PREDICTIVE FACTORS OF ARTERIAL CALCIFICATION SEVERITY IN COMPUTED TOMOGRAPHY ANGIOGRAPHY (CTA) LOWER LIMBS.

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LIST OF ABBREVIATIONS

AS	Agatston score
BKA	Below knee arteries
CS	Calcium score
CT	Computed tomography
CTA	Computed tomography angiography
CVS	Cardiovascular system
CVD	Cardiovascular disease
DM	Diabetes mellitus
ESRD	End stage renal disease
FRS	Framingham risk score
HDL	High density lipoprotein
HU	Hounsfield units
HUSM	Hospital Universiti Sains Malaysia
LDL	Low density lipoprotein
LIS	Laboratory information system
PACS	Picture Archiving and Communication System
PAD	Peripheral arterial disease
SFA	Superficial femoral arteries
TG	Triglyceride

ABSTRAK

Latar belakang kajian: CT angiogram kedua belah kaki sangat penting untuk membantu dalam menentukan tahap penyakit arteri periferi seseorang pesakit. Namun apabila terdapat kalsium yang termendap di dinding arteri, ia menyebabkan kesukaran untuk menginterpretasikan CT pesakit. Objektif kajian untuk menentukan faktor-faktor yang menyumbang kepada pemendapan kalsium di dinding arteri dalam pemeriksaan CT kedua belah kaki.

Metodologi: Kajian deskriptif keratan rentas ini dilakukan di Hospital Universiti Sains Malaysia untuk menganalisa jumlah kalsium yang termendap di dinding arteri dalam pemeriksaan CT kedua belah kaki yang dari Januari 2010 hingga Mei 2017. Semua pesakit yang ada kalsium yang termendap di dinding arteri dalam pemeriksaan CT kedua belah kaki akan dijadikan subjek dalam kajian ini. Tetapi pesakit yang sebahagian atau semua kakinya telah dipotong, kakinya terdapat besi dan apabila data klinikal yang diperlukan dalam kajian ini tidak terdapat di dalam fail pesakit, telah dikecualikan daripada kajian ini. Jumlah kalsium yang termendap di dinding arteri di kira menggunakan perisian komputer yang sama untuk mengira jumlah kalsium yang termendap di dinding arteri jantung. Jumlah kalsium yang termendap di dinding arteri di bahagikan kepada tinggi dan rendah menggunakan statistik lengkuk normal. Faktor-faktor yang mungkin menyumbang kepada pembentukan kalsium yang termendap di dinding arteri dikaji, iaitu simptom penyakit arteri periferi, sejarah medikal sakit jantung, strok, kegagalan buah pinggang, tahap kolesterol dalam darah, merokok atau tidak, kencing manis, darah tinggi dan tekanan darah sebelum pemeriksaan CT dilakukan. FRS di kira menggunakan kalkulator untuk mengira risiko penyakit jantung di laman sesawang Framingham Heart Study.

Keputusan: 51 subjek terlibat dalam kajian ini (n=51). Selepas dianalisa, faktor yang yang menyumbang kepada pemendapan kalsium di dinding arteri dalam pemeriksaan CT kedua belah kaki adalah umur yang lanjut, diabetes, PAD dan LDL. (R^2 =0.589, p<0.001 to 0.015). Terdapat hubungan yang signifikan di antara Framingham Score and Agatston Score. (r=0.555, p=0.001).

Kesimpulan: Umur, diabetes mellitus, PAD and LDL adalah faktor-faktor yang boleh digunakan untuk menyaring pesakit yang mempunyai pemendapan kalsium yang teruk di dinding arteri dalam pemeriksaan CT kedua belah kaki.

Kata kunci*: jumlah kalsium yang termendap di dinding arteri, CTA kedua belah kaki, Framingham score, Agatston score, penyakit arteri periferi*

ABSTRACT

Background: The use of multi-detector row technology has resulted in shorter acquisition time, increased volume coverage, lower dose of contrast medium, and improved spatial resolution for assessing small arterial branches. However the accuracy of the computed tomography angiography (CTA) examination may be degraded by the presence of calcified plaque. The objective of this study is to evaluate the factors that contribute to the severity of arterial calcification in CT lower limbs.

Methodology: A cross sectional study that conducted in Hospital Universiti Sains Malaysia. CT lower limbs with calcifications from January 2010 to May 2017 were selected for this study. Patients who were amputated, CT images with metallic stent, and those with inadequate clinical information were excluded from the study. The calcium score was analyzed using the standardized calcium score software that was used for coronary arteries. Calcium score was divided into low and high using normal distribution curve. Possible clinical factors that affect arterial calcification were studied such as peripheral arterial disease symptoms, history of acute coronary symptoms, cardiovascular disease, end stage renal disease, blood cholesterol level, smoker/non-smoker, treated for diabetes/hypertension and latest systolic blood pressure. Framingham Risk Score was calculated using General cardiovascular disease Risk Prediction calculator, from official website Framingham Heart Study.

