tr>
<tr>
<td>L1 divides lateral to psoas major muscle</td>
<td>43.6 (17/39)</td>
<td>45 (9/20)</td>
<td>42.1 (8/19)</td>
</tr>
<tr>
<td>Undivided L1 pierces with 4th lumbar a.</td>
<td>35.3 (6/17)</td>
<td>28.6 (2/7)</td>
<td>40.0 (4/10)</td>
</tr>
<tr>
<td>IH nerve pierces with 4th lumbar a.</td>
<td>21.6 (8/37)</td>
<td>26.3 (5/19)</td>
<td>16.7 (3/18)</td>
</tr>
<tr>
<td>IL nerve pierces with 4th lumbar a.</td>
<td>32.4 (11/34)</td>
<td>38.9 (7/18)</td>
<td>25.0 (4/16)</td>
</tr>
</tbody>
</table>

Key: n_f = occurrences of observed variable; n_s = sample size for observed variable; a. = artery; IH = iliohypogastric nerve; IL = ilioinguinal nerve.

4.1.2.3 Branching pattern

L1 was seen to divide into its terminal branches before piercing the TAM in 67.2% of cases (n = 39 out of 58). When L1 had divided before piercing the TAM, it did so either proximal to emerging from the lateral border of psoas major, as seen in 56.4% cases (n = 22), or distal to the lateral border, seen in 43.6% of cases (n = 17). In the event where L1 did not divide before exiting the abdominal cavity, L1 pierced the TAM at the same place as the fourth lumbar artery in 35.3% of cases (n = 6). When L1 divided into its terminal branches, it was noted that both the ilioinguinal and the iliohypogastric branches pierced TAM in company of the fourth lumbar artery in 8.1% of cases (n = 3), the iliohypogastric nerve only in 13.5% of cases (n = 5), and the ilioinguinal nerve only in 21.6% of cases (n = 8). Overall it was noted that either L1 itself (when it did not
divide) or one of its branches, pierced the TAM at a point in relation to the fourth lumbar artery in 40.7% of cases (n = 22).

4.1.2.4 Accidental findings

Several accidental anatomical findings were seen regarding the L1 aspect of the study. In two cases the iliohypogastric nerve had a root value of L1 while the ilioinguinal branch had a root value of L2. In two cases the L1 nerve branched twice to give three terminal branches which exited the abdominal cavity. Also, in two cases no L1 nerve could be found, but an enlarged T12 nerve seemed to compensate for the absence. Some single case of anomalies were seen: the iliohypogastric nerve originating from L1 and ilioinguinal from L1, L2; ilioinguinal nerve piercing psoas major; L1 dividing and remerging deep to psoas major before dividing again lateral to psoas major; and an accessory ilioinguinal was found between ilioinguinal nerve and the lateral femoral cutaneous nerve with a root value of L1, L2 while the iliohypogastric and ilioinguinal had a root value of T12, L1 on the same cadaver.

4.2 Ultrasound aspect

By evaluating the ultrasound anatomy, the efficacy of both the landmark technique and the ultrasound-guided TAP blocks could be improved. The ultrasound aspect looked at the depth of the TAP in live volunteers, as well as muscle thickness.

4.2.1 Comparisons between sides and BMI groupings

4.2.1.1 Left and right sides

The left and right sides were compared with a paired-samples t-test (Table 4.3). Two measurements showed a significant difference between left and right: the depth of the typical injection point ($t(42) = 3.907$), and the EOM thickness at the injection point ($t(42) = 2.791$). All other measurements showed no significant difference between the left and right sides and was pooled (n = 86). For the distance between the skin and the injection point, the mean distance on the right was 30.81 mm (SD 7.13 mm) and the mean distance on the left was 27.97 mm (SD 6.69 mm). For the EOM thickness at
the injection point, the mean on the right was 10.77 mm (SD 3.53 mm) and the mean on the left was 9.50 mm (SD 3.36 mm). A significant strong positive correlation was found between left and right for the depth of the injection point ($r(43) = 0.770, p = 0.000$). A significant moderate positive correlation was found between left and right for the EOM thickness at the injection point ($r(43) = 0.626, p = 0.000$). A one-way multivariate analysis of variance was done to evaluate the effect of handedness on the EOM thickness at the injection point between left and right. No significant difference could be found ($\text{Lambda}(2,15) = 0.793, p = 0.176$). The junction of the IOM and the TAM tendons appears as a hyperechoic dot on an ultrasound (Figure 4.3).

