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ORIGINAL ARTICLE

Association of coronary artery calcium score with calcification and degree of stenosis: An autopsy study

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Abstract

Introduction: Coronary artery disease (CAD) is a known cause of major cardiovascular events and calcium score (CS) has been developed as a marker of coronary atherosclerosis. Yet, the relationship between post mortem computed tomography (PMCT) CS with histologically observed calcification and the severity of coronary artery stenosis has not been widely explored and is still unclear. This study aims to determine the association between coronary artery PMCT CS with histologically observed calcification and degree of stenosis of coronary arteries in post-mortem cases. *Materials & Methods:* This was a cross-sectional study involving 101 subjects recruited from the National Institute of Forensic Medicine (IPFN) Hospital Kuala Lumpur (HKL) over a period of 15 months, from December 2012 until April 2014. PMCT CS of the coronary arteries was calculated using Agatston-Janowitz score. Histological presence of calcification was observed and the degree of stenosis was calculated using an image analysis technique. *Results:* PMCT CS increased with increasing severity of stenosis ($p<0.001$). PMCT CS showed a positive correlation with the presence of calcification ($r=-0.82$, $p<0.001$). *Conclusion:* Calcium score is strongly associated with coronary artery calcification and the degree of luminal stenosis in post mortem subjects. Thus, PMCT may be useful as a non-invasive tool in diagnosing CAD in the event that an autopsy is not possible.

Keywords: Coronary artery disease, post–mortem computed tomography, calcium score, calcification, stenosis, medico-legal autopsy

INTRODUCTION

Although autopsy is an age-old method for identifying the underlying pathology leading to death, and/or for detecting unnatural deaths, the number of autopsies performed worldwide are decreasing due to public resistance to autopsies, negative press attention, funeral delays, religious or cultural beliefs and fear of mutilation of the deceased's body.[1] Thus, post-mortem imaging which includes PMCT offers a method of investigation that can be used as a complementary tool to autopsy as in addition to yielding information about findings for later confirmation during an autopsy, it also guides the forensic pathologist to focus on a structure of interest which includes calcifications of the coronary arteries.[2,3]

Since coronary artery calcification unequivocally reflects CAD, numerous investigators have looked at the potential role of coronary calcium quantification by computed tomography (CT) as an accurate, cost-effective screening examination for coronary heart disease.[4-7] Coronary calcifications measured by CT are usually expressed as "Agatston score" and in numerous trials, coronary artery calcification (CAC) has been shown to be predictive of major cardiovascular events.[8]

Most studies investigated the use of coronary artery calcium score measured by the Agatston scoring method in living patients as a means of scoring the severity of coronary atherosclerosis in order to manage patients accordingly. However not much has been applied to the use of coronary

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artery calcium score in post mortem subjects which could act as a non-invasive tool in scoring coronary atherosclerosis. Therefore, the aim of this study was to evaluate the relationship between coronary artery calcium score (CACS) assessed by PMCT and examine its association with histological presence of calcification and degree of stenosis in post-mortem subjects.

MATERIALS AND METHODS

Case Selection

This study had been approved by the Institutional Ethics Committee [NMRR-11-1135-10262 and 600-RMI (5/1/6/01)]. The subjects recruited were from medico-legal autopsy cases brought to the National Institute of Forensic Medicine, Hospital Kuala Lumpur, Malaysia over a period of 15 months, from December 2012 until April 2014. They were divided into sudden death and sudden unnatural death cases. According to the World Health Organization (WHO), sudden death is defined as natural, nonviolent, unexpected death occurring within 24 hours of the onset of symptoms.[9] However, sudden unnatural causes comprised of trauma cases such as road traffic accident fatalities, homicide or suicide victims. Decomposed, severely charred and skeletonised bodies as well as bodies with severe trauma to the heart were excluded from the study.

Post-mortem CT scan

A dedicated 64-slice (Toshiba Aquilion 64 TSX-101A, Japan) multidetector computed tomography (MDCT) was used. Scans were performed in a cranio-caudal direction covering the heart from above the aortic arch via the apex till the diaphragm in 3.0 mm slice thickness with 3 mm interval axial sections using the following parameters: 120 kVp, Auto set mAs (Caredose), FOV 500 (LL), 1.0 x 32 raw detector collimation and 0.844/standard pitch. Quantification of calcium was based on a section by section analysis of CT images involving the left circumflex (LCX), left anterior descending (LAD), and right coronary arteries (RCA) using the Agatston-Janowitz score (CSCS -001A-Agatson's, Korea) on the Infinit Monitor (PACS version 3091, Korea). The PACS system utilised has a dedicated software to calculate the Agatston's score which is defined as any pixel within the region of interest with a CT density > 130 Hounsfield units in a fashion similar to other studies.10-14 Total CACS was computed as the sum of the Agatston score of calcified lesions within each artery (LCX, LAD and RCA). A CAC score cut off point of 160 was taken to categorise the data as this value has been shown to be useful in predicting coronary artery disease in previous studies.15,16 Only cases with luminal stenosis caused by calcified plaques were taken into account as the Agatston's score is only able to calculate calcified lesions.

