The Increase of The Relative Amount of Nodular Calcification In Femoral Plaques is Associated With Milder Lower Extremity Arterial Disease

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**Research Article**

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Abstract

Vascular calcification exists in different forms that reflect variable clinical and histological implications. Categories of calcification have not been quantified in relation to the clinical presentation of lower extremity arterial disease. The study analyzed 51 femoral plaques collected during femoral endarterectomy, characterized by > 90% stenosis. The plaques were longitudinally sectioned, stained with Hematoxylin and Eosin and digitized for a deep learning platform for quantification of the relative area of nodular calcification to the plaque section area. Vessel measurements and quantity of each calcification category was compared to the clinical risk factors and outcomes. Nodular calcification area proportion is associated with reduced risk of severely lowered toe pressure (< 30mmHg) (OR=0.910, 95%CI =0.835-0.992, p<0.05), severely lowered ankle brachial index (<0.4), (OR=0.912, 95%CI=0.84-0.986, p<0.05), and semi-urgent operation (OR=0.882, 95%CI=0.797-0.976, p<0.05). The analysis was adjusted by age, gender, hypertension, diabetes and dyslipidaemia. Increase of the relative amount of nodular calcification in femoral plaques with over 90% stenosis is associated with protection against severe LEAD, identified by severely lowered toe pressure and ankle brachial index and semi-urgent operations. Nodular calcification may contribute to a slower obstruction, hence milder obstructive ischaemic presentation.

Introduction

The amount of calcification in lower limb arteries has been associated to increased degree of stenosis, and higher cardiovascular morbidity and mortality [1], [2].

Vascular calcification is a bone-like formation continuum that presents different morphologies, which in turn impact differently on the plaque histology and the clinical presentation [3], [4]. It initiates as microcalcification, which predestines the plaque to an unstable state [5]. The propagation of microcalcification into macrocalcification is believed to have a stabilizing effect on the plaque, despite its potential stenosing impact [6]. Macrocalcification can occur in the form of a calcified plate, called sheet calcification (ShCa) or the form of osteoid metaplasia [4], [7]. Nodular calcification (NodCa) may be caused by a fracture of ShCa and is accompanied by surrounding fibrin deposits [7], [8]. The latter may also suggest an additional thrombotic origin of the nodular calcification [9]. NodCa may erupt through the protective cap of the plaque with an overlying thrombus to form a calcified nodule [10].

Semi-quantiﬁcation of calcification in lower limb arteries and its clinical implication has been analyzed using imaging scoring [1], [2],[11]. Histological quantification is a valuable instructive tool for understanding the clinical signiﬁcance and mechanistic of different plaque structures. To our knowledge, no study has analyzed the quantity of NodCa and ShCa in femoral atherosclerosis and their impact on LEAD presentation.

Interestingly, despite its dense fibrocalcific character, atherosclerosis in superficial femoral artery is reputable for its milder clinical presentation compared to other vascular territories [12]. This is partly by the virtue of the compensatory vascular remodeling observed in this artery, which compensates stenosis
even in sizable atherosclerotic lesions [12], [13]. Thus far, studies have not addressed the impact of different calcified morphologies on vascular remodeling.

Therefore, in this study, we quantify NodCa and ShCa in femoral plaque sections and analyze and compare the contribution of each category area proportion to the LEAD severity. We also analyze the effect of each quantified calcification category on regional vascular remodeling.

We applied a deep learning algorithm to obtain a consistent measuring method in all the studied sections and to facilitate quantification of the co-existing categories to be studied.

**Results**

Patients’ characteristics are shown in Table 1.
Table 1
Patients’ characteristics.

| Characteristic                                | Total       |
|-----------------------------------------------|-------------|
| N                                             | 51          |
| Sections containing NodCa                     | 49 (96)     |
| Sections containing ShCa                      | 48 (94.1)   |
| Average NodCa area proportion (%)             | 22.4 ± 12.3 |
| Average ShCa area proportion (%)              | 14.5 ± 11.8 |
| Age (years) (N =51)                           | 70.5 ± 6.9  |
| Risk factors                                  |             |
| Sex: male                                     | 28 (54.9)   |
| Diabetes type I or II                         | 14 (28.6)   |
| Hypertension                                  | 43 (84.3)   |
| Dyslipidemia                                  | 45 (88.2)   |
| Increased hs-CRP<sup>a</sup>                   | 13 (25.5)   |
| BMI = 25-30 (kg/m²)                           | 21 (42.9)   |
| BMI > 30                                      | 28 (57.1)   |
| Smoking status (N = 49)                       |             |
| Never                                         | 2 (4.0)     |
| Current smoker                                | 28 (54.9)   |
| Ex- smoker                                    | 19 (37.3)   |
| Co-morbidities                                |             |
| Coronary artery disease (N =49)               | 12 (24.5)   |
| Cerebrovascular disease                       | 3 (6.1)     |