Table 4.3: Difference between left and right sides.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Left</th>
<th>Right</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Depth: Skin to tendon junction</strong></td>
<td>34.55</td>
<td>37.02</td>
<td>0.066</td>
</tr>
<tr>
<td><strong>EOM thickness at tendon junction</strong></td>
<td>21.12</td>
<td>23.11</td>
<td>0.142</td>
</tr>
<tr>
<td><strong>Subcutaneous fat at tendon junction</strong></td>
<td>11.51</td>
<td>12.16</td>
<td>0.182</td>
</tr>
<tr>
<td><strong>Depth: Skin to injection point</strong></td>
<td>27.97</td>
<td>30.81</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>IOM thickness at injection point</strong></td>
<td>9.15</td>
<td>9.95</td>
<td>0.133</td>
</tr>
<tr>
<td><strong>EOM thickness at injection point</strong></td>
<td>9.50</td>
<td>10.77</td>
<td>0.008*</td>
</tr>
<tr>
<td><strong>Subcutaneous fat at injection point</strong></td>
<td>8.94</td>
<td>9.42</td>
<td>0.100</td>
</tr>
<tr>
<td><strong>Tendon junction to injection point</strong></td>
<td>33.60</td>
<td>33.18</td>
<td>0.765</td>
</tr>
</tbody>
</table>

*Significant difference using paired samples $t$-test ($\alpha=0.05$).
4.2.1.2 BMI groupings

All measurements (except for subcutaneous fat at tendon junction) for which a significant correlation with BMI value was observed, also showed a significant correlation with the BMI category (Table 4.4). This indicates that using BMI category alone is sufficient. Correlation was determined using a Pearson correlation test with $p \leq 0.05$ considered significant.

Figure 4.3: The tendon junction appears as a hyperechoic dot on the ultrasound image. Key: SF = Subcutaneous fat; EOM = External oblique muscle; IOM = Internal oblique muscle; TAM = Transversus abdominis muscle.
Table 4.4: Correlations between ultrasound measurements and BMI categories and values.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>BMI category r-value</th>
<th>BMI category p-value</th>
<th>BMI value r-value</th>
<th>BMI value p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth: Skin to tendon junction (n = 84)</td>
<td>0.337</td>
<td>0.002*</td>
<td>0.441</td>
<td>0.000*</td>
</tr>
<tr>
<td>EOM thickness at tendon junction (n = 84)</td>
<td>0.277</td>
<td>0.011*</td>
<td>0.349</td>
<td>0.001*</td>
</tr>
<tr>
<td>Subcutaneous fat at tendon junction (n = 81)</td>
<td>0.185</td>
<td>0.097</td>
<td>0.257</td>
<td>0.021*</td>
</tr>
<tr>
<td>Depth: Skin to injection point (n = 86)</td>
<td>0.489</td>
<td>0.000*</td>
<td>0.682</td>
<td>0.000*</td>
</tr>
<tr>
<td>IOM thickness at injection point (n = 85)</td>
<td>0.318</td>
<td>0.003*</td>
<td>0.411</td>
<td>0.000*</td>
</tr>
<tr>
<td>EOM thickness at injection point (n = 86)</td>
<td>0.358</td>
<td>0.001*</td>
<td>0.473</td>
<td>0.000*</td>
</tr>
<tr>
<td>Subcutaneous fat at injection point (n = 83)</td>
<td>0.430</td>
<td>0.000*</td>
<td>0.566</td>
<td>0.000*</td>
</tr>
<tr>
<td>Tendon junction to injection point (n = 85)</td>
<td>-0.121</td>
<td>0.270</td>
<td>-0.117</td>
<td>0.286</td>
</tr>
</tbody>
</table>

*Significance for correlation using Pearson correlation test (α=0.05).

