Lower extremity arterial calcifications assessed by multislice CT as a correlate to coronary artery disease

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Abstract

Background: Patients with peripheral artery disease (PAD) frequently have concomitant coronary artery disease (CAD) and display a higher risk for myocardial infarction, stroke, and death due to cardiovascular events. In order to prevent cardiovascular events, there is an increasing interest in new markers of atherosclerosis. Vascular calcifications (VC) are often present in the early stages of atherogenesis and could be considered an early marker. The aim of this study is to correlate the extent of lower limbs, aortic, and coronary arterial calcification diagnosed by non-contrast multislice CT with the severity of coronary artery disease diagnosed by conventional coronary angiography.

Results: There is borderline significant association between CAD (Gensini score) and each of total lower limb and aorto-iliac calcifications. There is significant association between the number of diseased coronaries and lower limb calcifications. Also, there is significant association between the coronary artery calcifications and lower limb arterial calcifications (total and segmental). Diabetes mellitus and hypertension are significantly associated with lower limb calcification (total, aorto-iliac, and infrapopliteal). Moreover, lower limb arterial calcifications (total and segmental) are positively correlated with increasing age.

Conclusion: Lower limb arterial calcifications, as diagnosed by non-contrast MSCT, is a noninvasive measure for evaluation of the atherosclerotic burden that correlate to the CAD; it can aid to refine risk stratification and the need for more aggressive preventive strategies.

Keywords: Lower limb arterial calcifications, Coronary calcifications, Coronary artery disease, Calcium score

Background

Patients with peripheral artery disease (PAD) frequently have concomitant coronary artery disease (CAD) and display a higher risk for myocardial infarction, stroke, and death due to cardiovascular events [1]. Patients with PAD harbor more extensive and calcified coronary atherosclerosis with accelerated disease progression [2]. The extent of coronary calcification in individual coronary arteries is related to the severity of coronary stenosis in these vessels [3].

In order to prevent cardiovascular events, there is an increasing interest in new markers of atherosclerosis. Vascular calcifications (VC) are often present in the early stages of atherogenesis and could be considered an early marker [4].

Both plain radiographs and ultrasound can detect vascular calcification. However, only CT-based methods allow for quantification and commonly used in clinical practice.

The associations between arterial calcification and mortality differ by vascular bed, suggesting that the location and severity of calcification in different vascular beds provides unique mortality information [5].
Many studies [6] underlined the high prognostic significance of VC. A score based on the quantification of calcifications in the intima and media of the coronary arteries (coronary artery calcification, CAC) detected by computed tomography (CT) has gained popularity. The CAC score is related to the occurrence of new cardiovascular events and CVD mortality [7] and provides added information to the Framingham risk score [8], stress echocardiography, or perfusion studies in the identification of high risk asymptomatic patients [9].

However, calcifications are often clinically assessed in a single district, e.g., the coronary arteries [6] or the femoral artery [10], and that might not reflect the overall peripheral calcium load.

Little research is available that examines the relationship between different locales of arterial calcification. However, some data are available that look at the relationship of peripheral vascular disease and CAD [11].

Observation of lower extremity and aortic arterial calcifications together with coronary artery calcification by multislice computed tomography (MSCT) and correlate it to the severity of coronary artery stenosis by conventional coronary angiography may hint at the extent of systemic atherosclerosis, which is useful in future risk assessment and clinical decision-making beyond traditional risk scores without additional cost or harm to cardiac patients.

The aim of this study is to quantify the extent of lower limbs, aortic, and coronary arterial calcifications by MSCT in patient with symptomatic coronary artery disease and to correlate the extent of lower limbs, aortic, and coronary arterial calcification with the severity of coronary artery stenosis diagnosed by conventional coronary angiography.

**Methods**

**Inclusion criteria**

A total number of 100 cases, who had ischemic heart disease, were enrolled.

**Exclusion criteria**

Patients with decompensated heart failure, acute pulmonary edema, and those with contra-indication to contrast administration (GFR less than 60 ml/min or documented anaphylactic reaction to angiographic contrast media) were excluded.

**Study design**

Our study is a case-control study.

**Clinical evaluation**

Full history taking and physical examination emphasizing cardiovascular risk factors were performed including the age and gender, smoking, hypertension, diabetes mellitus, dyslipidemia, and family history. For all patients, the body mass index (BMI) was calculated.