Results: A total of 51 patients was included in the study (n=51) with the mean age was 67. Majority of the participants were Malay (88.2%). The numbers of male and female participants were almost equal. Multivariate analysis showed that interrelated factors that affect log arterial calcium score included age, diabetes, peripheral arterial disease and low density lipoprotein (R^2 =0.589, $p<0.001$ to 0.015). There were significant moderate correlation between Framingham Score and Agatston Score. (r=0.555, p=0.001).

Conclusion: Age, diabetes mellitus, peripheral arterial disease and low density lipoprotein are the potential associated factors that can be used to screen for possibility of severe lower limb arterial calcifications. High Agatston score is associated with high Framingham risk score.

Keywords: *calcium score, CTA lower limb, Framingham risk score, Agatston score, peripheral vascular disease*

CHAPTER 1: INTRODUCTION

1.1 Introduction and literature review.

Peripheral arterial disease (PAD) is a common manifestation of atherosclerosis. The arteries are narrowed causing declining blood flow to patient's limbs. Lower limbs are not able to cope with the lesser supply to keep the demands of tissue during walking which contributes to symptom of intermittent claudication. Critical limb ischaemia is a severe manifestation of PAD and is characterized by severely diminished circulation, ischaemic pain, ulceration, tissue loss and/or gangrene, thereby making early detection of this disease is fundamental.

International study shows that the prevalence of PAD rises sharply with age and affects a substantial proportion of the elderly population. For example, a data from Framingham Study (1) showed the annual incidence of PAD increased from <0.4 per 1000 in men aged 35 to 45 years to 6 per 1000 in men aged 65+ years. A local data, based on a study conducted by Kadirvelu A et al (2) found that in a 301 consecutive patients aged 32 to 90 with established cardiovascular disease, ischaemic stroke or diabetes mellitus more than 5 years, the prevalence of PAD was 23%. PAD was found in 33% of patients with pre-existent cardiovascular disease, 28% in patients with ischaemic stroke and 24% in diabetic patients. PAD was also highly prevalent among the younger patients. PAD is highly prevalent among high-risk Malaysian patients and is not necessarily a disease of older age (2).

Pathophysiology of the arterial calcification can be classified into intimal atherosclerotic calcification, medial arterial calcification, and cardiac valve calcification (3). The medial calcification can be the major contributor of arterial calcification in lower extremity peripheral arterial disease (4). Experimental and clinical studies have shown that arterial calcification is an active and complex process in which the vascular smooth muscle cells are involved and a group of bone-associated proteins, including alkaline phosphatase, osteocalcin, osteopontin, and collagen-rich extracellular matrix are synthesized (5). Other regulatory factors include bone morphogenetic proteins, receptor activator of nuclear factor-kB ligand, tumor necrosis factor-alpha, fetuin-A, oxidative stress, hyperphosphatemia, and vitamin D (3).

A risk factor of peripheral arterial disease is atheroslecrotic plaque of fatty deposition within the mural lining of the vessel. Factors influence PAD includes cigarette smoking, diabetes mellitus, hypertension, dyslipidaemia, obesity, and alcohol consumption (6). Biologically, in diabetes, proatherogenic formation may include vascular inflammation and derangements in cellular components of vasculature, alteration in blood cells and hemostatic factors.

The Framingham Heart Study is an epidemiological project of the National Heart, Lung and Blood Institute and Boston University which pioneered many of the methods commonly used in risk estimation, and has been widely used for the assessment of cardiovascular disease (CVD) risk (1). The major and independent CVD risk factors demonstrated by Framingham Heart Study are cigarette smoking, elevated blood pressure, elevated serum total cholesterol and low-density lipoprotein cholesterol, diabetes mellitus, male gender and advancing age. A previous study showed that 10% to 27% of patients with a low CVS risk as assessed by the traditional Framingham equation, have a markedly increased calcium score and hence, significantly increased risk of CVS event (7). Framingham risk score (FRS) is a risk assessment tool for estimating a patient's 10 year risk of developing cardiovascular disease (1). The factors included in the FRS are age, gender, total cholesterol, HDL level, smoker, diabetes mellitus, systolic blood pressure and is the patient being treated for high blood pressure.

Computed tomographic angiography (CTA) is increasingly used for diagnostic imaging in patients with PAD. The use of multi-detector row technology has resulted in shorter acquisition time, increased volume coverage, lower dose of contrast medium, and improved spatial resolution for assessing small arterial branches (8). However the accuracy of the computed tomographic angiographic (CTA) examination may be degraded by the presence of calcified plaque as reported by several group of investigators (9-11).

Extensive vessel wall calcifications result in multiple problems in interpreting CTA lower limb, such as difficult or unable to produce maximum intensity projection images, and may lead to false-negative or false positive findings due to the "blooming" or high-attenuation artefacts. A prospective study done by Jurgen K Willmann et al (12), which compare CTA lower limbs and lower limb DSA, stated that the presence of vascular wall calcification causing overestimation or underestimation of the degree of stenosis. As a result, wall calcifications in CTA often limited the diagnostic value of CTA and the patient would need additional imaging studies such as lower limbs digital subtraction angiography (DSA) which is invasive compared to CTA.

