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Anatomical variations in the origins of the lateral circumflex femoral arteries in a South African sample: A cadaver study

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ABSTRACT

Background: Clinically, the lateral circumflex femoral artery (LCFA) is used in a variety of procedures, these include anterolateral thigh flaps, aortopopliteal bypass, coronary artery bypass grafting and extracranial-intracranial bypass surgeries. Variations in the anatomy of the LCFA, profunda femoris artery (PFA) and their branches have been noted in several studies. There are numerous clinical implications related to the inadequate anatomical knowledge of this region.

Methods: The study sample consisted of 55 cadavers of different ages, ancestry groups and varying body mass index. A total of 90 legs were dissected. The anterior thigh compartment was dissected and the common femoral artery (CFA), superficial femoral artery (SFA), PFA and LCFA further exposed. The course and variations were noted and results documented for further analysis.

Results: The LCFA was present in all the legs dissected. The origin of the LCFA was found to be the PFA in 75.6% of the left legs and 82.2% of the right legs dissected.

In two cases, a male and female limb, the branching of the PFA from the CFA was observed directly from the external iliac artery, deep to the inguinal ligament.

A variation was noted in a female cadaver, where the branches of the LCFA on the left limb had different points of origin. Another variation was noted on a male cadaver, the left limb had two different points of branching for the ascending and descending branches of the LCFA, a common trunk was absent.

Conclusion: This study indicates that the most common site of origin for the LCFA was the PFA. The branching of the PFA from the CFA was found to be variable in 2.2% of the cases, where the PFA was a direct branch of the external iliac artery. The study also noted variations relating to the origin of the transverse branch of the LCFA, this branch was absent in 4.4% of the limbs dissected.

Knowledge of the variations related to the origin of the PFA, LCFA and their branches is important in reducing the chances of intra-operative bleeding, during different surgical procedures.

1. Introduction

The lateral circumflex femoral artery (LCFA) is an artery that branches from the profunda femoris artery (PFA) and at times directly from the superficial femoral artery (SFA) (Fig. 1a and b). The LCFA is known for contributing to the blood supply of the muscles in the lateral aspect/compartment of the thigh, and to a lesser degree contributes to the blood supply of the neck of the femur and femoral head. Around the shaft of the femur, the LCFA encircles the superior part of the femoral shaft and anastomoses with the medial circumflex artery of the thigh (MCFA) [1]. In its course, the LCFA passes laterally deep to the sartorius and rectus femoris muscles, dividing into the ascending, transverse and descending arteries (Fig. 2). The ascending branch supplies the anterior part of the gluteal region, transverse branch winds around the femur, while the descending branch joins genicular peri-articular anastomosis [1]. Therefore, the LCFA gives blood supply to the head and neck of the femur, greater trochanter, the vastus lateralis and the knee [2]. In a study conducted by Metwally et al. [3], the LCFA was found to be a contributing source of blood supply to the sciatic nerve in 25% of the 20 dissected cadaver legs.

The common femoral artery (CFA) which is frequently accessed by radiologist and surgeons, is easily accessible to catherization. Knowledge of the anatomy and possible variations of the PFA, LCFA and MCFA

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List of abbreviations

LCFA	Lateral circumflex femoral artery
PFA	Profunda femoris artery
CFA	Common femoral artery
SFA	Superficial femoral artery
MCFA	Medial circumflex femoral artery
LFCN	Lateral femoral cutaneous nerve
BMI	Body mass index
I	Inferior
D	Distal
M	Medial
L	Lateral
S	Superior
P	Proximal
LCFAa	Ascending branch of the lateral circumflex femoral artery
LCFAd	Descending branch of the lateral circumflex femoral artery
LCFAt	Transverse branch of the lateral circumflex femoral artery

are important for clinicians [4,5]. The PFA is used for arteriography, ultrasound and Doppler imaging, digital subtraction angiography and magnetic resonance imaging. Some of the current uses of the PFA include the use for haemodialysis where it supplants the use of the SFA. The branches of PFA are also used as long vascular pedicle during breast reconstruction after mastectomy in cases of carcinoma of breast [6].