All ultrasound measurements were analysed for a difference between healthy and overweight BMI groups using an independent samples t-test (Table 4.5). All measurements except for two showed a statistically significant difference between healthy and overweight BMI groups (significance p ≤ 0.05). No significant difference was seen for subcutaneous fat at the tendon junction, as well as the distance between the tendon junction and the injection point.
Table 4.5: Differences between healthy and overweight using an independent samples t-test.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Healthy</th>
<th>Overweight</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mm)</td>
<td>SD (mm)</td>
<td>Mean (mm)</td>
</tr>
<tr>
<td>Depth: Skin to tendon junction</td>
<td>32.72 8.55</td>
<td>38.85 8.75</td>
<td>0.002*</td>
</tr>
<tr>
<td>EOM thickness at tendon junction</td>
<td>19.61 8.68</td>
<td>24.62 8.93</td>
<td>0.011*</td>
</tr>
<tr>
<td>Subcutaneous fat at tendon junction</td>
<td>11.44 2.99</td>
<td>12.53 2.86</td>
<td>0.097</td>
</tr>
<tr>
<td>Depth: Skin to injection point</td>
<td>25.85 6.13</td>
<td>32.77 6.34</td>
<td>0.000*</td>
</tr>
<tr>
<td>IOM thickness at injection point</td>
<td>8.48 3.07</td>
<td>10.61 3.33</td>
<td>0.003*</td>
</tr>
<tr>
<td>EOM thickness at injection point</td>
<td>8.86 3.03</td>
<td>11.34 3.42</td>
<td>0.001*</td>
</tr>
<tr>
<td>Subcutaneous fat at injection point</td>
<td>8.20 2.22</td>
<td>10.22 20.08</td>
<td>0.000*</td>
</tr>
<tr>
<td>Tendon junction to injection point</td>
<td>35.38 12.45</td>
<td>32.31 13.06</td>
<td>0.270</td>
</tr>
</tbody>
</table>

*Significant difference using independent samples t-test (α=0.05).

4.2.2 Measurement sites

Comparing the corresponding measurements between the two measurement sites, overall the measurements done at the tendon junction was thicker than at the injections point. However, as the measurement was done at the tendon junction for the TAM and the IOM, no muscle thickness is possible for the IOM at the tendon junction. Data was divided into healthy and overweight BMI groups and a clear similarity between BMI group charts can be seen on the graphs (Figure 4.4; Figure 4.5).
The distance between the tendon junction and the injection point on average was 33.83 mm (SD 12.78 mm) and the injection point was found to be more superficial than the tendon junction in 67% of cases. Intra-rater and inter-rater statistics showed significant strong correlations for all ultrasound measurements. A summary of the mean distances for all ultrasound measurements for both healthy and overweight BMI groups can be seen below (Figure 4.6).
Figure 4.6: Mean distances (in mm) seen for ultrasound measurements between healthy BMI (top) and overweight BMI (bottom).
Chapter 5: Discussion

The current study evaluated the anatomy of the nerves running in the TAP. The cadaveric aspect evaluated the TAP plexus and the L1 nerve and its terminal branches. The ultrasound aspect evaluated the depth of the TAP and the overlying muscle and fat layers. In this chapter the results, shown in chapter 4, is discussed, compared to previous literature, and put into a practical or clinical context as far as possible.

5.1 Cadaver aspect

5.1.1 TAP component

5.1.1.1 Number of nerves

Although no definitive literature could be found on the nerve density in the TAP between the costal margin and the iliac crest, in the current study it was found that the nerve density influences the branching patterns of the TAP block. Furthermore, an increase was seen in the number of nerves at the linea semilunaris when compared to the MAL. This increase is consistent with at least one nerve branching (without merging with another) before the nerve reaches the linea semilunaris. However, these findings do not imply that the current technique should be changed as no correlation was seen between number of nerves observed and number of nerves pierced.

Alternatively, two adjacent nerves branching and the branches then merging (Figure 4.2) would also result in the increase of the number of nerves. A large number of nerves at the MAL is also associated with a large number of nerves at the linea semilunaris, indicating that the increase in the number of nerves is consistent. Therefore, the current study has shown that nerve branching is an indicator of nerve density, both at the MAL and at the linea semilunaris. No other literature could be found on the nerve density.
5.1.1.2 Needle depth

Needle depth is of the utmost importance in the blind TAP block. If the needle is inserted too superficial, the block may be ineffective, requiring increased post-operative opioid usage. If the needle is inserted too deep, the block will also be ineffective, and stands the chance of piercing the peritoneum and the abdominal organs (particularly the liver, kidneys, or intestines) (Lancaster and Chadwick, 2010; Walker, 2010; Young et al., 2012).

