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Title: Feasibility of *in vivo* Imaging of Fibroblast Activation Protein in Human Arterial Walls

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Disclosure

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ABSTRACT

Increased expression of fibroblast activating protein (FAP) in fibrous caps may contribute to progression of atherosclerotic plaques. **Methods** Forty-one patients who underwent gallium-68-conjugated quinoline-based FAP inhibitor (^{68}Ga-FAPI-04) PET/CT for non-cardiovascular indications were retrospectively analyzed. Correlations were assessed between the uptake of ^{68}Ga-FAPI-04 in large arterial walls (SUV_{max} and target-to-background ratio, TBR) and degree of calcification and cardiovascular risk factors. **Results** Focal arterial uptake of ^{68}Ga-FAPI-04 or calcification was detected in 1,177 arterial segments in all 41 patients. TBR was negatively correlated with the degree of calcification (Hounsfield Units, HU) (r = -0.27, P < 0.01). Mean TBR in higher-risk patients was greater than lower-risk patients (2.2 ± 0.3 vs. 1.8 ± 0.3, P < 0.01). Immunohistochemical labeling of carotid plaques exhibited prominent FAP expression in a thin fibrous cap and moderate FAP expression in a thick cap. **Conclusion** ^{68}Ga-FAPI-04 PET/CT might have potential for imaging fibroblastic activation in the arterial wall.

Keywords: ^{68}Ga-FAPI-04; fibroblast activating protein; PET/CT; active arterial wall

INTRODUCTION

Atherosclerosis is the primary cause of cardiovascular disease, defined by the chronic, progressive accumulation of lipids and fibrous elements in large arterial walls. The major contributors to plaque vulnerability include a large necrotic core, a thin fibrous cap, expansive remodeling, neovascularization, plaque hemorrhage, and adventitial inflammation (*1*). The identification of specific biomarkers of plaque vulnerability remains highly important, yet difficult (*2*). Destabilization of fibrous caps is mediated by collagen degeneration and the activity of extracellular proteases (*1*). Fibroblast activation protein (FAP) is a type II membrane-bound serine protease (*3*). Preliminary *ex vivo* analysis detected higher FAP expression in human atherosclerotic aortic plaques than in plaque-free arterial walls; particularly, FAP expression increased in thin-capped compared with thick-capped atheromas (*4,5*). Recently, the development of positron emission tomography (PET) imaging using several ^{68}Ga-labeled FAP inhibitors introduced the possibility of non-invasive, *in vivo* visualization of human FAP expression (*6*). In this study, we aimed to quantify the arterial fibroblast activation via a gallium-68-conjugated quinoline-based FAP inhibitor (^{68}Ga-FAPI-04) PET/CT imaging in correlation with cardiovascular risk factors.

MATERIALS AND METHODS

Patients

Forty-one patients (10 females and 31 males; 59 ± 11 years) with suspicious hepatic lesions (*n* = 27) or immunoglobulin G4-related disease (*n* = 14) underwent ^{68}Ga-FAPI-04 PET/CT imaging between January 2019 and January 2020. The baseline characteristics and cardiovascular risk factors were documented (see Table 1). The exclusion criteria included pariaortitis, vasculitis, and chemotherapy within four weeks. The study protocol complied with the tenets of the Declaration of Helsinki and its later amendments. The study protocol was approved by the institutional review board of Peking Union Medical College Hospital, and all subjects signed written informed consents form before imaging.

Radiopharmacy and PET/CT Scans

Radiolabeling with ^{68}Ga-FAPI-04 was performed as previously described (*7,8*). All subjects underwent PET/CT scans on dedicated PET/CT scanner (Polestar m660, SinoUnion, China) after an uptake time of 42 - 70 min following intravenous injection of ^{68}Ga-FAPI-04 (92.5 - 260 MBq). Following an unenhanced low-dose CT scan (120 keV, 30 - 50 mA), PET images were obtained from the tip of the skull to the mid-thigh in 3-D mode with a bed time of 2 minutes.