In clinical practice, the branches of the LCFA have a variety of functions where it is used in anterolateral thigh flaps [7], aortopopliteal bypass [8,9] coronary artery bypass grafting [10] and extracranial-intracranial bypass surgeries [11]. Arterial grafts provide better patency rates for coronary artery bypass grafting than saphenous veins in both the short and long term [12]. Sound anatomical knowledge of the branching patterns of the CFA, SFA, PFA and LCFA is imperative in order to perform the aforementioned procedures, to ensure better clinical outcomes and reduced intraoperative complications. Variations in the anatomy of the LCFA have been noted in considerable number of studies, with varying branching patterns and origins as well as clinical implications. These variations have however not been studied extensively in the South African population. De Beer [13] investigated such variations in the South African sample, however, the study was limited to one ethnic group. Previous studies by Prakash et al. [5], Uzel et al. [14] and Sinkeet et al. [15] have documented differences in the Turkish, Indian and Kenyan sample, respectively, thereby creating the need to explore if such differences exist in our sample. Such findings may assist in the reduction of intraoperative complications associated with the LCFA surgical procedures.

Compelling evidence from previous studies [16,17] implicates injury to the lateral femoral cutaneous nerve (LFCN) and LCFA as major drawback to the anterior approach in total hip arthroplasty. Insufficient knowledge of the anatomy of the anterior thigh relating to the anterior approach to hip arthroplasty could possibly lead to intra-operative bleeding if the ascending branch of the LCFA is transected by mistake [18,19]. Therefore, a sound knowledge of the anatomy of the region is imperative as patient lives could be lost if the surgeon transects vessels which could otherwise be circumvented. This article therefore explores the anatomy of the LCFA in a South African cadaver sample.

2. Materials and methods

Ethical clearance for this study was sought and obtained (274/2014) prior to the commencement of the data collection phase of this study. All the adult cadavers used in this study were obtained and dissected under the rules and regulations defined within the South African National Health Act 61 of 2003. All data was collected from full body cadavers. The cadavers were handled with respect and care at all times, and properly safeguarded. No information which could possibly reveal the identities of the cadavers was obtained.

Access to personal information regarding the cadaver's age, sex, and ancestry was obtained from hospital records and noted, this information was restricted to the author. The height and weight of each cadaver was obtained post-mortem, prior to embalming and should therefore be as accurate a reflection of the height and weight of the individual.

In this study, 55 embalmed formalin fixed adult cadavers were dissected from the Department of Anatomy, University of Pretoria. Cadaver shortages are a common challenge currently experienced by medical schools, as a result in some cadavers only one limb was useable. The study sample was therefore combined to make a total of 90 legs

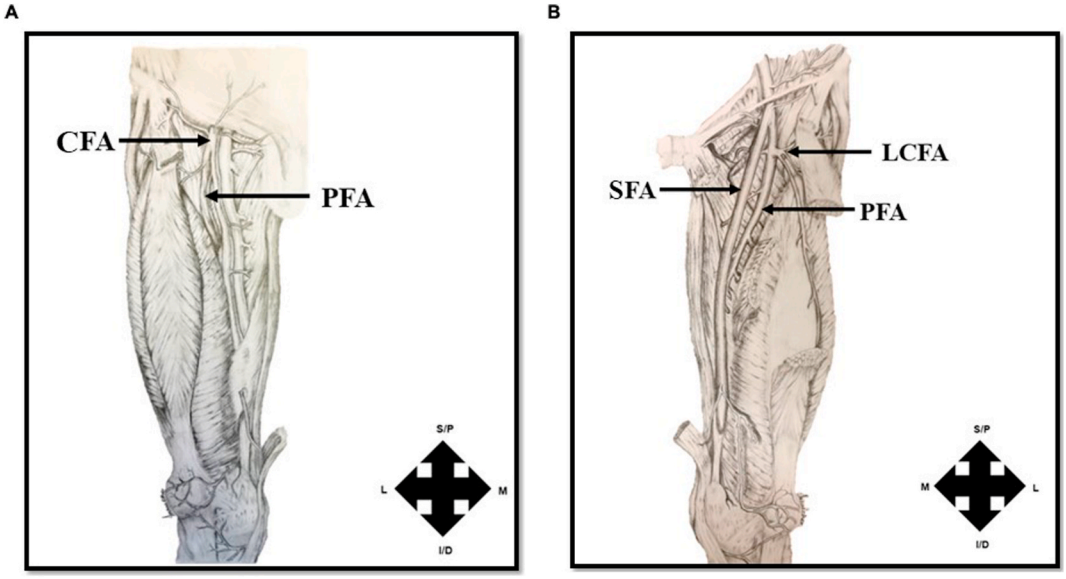

Fig. 1. a and b View of the anterior compartment of the thigh showing the common femoral artery (CFA), superficial femoral artery (SFA), profunda femoris artery (PFA) and the lateral circumflex femoral artery (LCFA). Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal.