A study by McDermott et al. (2012) using the blind technique on adult patients, found the needle was positioned in the correct plane in 59.7% of injections, whereas the current study found only 7.6% of needles in the correct position. Furthermore, the study by McDermott et al. (2012) found the needle tip piercing the TAM or even the peritoneal cavity in 30.6% of cases, while the current study observed this phenomenon in the majority of cases with 79.3% (Figure 5.1). It is important to note that the study by McDermott et al. (2012) used live patients (n = 72 needles) and did not use the triangle of Petit as a landmark. The current study inserted 92 needles into embalmed cadavers using the TAP technique. Typically cadavers are used to assess underlying anatomy, while living patients are of special interest in terms of needle depth. Although embalming may have caused a limited increase in rigidity, it could be responsible for giving just enough rigidity to produce a result that differed from the live patients. Embalming using the Thiel method, instead of the traditional formalin method, could reveal different results as the Thiel method makes the cadavers more flexible (Eisma and Wilkinson, 2014).
It was anticipated to observe a significant difference between the healthy and overweight categories for needle depth in the cadavers, however the statistics did not support this expectation. The lack of difference between the healthy and overweight categories for needle position is similar to the findings of McDermott et al. (2012) in live patients (Figure 5.1). In both studies, it is possible that the “pops” felt when piercing the fascial layers were distinct and in so doing, effective enough to alert the clinician that the needle had already passed through to the TAP. However, it is also possible that no distinct “pop” was felt when going through the TAM, leading to the increased TAM and peritoneal piercings. The lack in TAM “pop” could also explain the increase in TAM piercings, as this study did not examine the peritoneum for any punctures. This explanation also supports the findings of a previous study which suggested the “double pop” method is effective (McDonnell et al., 2007).

The lack of significant difference between BMI groups could also indicate that the blind TAP block method does not need to be modified for overweight BMI individuals. However, from the ultrasound data of the current study it is seen that there is a significant difference between the distance the needle is inserted in healthy and overweight BMI individuals. Therefore, there is a possibility that the pops felt are effective enough to be deep, but another method should be determined to limit the
depth of the needle tip so as to avoid piercing the peritoneum or injecting the anaesthetic into the TAM.

The current study revealed that with blind needle insertion, using the triangle of Petit landmark, the needle is between 4.56 mm to 6.83 mm from the closest nerve 95% of the time when the needle pierced the TAM. Therefore, in approximately 95% of cases, the needle will not damage a nerve should the needle be inserted too deeply. Assuming the needle is in the correct plane, the nerve will not be damaged, but will be close enough to increase the likelihood of effective anaesthesia. This correlates well with the needle piercing a nerve in 6.5% of cases in the current study. No other literature could be found comparing the incidence rate of nerve damage to the distance to the closest nerve, or to the TAP plexus branching patterns. Piercing a nerve would have caused at the very least a temporary decrease in innervation to the anterior abdominal wall from the point of injury.

5.1.1.3 Branching pattern

Although no related literature could be found, the current study showed a high number of possible variations for the branching patterns of the TAP nerve plexus, with the nerves closest to the needle often giving off multiple branches. These variations have clinical implications as damage to the nerve closest to the needle will potentially cause long-term temporary decreased innervation to a larger area when compared to a single unbranched nerve. In the event where there are significant interactions between adjacent nerves, such as mergers, piercing a nerve will not have such a noticeable effect as the adjacent nerve is able to compensate for the damage.

The position of the merger also determines the extent of the impaired innervation. When the nerves only merge at the linea semilunaris, the area from the point of the nerve damage to the linea semilunaris will be effected, but not the rectus abdominis. Therefore, if the merger is closer to the linea semilunaris than the MAL, a smaller area will be affected by the nerve damage. In the current study, there were also cases of a single nerve branching, only for all of the branches to merge again. If this nerve is damaged, a greater area will be affected with resultant loss of sensory innervation over the inferolateral abdominal region.
5.1.2 L1 component

Very little literature could be found on the anatomical variations regarding the L1 nerve and its terminal branches. One study by Anloague and Huijbregts (2009) noted anatomical variations in the iliohypogastric nerve in 20.6% of observations, with no variations in the ilioinguinal nerve. The current study found variations in both nerves, specifically concerning the root contributions.

5.1.2.1 Root contributions

A previous study by Klaassen et al. (2011) assessed the root contributions of the iliohypogastric nerve and the ilioinguinal nerve separately. Overall, the root contributions seen in the current study agree with the data for the ilioinguinal nerve data, but the article for the study by Klaassen et al. (2011) did not publish all of the data for the iliohypogastric nerve and therefore cannot be used for comparison of root contributions by L1 and L2, L2 and L3, as well as T12, L1 and L2 (Figure 5.2). The frequency for T12 and L1 as a root contribution to L1 seen in the current study (17% from 54 cases) seems to correlate well with the iliohypogastric and ilioinguinal nerves in the complementary study by Klaassen et al. (2011) (14% out of 200 cases for each respectively).
Different studies showed the root contribution to the iliohypogastric nerve was both T12 and L1 in 34% for a study by Baird (1983), and 13.3% for a study by Gandhi et al. (2013). The latter is closer to the frequency seen in the current study (17% for L1 in general) as well in the study done by Klaassen et al. (2011).