Data presented as mean ± SD or number (%), data obtained from the 51 patients, otherwise, N represents the number of data available. Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; GFR, glomerular filtration rate; ACE, Angiotensin-converting enzyme; AT, Angiotensin II; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; LEAD, lower extremity arterial disease; TP, toe pressure; ABI, ankle brachial index.

<sup>a</sup> Females’ values > 2.5 mg/L and males’ values > 3 mg/L.
| Characteristic                                      | Total   |
|----------------------------------------------------|---------|
| ACE inhibitors / AT blockers                       | 38 (74.5) |
| Aspirin                                            | 36 (70.6) |
| Clopidogrel                                        | 7 (13.7)  |
| Statins                                            | 38 (74.5) |
| Warfarin                                           | 3 (5.9)   |
| Lab parameters                                     |         |
| Hemoglobin (g/L) (N =50)                           | 139.0 ± 13.9 |
| Total leukocyte count (10E9/L) (N =50)              | 7.9 ± 1.8 |
| Thrombocytes (10E9/L) (N = 50)                     | 267.2 ± 65.6 |
| Total cholesterol (mmol/L)                         | 4.3 ± 1.3 |
| LDL-cholesterol (mmol/L) (N = 50)                  | 2.1 ± 0.8 |
| HDL-cholesterol (mmol/L)                           | 1.3 ± 0.5 |
| Triglycerides (mmol/L)                             | 1.8 ± 2.3 |
| LEAD symptoms severity (Fontaine class)           |         |
| Claudication (II,IIA,IIB)                          | 34 (66.6) |
| Rest pain(III)                                     | 12 (23.5) |
| Ischemic ulcer or gangrene (IV)                    | 5 (9.8)   |
| Classification of the intervention                 |         |
| Semiurgent operation                               | 9 (17.6)  |
| Elective operation                                 | 42 (82.4) |
| TP readings (N =43)                                |         |
| TP < 30 mmHg                                       | 12 (72.1) |
| TP ≥ 30 mmHg                                       | 31 (27.9) |
| ABI (N = 41)                                       |         |

Data presented as mean ± SD or number (%), data obtained from the 51 patients, otherwise, N represents the number of data available. Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; GFR, glomerular filtration rate; ACE, Angiotensin-converting enzyme; AT, Angiotensin II; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; LEAD, lower extremity arterial disease; TP, toe pressure; ABI, ankle brachial index.

* Females’ values > 2.5 mg/L and males’ values > 3 mg/L.
Proportion of NodCa area on the slide sections did not associate with any of the patient’s baseline characteristics. Patients with normal BMI had higher area proportion of ShCa than overweight or obese patients, 20.0 ± 15.1 vs 10.5 ± 6.9, respectively, \( p < 0.05 \) (Supplementary Table S1).

Area proportion of ShCa correlated positively with serum HDL cholesterol \( (R = 0.419, p < 0.005) \), and negatively with serum LDL cholesterol \( (R = -0.346, p < 0.05) \), and serum triglyceride levels \( (R = -0.371, p < 0.01; \) supplementary Table S2).

Analysis of covariance for the association of vessel diameter to the relative amount of NodCa and ShCa along with other confounding factors; age, gender, BMI, smoking, hypertension, diabetes, GFR, and hs-CRP showed that the area proportion of NodCa associated significantly to the vessel diameter \( (F = 5.700, p < 0.05) \), however, ShCa association was non-significant \( (F = 2.43, p = 0.626, \text{Table 2}) \).

### Table 2
Analysis of covariance of NodCa and ShCa area proportions in association to the vessel diameter.

| Category | NodCa | ShCa |
|----------|-------|------|
| N        | 49    | 48   |
| F        | 10.19 | 5.282|
| \( p \)  | \(< 0.005\) | \(< 0.05\) |
| \( F^* \)| 6.226 | 4.776|
| \( p^* \)| \(< 0.05\) | \(< 0.05\) |
| \( F^\dagger \)| 5.700 | 2.43 |
| \( p^\dagger \)| \(< 0.05\) | 0.626|

*adjusted by demographic determinants of vessel diameter: gender, age and BMI

†adjusted by modulants of vascular remodeling: smoking, hypertension, diabetes, renal impairment (GFR), and inflammatory state (hs-CRP), along with the demographic factors, gender, age and BMI.