dissected, which consisted of both male (n = 73) and female (n = 17) cadavers. The study sample consisted of cadavers of different ages (range 27–90, SD = 17.47), varying body mass index (BMI) (range of ≥18 – ≥30 kg/m^2) and different ancestry groups as variations in the course of the CFA, SFA, PFA and LCFA, based on ancestry, were not anticipated.

A standard dissecting method was developed and applied to all the cadavers used in this study. The skin overlying the anterior compartment of the thigh was incised, dissected and reflected medially. The subcutaneous fat, translucent fascia and superficial vessels were fully dissected and the sartorius muscle and quadriceps muscle identified. The sartorius muscle and the rectus femoris muscle were transacted, to further expose the CFA, SFA, PFA and the LCFA (Fig. 2). The FA, PFA and their branches were identified and the LCFA followed along its course (Fig. 1b). Various measurements were taken with Vernier callipers and recorded. These measurements, recorded in mm, were taken in relation to the CFA where it is first visualised entering the anterior compartment of the thigh, coursing posterior to the inguinal ligament, and lying within the femoral triangle. The first measurement was taken from the entry of the CFA into the anterior compartment of the thigh to where the PFA branches from the CFA. The second set of measurements included the distance from the pubic tubercle to the branching of the LCFA from the PFA or the SFA origin. The third set of measurements were taken as the distance from the origin of the PFA to the branching of the LCFA. The other measurements included the distance from the origin of the LCFA to the trifurcation into ascending, descending and/or transverse branch. Lastly, the distance from LCFA trifurcation to where the transverse branch emanated from the ascending and/or descending branch. The last set of measurements were not taken in all cases, as the transverse branch was not always the main source for the ascending branch (Fig. 1b).

Cadavers bearing any visible trauma in the hip joint region were excluded from the study, as well as cadavers bearing any conspicuous scars in the anterior thigh compartment, due to possible previous surgery. The exclusion criteria also considered those cadavers on whom the CFA, SFA, PFA and/or the LCFA has been transacted or removed by previous dissectors.

3. Results

In this study where a total of 90 legs (45 left legs and 45 right legs) were dissected, the LCFA was present in all the legs. Due to cadaver shortages, an unequal number of male and female cadaver legs were dissected with 73 legs belonging to male cadavers and 17 legs of female cadavers (Table 1). Standard descriptive statistics were calculated per age group, sex, BMI, height and weight ranges. The origin of the LCFA was found to be from two sources namely, the PFA and to a lesser degree the SFA. In our study, the origin of the LCFA was found to be the PFA in 75.6% (34 of 45 legs) of the left legs and 82.2% (37 of 45 legs) of the right legs dissected (Table 2).

The origin of the LCFA was found to be variable, in 24.4% (11 of 45 legs) of the left legs dissected, the LCFA was a direct branch of the SFA while the number was 17.8% (8 of 45 legs) on the right (Fig. 3). In this group, 18 legs were from male cadavers with only one from a female cadaver.

In two cases, one male (right) and one female (left) limb, the branching of the PFA from the CFA occurred posterior to the inguinal ligament (Fig. 4).

A variation was noted in a female cadaver, where the branches of the LCFA on the left limb had different points of origin. The descending branch was noted as emanating from the SFA, while the ascending branch had both an SFA and PFA origin (Fig. 5). Another variation was noted on a male cadaver, the left limb had two different branching points for the ascending and descending branches of the LCFA, a common trunk was absent.

4. Discussion

In performing surgical procedures relating to the femoral triangle and hip replacement, knowledge of the origin and distribution of the PFA, are critical as injury to these vessels have clinical implications. Injury to the vessels in this region may lead to severe secondary

Table 1
The origin of the lateral circumflex femoral artery in the cadaver sample.