A root contribution of L1 only for different studies can be seen below (Table 5.1). Conflicting data is seen on the L1 only root contribution as reported by various authors (Baird, 1983; Gandhi et al., 2013; Klaassen et al., 2011). This could be due to a variation between populations as none of the other studies were done on a South African population. It is unclear why seemingly population specific variations have been noted by Baird (1983), Gandhi et al. (2013), Klaassen et al. (2011). It is unlikely that population group would affect the L1 spinal nerve to have different root values. These differences might simply be due to normal variation, however it should be confirmed by future studies. Also, when analysing the iliohypogastric and ilioinguinal nerves, if they are from a single L1 trunk (as seen in all the samples from the current study), it could be difficult in assessing the root contribution for separate nerves in a cadaver. In the current study, two cases were seen where the iliohypogastric nerve originated from the L1 root only while the ilioinguinal root originated from the L2 root only. However, these two cases were the only ones where there was a clear difference.

**Figure 5.2: L1 root contributions between studies (Klaassen et al., 2011).** Key: IH = iliohypogastric nerve, IL = ilioinguinal nerve.
in the nerve root origin for the iliohypogastric and ilioinguinal nerves (2 cases out of 58).

Table 5.1: Comparison of single L1 root contribution between the current study and results by others (Baird, 1983; Gandhi et al., 2013; Klaassen et al., 2011).

<table>
<thead>
<tr>
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<th>L1 and branches</th>
<th>Iliohypogastric nerve</th>
<th>Ilioinguinal nerve</th>
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<tr>
<td>Baird, 1983</td>
<td>-</td>
<td>32%</td>
<td>-</td>
</tr>
<tr>
<td>Gandhi et al., 2013</td>
<td>-</td>
<td>86.6%</td>
<td>-</td>
</tr>
<tr>
<td>Klaassen et al., 2011</td>
<td>-</td>
<td>10%</td>
<td>65%</td>
</tr>
<tr>
<td>Current study</td>
<td>71%</td>
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</tr>
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</table>

There were several aspects of the L1 component on which no relating literature could be found. No literature could be found on the frequency of the iliohypogastric and ilioinguinal nerves coming from a single trunk and dividing or not, while the current study saw the L1 nerve dividing in 67.2% of cases. It also proved difficult to find any previous studies reporting whether the L1 nerve divides deep to the psoas major muscle or lateral to it.

5.1.2.2 Branching pattern

In general, the current study confirmed the most constant position of the iliohypogastric and ilioinguinal nerves in the posterior abdomen is on the anterior surface of the quadratus lumborum muscle, as seen in a previous study (Reinpold et al., 2015). However the study by Reinpold et al. (2015) did not mention any cases where the L1 nerve did not divide before piercing the TAM, while the current study found L1 did not divide in 32.8% of cases (19 out of 58). This difference could also possibly be due to difference in population group as the current study was done on a South African population. However, as previously mentioned, differences might just be due to normal variation. The high number of undivided L1 nerves entering the TAP could make a single L1 block injection into the TAP easier for the South African population.
In the current study, it was also observed that either L1 itself or at least one of its branches pierced the TAM with the fourth lumbar artery in 40.7% of noted cases. This indicates the formation of a small communicating channel formed between the posterior abdominal wall in the abdominal cavity, and the TAP. Stenosis of the channel could lead to compression and damage to both structures. The arterial branches entering the TAP with the nerves are in the same danger as the nerves to be damaged by a needle. Despite almost half of noted cases showing the nerves entering the TAP accompanying the fourth lumbar artery, no previous literature on this finding could be found.

5.1.2.3 Impact of accidental findings

The accidental findings seen in the current study (as described in section 4.1.2.4 Accidental findings) almost exclusively has an impact on the innervation region of the anterolateral abdominal wall. Increased nerve branches will increase the nerve density as seen in the TAP component. There were also two cases where T12 took over the innervation as no L1 could be found.