Calcium score (CS) is the score used to quantify calcification in coronary arteries. Agatson score, is the calcification scoring method used in cardiac CT angiography (CCTA). Calcium score was determined and expressed as Agatston score according to the method described by Agatston et al. (13). The clinical implication of the coronary artery CS is well documented and demonstrated. Previous study done by

Tamar S. Polonsky et al (14) shows that coronary artery calcium score (CACS) has been shown to predict future coronary heart disease (CHD) events.

However little is known about the association between lower limbs arterial calcification and clinical outcomes. Huang CL et al (15) concluded that lower extremity arterial calcification was associated with amputation and mortality in patients with symptomatic PAD. A cross-sectional study done by C.E. Aubert et al (16) used CS of below knee artery to determine the impact of calcification on false negative tests (arteriopathy present despite normal screening tests). The screening tests used were pulse palpation and ankle brachial index. This study showed that below knee vascular calcification gave a high rate of false negative results for ankle brachial index. Guzman, Raul J et al (17) concluded that in PAD patients, high CS is associated with the severity of the disease and is valuable as a marker of amputation risk. Ouwendijk R et al (11) justified that diabetes mellitus, cardiac disease, and elderly age (older than 84 years) were independently predictive for the presence of vessel wall calcifications. Huang et al (15) justified that subjects with diabetes mellitus and ESRF had higher CS.

All of the studies mentioned above used CS for lower limbs, however the subjects were only those with PAD symptoms. Guzman, Raul J. et al (17) acknowledge the limitation of their study by integrating CS used in coronary artery for lower limbs. There are other scoring methods such as the volumetric method of Callister, T. Q. et al (18) or the mass scoring method initially suggested by Detrano et al (19). Future work will be needed to compare between these few CS methods.

To the best of our knowledge this is the first study on the correlation between Framingham score and Agatston score. Previous study by Tochi M. Okwuosa et al (20) focused on the correlation between the coronary artery calcium (CAC) score and Framingham risk score (FRS). Their study showed that the prevalence and amount of CAC increased with higher FRS. CAC ≥300 was observed in 1.7% and 4.4% of those with FRS 0–2.5% and 2.6–5%, respectively. Likewise, CAC ≥300 was observed in 24% and 30% of those with FRS 15.1–20% and >20%, respectively. Trends were similar when stratified by age, gender and race/ethnicity. Another study by Ellis CJ et al (7), showed approximately 10% to 27% of patients with a low CVS risk as assessed by the traditional Framingham equation, have a markedly increased calcium score and hence, actually, a significantly increased risk of a CVS event.

1.2 Rationale of the study:

The aim of our study was to evaluate the factors that contributes to lower extremity calcium score and to determine the correlation between the Framingham score and Agatston score. We tried to predict which patients will have severe lower limb arteries calcification and will gain less benefit from CTA lower limbs. This is crucial, in order to avoid unnecessary radiation to the patient and potentially to be able to reduce the possibility of false positive or false negative result in CTA lower limb.

CHAPTER 2: STUDY PROTOCOL

2.1 TITLE:

PREDICTIVE FACTORS OF ARTERIAL CALCIFICATION SEVERITY IN COMPUTED TOMOGRAPHY ANGIOGRAPHY (CTA) LOWER LIMBS.

2.2 GENERAL OBJECTIVE:

To evaluate the factors that contribute to arterial calcification in CTA lower limbs.

2.3 SPECIFIC OBJECTIVES:

1. To identify the associated factors that are related to arterial calcium score (CS).

2. To determine the correlation between Framingham risk score and Agatston score.

2.4 METHODOLOGY

Study design:

This was a cross sectional study and was conducted at Hospital Universiti Sains Malaysia (HUSM).

Study duration and location:

Study duration was from January 2016 until November 2017 in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan.

Study population and sample:

Reference population were patients that have lower limb arterial calcification. Source population were CT lower limb images in picture archiving and communication system (PACS) in HUSM from January 2010 until May 2017.

Inclusion criteria: All CT lower limbs with arterial calcification.

Exclusion criteria:

1. Type I diabetes mellitus.
2. CT study with no non contrast images.
3. Previous lower limb vascular intervention or lower extremity amputation.
4. Images with extensive metal artifact or missed arterial segments
5. Inadequate data for Framingham score.
6. Non-transferrable PACS images to CT scan workstation for CS calculation.

Sample size calculation.

1. For first objective, using G*Power tool (21) using F tests linear multiple regression: Fixed model, R^z increase, with the significance level (alpha) 0.05 and the power of study (1- β) of 80%, and the number of tested predictors are 19, the actual power is 0.80 and total sample size obtained is 153.
2. For second objective, using formula for sample size for correlation analysis,correlation coefficient, r = 0.3 (from previous study done Huang, Chi Lun et al (15)) with the significance level (alpha) 0.05 and the power of study (1- β) of 80%, total sample size obtained is 85. The largest sample size is n =153 patients. After adding 10% estimated missing data, we get; n = 153+ (0.1 × 153) = 168. Therefore, a total patient to be sampled is 168. Sampling method was all patient who has calcifications in their CTA lower limb done from January 2010 until May 2017.