Side	Origin of the LCFA			
	PFA		SFA	
	No.:	%	No.:	%
Left Leg	34/45	75.6	11/45	24.4
Right Leg	37/45	82.2	8/45	17.8

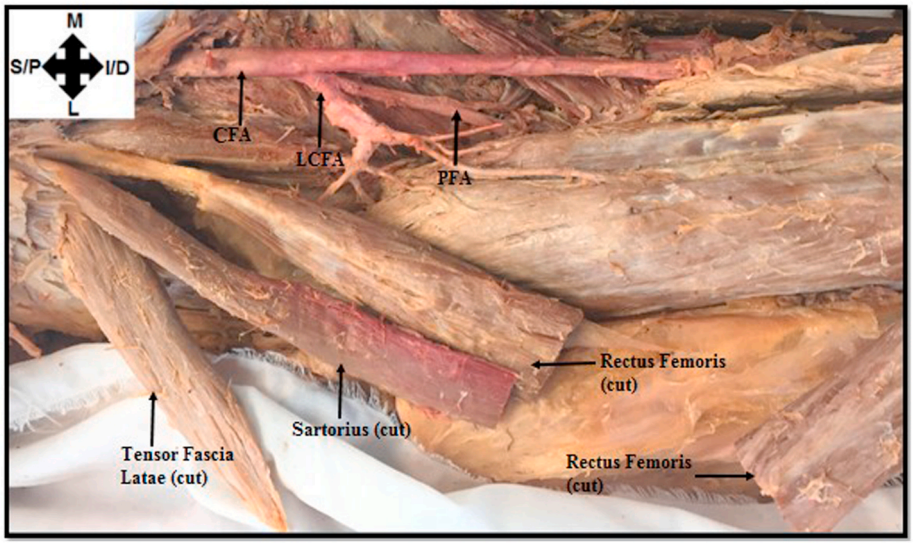

Fig. 2. Superior view of the anterior compartment of the thigh after transecting the sartorius and rectus femoris muscles. The LCFA can be seen bifurcating directly from the PFA. Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal; CFA-Common Femoral Artery; PFA-Profunda Femoris Artery; LCFA-Lateral Circumflex Femoral Artery.

Table 2
The prevalence of the LCFA in the male and female cadaver sample.

Side	Sex			
	Male		Female	
	No.:	%	No.:	%
Left Leg	37/45	82.2	8/45	17.8
Right Leg	36/45	80.0	9/45	20.0

haemorrhage while performing femoral artery puncture [6,20,21]. In our study, the most common site of origin of the PFA was found to be CFA in 97.8% of cases (88/90 limbs) dissected. The variation was found in two limbs, one female (left limb) and the other a male cadaver (right limb), in these two cases the PFA was found branching directly from the external iliac artery. Knowledge of the site of origin of the PFA is important for clinicians as it assists in avoiding iatrogenic femoral arterio-venous fistula while performing femoral artery puncture [21]. The LCFA, a branch of the PFA and FA, was found originating from the PFA in 75.6% of the left legs and 82.2% of the right legs dissected. The left side origin is similar to the pooled prevalence of 77.6% documented by Tomaszewski et al. [22] following a systematic review of 26 articles, however, a lower right percentage of 73.9% was documented. The transverse branch of the LCFA was absent in 4.4% of the limbs dissected.

In the 90 cadaver limbs dissected, 51 (25 left and 26 right limbs) were of white ancestry, 30 (15 left and 16 right limbs) of black ancestry and 8 (5 left and 3 right limbs) of coloured ancestry. The age groups varied, with 14 of the cadavers being younger than the age of 50, 39 being 50 years and more to a maximum of 90 years. Data for age was absent for two male cadavers. A wide-ranging BMI distribution was apparent, 12 of the cadaver sample were found to be underweight (BMI >18 kg/m^2) with two cadavers being obese (BMI >30 kg/m^2), the remainder of the cadaver sample had a weight distribution ranging within normal limits (<18 kg/m^2 -25 kg/m^2), while five of were overweight (BMI >25 kg/m^2 but ≤ 30 kg/m^2). Sayed-Noor et al. [23] documented an increased risk of infection following hip arthroplasty, resulting in reoperation (at 2 years) and revision (at 5 years) in patients of different overweight and obese categories. This cohort of patients were also noted by Barett et al. [24] as requiring hip surgery 10 years earlier than patients will normal BMI.