5.2 Ultrasound aspect

5.2.1 Comparison between sides and BMI groupings

5.2.1.1 Left and right sides

A significant difference was seen between left and right for the depth of the injection point and the EOM thickness at the injection point. As no other significant differences were seen, it is likely that the depth of the injection point differed significantly was the EOM thickness taken at that same site. While the ultrasounds were being taken, the anaesthesiologist found it easy to positively identify left-handed individuals simply by looking at the muscle thickness. Even though the statistical tests showed no significant relationship between handedness and EOM thickness, the right side was consistently thicker in right-handed individuals.

A study by Sugaya et al. (2014) evaluated the thickness of the EOM, IOM, and TAM 15 mm anterior to the TAM tendon. The results were compared to the current study’s
results for the EOM and IOM thickness at the injection point (Figure 5.3). The study by Sugaya et al. (2014) showed much less of a difference between sides when compared to the current study. The current study also showed markedly thicker muscles overall when compared to the study by Sugaya et al. (2014). The observed differences could be explained by the difference in population groups and sex as the study by Sugaya et al. (2014) was done in Japan evaluating only males. The current study evaluated both males and females in a South African sample. The demographics, apart from sex, was similar in both studies. In the Japanese study, the mean age was 23.8 years, height was 171.5 cm, and weight was 64.1 kg. In the current study, the mean age was 22.8 years, height was 170.1 cm, and weight was 74.7 kg. The difference in weight could be due to muscle or fat differences. If population is the cause of the difference between the studies, the depth of needle insertion for a TAP block could require modifications according to region of the world, but further investigations are needed.

![Figure 5.3: Muscle thickness according to sides between the current study and a study by (Sugaya et al., 2014).](image)

The hyperechoic dot of the IOM and the TAM junction could be a valuable landmark for the ultrasound-guided TAP block. Further studies are needed to test if a better injectate spread can be obtained by inserting the needle just anterior to this hyperechoic dot.
5.2.1.2 BMI groupings

Significance was seen between measurements in both the BMI values and the BMI categories for measurements. The usage of BMI categories could therefore be justified to simplify the analysis. Practically, this allows a clinician doing an ultrasound-guided TAP block to simply take into account the BMI category before attempting the needle insertion.

A statistically significant difference between healthy and overweight BMI groups was seen for all but two measurements: subcutaneous fat at tendon junction, and the distance between the tendon junction and the injection point. Practically this means that if a block was to be administered more posteriorly (closer to the tendon junction of the IOM and the TAM), the distance posterior to the original injection point would not be affected by the patient’s BMI. The lack of significant difference in subcutaneous fat at the tendon junction indicates the reduction of subcutaneous fat more posteriorly is more dramatic in overweight individuals than in healthy BMI individuals. Although there is no difference for subcutaneous fat, EOM thickness was seen to be significantly different between healthy and overweight individuals, this has little to no practical implications as the depth to the tendon junction differs significantly between healthy and overweight individuals.

The EOM thickness at the injection point was markedly thinner in individuals with a healthy BMI when compared to individuals of an overweight BMI. This poses a possible problem during the blind TAP block as it will be easier to pass through the EOM in healthy BMI individuals without being aware of the situation. It has been suggested due to the difficulty in identifying the three muscle layers in obese patients, it should be kept in mind that the IOM is the thickest of the three (Hadzic and NYSORA, 2012). However, in the current study, the EOM was thicker than the IOM in both healthy and overweight BMI individuals. Although further investigation of this in obese BMI individuals is necessary, this study reveals the need to consider the anatomy of the region even with ultrasound-guidance. With the mean depth of the TAP as 26.9 mm (maximum 43.9 mm, SD 6.8 mm) for healthy BMI, and 34.2 mm (maximum 44.8 mm, SD 6.3 mm) for overweight BMI, it would be wise not to exceed a depth of 40 mm unless the TAP can clearly be seen to be deeper on the ultrasound.
5.2.2 Measurement sites

An increased depth is seen at the tendon junction when compared to the typical injection point. The increased depth for the IOM and TAM junction, due to larger EOM thickness, limits the use of tendon junction site posteriorly as a novel site for blind TAP blocks. However, the hyperechoic dot formed by the tendon junction is a potentially valuable landmark during ultrasound-guided TAP blocks as the distance between the tendon junction and injection point is relatively stable.

5.3 Clinical relevance of this study

The current blind TAP block can be improved by keeping in mind the anatomy of the nerve plexus found in the TAP. The L1 blocks could potentially be improved by moving the needle insertion further posterior to the ASIS, although further studies are needed. Here, a single injection into the TAP could be administered to use less injectate.