Study subject.

Written subject's consent were not needed since it was a retrospective study. For some of the information needed which were unavailable in the patient's folder, phone calls were made and verbal consents were obtained from the patients.

Variable definition

Agatston score:

Calcium scores for lower limb arteries were determined and expressed as Agatston score according to the method described by Agatston et al. (13). This method was chosen because it is widely use and it provides a simple transition from coronary calcium scoring. Any structure which had densities of 130 Hounsfield units (HU) or more and having an area of 1 mm^2 or more was segmented as calcified focus and those foci overlying the anatomic site of coronary arteries were considered to represent calcified plaques. Using an area of at least 1 mm^2 - comprising of at least 2 pixels - ensured one not including single pixel - which represents image noise - in measurements. In each segmented calcified focus, based on its peak density, a density score of 1 through 4 was assigned. The stratified density scores 1, 2, 3 and 4 represented the highest densities 130-199 HU, 200-299 HU, 300-399 HU and ≥ 400 HU, respectively. The most important determining factors in calculating calcium score of each plaque were the measured area of each calcified plaque and its density. The total Agatston score (AS) of each individual was calculated by summing the scores of every calcified focus through all of the coronary arteries (13).

Lower limbs CS:

The CS for bilateral common iliac, external iliac, superficial femoral arteries (SFA) and below-knee arteries (BKA) including anterior tibial, posterior tibial, and peroneal arteries were performed using standardized calcium scoring software for coronary arteries. On cross-sectional images through the lower extremities, area of calcification with a cross sectional sectional area >1mm and a density of >130 Hounsfield units (HU) were identified automatically. Regions of interest with calcification were manually selected and labelled. The measurement started at the common iliac artery and ended at the widest portion of the tibial and fibular malleoli at the ankle. Calcium score for each area of interest along the course of the SFA, bilateral common iliac, external iliac, superficial femoral arteries (SFA) and below-knee arteries (BKA) calcification score was determined and expressed as Agatston score according to the method described by Agatston et al. (13)

Framingham risk score (FRS):

Risk assessment tool for estimating a patient's 10 year risk of developing cardiovascular disease. The factors include in FRS are age, gender, total cholesterol, HDL level, smoker, diabetes mellitus, systolic blood pressure and is the patient being treated for high blood pressure. General CVD Risk Prediction calculator, from official website Framingham Heart Study (1) was used to calculate the Framingham risk score. We used the subject's data, such as gender, age, systolic blood pressure, treatment for hypertension, current smoker, diabetes, HDL and total cholesterol level and inserted into the calculator to get the risk (%) of the subject will develop cardiovascular disease in 10 years' time.

Diabetics, cerebral vascular disease, PAD, ESRD: Documented in the clinical folder or under treatment for the diseases.

Smoking history: Smoker or non-smoker based on clinical folder.

Systolic blood pressure (SBP): Record from the latest clinic appointment before the CT examination.

Research/measurement tool

The CT lower limb were done using MDCT scanner (SOMATOM Sensation cardiac 128; Siemens AG, Munich, Germany). Scans were performed using a 3-mm-increment, mAs=200, and kV=120 with a field of view of 350 to 380mm, yielding typical special resolution 0.7 x 0.7 x 3.0mm3. The scan duration of the lower limb was approximately 12 to 15 seconds. From the acquired raw data, the scan was reconstructed in 3mm slices. The average number of slices of lower extremities was approximately 350. The scoring of calcification for bilateral common iliac, external iliac, superficial femoral arteries (SFA) and below-knee arteries was performed using standardized calcium scoring software at the CT console HUSM.

Data collection:

The following information were recorded on a patient data sheet form developed for this study: gender, age, and reason for CTA lower limbs. All CTA lower limbs done from January 2010 to May 2017 were analyzed using PACS BARCO at HUSM. All CTA with calcifications were included as subjects. Arterial calcification measurement were done and scored. Clinical factors were traced from patient's clinical folder. Some of the information were obtained by phone calls during which verbal consent were obtained from the patients. The data that were collected: total cholesterol, HDL level, smoker, diabetes mellitus, systolic blood pressure, is the patient being treated for high blood pressure, cerebral vascular disease, peripheral arterial disease and end stage renal disease. Latest blood investigation results of the subjects; which were obtained before the CT done, were traced from the LIS (laboratory information system) hospital system: total cholesterol, HDL, LDL, TG, creatinine, urea, and calcium level.