Abbreviations: NodCa, nodular calcification area proportion; ShCa, sheet calcification area proportion.
Proportion of NodCa and ShCa areas on each individual slide sections are shown in figure 2.

Higher NodCa area proportion associated with reduced likelihood of having severely lowered TP (<30 mmHg), (OR = 0.903, 95% CI = 0.843-0.967, \( p < 0.005 \)) and severely lowered ABI (< 0.4) (OR = 0.925, 95% CI = 0.873-0.980, \( p < 0.001 \)). Multivariate logistic analysis including age, gender, hypertension, diabetes and dyslipidaemia along with NodCa showed that NodCa association remained significant; (OR = 0.910, 95% CI = 0.835-0.992, \( p < 0.05 \)) (OR = 0.912, 95% CI = 0.84-0.986, \( p < 0.05 \)) for TP and ABI, respectively. Additionally, higher NodCa area proportion was associated with reduced risk of semi-urgent operation (OR = 0.924, 95% CI = 0.868-0.984, \( p < 0.05 \)), adjusted analysis to the above-mentioned confounders was also significant, (OR =0.882, 95% CI =0.797-0.976, \( p < 0.05 \)). Higher NodCa area proportion was not associated with Fontaine class III, IV (rest pain and ischaemic ulcer). ShCa area proportion was not associated with any of the mentioned LEAD severity indicators (Table 3).

### Table 3
Binary logistic regression of LEAD severity indicators in association with NodCa and ShCa area proportions.

| Category                        | NodCa |                   |       | ShCa   |                   |       |
|--------------------------------|-------|-------------------|-------|--------|-------------------|-------|
|                                | OR    | 95% CI            | \( p \) | OR     | 95% CI            | \( p \) |
| Fontaine class III, IV         | 0.975 | 0.931-1.021       | 0.285 | 1.020  | 0.970-1.072       | 0.446 |
|                                | *     | 0.965             | 0.915-1.019 | 0.201 | 1.012  | 0.961-1.067       | 0.651 |
|                                | †     | 0.948             | 0.890-1.011 | 0.101 | 1.028  | 0.965-1.095       | 0.388 |
| TP < 30 mmHg                   | 0.903 | 0.843-0.967       | < 0.005 | 1.008  | 0.949-1.071       | 0.797 |
|                                | *     | 0.913             | 0.846-0.985 | < 0.05 | 1.015  | 0.948-1.086       | 0.673 |
|                                | †     | 0.910             | 0.835-0.992 | < 0.05 | 0.997  | 0.92-1.075        | 0.938 |
| ABI < 0.4                      | 0.925 | 0.87-0.980        | < 0.01 | 1.025  | 0.972-1.080       | 0.360 |
|                                | *     | 0.927             | 0.870-0.988 | < 0.05 | 1.023  | 0.969-1.079       | 0.413 |
|                                | †     | 0.912             | 0.84-0.986 | < 0.05 | 1.034  | 0.96-1.109        | 0.347 |
| Semi-urgent operations         | 0.914 | 0.843-0.992       | < 0.05 | 1.031  | 0.969-1.096       | 0.339 |
|                                | *     | 0.904             | 0.841-0.972 | < 0.01 | 1.060  | 0.998-1.126       | 0.058 |
|                                | †     | 0.882             | 0.797-0.976 | < 0.05 | 1.057  | 0.985-1.135       | 0.125 |

* adjusted by gender and age
† adjusted by gender, age, BMI, diabetes, hypertension and dyslipidaemia

Abbreviations: LEAD, lower extremity arterial disease; NodCa, nodular calcification area proportion; ShCa, sheet calcification area proportion; TP, toe pressure; ABI, ankle brachial index.
Discussion

We have, for the first time, quantified the amount of NodCa and ShCa on femoral artery plaque sections. The results revealed that the increase of the relative amount of NodCa is associated with reduced vascular ischemia of the lower limb, even at over 90% level of stenosis. Additionally, higher relative amount of NodCa is associated with reduced risk of semi-urgent operation. The latter may implicate a slower obstruction and eventually a milder LEAD presentation.