Based on the results of the study, the needle should be advanced at least 2 cm in healthy BMI individuals and at least 3 cm in overweight BMI individuals in order to reach the correct site of injection for TAP block. BMI has no influence on the course of the thoracolumbar nerves. The differences between right and left will be compensated for by the spread of anaesthetic agent between the tissues.

5.4 Limitations and future directions

The current study used cadavers embalmed by the traditional formalin method. Although the embalming technique should have only minimal effect on the abdominal muscle layer rigidity, this could be evaluated by repeating the cadaver aspect of the study on cadavers embalmed using the Thiel method. The Thiel method of embalming causes a decrease in embalming-induced rigidity (Eisma and Wilkinson, 2014). If the same results are obtained in cadavers embalmed with the Thiel method, this would support that the embalming has little to no effect on the study.

The branching pattern of the TAP dissections could be analysed more effectively by using geometric morphometric analyses and transfiguration software. However, due to time constraints, this could not be implemented with the current study as the
cadavers had to be used for dissection by undergraduate students. The same techniques could be implemented to analyse the exact position where the L1 nerve and its branches pierce the TAM. Additionally, an injectate study on a modified L1 block, using fresh cadavers, could be done by inserting the needle more posterior to the ASIS into the TAP.

As the average distance between the tendon junction and the typical injection point remains constant, further studies using the hyperechoic dot as a confirmatory landmark are needed. Additional research is also needed in order to confirm differences in abdominal muscle thickness at the TAP injection point across regions. The current study used healthy and overweight cadavers and live volunteers. A supplementary study, with similar aims but done on underweight and obese individuals, would give a larger view of the TAP and L1 blocks across a South African population. Adding waist-to-hip ratio to the ultrasound aspect on live volunteers could give more clarity on the muscle layer thickness as well as the subcutaneous fat thickness. Ideally this should be correlated with the cadaver aspect of the study, but due to deformation and compression during embalming, this would not be accurate.

By using the results from the current study, a modified technique could be devised for the blind TAP block. A cadaver study, preferably using Thiel embalmed cadavers, would be the ideal starting point to attempt a modified blind TAP block before moving on to live patients.
Chapter 6: Conclusion

This study aimed to determine the course and branching patterns of the thoracolumbar nerve plexus in the TAP. In this regard, the study found the thoracolumbar plexus exhibits great variety in terms of branching and nerve interactions. Several different nerve interactions were seen, including nerve branching, and mergers and a vast array of combinations. A general increase in the number of thoracolumbar nerves were seen at the linea semilunaris, coming from the MAL.

No relationship could be found between BMI and TAP plexus positioning, indicating the blind TAP block for normal and overweight BMI individuals could be equally effective, although further study will be needed to confirm this. The L1 nerve has a variable root contribution with about 30% having a different root contribution than just L1. While the current study saw the L1 nerve dividing in 67.2% of cases, no other literature could be found describing the frequency of the iliohypogastric and ilioinguinal nerves coming from a single trunk and dividing or not.

The study also aimed to determine the general position where L1 split into its two terminal branches and where these branches enter the TAP. It was found that the L1 nerve often does not split into its two terminal branches before piercing the TAM (32.8%). The nerve or its branches also pierced the TAM together with the fourth lumbar artery in about 40.7%. Despite almost half of noted cases showing the nerves entering the TAP accompanying the fourth lumbar artery, no previous literature on this finding could be found. This could be a finding specific to a South African population group and requires further investigation.

The ultrasound aspect of this study evaluated the depth of the TAP at the injection position as well as the thickness of the overlying muscle layers and subcutaneous fat. A noted difference was seen between the muscle layer thickness of the current study and a Japanese study, suggesting that muscle thickness in a South African population should be taken into account during a blind TAP block.

The tendonous junction of the IOM and the TAM appears as a hyperechoic dot on an ultrasound. The hyperechoic dot could prove to be a valuable landmark to validate the correct positioning of the needle tip in the ultrasound-guided block.
From the study, it is suggested that the blind TAP block can be administered by inserting the needle at the apex of the triangle of Petit. The needle should be advanced at least 2 cm in healthy BMI individuals, and 3 cm in overweight BMI individuals, while using the traditional “pops” for the blind TAP block in order to confirm TAP block needle placement.