Image Analysis

We performed an active segments analysis (target-to-background ratio [TBR] $\geqslant$ 1.6) in the five major arteries, including the aortic arch, ascending aorta, thoracic aorta, abdominal aorta, and the iliac arteries (*9*). The regions of interest (ROIs) were manually drawn around each active segment (10 mm diameter), and SUV_{max} was determined from transaxial PET/CT images. The TBR of each active segment was derived as the segment's SUV_{max} divided by the $SUV_{bloodpool}$ (average SUVmean of three ROIs within the vena cava). We calculated the mean TBR of all active arterial segments for assessment of overall burden for each patient (*9*). The radioactivity in calcified arterial segments with a minimum density of 130 Hounsfield Units (HU) on non-contrast CT images were also assessed (*10*). Two experienced nuclear medicine physicians (J.Ning and J.Li) assessed the PET/CT images. Discrepancies were re-assessed by consensus of two readers. All analyses were conducted using HERMES Hybrid 3D (Hermes Medical Solutions, London, UK).

Immunohistochemistry to Assess FAP Expression in Carotid Arterial Plaques

Cryosections of tissue samples containing carotid plaques were obtained from patients who underwent endarterectomy secondary to carotid artery stenosis. Fibrous caps were identified as collagen-rich tissues visualized with elastin Masson's trichrome stain separating the lumen and the necrotic core. Immunohistochemistry assessed the FAP expression with anti-FAP antibody (1:300, SP325 Abcam, UK).

Statistics

Parametric variables are expressed as mean ± SD or median (first quartile, third quartile). Arterial segments were categorized based on calcification [noncalcified (< 130 HU), mildly calcified (130 – 399 HU), and severely calcified segments (≥ 400 HU)]. Patients were divided into high-risk (prevalence of ≥ 4 cardiovascular risk factors) and low-risk (< 4 cardiovascular risk factors) groups. FAPI uptake was compared among the three calcification groups by one-way analyses of variance (ANOVAs). The variation of mean TBR of each patient in different cardiovascular risk factor groups and high-risk or low-risk groups was assessed by unpaired t-tests. Inter-observer reliability was done in all patients with intraclass correlation efficient with a two-way random model applying absolute agreement. All statistical analyses were performed using SPSS Statistics (Version 25, IBM Corporation, Armonk, New York). *P* values < 0.05 denoted statistical significance.

RESULTS

^{68}Ga-FAPI-04 Uptake of Active Arterial Segments and Relationship with Calcification

A total of 1,177 arterial segments of focal uptake of ^{68}Ga-FAPI-04 or calcification were identified in all 41 patients. The mean SUV_{max} and mean TBR for ^{68}Ga-FAPI-04 were 1.6 ± 0.5 and 2.0 ± 0.7, respectively. Among all of the assessed arterial segments, the abdominal aorta exhibited the highest number of segments (*n* = 379), followed by the thoracic aorta (*n* = 272) and the ascending aorta (*n* = 203). Analysis of all 1,177 segments showed a significant correlation between the extent of calcification (HU) and the intensity of ^{68}Ga-FAPI-04 uptake (TBR) (r = -0.27, P < 0.01; Fig.1A). Non-calcified segments presented with significantly higher uptake (TBR = 2.2 ± 0.6; *n* = 603) than mildly calcified segments (TBR = 1.9 ± 0.8; *n* = 220) (P < 0.01). Severely calcified segments exhibited the lowest uptake of ^{68}Ga-FAPI-04 (TBR = 1.7 ± 0.6; *n* = 354) (P < 0.01) (Fig.1B). Correlation coefficient was 0.89 (0.80,0.95) for TBR with 95% confidence intervals for inter-observer agreement.

Relationship Between Arterial ^{68}Ga-FAPI-04 Uptake and Cardiovascular Risk Factors

The mean number of active arterial segments per patient was 29 ± 13 (range: 8 - 78). In the per-patient analysis, the mean TBR for ^{68}Ga-FAPI-04 was 1.9 ± 0.4. The mean individual TBR value was found to be significantly higher in overweight or obese patients (BMI ≥ 24.0, 2.2 ± 0.4; n = 10) than in those with normal weight (1.8 ± 0.3; n = 21). There was no significant difference of ^{68}Ga-

FAPI-04 uptake in other cardiovascular risk factor groups (Fig. 2), including male sex, older age, hypertension, diabetes mellitus, dyslipidemia, smoking habits, and past cardiovascular events. The mean TBR and the number of identified arterial segments in the high-risk patients (≥ 4 cardiovascular risk factors, TBR_{mean} 2.2 ± 0.3, segment number 36 ± 17; n = 15) was significantly higher than that in the low-risk patients (1.8 ± 0.3, 24 ± 9; n = 26) (both $P < 0.01$). Figure 3 showed examples of radiotracer uptake patterns of ^{68}Ga-FAPI-04 in the arterial wall. All patients in the figure were over 60 years old with >4 cardiovascular risk factors.