In our study, the average distance for the majority 88 of the 90 (97.8%) legs dissected, showed the distance from the CFA (where it is seen distal to the inguinal ligament) to the branching of the PFA to be 38.9 mm, with the length for most cases being between >40 and 50 mm. The normal distance of the origin of the PFA from the midpoint of the inguinal ligament is documented by Standring [2] as 35–40 mm. Similar findings were made by Manjappa and Prasanna [25] who documented the mean distance of 35.6 mm from the origin of the PFA at the posterolateral aspect of the CFA. In 2.2% (2/90) of the hips dissected, the PFA branched posterior to the inguinal ligament, directly from the external iliac artery. The average distance for the left side measurements was documented to be 36.8 mm for 49% (44/90) hips dissected and 44 mm for the right side measurements of 49% (44/90) hips. The largest measurement for the left side was 64 mm with the lowest measurement being 9 mm. The largest measurement for the right side measurement was documented at 71 mm with the lowest being 10 mm (Table 3). Advantages are found in the high origin of the PFA from the CFA as these vessels can be used for catherization and further investigations of any arterial system of the body [26].

The distance from the origin of the LCFA as a branch of the PFA was found to be between 0 and 10 mm in the majority of the recorded cases. The lowest measurement recorded (left limb) was found to be 2 mm with

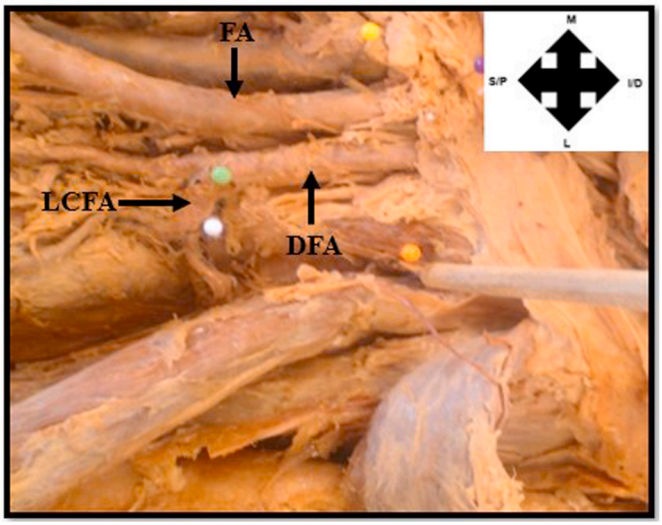

Fig. 4. Superior view of the anterior compartment of the thigh showing the bifurcation of the PFA directly from the external iliac and not the FA. Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal; FA-Femoral Artery; PFA-Profunda Femoris Artery; LCFA-Lateral Circumflex Femoral Artery.

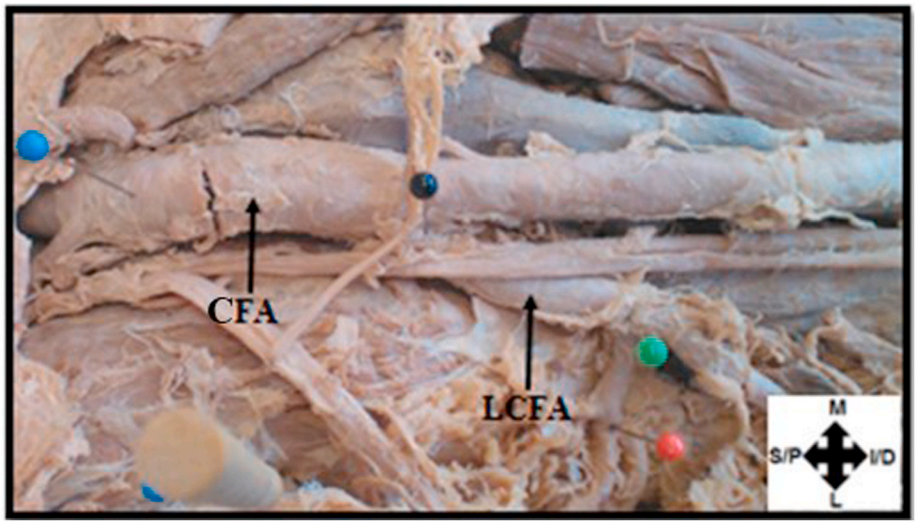

Fig. 3. Superior view of the anterior thigh compartment showing the bifurcation of the LCFA directly from the FA. Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal; CFA-Common Femoral Artery; LCFA-Lateral Circumflex Femoral Artery.