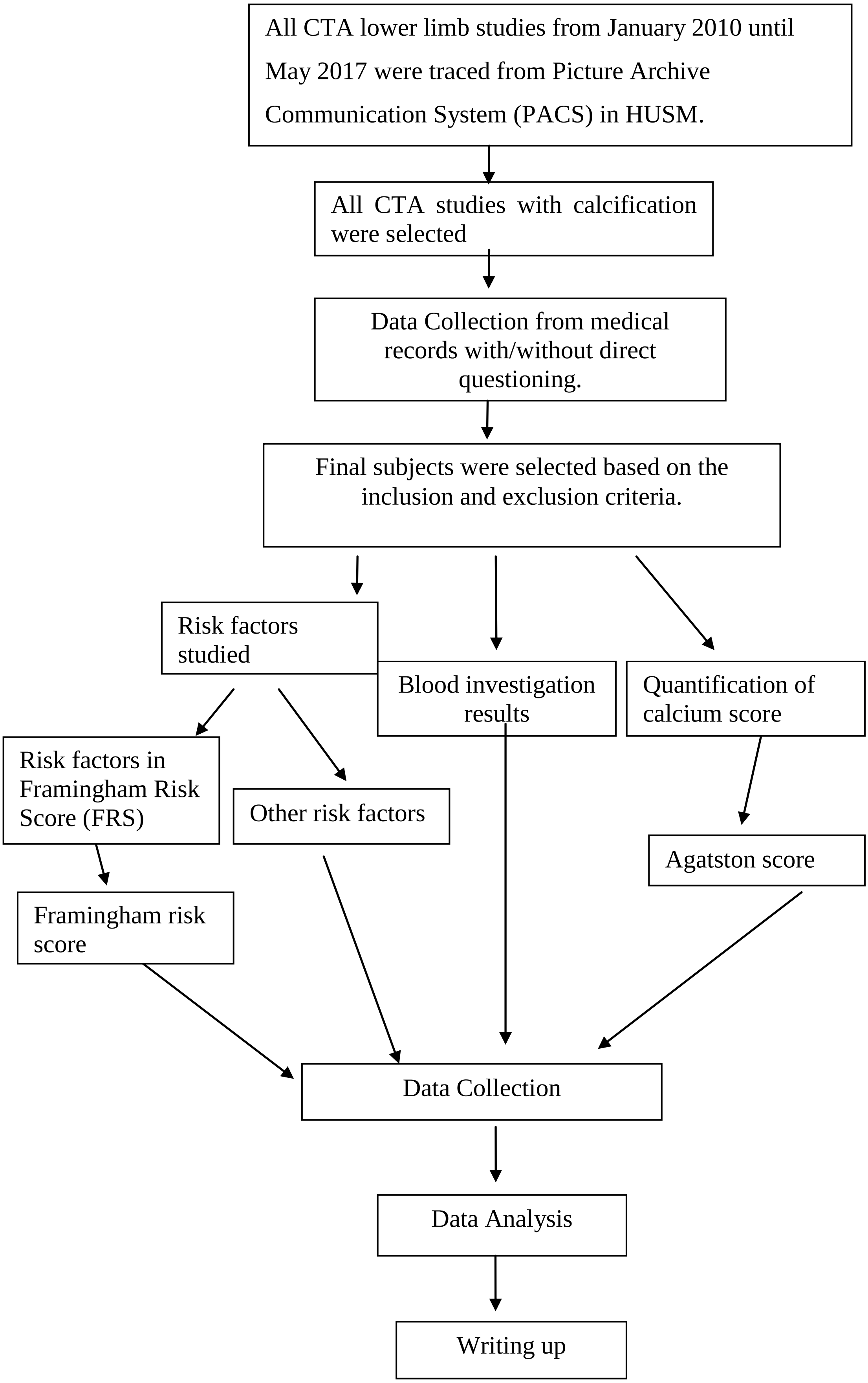
Statistical Analysis

The statistical analyses used in this study were descriptive statistics (mean and standard deviation), frequency, and simple and multiple linear regression analysis and correlation analysis. The data were analyzed using IBM SPSS 22.0 for windows. Before processing and analyzing, the data were checked and cleaned to avoid any missing data. The power for the study was set as 80% and values below 5% ($p<0.05$) were considered statistically significant.

Ethical issues/consideration/approval

Ethical approval were applied from the HUSM ethical board, and approval from Director of HUSM regarding utilizing patient's clinical information and images in PACS.

STUDY FLOW CHART



References

1. Framingham Heart study. A project of the National Heart, Lung, and Blood Institute and Boston University 2017 [cited 2017 13 November 2017]. Available from: https://www.framinghamheartstudy.org/risk-functions/cardiovascular-disease/10-year-risk.php.

2. Kadirvelu A, H Chee K, S Tan K, T Tan C, Lang C. Prevalence of peripheral artery disease in urban high-risk Malaysian patients2003. 369-72 p.

3. Sage AP, Tintut Y, Demer LL. Regulatory Mechanisms in Atherosclerotic Calcification. Nature reviews Cardiology. 2010;7(9):528-36.

4. Christopher David S, Jonathan Gavin B, Shehzad I, Sharon R, Melwyn P. Medial Artery Calcification as an Indicator of Diabetic Peripheral Vascular Disease. Foot & Ankle International. 2008;29(2):185-90.