FAP Expression in Human Carotid Atherosclerotic Plaques

Collagen tissue was assessed by Masson's trichrome staining (Figure 4, in blue) of human carotid arterial plaques. Based on the fibrous cap thickness, the specimens were characterized as thin-capped (< 65 mm) or thick-capped (≥ 65 mm) plaques. Immunohistochemical labeling with an anti-FAP antibody in crossed sections demonstrated a prominent FAP expression in the thin fibrous cap vs. relatively lower in the thick fibrous cap (Fig. 4). Specific FAPI expression localized in denatured collagen fibers (Fig. 4).

DISCUSSION

To our knowledge, this is the first non-invasive study to describe the expression of FAP in the human arterial walls via ^{68}Ga-FAPI-04 PET/CT imaging. In this retrospective study of a non-cardiovascular cohort, we observed significantly elevated uptake in non-calcified active arterial segments compared to advanced chronic lesions presenting extensive calcification; and elevated ^{68}Ga-FAPI-04 uptake in patients with increased cardiovascular risk factors. We also found increased arterial uptake values in high-risk patients than low-risk patients. Obesity presented a relatively more prominent impact on arterial uptake, in comparation to other cardiovascular risk factors. This observation might be related to increased image noise in obese patients.

The role of FAP in atherosclerosis is complex. Evrard et al. detected a significant number of endothelial-lineage-derived cells expressing FAP in rupture-prone, thin-capped plaques more than stable plaques in atherosclerosis-prone mice and *ex vivo* human aortic plaques (*11*). Nonetheless, Monslow et al. demonstrated co-localization of FAP and vascular cell adhesion molecule 1, which marked vascular smooth muscle cells with a proliferative and matrix-producing tendency in atherosclerotic mice (*12*). In accordance with pioneering results, elevated FAP expression in thin fibrous cap and fibrosis, collagen-rich tissue in intima was both detected in our immunohistological findings. The role of FAP in both remodeling and stabilizing the extracellular matrix in atherosclerosis is under further investigation. The interpretation of the ^{68}Ga-FAPI-04 signal in arterial walls is challenging. We found increased FAPI uptake in non and low-level calcified lesions

compared to higher-level calcified lesions, which might indicate aggravated fibroblast activation is irrelevant to arterial calcification burden. Nonetheless, there is a need for further evidence of arterial fibrosis quantification and calcification activation.

The retrospective and non-cardiovascular nature of this study led to several inevitable limitations: 1. The discriminatory value of FAPI uptake needs to be further validated in a cardiovascular cohort. 2. None of the patients had concurrent histological evidence, autoradiograph, or other *in vivo* enhanced imaging approaches which may further facilitate identification of atherosclerotic plaques (*13*). 3. Scans were performed using a routine protocol which was not optimal for vessel imaging (*14*). 4. Coronary arterial lesions were not assessed due to non-cardiac-gated PET scans, with increased motion effects and partial volume effects. 5. Due to the inherent limitation of non-contrast CT, non-calcified segments could be underestimated. 6. A novel whole arterial segmentation may allow a more global impression of tracer activity across vessel beds (*15*). Overall, our preliminary study provides a potentially feasible method to image atherosclerosis *in vivo* by ^{68}Ga-FAPI-04 PET/CT. Prospective studies using ^{68}Ga-FAPI-04 PET imaging in symptomatic atherosclerotic cohorts are warranted.

CONCLUSION

^{68}Ga-FAPI-04 PET/CT might have a potential for imaging fibroblast activation in the arterial wall, which could provide new insights into the pathological mechanisms. Further studies to investigate the performance of FAP imaging in symptomatic atherosclerosis cohorts are highly warranted.

CONFLICT OF INTEREST STATEMENT

No potential conflicts of interest relevant to this article exist.

KEY POINTS

QUESTION: How is the performance of ^{68}Ga-FAPI-04 PET/CT for imaging of arterial walls in humans?

PERTINENT FINDINGS: In this retrospective analysis of 41 patients, we observed elevated ^{68}Ga-FAPI-04 uptake in patients with increased cardiovascular risk factors.

IMPLICATIONS FOR PATIENT CARE: ^{68}Ga-FAPI-04 PET/CT has potential as a feasible method of imaging fibroblastic activation in the arterial wall, and could provide new insights into the pathological mechanisms driving its progression.