NodCa and ShCa typically coexist in the same plaque, according to the continuum of vascular calcification. To understand their clinical relevance, the quantification of NodCa and ShCa lesions is needed. Quantifying of more than one feature accurately and consistently is difficult to attain using the conventional subjective scoring method. Therefore, we applied deep learning algorithm analysis for the localization and quantification of these calcified structures. The quantification was optimized and validated by the high sensitivity and specificity, with around 2% area error.

NodCa is believed to fracture from sheet calcification [15]. This possibly explains the highest prevalence of NodCa in artery with more dynamic activity like superficial femoral artery compared to coronary and carotid arteries [10]. Furthermore, smooth muscle cells of femoral artery have an inherently different molecular makeup from other vascular beds; their higher expression of TGFβ promotes mineralizing activity [16]. This may explain the prevailing dense calcification observed in this study sections.

All study samples were obtained from operated patients, and they had over 90% including occlusion, identified histologically, and confirmed from preoperative magnetic resonance angiography. Interestingly, distal limb perfusion in these patients, quantified by measurements of toe pressure and ankle brachial index, varied in their severity. This clinical variation is reflected in a variable presentation and relative quantity of the calcified structures, ShCa and NodCa in the patients’ lesions. The observed clinical variation, however, was mainly associated to the quantified NodCa according to this study analysis.

In the current study, NodCa was encountered in 96% of sections with over 90% level of stenosis. Such a high prevalence can be attributed to the good exposure of the longitudinally sectioned plaques. Despite the high prevalence of NodCa in the studied obstructed/semi-obstructed lesions, quantification of NodCa in these samples has revealed that its relative amount contributed to a milder presentation of LEAD. The milder presentation was assessed by the negative association of the quantified NodCa with severely lowered TP and ABI. Furthermore, increase of the relative amount of NodCa was associated with decreased risk of semi-urgent intervention. The association of the higher relative amount NodCa with milder LEAD in obstructed/semi-obstructed samples may be implicated by a slow rate of stenosis progression [17]. No association was observed between the quantified ShCa and the clinical presentation of LEAD.

The applied quantifying approach has demonstrated that the increase of the relative amount of NodCa contributed significantly to the increase of the measured vessel diameter in samples with over 90% stenosis. Larger arteries at the obstructive/semi-obstructive level may incubate larger plaques and likely
more NodCa. However, the quantification in this study is made relative to the plaque area. Furthermore, the analysed association, after adjustment to the demographic determinants of vessel size: gender, age and BMI; and modifiers of vascular remodeling: smoking, hypertension, diabetes, GFR, and hs-CRP, remained significant [18], [19]. This indicates that NodCa may have some contribution to an expansive vascular remodeling. No association was noted between the amount of ShCa and the measured vessel diameter in these samples.

We postulate, that the loss of the compact calcified structure by the fragmentation of NodCa, and the separation of NodCa fragments by fibrin that collects from the leaky capillaries may enhance the expansive vascular remodeling [15]. Expansive vascular remodeling is proved to delay stenosis progression [20]. This possibly indicates a slower obstruction of vessels that incubate abundant NodCa in their lesions, presented by reduced propensity to semi-urgent intervention [20].

Previous studies on the clinical association of the quantied NodCa in LEAD have not, to our knowledge been published. However, the presence of calcified nodules on femoral plaque samples has been also observed to protect against post-operative major amputation and/or re-intervention of the re-vascularized limb [21]. In the current study, the patients were not analyzed post-operatively.

The amount of ShCa was significantly reduced in normal BMI patients compared to overweight/obese patients. In line with this finding, the amount of ShCa inversely correlated with serum LDL cholesterol and triglyceride, and positively correlated with serum HDL cholesterol in the studied patients. Level of lipids in serum is believed to increase mainly the deposition of early calcification in the form of microcalcification and has been demonstrated histologically [22]–[24].

The strength of the study is the rigorous clinical examination results obtained pre-operatively. We developed a reliable neural network method to quantify different forms of arterial calcification. The trained algorithm attained high sensitivity and specificity. The study material consisted of plaques harvested during surgical intervention; therefore, they represent a later stage of the disease. No conclusion could be drawn from this material about less severe stage of disease. Our findings about NodCa and disease severity and vessel diameter represent a cross-sectional study and to our knowledge the first study to quantify the relative amount of NodCa and ShCa.