Autopsy & Histology

The autopsy was performed by a medical officer attached to the Institute or a Forensic Pathologist. The LAD, LCX and RCA were cross-sectioned at a 5 mm interval and examined for atherosclerotic lesions. Sections were then taken from the worst affected area at the proximal (1), middle (2) and distal third (3) of each artery. If there were no obvious lesions, representative sections were taken at similar segments as stated above. For each sampling, a 5-mm arterial segment was taken, as in standard histopathology practice. The sections were fixed in 10% formalin and processed to form a paraffin-embedded tissue block. The arteries with significant calcification were subjected to a decalcification process by incubating with 15% formic acid solution until soft. The tissue blocks were then sectioned at a 4 μm thickness and stained with Haematoxylin and Eosin (H&E).

Photomicrographs were taken of the sections and analysed using an image analysis software (NIS-Element, Nikon, Japan). The presence of calcification was defined as the tissue area that stained deep purple on H&E stain. Its absence or presence was noted for each arterial segment. The degree of luminal stenosis was determined by calculating the percentage of the luminal diameter divided by the diameter of the internal elastic as described in a previous study.[17] For analysis, the luminal stenosis result was divided into three categories: mild (< 25% occlusion), moderate (25 - 75%) and severe (with >75% occlusion.

STATISTICAL ANALYSIS

The statistical analysis package for social sciences (SPSS) for Windows, version 16.0 (Chicago, IL, USA) was used for all analyses. Continuous variables were presented as the mean value ± SD. Discrete variables were presented as percentages. Association of coronary artery calcium score with the histological presence of calcification and the degree of stenosis was calculated using Pearson's correlation and chi-square test. A P value < 0.05 was considered significant.

RESULTS

Demographic data

A total of 101 cases were included in this study, of which 87 were males and 14 were females. The mean age ± SD was 38.9 ±11.6 years. The subjects were grouped according to the manner of death which were natural (n=36), motor vehicle accidents (n=52) and homicide and suicide (n= 13). Malaysians contributed 65.35% (n=66) of cases while the remaining (34.65%) cases were from Indonesia, Myanmar and Pakistan. Amongst the Malaysians, Chinese were predominant 28.71% (n=29), followed by Malays 18.81% (n=19) and Indians 17.82% (n= 18). Summary of the demographic data is as shown in Table 1.

PMCT CS and histological analysis

In total, 909 coronary artery sections were sampled, which included representative sections from the LAD, LCX and RCA. This study found that the LAD was most frequently and most extensively calcified, followed by the RCA using PMCT CS as shown in Table 2. Coronary artery calcification detected on PMCT and calcification seen on histology is demonstrated in Fig. 1.

PMCT CS vs histological evaluation of the degree of stenosis and calcification

PMCT CS was significantly higher in those with severe ($p<0.001$) when compared to moderate ($p<0.001$) or mild ($p<0.01$) stenosis, Table 3. PMCT CS also showed a strong correlation with the presence of calcification on histology, Table 4. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of PMCT CS in detecting luminal narrowing and calcification of the arteries are presented in Table 5.

DISCUSSION

This study showed that the LAD was most frequently and most extensively calcified, followed by the RCA using both PMCT CS and histology due to the local hemodynamic and anatomic particularities of the left coronary system (LCA) vs RCA which is responsible for the predilection of atherosclerosis development in the LCA. Pathological anatomical studies have demonstrated that coronary lesion formation starts in the first 2 cm of the left coronary artery followed by the proximal right coronary arteries.[12] Predilection for LCA also include greater wall stress throughout the cardiac cycle in LCA, torsion generating helical flow patterns and increased branching of the LCA as compared to the RCA causing a disturbed flow and ultimately leading to atherosclerosis.[18-25] This is consistent with reports on the natural history of coronary calcium detected by EBCT and with data from histopathologic and angiographic series.[26-28]

This study is novel as it used CT to determine and calculate calcium score in post mortem subjects. CT is a non-invasive extremely sensitive modality for the detection of calcium,