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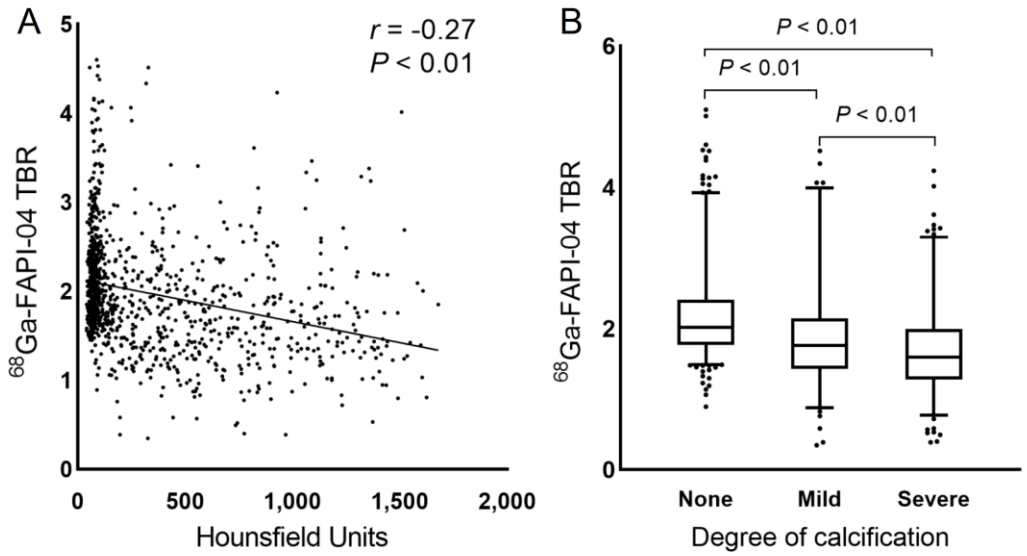
234 **FIGURE LEGENDS**



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236 **FIGURE 1.** ^{68}Ga-FAPI-04 uptake correlates with the degree of calcification in the per-segment

237 analysis (n = 1,177).