**Conclusion**

Femoral plaques with over 90% stenosis demonstrate variable clinical severity of LEAD. The applied deep learning analyzing tool allows for categorical quantification of calcification types in the plaque's sections. The quantifying approach is more reliable than simply indicating the presence of calcification category in terms of clinical significance assessment. This quantifying approach for calcification categories has revealed that the relative predominance of NodCa indicated a slowly progressing obstruction diagnosed by alleviated ABI and TP readings and reduced semi-urgent surgical intervention and inferred by a possible expansive vascular remodeling contribution. These results improve our understanding of the
complex pathophysiology of LEAD and pave way for personalized treatment options targeted at the specific pathological findings of endarterectomy patients.

**Methods**

**Cohort description**

Femoral plaque samples were collected during endarterectomy of the femoral artery bifurcation between October 2014 and January 2017 \[4\]. The severity of LEAD of the patients (N=90) was determined by ankle brachial index (ABI), toe pressure (TP), Fontaine classification of LEAD symptoms, and urgency of the operation. Preoperative magnetic resonance angiography images were used to confirm the severity of the stenosis (Fig. 1).

A and B are preoperative magnetic resonance images of two male patients that present obstructive level of stenosis of femoral artery (blue arrow) caused by the atherosclerotic lesions C and D respectively. Obstruction in these lesions is demonstrated histologically (arrowhead). Lesion C is dominated by NodCa, closely seen in the magnified image (thin arrow) surrounded by fibrin (white asterisk), while lesion D has mainly ShCa (black asterisk) in the magnified image. The trained algorithm (E) and (F) recognizes and calculates area proportion of NodCa (blue color) and ShCa (red color) to the sectioned plaque (green color) area. The calculated area proportion in E is 0.26 for NodCa and 0.10 for ShCa, while in F is 0.06 for NodCa and 0.40 for ShCa.

We compared the area proportion of NodCa and ShCa observed in the plaque tissue to the clinical characteristics listed above. We analyzed endarterectomy samples, which fulfilled two criteria:1) more than 90% stenosis including obstruction, was observed both histologically and by magnetic resonance angiography, and 2) internal elastic lamina for vessel diameter measurement was identifiable in the plaques’ histological section.

Plaques were formalin-fixed, decalcified, and longitudinally sectioned into two halves. Sections were stained with Hematoxylin and eosin stain for histomorphometry analysis. Sample processing and laboratory analyses are described in detail elsewhere \[4\].

The patients signed a written informed consent to the study before endarterectomy operation. The study is approved by the Ethics Committee of medicine of the Hospital District of Helsinki and Uusimaa (ASO-project Drno 78/13/03/00/2014) and all methods were performed in accordance with the relevant guidelines and regulations.

**Deep learning algorithm training**

Hematoxylin and eosin-stained slides of femoral plaques longitudinal sections were digitized with a whole-slide scanner (3D HISTECH Pannoramic 250 Flash III, 3DHistec, Budapest, Hungary) with 20x objective and a pixel size of 0.23µm. The slides were then uploaded to a cloud-based image deep learning platform (Aiforia Create, Aiforia Technologies Oy, Helsinki, Finland).
To quantify each of the predefined calcification categories, two sequential algorithms were developed; the first algorithm, the plaque tissue algorithm recognized and quantified the area of plaque tissue from slide background. The algorithm was set to region context size of 50 µM, the complexity level of Complex and the default specifications. Upon this algorithm, a stratified second algorithm, the calcification algorithm, was built for quantification of the calcification categories, NodCa and ShCa. While developing the algorithms, accuracy was assessed through 1) Verification of each annotation 2) Analysis of the untrained regions and whole section slides. Calcification algorithm was fine adjusted on the following parameters: iterations = 7000, field of view = 100 x, image augmentation range (-10 to 10), aspect ratio=1, maximum sheer=1, luminance range (-30 to 30), contrast range (-30 to 30), maximum white balance change= 10, and noise= 2. The algorithm quantified ShCa and NodCa as the area of every recognized structure of the category in mm², the collective area of each category, and the area proportion of the calcification category to that of the plaque section tissue area (Fig. 1). To optimize the analysis, visual validation of the final algorithm analysis was conducted, whereafter, tiny lesions that were part of the continuum yet did not contribute to an actual nodule, were excluded. This was done after visually determining the cut-off size limit of the lesion per each slide. The precision of the finalized algorithm was 97.53%, sensitivity was 97.66% and total area error (false positive and negative) was 2.06%.

The maximum width of the vessel diameter in the most stenosed part was measured to assess the vascular remodeling in relation to the area proportion of NodCa and ShCa. This was done using the measurement tool in the Aiforia platform (supplementary figure S1).