**Non-contrast computed tomography for calcium scoring**

**Coronary calcium score**

A non-contrast ECG-gated CT examination of the heart was performed for 85 patients in order to detect and quantify coronary calcifications through the volume extended from below the carina to the apex of the heart using the multislice aquillion 64 CT scanner of Toshiba (Tokyo, Japan), with 64 channels. Acquisition parameters were ECG-gated study at 75% of the RR interval, 400 ms gantry rotation, 64 × 3 mm collimation, 80 mA, and 120 kV. The radiation dose of the CT coronary calcium score, according to this technique, is about 1 mSv in average.

The reconstructed axial images were transferred to a dedicated workstation (Vitrea 5, Vital Images, USA) for offline analysis. Calcium scores were calculated with the dedicated cardiac software and expressed as Agatston score. Using the sequential axial images, any tissue above 130 HU occupying a minimum of 0.5 mm² that could be identified along the anatomical course of a coronary artery was considered as coronary artery calcification, and hence, highlighted and analyzed (Fig. 1).

**Calcium score of the abdominal aorta and both lower limb arterial systems**

A non-contrast CT examination of the abdominal aorta and both lower limb arterial systems was performed for all patients in order to detect...
and quantify arterial wall calcifications through the volume extended from the level of the diaphragm down to the mid foot level using the multislice aquilion 64 CT scanner of Toshiba (Tokyo, Japan), with 64 channels (Figs. 2 and 3). Acquisition parameters were 400 ms gantry rotation, 64 × 10 mm collimation, 80 mA, and 120 kV. The radiation dose, according to this technique, is about 3 mSv in average.

The reconstructed axial images were transferred to a dedicated workstation (Vitrea 5, Vital Images, USA) for offline analysis. There were no current established dedicated protocols to calculate the lower limb arterial calcifications, and hence, the cardiac protocols were utilized to quantify the calcium score that was also expressed as Agatston score. The lower limb arterial systems were segmented into aorto-iliac, femero-popliteal, and infra-popliteal regions, and each segment was assessed separately on either side. Using the sequential axial images, any tissue above 130 HU occupying a minimum of 0.5 mm² that could be identified along the anatomical course of the abdominal aorta and the lower limb arterial systems was considered as athermanous calcification, and hence, highlighted and analyzed.

Coronary angiography
All patients underwent conventional coronary angiography. Results were reviewed, and severity of the CAD was evaluated using the following.

CASS score Coronary artery disease is divided into 4 groups according to the number of diseased vessels involved (the diseased vessel is defined as 50% or greater stenosis for LMT, 70% or greater stenosis for LAD or LCX or RCA). The four groups are as follows: no significant vessel involvement, one significant vessel involvement, two significant vessel involvement, and three or more significant vessel involvement. The left main stenosis was counted as two diseased vessels, but the LAD, LCX, and RCA were counted as one vessel for each [12].

Gensini score Scoring system that was based on the percentage of luminal narrowing (25%, 1 point; 50%, 2 points; 75%, 4 points; 90%, 8 points; 99%, 16 points; and total occlusion, 32 points). Each coronary lesion score would be calculated using the equivalent of the percentage luminal narrowing multiplied by coefficient of coronary segment. The coefficient factors of the coronary segments are as follows: 5 for the left main coronary artery; 2.5 for the proximal LAD and proximal LCX (3.5 if LCX is dominant); 1.5 for the mid region of the LAD; 1 for the distal LAD, the first diagonal, the proximal RCA, the mid RCA, the distal RCA, the posterior descending, the mid LCX (2 if LCX is dominant), the distal LCX, and the obtuse margin, ramus intermedium; and 0.5 for the second diagonal and the postero-lateral branch. The Gensini score was expressed as the sum of the scores for all the individual coronary segment scores.

Statistical methodology
All data were analyzed using the SPSS version 24 statistical software and R statistical package version 3.4.1, with two-tailed \( p \) value < 0.05 indicating statistical significance.

 Normally distributed numerical values were reported as mean ± SD. For variables with a skewed distribution, the data were expressed as median and inter-quartile range. Qualitative variables were presented as counts and percentages.