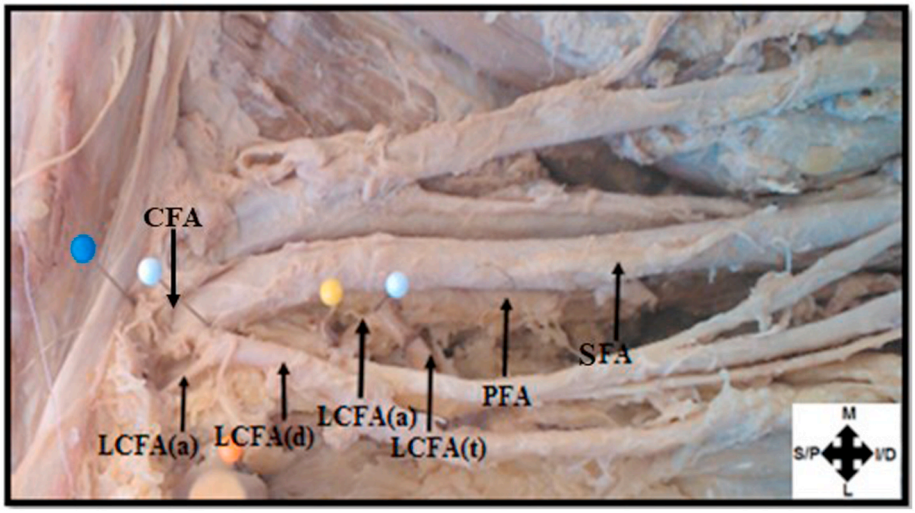

Fig. 5. Superior view of the anterior thigh compartment showing the different origin of the branches of the LCFA. The ascending branch (LCFAa) emanates from both the CFA and the PFA, the descending branch (LCFAd) is emanating from the CFA. Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal; CFA-Common Femoral Artery; SFA-Superficial Femoral Artery; PFA-Profunda Femoris Artery; LCFA-Lateral Circumflex Femoral Artery; LCFA(t)-Transverse branch of the Lateral Circumflex Femoral artery.

Table 3
Distance of common femoral artery coursing posterior to inguinal ligament to the profunda femoris artery branching.

Distance (mm_s)	No. of cases on the left side	No. of cases on the right side	Total cases	
			No.:	Percentage
<10	1	-	1/88	1.1
10–20	5	1	6/88	6.8
>20-30	9	11	20/88	22.7
>30-40	12	7	19/88	21.6
>40-50	10	17	27/88	30.7
>50-60	6	5	11/88	12.5
>60-70	1	2	3/88	3.4
>70-80	–	1	1/88	1.1

the highest measurement being 51 mm (left limb) (Table 4). These results compare with Dixit et al. [20] who found the largest measurement of this distance to be in the range of 51–60 mm in eight of the recorded cases. The distance between the origin of the LCFA from the PFA is clinically significant in surgical or angiographic interventions, for health professionals dealing with the PFA and its circumflex femoral branches [5,27].

The most common site of origin for the LCFA was found to be the PFA, this is in line with published literature [4,5,10,13,14,20,26,28–38]. In our study 78.9% of these vessels branched from the PFA. Our findings are closely related to those of Fukuda et al. [10], Uzel et al. [13], Boonkham and Plakornkul [30] and Rajani et al. [38], who found these results to be 78.6%, 77.3%, 77% and 75.8%, respectively (Table 5). In their study, Fukuda et al. [10] dissected 131 limbs, while Uzel et al. [13] dissected 110 limbs and Boonkham and Plakornkul [30] dissected 113 limbs (74 male and 39 female cadavers). It is evident from various studies conducted that the origin of the LCFA is primarily the PFA, with ranges between 56.7% and 92.3% (Table 5). Knowledge of the site of origin of the LCFA is important as this vessel contributes to the blood supply of the femoral neck, muscles and skin over the thigh. The increased blood supply to this region may decrease the incidence of flap necrosis [39]. The uses of the LCFA also extend to the reconstruction of large defects in the face, secondary to gunshot wounds [40].