5. Demer LL, Tintut Y. Vascular Calcification. Circulation. 2008;117(22):2938.

6. Allison MA, Criqui MH, McClelland RL, Scott JM, McDermott MM, Liu K, et al. The effect of novel cardiovascular risk factors on the ethnic-specific odds for peripheral arterial disease in the Multi-Ethnic Study of Atherosclerosis (MESA). Journal of the American College of Cardiology. 2006;48(6):1190-7.

7. Ellis CJ, Legget ME, Edwards C, Van Pelt N, Ormiston JA, Christiansen J, et al. High calcium scores in patients with a low Framingham risk of cardiovascular (CVS) disease: implications for more accurate CVS risk assessment in New Zealand. The New Zealand medical journal. 2011;124(1335):13-26.

8. Rubin GD, Shiau MC, Leung AN, Kee ST, Logan LJ, Sofilos MC. Aorta and iliac arteries: single versus multiple detector-row helical CT angiography. Radiology. 2000;215(3):670-6.

9. Catalano C, Fraioli F, Laghi A, Napoli A, Bezzi M, Pediconi F, et al. Infrarenal aortic and lower-extremity arterial disease: diagnostic performance of multi-detector row CT angiography. Radiology. 2004;231(2):555-63.

10. Ofer A, Nitecki SS, Linn S, Epelman M, Fischer D, Karram T, et al. Multidetector CT Angiography of Peripheral Vascular Disease: A Prospective Comparison with Intraarterial Digital Subtraction Angiography. American Journal of Roentgenology. 2003;180(3):719-24.

11. Ouwendijk R, de Vries M, Pattynama PM, van Sambeek MR, de Haan MW, Stijnen T, et al. Imaging peripheral arterial disease: a randomized controlled trial comparing contrast-enhanced MR angiography and multi-detector row CT angiography. Radiology. 2005;236(3):1094-103.

12. Willmann JK, Baumert B, Schertler T, Wildermuth S, Pfammatter T, Verdun FR, et al. Aortoiliac and Lower Extremity Arteries Assessed with 16–Detector Row CT

Angiography: Prospective Comparison with Digital Subtraction Angiography. Radiology. 2005;236(3):1083-93.

13. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. Journal of the American College of Cardiology. 1990;15(4):827-32.

14. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, et al. Coronary Artery Calcium Score and Risk Classification for Coronary Heart Disease Prediction: The Multi-Ethnic Study of Atherosclerosis. JAMA : the journal of the American Medical Association. 2010;303(16):1610-6.

15. Huang C-L, Wu IH, Wu Y-W, Hwang J-J, Wang S-S, Chen W-J, et al. Association of Lower Extremity Arterial Calcification with Amputation and Mortality in Patients with Symptomatic Peripheral Artery Disease. PLoS ONE. 2014;9(2):e90201.

16. Aubert CE, Cluzel P, Kemel S, Michel PL, Lajat-Kiss F, Dadon M, et al. Influence of peripheral vascular calcification on efficiency of screening tests for peripheral arterial occlusive disease in diabetes--a cross-sectional study. Diabetic medicine : a journal of the British Diabetic Association. 2014;31(2):192-9.

17. Guzman RJ, Brinkley DM, Schumacher PM, Donahue RMJ, Beavers H, Qin X. Tibial artery calcification as a marker of amputation risk in patients with PAD. Journal of the American College of Cardiology. 2008;51(20):1967-74.

18. Callister TQ, Cooil B, Raya SP, Lippolis NJ, Russo DJ, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. Radiology. 1998;208(3):807-14.

19. Detrano R, Kang X, Mahaisavariya P, Tang W, Colombo A, Molloi S, et al. Accuracy of quantifying coronary hydroxyapatite with electron beam tomography. Investigative radiology. 1994;29(8):733-8.

20. Okwuosa TM, Greenland P, Ning H, Liu K, Bild DE, Burke GL, et al. Distribution of coronary artery calcium scores by Framingham 10-year risk strata in the MESA (Multi-Ethnic Study of Atherosclerosis) potential implications for coronary risk assessment. Journal of the American College of Cardiology. 2011;57(18):1838-45.

21. Bruin J. newtest: command to compute new test {@ONLINE} 2011 [cited 2017 14 November 2017]. Available from: https://stats.idre.ucla.edu/stata/ado/analysis/.

CHAPTER 3: MANUSCRIPT

3.1. TITLE: PREDICTIVE FACTORS OF ARTERIAL CALCIFICATION SEVERITY IN COMPUTED TOMOGRAPHY (CTA) LOWER LIMBS.

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3.2. ABSTRACT

ABSTRACT

Background: The use of multi-detector row technology has resulted in shorter acquisition time, increased volume coverage, lower dose of contrast medium, and improved spatial resolution for assessing small arterial branches. However the accuracy of the computed tomography angiography (CTA) examination may be degraded by the presence of calcified plaque. The objective of this study is to evaluate the factors that contribute to the severity of arterial calcification in CT lower limbs.