Spearman’s correlation was used to test association between continuous variables (Gensini score, coronary, and peripheral calcification) as the data were skewed even after trying variable transformation. For the same reason, comparisons of continuous data between two groups were made using Mann-Whitney \( U \)-test. Furthermore, comparisons between three (e.g., tertiles of Gensini) or more (e.g., number of significantly stenosed vessels categorized into 0, 1, 2, and 3 or more) groups were made using Kruskal-Wallis test.

Results
The study included 100 patients who underwent conventional coronary angiography and non-contrast CT study to detect and calculate the lower limb calcifications. Only 85 patients underwent coronary calcium score, while the remaining 15 patients had stent...
deployment that interferes with accurate assessment of the coronary calcifications.

**Descriptive data**

**Demographic data of studied patients**
The baseline characteristics of all patients were given in Table 1.

**Clinical presentation**
Patients presented with stable and unstable coronary syndromes as are shown in Table 2.

**Coronary angiography**
Patients were classified according to number of diseased coronary vessels as non-significant disease in 12 patients (12%), single vessel disease in 38 patients (38%), two vessel disease in 33 patients (33%), and multi-vessel disease in 17 patients (17%). Gensini score ranged between 20 and 79 with a median of 37. The details of coronary artery disease are mentioned in Table 3.

**Non-contrast CT scan of the coronary arteries and the lower limb arterial systems**
Sixty-nine percent of the studied patients had lower limbs arterial calcifications detected by MSCT. Distribution of lower limb arterial calcifications among arterial segments of the studied patients was as follows: 66% of

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**Table 1**
| Clinical features           | Value          |
|-----------------------------|----------------|
| Age (year)                  | 54.8 ± 11.2    |
| Male gender                 | 71 patients (71%) |
| BMI                         | 29.4 ± 5.52    |
| Smoking                     | 54 patients (54%) |
| Hypertension                | 54 patients (54%) |
| Diabetes Mellitus           | 42 patients (42%) |
| Dyslipidemia                | 54 patients (54%) |
| Family history of CAD       | 33 patients (33%) |
the aorto-iliac segments, 38% of the femoro-popliteal segments, and 20% of the infra-popliteal segments.

**Correlation of coronary artery disease with lower limb and coronary artery calcifications**

**Correlation of Gensini score to lower limb and coronary artery calcification**

Total coronary Agatston, total lower limb calcium score, aorto-iliac calcium score, and femoro-popliteal calcium score were positively correlated with Gensini score (p values = 0.002, 0.003, 0.002, and 0.007 respectively). There was no significant correlation between Gensini and the infra-popliteal calcium score (p = 0.386) (Table 4).

**Association of Gensini score with coronary and lower limb calcification (by Kruskal-Wallis test)**

There was a borderline association in total lower limb calcium score and aorto-iliac calcium score scores among the tertiles of Gensini with the total lower limb calcium score and aorto-iliac calcium score being lowest in the first Gensini tertile and highest in the third Gensini tertile (p values = 0.06 and 0.05, respectively)

Also, there was significant association regarding the total coronary Agatston score (p value = 0.02).

There was no significant association regarding femoro-popliteal and infra-popliteal calcium scores among the tertiles of Gensini score (p values = 0.13 and 0.14, respectively)

**Association of CASS score with lower limb and coronary artery calcification (by Kruskal-Wallis test)**

There was a significant association in the total coronary Agatston, total lower limb calcium score, femoro-popliteal calcium score, and infra-popliteal calcium score with the number of significant coronary vessel disease; these scores were lowest in the group with non-significant coronary vessel involvement, higher in the group with single significant coronary vessel involvement, and highest in the group with two or more significant coronary vessel involvement.

There was borderline association regarding aorto-iliac segment calcium score (p value = 0.05).

**Correlation of the lower limb arterial calcifications to the coronary calcium score and to the cardiovascular risk factors**

A. Coronary calcium score: Total lower limb, aorto-iliac, femoro-popliteal, and infra-popliteal calcium scores were positively correlated with total coronary Agatston score (p values = < .001, < .001, < .001, and .011, respectively).

B. Age: Total coronary Agatston, total lower limb, aorto-iliac, femoro-popliteal, and infra-popliteal calcium scores were positively correlated with increasing age (p values = .001, < .001, < .001, < .001, and .002, respectively).

C. Diabetes mellitus: Total lower limb Agatston, aorto-iliac, and infra-popliteal calcium scores were significantly higher in diabetics in comparison with the non-diabetic patients (p values = .001, .003, and .004, respectively).