A variation was noted in two cadavers, where the branches of the LCFA had different points of origin. In the female cadaver this variation was found on the left limb, where the descending branch was noted as emanating from the CFA, while the two ascending branches had both a CFA and PFA origin (Fig. 5). Another variation was noted on a male cadaver, the left limb had two different branching points for the ascending and descending branches of the LCFA, a common trunk was absent. Rajani et al. [38] mentions such a variation in their study, where

Table 4
Distance of the origin of the lateral circumflex femoral artery from the profunda femoris artery.

Distance (mm_s)	No. of cases on the left side	No. of cases on the right side	Total cases	
			No.:	Percentage
0-10	12	12	24/69	34.8
>10–20	10	11	21/69	30.4
>20–30	6	10	16/69	23.2
>30–40	3	3	6/69	8.7
>40–50	1	–	1/69	1.4
>50–60	1	–	1/69	1.4

Table 5
The origin of the lateral circumflex femoral artery in various populations.

Reference	Population	Frequency of origin of circumflex femoral arteries (%)	
		Femoral Artery	Profunda Femoris Artery
Adachi, 1928	German	18.3	78.2
Siddharth et al., 1985	Indian	16.0	71.0
Lippert and Pabst, 1985	German	19.0	76.0
Boonkham and Plakornkul, 1987	Thai	21.3	77.0
Perera, 1995	Colombian	14.6	85.4
Massoud and Fletcher, 1997	American	2.8	81.0
Fukuda et al., 2005	Japanese	21.4	78.6
Choi et al., 2007	Chinese	13.2	86.8
Uzel et al., 2008	Turkish	22.7	77.3
Tansatit et al., 2008	–	43.3	56.7
Samarawickaina et al., 2009	Sri Lankan	7.7	92.3
Prakash et al., 2010	Indian	18.8	81.3
Dixit et al., 2011	Indian	7.9	77.2
Sinkeet et al., 2012	Kenyan	2.4	65.5
Ogeng'o et al., 2013	Kenyan	27.9	66.7
Aghera et al., 2014	Indiana	18.6	80.4
Rajani et al., 2016	Indian	22.7	75.8
Current study, 2020	**South African**	**21.1**	**78.9**

a separate descending branch of the LCFA was found originating from either the CFA or PFA in six limbs. The descending branch of the LCFA has been used successfully as a high flow circuit for extra intracranial bypass surgery, it is therefore worth noting the variations that exist in the origin of these vessels.

In this study, it was further noted that at the trifurcation of the LCFA, the transverse branch either originated from the ascending or descending branch, therefore an extra set of measurements was taken (Fig. 6). The distance from the LCFA trifurcation to the origin of the transverse branch was recorded and the data analysed. We noted that in 65.6% (59/90) of the recorded cases, the transverse branch originated from the ascending branch of LCFA while in 10% (9/90) of the limbs dissected, the transverse branch originated from the descending branch of the LCFA. Our results also showed that the transverse branch of the LCFA was not always present as was the case in 4.4% (4/90) of the limbs dissected. The trifurcation of the LCFA is therefore only noted in 20% (19/90) of the limbs dissected. The study findings are comparable to the results documented by Gremigni [41], who found the trifurcation occurring in only 16% of the study sample. The findings relating to the origin of the transverse branch of the LCFA could not be compared to published literature as the research on this origin is absent. Knowledge of variations relating to the positioning of the LCFA are of utmost importance to vascular surgeons carrying out diagnostic and therapeutic procedures around the femoral triangle, to radiologists for avoiding misinterpretations of radiographs and anatomists for rare variations relating to the anterior compartment of the thigh [42].