**Data analysis**

Area proportions of NodCa and ShCa was analyzed in relation to patients’ continuous and categorized binomial variables. Continuous data analysis of the patients is presented as the mean (± standard deviation). Data were analyzed for normal distribution by Shapiro-Wilk test. Normally distributed data were analyzed by two-tailed t-test and Pearson correlation test, while non-normally distributed data were analyzed by Mann-Whitney U and Spearman rank analysis. Analysis of covariance was set to assess vessel diameter association with area proportion of NodCa and ShCa along with other confounding factors; age, gender, body mass index (BMI), smoking, hypertension, glomerular filtration rate (GFR) and inflammatory state (high sensitivity C-reactive protein (hs-CRP)). Our data fit the assumption of logistic regression analysis for the association of area proportion of NodCa and ShCa with the clinical parameters of LEAD, adjusted by hypertension, diabetes and dyslipidaemia. P value < 0.05 was considered statistically significant. Data were analyzed using SPSS 25 (Armonk, NY: IBM Corp).

The age was categorized by the median into patients older or younger than 70.5 years. was categorized by the cutoff point of normal and overweight (25 kg/m²) into normal, and overweight or obese (combined). Laboratory measurements were categorized according to the standardized age/gender-relevant reference values that are adopted in the analyzing facility, Helsinki University Hospital Laboratory Services (HUSLAB). Hs-CRP was considered increased for females when levels exceeded 2.5 mg/L and for males when levels exceeded 3 mg/L. GFR was considered impaired if it was less than the following
values measured in ml/min \(/1.73 \text{m}^2\): 77 for patients aged 50-59 years, 69 for patients aged 60-69 and 59 for patients aged 70 years and older. Leukocytosis was labelled for readings higher than 8.2 E9/L, while anaemia was deduced from females’ haemoglobin readings < 117 g/L and from males’ readings < 134 g/L. The categorization of ABI and TP parameters was based on the clinical reference [14]. ABI less than 0.4 and TP less than 30 mmHg were deemed as severe disease indicators. Fontaine class was categorized into two groups, patients with claudication were deemed to have mild symptoms and patients with rest pain, ischaemic ulcer or gangrene were considered to have severe symptoms [4]. Surgical interventions were classified into elective operations, semi-urgent operations were determined if an exceeded intervention was mandated within 4 weeks of their clinical evaluation.

**Abbreviations**

NodCa: nodular calcification ShCa: sheet calcification LEAD: lower extremity arterial disease BMI: body mass index Hs-CRP: high-sensitivity C-reactive protein GFR: glomerular filtration rate LDL: low-density-lipoprotein HDL: high-density-lipoprotein TP: toe pressure ABI: ankle brachial index ACE: angiotensin-converting enzyme AT: angiotensin II

**Declarations**

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**Conflict of interest**

Johan Lundin is the founder and the consultant of Aiforia Oy. All other authors declare that there is no conflict of interest in this study.

**Author contributions**

The study was conceived and designed by IL, JS, and ML-L. MA developed the algorithm, analyzed the data and wrote the manuscript. IL supervised the algorithm development and statistical analyses. ML and MA analyzed the magnetic resonance images. JT and ML collected the clinical data. NL and JL are consultants for algorithm training. AA and MV provided the samples. MIM did specimen sectioning and
was the histopathological consult, JS provided funding for the study. IL, JS and ML-L developed the manuscript, all authors contributed to and approved of the final manuscript.

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**Figures**
Figure 1

Obstructive femoral samples from diagnostic images to quantified histomorphometry.

A and B are preoperative magnetic resonance images of two male patients that present obstructive level of stenosis of femoral artery (blue arrow) caused by the atherosclerotic lesions C and D respectively. Obstruction in these lesions is demonstrated histologically (arrowhead). Lesion C is dominated by NodCa, closely seen in the magnified image (thin arrow) surrounded by fibrin (white asterisk), while lesion D has mainly ShCa (black asterisk) in the magnified image. The trained algorithm (E) and (F) recognizes and calculates area proportion of NodCa (blue color) and ShCa (red color) to the sectioned plaque (green color) area. The calculated area proportion in E is 0.26 for NodCa and 0.10 for ShCa, while in F is 0.06 for NodCa and 0.40 for ShCa.

Figure 2

The collective area proportion of nodular calcification (NodCa) and sheet calcification (ShCa) of the 51 patients with > 90% stenosis included in the study

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