Methodology: A cross sectional study that conducted in Hospital Universiti Sains Malaysia. CTA lower limbs with calcifications from January 2010 to May 2017 were selected for this study. Patients who were amputated, CT images with metallic stent, and those with inadequate clinical information were excluded from the study. The calcium score was analyzed using the standardized calcium score software that was used for coronary arteries. Calcium score was divided into low and high using normal distribution curve. Possible clinical factors that affect arterial calcification were studied such as peripheral arterial disease symptoms, history of acute coronary symptoms, cardiovascular disease, end stage renal disease, blood cholesterol level, smoker/non-smoker, treated for diabetes/hypertension and latest systolic blood pressure. Framingham Risk Score was calculated using General cardiovascular disease Risk Prediction calculator, from official website Framingham Heart Study.

Results: A total of 51 patients was included in the study (n=51) with the mean age was 67. Majority of the participants were Malay (88.2%). The numbers of male and female participants were almost equal.Multivariate analysis showed that interrelated factors that affect log arterial calcium score included age, diabetes, peripheral arterial disease and low density lipoprotein(R^2 =0.589, $p<0.001$ to 0.015). There were significant moderate correlation between Framingham Score and Agatston Score. (r=0.555, p=0.001).

Conclusion: Age, diabetes mellitus, peripheral arterial disease and low density lipoproteinare the potential associated factors that can be used to screen for possibility of severe lower limb arterial calcifications. High Agatston score is associated with high Framingham risk score.

Keywords: *calcium score, CT lower limb, Framingham risk score, Agatston score, peripheral vascular disease*

3.3. INTRODUCTION

The prevalence of peripheral arterial disease rises sharply based on international and local data. A data from Framingham Study(1) show PAD annual incidence increased from <0.4 per 1000 in men aged 35 to 45 years to 6 per 1000 in men aged 65+ years. A local data, based on a study conducted by Kadirvelu A et al (2) found that in a 301 consecutive patients aged 32 to 90 with established cardiovascular disease, ischaemic stroke or diabetes mellitus more than 5 years, the prevalence of PAD was 23%.Risk factors attribute to peripheral arterial disease is atheroslecrotic plaque of fatty deposition within the mural lining of the vessel. Factors influence PAD includes cigarette smoking, diabetes mellitus, hypertension, dyslipidaemia, obesity, and alcohol consumption (3).

The Framingham Heart Study is a well-known and an established epidemiological project that has been widely used for the assessment of cardiovascular disease (CVD) risk (1). A previous study showed that 10% to 27% of patients with a low CVS risk as assessed by the traditional Framingham equation, have a markedly increased calcium score and hence, significantly increased risk of CVS event(4). Framingham risk score (FRS) is a risk assessment tool for estimating a patient's 10 year risk of developing cardiovascular disease (1). The factors include in the FRS are age, gender, total cholesterol, HDL level, smoker, diabetes mellitus, systolic blood pressure and is the patient being treated for high blood pressure.

Computed tomography angiography (CTA) is essential as a diagnostic imaging in PAD patients. However the presence of calcified plaque is troublesome as reported by several group of investigators(5-7). Extensive vessel wall calcifications give rise to several disadvantages such as difficult or unable to produce maximum intensity projection images, and may lead to false-negative or false positive findings due to the artefacts. A prospective study done by Jurgen K Willmann et al(8) which compared the CTA lower limbs and lower limb DSA, stated that the presence of vascular wall calcification causedoverestimation or underestimation of the degree of stenosis. As a result, wall calcifications in CTA often limited the diagnostic value of CTA and the patient would need additional imaging studies such as lower limbs digital subtraction angiography (DSA) which is invasive compared to CTA.

Calcium score (CS) is the score used to quantify calcification arteries. Agatson score is the method described by Agatston et al (9) which is a scoring method used in cardiac CT angiography (CCTA). The clinical implication of the coronary artery CS is well documented and demonstrated. A study done by Tamar S. Polonsky et al (10) showed that coronary artery calcium score (CACS) has been shown to predict future coronary heart disease (CHD) events.

There are few previous studies that used CS for lower limbs arterial calcification. Huang CL et al (11) studied the association of lower extremity arterial calcification with amputation and mortality in patients with symptomatic PAD. They concluded that the severe the lower extremity arterial calcification, the higher chances of patient to develop complications such as amputation. Whereas a cross-sectional study done by C.E. Aubert et al (12) used CS of below knee artery to determine the impact of calcification on false negative tests (arteriopathy present despite normal screening tests). The screening tests used are pulse palpation and ankle brachial index. This study showed that below knee vascular calcification gave a high rate of false negative results for ankle brachial index. All of the studies mentioned above used CS for lower limbs, however the subjects were only those with PAD symptoms. Regarding the factors that contributed to arterial calcification, in previous study, diabetes mellitus, cardiac disease, and elderly age (older than 84 years) are independently predictive for the presence of vessel wall calcifications(7). Huang et al (11) justified that subjects with diabetes mellitus and ESRF had higher CS.

There are few other methods to score arterial calcification of lower limbs, but these are less popular than CS. Volumetric method that is described by Callister, T. Q. et al (13) and the mass scoring method initially suggested by Detrano et al (14). Future work will be needed to compare between these few CS methods.