5. Limitations

Several limitations were encountered during the data collection phase of our study. The methods were adapted to suit and accommodate the fixed and hardened nature of cadaver material. The first adaptation was in the attempt to follow the CFA, SFA, PFA and the LCFA along their course, the rectus femoris and sartorius muscles had to be transected, as these muscles are hardened. Cadaver material is a scarce resource, as a result data collection was restricted by the limited number of cadavers. The cadavers used in the Department of Anatomy, University of Pretoria are shared by students in various medical disciplines, as a result damage to some vessels which were important to our study occurred further limiting our sample size. The last limitation documented was an absence of data for ages in two male cadavers.

6. Future direction of the study

In the future, the study will look at the CFA, SFA, PFA and the LCFA on unembalmed human tissue or “fresh material”.

7. Conclusion

In our study, the most common origin of the LCFA was found to be PFA. Variations in the branching pattern of the LCFA is common in various populations. Knowledge of these variations related to the LCFA are important in clinical practice during vascular diagnostic intervention and surgeries as the unusual origin of the LCFA may cause accidental injury during surgery [43]. The frequency of variations relating to vessels in the anterior proximal thigh implies that surgeons working with this sample should exercise caution when performing procedures such as total hip arthroplasty.

The PFA was found branching from the CFA in 97.8% of the cases. This distance was found to be an average of 38.9 mm for the combined left and right side measurements. The highest measurement was recorded as 64 mm and 71 mm for the left and right side, respectively.

The development of the vasculature in the lower limb precedes the morphological and molecular changes that occur in the limb mesenchyme, hence the vascular variations are more of a rule than an exception [27]. A knowledge of the variations related to the origin of the PFA, LCFA and their branches is important in reducing the chances of intra-operative bleeding.

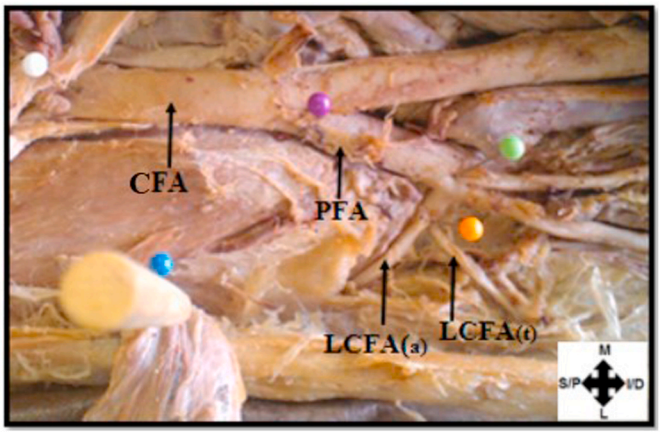

Fig. 6. Superior view of the anterior thigh compartment showing the bifurcation of the transverse branch, LCFA(t), from the ascending branch, LCFA(a). Key: I-Inferior; D-Distal; M-Medial; L-Lateral; S-Superior; P-Proximal; CFA-Common Femoral Artery; PFA-Profunda Femoris Artery; LCFA-Lateral Circumflex Femoral Artery.

Declarations of interest

None.

CRediT authorship contribution statement

N. Mogale: Conceptualization. **S.A.S. Olorunju:** Data curation. **S. Matshidza:** Conceptualization. **N. Briers:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors wish to sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially improve patient care and increase mankind’s overall knowledge. Therefore, these donors and their families deserve our highest gratitude. The technical staff at the Department of Anatomy, School of Medicine, University of Pretoria, is acknowledged for their assistance during the data collection phase of this research. Ms Jade Naicker is acknowledged for her annotation of Fig. 1a and b.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tria.2020.100098.

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Ethical statement

Ethical clearance for this study was sought and obtained (274/2014) prior to the commencement of the data collection phase of this study. All

the adult cadavers used in this study were obtained and dissected under the rules and regulations defined within the South African National Health Act 61 of 2003. All data was collected from full body cadavers. The cadavers were handled with respect and care at all times, and properly safeguarded. No information which could possibly reveal the identities of the cadavers was obtained.

Access to personal information regarding the cadaver's age, sex, and ancestry was obtained from hospital records and noted, this information was restricted to the author. The height and weight of each cadaver was obtained post-mortem, prior to embalming and should therefore be as accurate a reflection of the height and weight of the individual. The Anatomical Quality Assurance (AQUA) Checklist for reporting anatomical study ethics were referenced for this study [44].

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