TABLE 1: Demographic data of subjects

Parameters	Subjects (n=101)
[a]Age (years)	38.9 ± 11.6
Gender:	
Males	86.1%
Females	13.9%
Ethnicity:	
Malay	18.8%
Chinese	28.7%
Indian	17.8%
Others	34.7%
Cause of death:	
Natural	35.6%
Accidental	51.5%
Homicide or suicide	12.9%

NOTE: [a]Data expressed as mean ± SD

TABLE 2: PMCT CS (mean ± SD) of the coronary vessels

	PMCT CS (Mean ± SD)
All Coronary Vessels (n=909)	11.96 ± 62.50
LAD (n=303)	19.26 ± 91.51
LCX (n=303)	7.72 ± 42.03
RCA (n=303)	8.89 ± 39.02
LAD1 (n=101)	47.31 ± 137.15
LAD2 (n=101)	10.09 ± 72.34
LAD3 (n=101)	0.38 ± 2.05
LCX1 (n=101)	10.63 ± 48.70
LCX2 (n=101)	8.42 ± 44.49
LCX3 (n=101)	4.13 ± 31.02
RCA1 (n=101)	8.46 ± 39.02
RCA2 (n=101)	10.47 ± 40.20
RCA3 (n=101)	7.75 ± 38.14

NOTE: Data expressed as mean ± SD. PMCT CS = post-mortem computed tomography calcium score; LAD = Left Anterior Descending; LCX = Left Circumflex; RCA = Right Coronary Artery; LAD1 = Proximal Left Anterior Descending; LAD2 = Mid Left Anterior Descending; LAD3 = Distal Left Anterior Descending; LCX1 = Proximal Left Circumflex; LCX2 = Mid Left Circumflex; LCX3 = Distal Left Circumflex; RCA1 = Proximal Right Coronary Artery; RCA 2 = Mid Right Coronary Artery; RCA 3 = Distal Right Coronary

TABLE 3: Association between the degree of stenosis and CS=calcium score (mean ± SD)

Degree of stenosis	CS (Mean ± SD)	[a]p value
Mild	29.7 ± 4.73	<0.01
Moderate	121.8 ± 23.67	<0.001
Severe	179.4 ± 64.14	<0.001

NOTE: One-way ANOVA was applied. [a]Post-hoc test Bonferroni's procedure: All mean of calcium score between no occlusion and category of stenosis were significantly different.

TABLE 4: Association between the histological presence of calcification and calcium score in coronary arteries

Calcium Score (n=909)	Presence of calcification (n=909)		p value
	No (%)	Yes (%)	
< 160	91.6	8.4	<0.001
≥ 160	4.3	95.7	

NOTE: Data expressed as percentage

TABLE 5: Calcium score test performance in detecting degree of stenosis and presence of calcification

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Overall accuracy
Degree of stenosis	42.9	98.1	26.1	99.1	96.8
Presence of calcification	77.1	99.9	98.7	97.4	91.7