D. Hypertension: Total lower limb, aorto-iliac, and infra-popliteal calcium scores were significantly higher in hypertensive patients in comparison with normotensive patients (p values = .003, .02, and .02, respectively).

E. Dyslipidemia: There was no significant correlation between dyslipidemias and non-

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**Table 2** Clinical presentation of studied patients

| Clinical presentation | Percentage |
|-----------------------|------------|
| Non STE ACS           | 35 patients (35%) |
| STEMI                 | 34 patients (34%) |
| Stable CAD            | 27 patients (27%) |
| ICM                   | 3 patients (3%) |
| SCD survivor          | 1 patient (1%) |

*Non STE ACS* non ST segment elevation acute coronary syndrome, *STEMI* ST segment elevation myocardial infarction, *ICM* ischemic cardiomyopathy, *SCD* sudden cardiac death

**Table 3** Angiographic characteristic features of the studied patients

| Angiographic characteristic features | Percentage |
|-------------------------------------|------------|
| Total Gensini                       | 37 (20, 79) |
| Left main stenosis                  | 5 (5%)     |
| LAD stenosis                        | 77 (77%)   |
| LCX stenosis                        | 29 (29%)   |
| RCA stenosis                        | 44 (44%)   |
| Ramus stenosis                      | 4 (4%)     |
| Number of vessels with significant stenosis |         |
| 0                                   | 12 (12%)  |
| 1                                   | 38 (38%)  |
| 2                                   | 33 (33%)  |
| ≥ 3                                 | 17 (17%)  |

**Table 4** Correlation of Gensini score to lower limb and coronary artery calcification

| Variables                      | Number of patients | r value | p value |
|-------------------------------|--------------------|---------|---------|
| Total coronary Agatston       | 85                 | .333    | .002    |
| Total lower limb calcium score| 100                | .299    | .003    |
| Aorto-iliac calcium score     | 100                | .307    | .002    |
| Femoro-popliteal calcium score| 100                | .268    | .007    |
| Infra-popliteal calcium score | 100                | .088    | .386    |
discrimination dyslipidemic patients regarding lower limb calcium scores \( (p \text{ value } = 0.79) \).

F. There was no significant correlation between smokers and non-smoker patients regarding lower limb calcium scores \( (p \text{ value } = 0.32) \).

Discussion

Coronary artery disease (CAD) is the leading cause of death in the world and is predicted to remain so for the next years. Noninvasive measures for evaluation of the atherosclerosis have emerged as adjuncts to traditional cardiovascular disease risk factors in an attempt to refine risk stratification and the need for more aggressive preventive strategies [13]. Different screening methods have emerged for this purpose, including stress ECG, stress echocardiography, and myocardial scintigraphy as well as the coronary calcium score using the MSCT.

To the best of our knowledge, we are the first to investigate the correlation between lower limb calcification using non-contrast CT and coronary artery disease severity through gold standard coronary angiography using Gensini score in ischemic heart disease patients.

Association of lower limb arterial calcification with coronary artery disease

In our study, there was borderline significant association between coronary artery disease (Gensini score) and each of total lower limb and aorto-iliac calcification. On the other hand, there was no significant association with each of femoro-popliteal and infra-popliteal calcifications.

Moreover, our study demonstrated that there was a significant association between the number of diseased coronaries and lower limb arterial calcification.

These results were compatible with results of many researchers as Shin et al. [14], Ouwendijk et al. [15], Reaven et al. [16], Criqui et al. [17], and Kim et al. [18].

Shin et al. [14] studied cardiac and peripheral arterial calcification by computed tomography in 103 PAD patients. The patients were categorized as non-significant CAD, single CAD, or multivessel CAD. He found that PAD patients with multivessel CAD had significantly higher peripheral arterial calcifications than those with non-significant CAD \( (p < 0.001) \).

Ouwendijk et al. [15] studied the association between vessel wall calcification (in aorto-iliac, femoro-popliteal, and infra-popliteal vessels) by CT angiography and history of cardiac disease (including chest pain, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, and myocardial infarction) in 145 patients and found that cardiac disease was independently predictive for the presence of vessel wall calcifications \( (p = .05) \).

Reaven et al. [16] studied coronary artery disease and abdominal aorta calcification by electron beam computed tomography in 309 patients and showed that subjects with coronary artery or abdominal aorta calcification had a strikingly higher prevalence of coronary artery disease and all combined cardiovascular disease.