Overall, this study highlighted the anatomy surrounding the TAP and showed great significance for the South African population. This study contributed the effectiveness of both the blind and the ultrasound-guided TAP block.
Chapter 7: References


Appendices

Appendix A: Participant’s information leaflet & informed consent form for non-intervention study

Anatomical variations of the thoracolumbar nerves with reference to TAP block

Dear Participant

1) INTRODUCTION

You are invited to volunteer for a research study. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study, you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator. You should not agree to take part unless you are completely happy about all the procedures involved.

2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of this study is to determine the position of the thoracolumbar nerves. These nerves give sensation to the sides and the front of the abdomen. By doing so, we wish to learn more about the exact position and course of the nerves so that anaesthesiologists will know where to block these nerves for regional anaesthesia in order to provide adequate pain relief for abdominal surgical patients post-operatively.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study involves evaluation of the sides of the abdomen by sonar to measure the distances between the space where the nerves run and the surface of the skin. The procedure will be performed by a qualified anaesthesiologist who will only obtain an image from the abdominal region. We will also document your sex, age, weight (by means of an electronic scale) and height (by tape measure), and if you are left or right handed. All height and weight measurements as well as the ultrasound procedure will be done in private and closed off space which will only be accessed by the researchers.
and the anaesthesiologist other than yourself. Please note that you are required to wear clothing that will allow the abdomen to be visualised, as indicated in figures 1 and 2 below.

Figure 1: Area for ultrasound on illustration (Netter, 2010)

Figure 2: Area for ultrasound on live subject (med.umich.edu)

4) RISK AND DISCOMFORT INVOLVED

There is no risk or discomfort involved in this study. No needles will be used and the sonar will not cause any pain. The only discomfort will be the time for travel and the time taken to find the nerve by sonar. If you are a patient, this study will in no way influence your medical treatment.
5) POSSIBLE BENEFITS OF THIS STUDY

You will not have any direct medical benefit, but this study will allow us to do regional anaesthesia on this nerve more accurately and with less possible complications. All participants will receive R50-00 as travel compensation.

6) WITHDRAWING FROM THE STUDY

You may at any time withdraw from this study without stating a reason. If you are a patient, you will still receive your treatment as prescribed by your doctors regardless of your decision to participate or not to participate in this study.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving humans/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

8) INFORMATION

If you have any questions concerning this study, you should contact:

Ms Bianca Smit cell: 076 309 3640 or Ms Nanette Briers tel: 012 319 2631

9) CONFIDENTIALITY

All information obtained whilst in this study will be regarded as confidential. Results will be published or presented in such a fashion that participants remain unidentifiable.
10) CONSENT TO PARTICIPATE IN THIS STUDY

I, ..................................................... (name of participant), have read or had read to me in a language that I understand, the above information before signing this consent form. The content and meaning of this information have been explained to me. I have been given opportunity to ask questions and am satisfied that they have been answered satisfactorily. I understand that if I do not participate, it will not alter my management in any way. I hereby volunteer to take part in this study. I have received a signed copy of this informed consent agreement.

........................................................   .......................
Participant signature                     Date

........................................................   .......................
Person obtaining informed consent        Date

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Witness                                   Date
Appendix B1: Cadaver data capture sheet

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<th>Table (Cadaver)</th>
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Appendix B2: Participant data capture sheet

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The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

Approval Certificate
New Application

Ethics Reference No 73/2014

Title Anatomical variations of the thoracolumbar nerves with reference to transverse abdominal plane (TAP) block.

Dear Ms Bianca Smit

The New Application as supported by documents specified in your cover letter for your research received and was approved by the Faculty of Health Sciences Research Ethics Committee on the 14/05/2014.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years.
- Please remember to use your protocol number (73/2014) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

___________________________________
Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Tel:012-3541330 Fax:012-3541367 Fax2Email: 0866515924 E-Mail: fhsethics@up.ac.za
Web: //www.healthethics-up.co.za H W Snyman Bld (South) Level 2-34 Private Bag x 323, Arcadia, Pta, S.A., 0007
Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No.: 73/2014

Title: Anatomical variations of the thoracolumbar nerves with reference to TAP block

Dear Ms Bianca Smit

The Amendment as described in your documents specified in your cover letter dated 27/07/2015 received on 30/07/2015 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29/08/2015.

Please note the following about your ethics amendment:

- Please remember to use your protocol number (73/2014) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics amendment is subject to the following:

- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

[Signature]

Dr R Sommers, MBChB, MMed (Int); MPharMed.
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