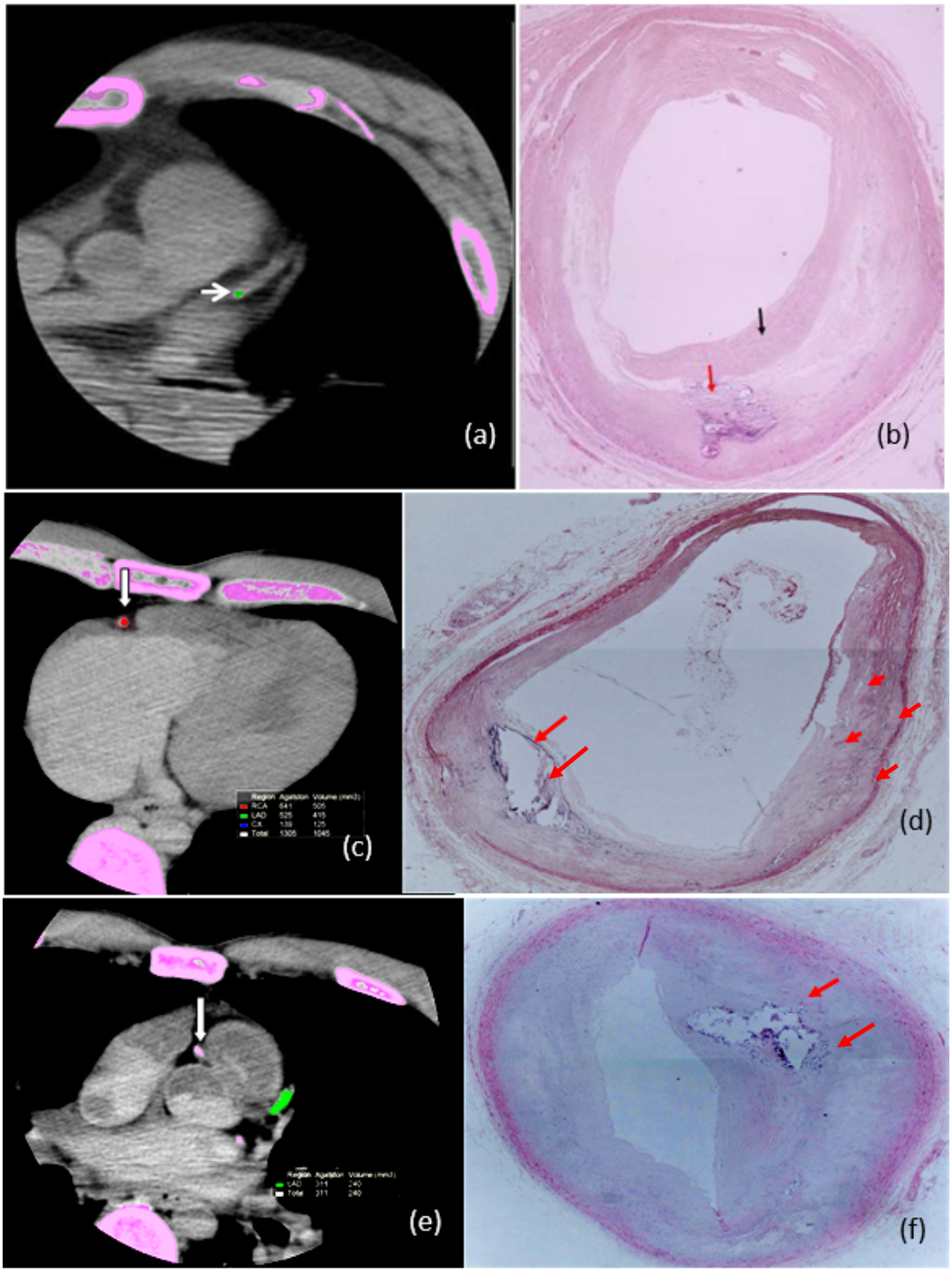

FIG. 1: (a) & (b): Comparison between PMCT and histological appearance of coronary artery calcification. Left anterior descending coronary artery (a) calcification on PMCT (white arrow), and (b) calcification on histology (red arrow), and the fibrous cap of the atherosclerosis is denoted by the black arrow. (c) & (d): Right coronary artery (c) calcification on PMCT (white arrow) and (d) calcification on histology; there is one large calcified area (long red arrow) and streaks of calcifications (short red arrows). (e) & (f): another right coronary artery (e) calcification on PMCT (white arrow) and (f) on histology (red arrow).

exhibiting much greater detection rates than either fluoroscopy or conventional/digital radiography. Furthermore, the fast temporal and spatial resolutions of CT scanners provide the ability to precisely localise calcific deposits in the coronary arterial system non-invasively. Reports have also shown that CT analysis allows for precise estimation of the calcium content of plaques which was compared with histology as the gold standard when compared with other imaging modalities like ultrasound.[29]

We found that PMCT CS has a high specificity in detecting coronary artery calcification but its sensitivity is low. This can be due to the fact that areas of calcification with a Hounsfield unit of below 130 is (coronary artery calcification with a Hounsfield unit below 130 are) not detected by the software. Since CAC is an indisputable marker for CAD, CAC quantification by CT has been proven to have real value as a risk predictor for coronary heart disease in living patients, though not many studies have been done in post mortem subjects.

CONCLUSION

In conclusion, coronary artery calcium score is strongly associated with the area of calcification and the degree of luminal stenosis (stenosis) calculated histologically. Further studies should be done looking at Future studies should look at the association of calcium score calculated using PMCTA versus plaque stability which will make PMCTA a robust technique capable of providing both quantitative measurements of stenosis, as well as qualitative assessment of plaque morphology.

Conflict of interest: The authors of this manuscript do not believe that there is a conflict of interest that could potentially be construed to affect the material contained in the manuscript that is being submitted to the Journal.

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LIST OF ABBREVIATIONS

CAD	- coronary artery disease
CS	- calcium score
PMCT CS	- post mortem computed tomography calcium score
PMCT	- post mortem computed tomography
CT	- computed tomography
CAC	- coronary artery calcification
CACS	- coronary artery calcium score
LCX	- left circumflex artery
LAD	- left anterior descending artery
RCA	- right coronary artery
H&E	- Haematoxylin and Eosin
LCA	- left coronary system
EBCT	- electron beam computerized tomography

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