Criqui et al. [17] studied abdominal aortic calcifications (AAC) and coronary artery calcification (CAC) by computed tomography in 120 stable OCAD (luminal narrowing \( \geq 50\% \)) patients and 120 controls without OCAD and found that the prevalence of AC and CAC was significantly higher in OCAD patients than in controls \((64\% \text{ vs. } 48\%, p = 0.019; 57\% \text{ vs. } 32\%, p < 0.001, \text{ respectively})\).

Tatsunori and Nemoto [19] investigated 197 patients with PAD who underwent coronary angiography and lower extremity computed tomography angiography; they calculated syntax score from coronary angiography while the lower limb artery calcification was expressed as calcium score in similar methods to Agatston score. They found that lower limb arterial calcification was a stronger predictor for cardiovascular death rather than syntax score among patients with peripheral artery disease.

And finally, Xiaoteng et al. [20] investigated the correlation of the extent of aortic arch calcification detectable on chest X-rays with the severity of coronary artery disease in patients with acute coronary syndrome in 1418 patients, and they found that the extent of aortic arch calcification detectable on chest X-rays might provide valuable information in predicting coronary artery disease severity in acute coronary syndrome patients.

Association of lower limb arterial calcification with coronary artery calcification

In our study, the lower limbs arterial calcification (total and segmental) was significantly associated with the coronary artery calcification.

These results were agreed with results of Shin et al. [14], Costacou et al. [11], Patsch et al. [21], Churchill et al. [22], and Takasu et al. [23].

Shin et al. [14] evaluated coronary and lower extremity arterial calcification (LEAC) by computed tomography in 103 PAD patients. Patients were categorized as non-significant CAD, single CAD, or multivessel CAD. He
found that LEAC significantly correlated with CCS ($r = 0.831, p < 0.001$).

Costacou et al. [11] studied computed tomography of lower extremity and coronary artery calcifications in 121 patients and showed that lower extremities arterial calcification and the heart share many of the same risk factors; lower extremity arterial calcification is an independent correlate of the later presence of coronary artery calcification.

Patsch et al. [21] studied lower leg and coronary arterial calcifications by computed tomography in 46 patients and found that a significant positive correlation between lower leg arterial calcification and coronary artery calcification ($r = 0.6; p < 0.01$).

Churchill et al. [22] evaluate coronary artery and ascending and descending thoracic aorta calcifications by computed tomography in 1739 patients and found that ascending and descending thoracic aortic calcium have similar associations with traditional cardiovascular risk factors in diabetics and are independently associated with coronary artery calcium.

Takasu et al. [23] quantified coronary artery calcification (CAC) and descending thoracic aortic calcification (DTAC) by non-enhanced cardiac CT in 6814 patients aged 45–84 years old and concluded that DTAC was found to be a strong predictor of CAC independent of cardiovascular risk factors.

**Association of coronary artery calcification with coronary artery disease**

Quantification of the coronary artery calcification has been extensively studied for its ability to predict coronary artery disease.

Our study demonstrated that the coronary artery calcification was significantly associated with the coronary artery disease. In addition, there was a significant association between the number of diseased coronaries and total coronary artery calcifications.

These results were compatible with results of many researchers as Almasi et al. [24], Iwasaki and Matsumoto [25], Budoff et al. [26], Gokdeniz et al. [27], and Rosen et al. [3].

Almasi et al. [24] studied 202 consecutive patients who underwent conventional coronary angiography to assess the severity of coronary artery disease by Gensini score and computed tomography to measure CAC by Agatston algorithm and confirmed the strong relationship between the CAC and the presence and severity of stenosis in coronary arteries assessed by both the number of diseased vessels and also by the Gensini score.

Iwasaki and Matsumoto [25] studied coronary artery calcification (CAC) and significant coronary stenosis using coronary computed tomographic angiography (CCTA) in 651 patients and found that stepwise increased risk of coronary events associated with increasing CAC was caused by increasing incidence of significant stenosis.

Budoff et al. [26] evaluated coronary artery calcification by computed tomography and coronary artery disease by coronary angiography in 1851 patients. The overall sensitivity for CAC to predict obstructive disease on angiography was 95%, and the specificity was 66%. They also found that CAC provides incremental and independent power in predicting the severity and extent of significant CAD in symptomatic patients.