To the best of our knowledge this is the first study on the correlation between Framingham risk score and Agatston score. Previous study by Tochi M. Okwuosa et al(15) focused on the correlation between the coronary artery calcium (CAC) score and Framingham risk score (FRS). Their study showed thattheprevalence and amount of CAC increased with higher FRS. Another study by Ellis CJ et al (4) showed approximately 10% to 27% of patients with a low CVS risk as assessed by the traditional Framingham equation, have a markedly increased calcium score and hence, actually, a significantly increased risk of a CVS event.

In this study, we investigatedthe factors that contribute to arterial calcification in CT lower limbs and the correlation between Framingham risk score and Agatston score. By doing this study, we would be able to predict factors that gives rise to severe calcification in CTA lower limbs. Potentially it will reduce the possibility of false positive or false negative result in CTA lower limb.

3.4 METHODOLOGY

This was a cross sectional study conducted at Hospital Universiti Sains Malaysia (HUSM).Reference population was patients that have lower limb arterial calcification on CT studies. CT lower limb images were retrieved from picture archiving and communication system (PACS) in HUSM from January 2010 to May 2017. Ethical approval was obtained from the ethic committee (JEPeM-USM code USM/JEPEM/15110450).

All CT lower limbs with arterial calcification were included.Individuals with a history of lower extremity amputation, CTA study with no non-contrast images, images with extensive metal artefact or missed arterial segments were excluded. Subjects with Type I diabetes mellitus and previous lower limb vascular intervention, inadequate data for Framingham score and non-transferable PACS images to CT scan workstation for calcium score calculation were also excluded from the study.

Profile of the subjects were recorded in the data collection sheet. The data include gender, age, race and medical history of symptoms of PAD, ACS, CVA and ESRD. Laboratory results were also documented include total cholesterol, HDL, LDL, TG,creatinine, urea, and calcium level. The data was taken from the medical record and LIS (laboratory investigation system). For missing information phone interview was made to acquire the information during which verbal consents were also obtained from the patients.

A subject was defined as diabetics when he/she was under treatment for diabetes. Smoking history was classified into two groups, smoker or non-smoker based on the clinical notes. Systolic blood pressure (SBP) was taken from the measurement in the latest clinic appointment before the CTA examination. History of acute coronary syndrome event, cerebral vascular accident, symptoms of PAD and end stage renal disease were taken as positive if there is documentation in the patient's medical record that the patient is treated for the listed disease.

General CVD Risk Prediction calculator, from official website Framingham Heart Study was used to calculate the Framingham risk score(1).Data such as gender, age, systolic blood pressure, treatment for hypertension, current smoker, diabetes, HDL and total cholesterol level were used to calculate the risk (%) of the subject developing CVD in 10 years time. (Figure 1 and 2).

The CT lower limb was performed using MDCT scanner (SOMATOM Sensation cardiac 128; Siemens AG, Munich, Germany). Scans were performed using a 3-mm-increment, mAs=200, and kV=120 with a field of view of 350 to 380mm, yielding typical special resolution 0.7 x 0.7 x 3.0mm^3. The scan duration of the lower limb was approximately 12 to 15 seconds. From the acquired raw data, the scan was reconstructed in 3mm slices. The average number of slices of lower extremities was approximately 350. The scoring of calcification for bilateral common iliac, external iliac, superficial femoral arteries (SFA) and below-knee arteriesincluding anterior tibial, posterior tibial, and peroneal arteries were performed using standardized calcium scoring software at the CT console, HUSM.

On cross-sectional images through the lower extremities, area of calcification with a cross sectional sectional area more than 1mm and a density of more than 130 Hounsfield units (HU) were identified automatically. Regions of interest with calcification were manually selected and labelled. The measurement started at the common iliac artery and ended at the widest portion of the tibial and fibular malleoli at the ankle. Calcium scores for each area of interest along the course of the bilateral common iliac, external iliac, superficial femoral arteries (SFA) and below-knee arteries (BKA) were determined and expressed as Agatston score according to the method described by Agatston et al.(9).This method was chosen because it is widely use and it provides a simple transition from coronary calcium scoring.Example to calculate calcium scoring is shown in Figure 3 to 5. However there was no specified calcium score software to calculate calcium scoring for lower limb arteries. Hence, the table for the cardiac artery, was modified; LM=common iliac artery, LAD=external iliac artery, CX=superficial femoral artery and RCA=below knee arteries. The final CS for each subjects was the sum of the individual scores from all the arterial segments from common iliac to below knee arteries (ended at the widest portion of the tibial and fibular malleoli at the ankle).

Data were assessed for normal distribution. Categorical data was presented as frequency and percentages; continuous data was presented as mean ± standard deviation. Univariate linear regression was used to study the factors associated with the CS. Multiple linear regression analysis was performed to determine the independent parameters correlated with CSstatistically significant at $p<0.05$. The data were analysed using IBM SPSS 22.0 for windows. Subjects were divided into low CS and high CS based on the sum CS using normal distribution curve (bell shaped distribution).