Gokdeniz et al. [27] studied coronary artery disease by Gensini score, and coronary calcium score (CAC) by computed tomography in 240 patients, found that Gensini score was significantly correlated with total CAC score.

Rosen et al. [3] studied coronary artery calcification and the severity of coronary disease by coronary angiography in 6814 subjects of The Multi-Ethnic Study of Atherosclerosis (MESA) and found that there is a significant relationship between the extent of calcification and mean degree of stenosis in individual coronary vessels; however, 16% of the coronary arteries showed significant stenoses without calcifications at baseline.

**Analysis of risk factors of coronary artery and lower limb arterial calcification**

In our study, we demonstrated that diabetes mellitus and hypertension were significantly associated with lower limb calcifications (total, aorto-iliac, and infra-popliteal). Lower limb arterial calcifications (total and segmental) were also positively correlated with increasing age. These results were compatible with results of Ouwendijk et al. [15], Allison et al. [28], and Chowdhury et al. [29].

Ouwendijk et al. [15] studied the association of vessel wall calcifications (in aorto-iliac, femero-popliteal, and below the knee) by CT angiography and cardiovascular risk factors in 145 patients and found that diabetes mellitus and age were independently predictive for the presence of vessel wall calcifications (all $p = 0.05$).

Allison et al. [28] studied 650 asymptomatic subjects referred to assess proximal and distal aorta and iliac vessels for atherosclerotic calcification and found that age and hypertension were the dominant risk factors for systemic calcified atherosclerosis.

Chowdhury et al. [29] studied 220 PAD patients that underwent CT to determine lower limb arterial calcification (LLAC) and found that diabetes mellitus was more common in patients in the highest quartile of LLAC scores ($p = 0.039$).

**Conclusion**

There is significant association between the coronary artery calcification and lower limb arterial calcification.
(total and segmental). There is borderline significant association between coronary artery disease (Gensini score) and each of total lower limb and aorto-iliac calcifications. On the other hand, there is no significant association between coronary artery disease (Gensini score) and each of femoro-popliteal and infra-popliteal calcification. There is significant association between the number of diseased coronaries and lower limb calcification.

Diabetes mellitus and hypertension are significantly associated with lower limb calcification (total, aorto-iliac, and infra-popliteal). Moreover, lower limb arterial calcification (total and segmental) is positively correlated with increasing age.

Limitations
The present study is limited by the relatively small number of patients that could not be used as a standard reference value for the whole population.

Another limitation is the lack of reference values for non-contrast CT parameters of lower limb arterial calcifications.

Abbreviations
CAD: Coronary artery disease; PAD: Peripheral arterial disease; VC: Vascular calcification; CAC: Coronary artery calcifications; CT: Computed tomography; MSCT: Multislice computed tomography; GFR: Glomerular filtration rate; BMI: Body mass index; ECG: Electrocardiogram; HU: Hounsfield unit; CASS: Coronary artery surgery study; STE ACS: ST segment elevation acute coronary syndrome; STE MI: ST segment elevation myocardial infarction; ICM: Ischemic cardiomyopathy; SCD: Sudden cardiac death; AAC: Abdominal aortic calcification; OCAD: Obstructive coronary artery disease; AC: Aortic calcifications; DTAC: Descending thoracic aortic calcifications; CCTA: Coronary computed tomography angiography; MESA: Multi-Ethnic Study of Atherosclerosis; LLAC: Lower limb arterial calcifications

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Authors’ contributions
GA contributed the idea of the study, collected the patients, and was a contributor in writing the manuscript. HH performed and interpreted the angiographic studies. AH performed and interpreted the angiographic studies. MA performed and interpreted the CT images. DL was responsible for the data analysis and statistics. EB was a major contributor in writing the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All procedures performed in this study were in accordance with the ethical standards, approved by the ethics committee of Cairo University Hospitals, and complied with the Declaration of Helsinki 1964 and its latter amendments. Written informed consent was obtained from all individual participants included in this study. The ethics committee reference numbers are not available, as the study was started 2 years ago and ended 15 months later; the numbers were not collected at the time of start and could not be reached now.

Consent for publication
All patients included in this research gave written informed consent to publish the data contained within this study.

Competing interests
The authors declare that they have no competing interests.

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