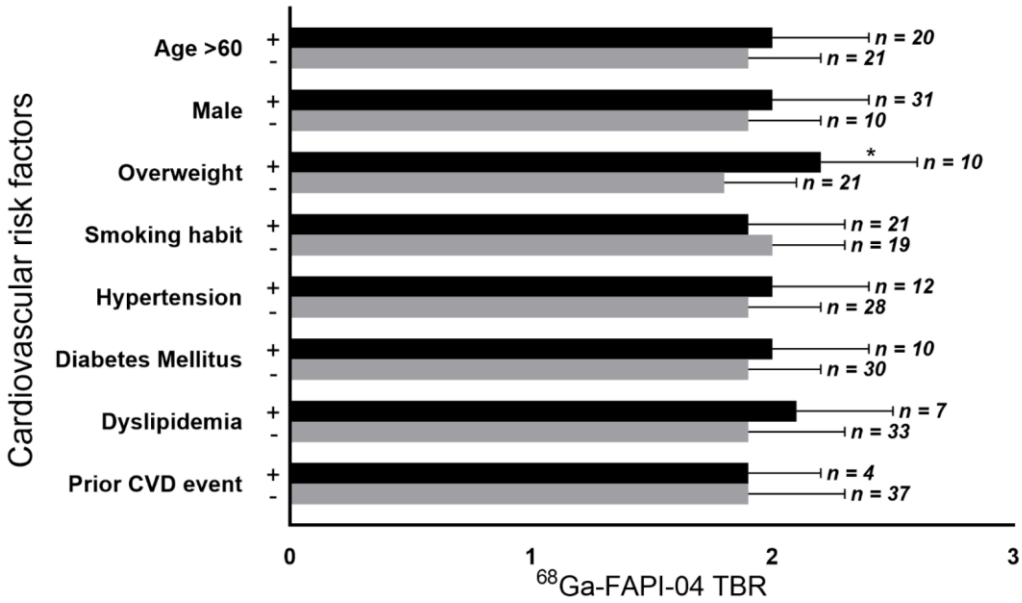
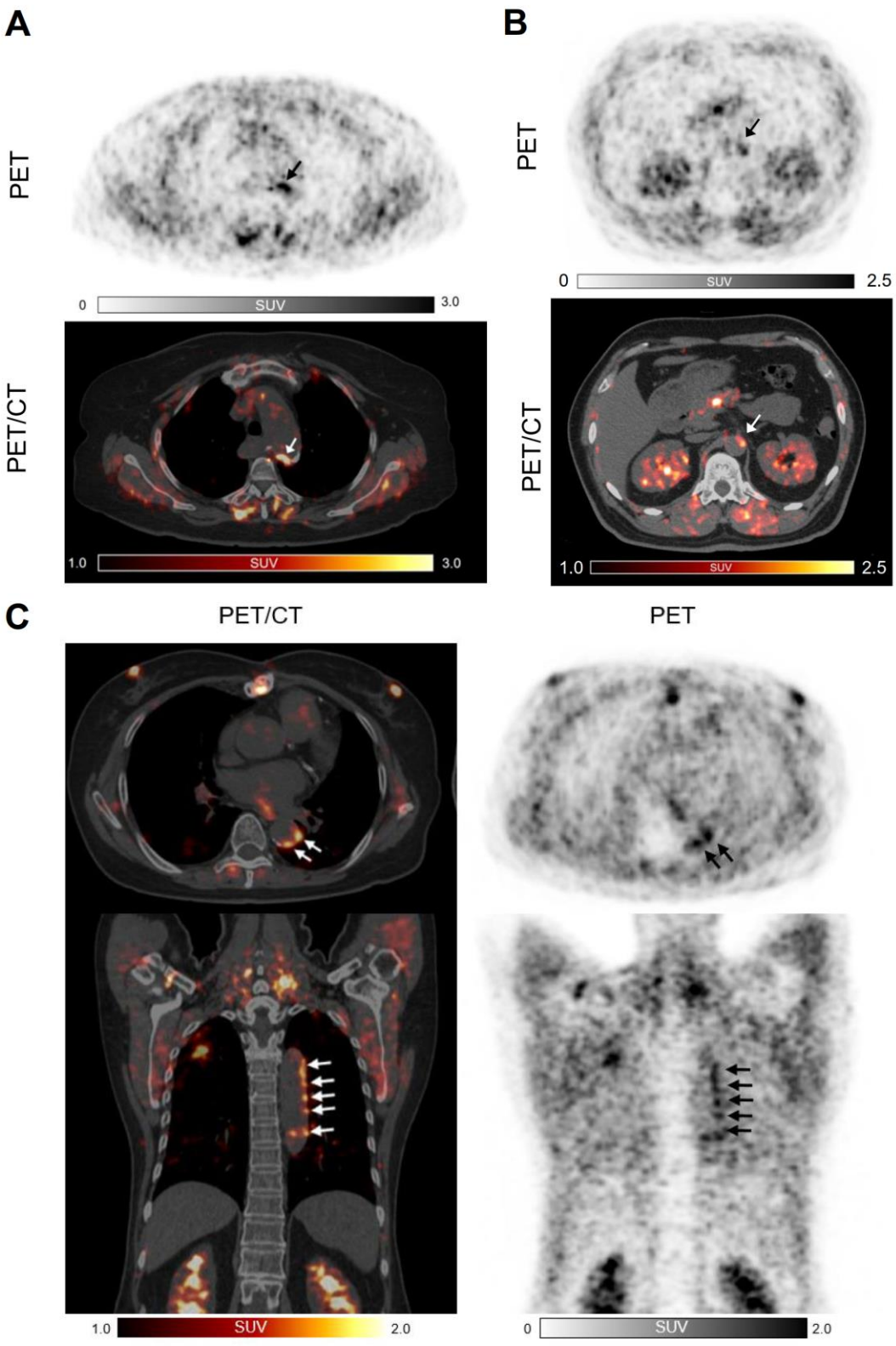


FIGURE 2. Comparison of overall arterial ^{68}Ga-FAPI-04 burden with respect to cardiovascular risk factors. * indicates a statistically significant difference ($P < 0.05$) in ^{68}Ga-FAPI-04 TBRs between patients who were overweight/obese or normal weight based on their body mass index.


A
PET
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SUV
2.5
C
PET/CT
PET
1.0
SUV
2.0
0
SUV
2.0

243

FIGURE 3. Three examples of ^{68}Ga-FAPI-04 uptake of active arterial segments. All three patients were over 60 years old with a history of hypertension and dyslipidemia. Patient A and C also had diabetes mellitus, and experienced myocardial infarction and percutaneous coronary intervention treatment. Patient A was obese (body mass index = 30.0) while patient B had a history of heavy smoking.

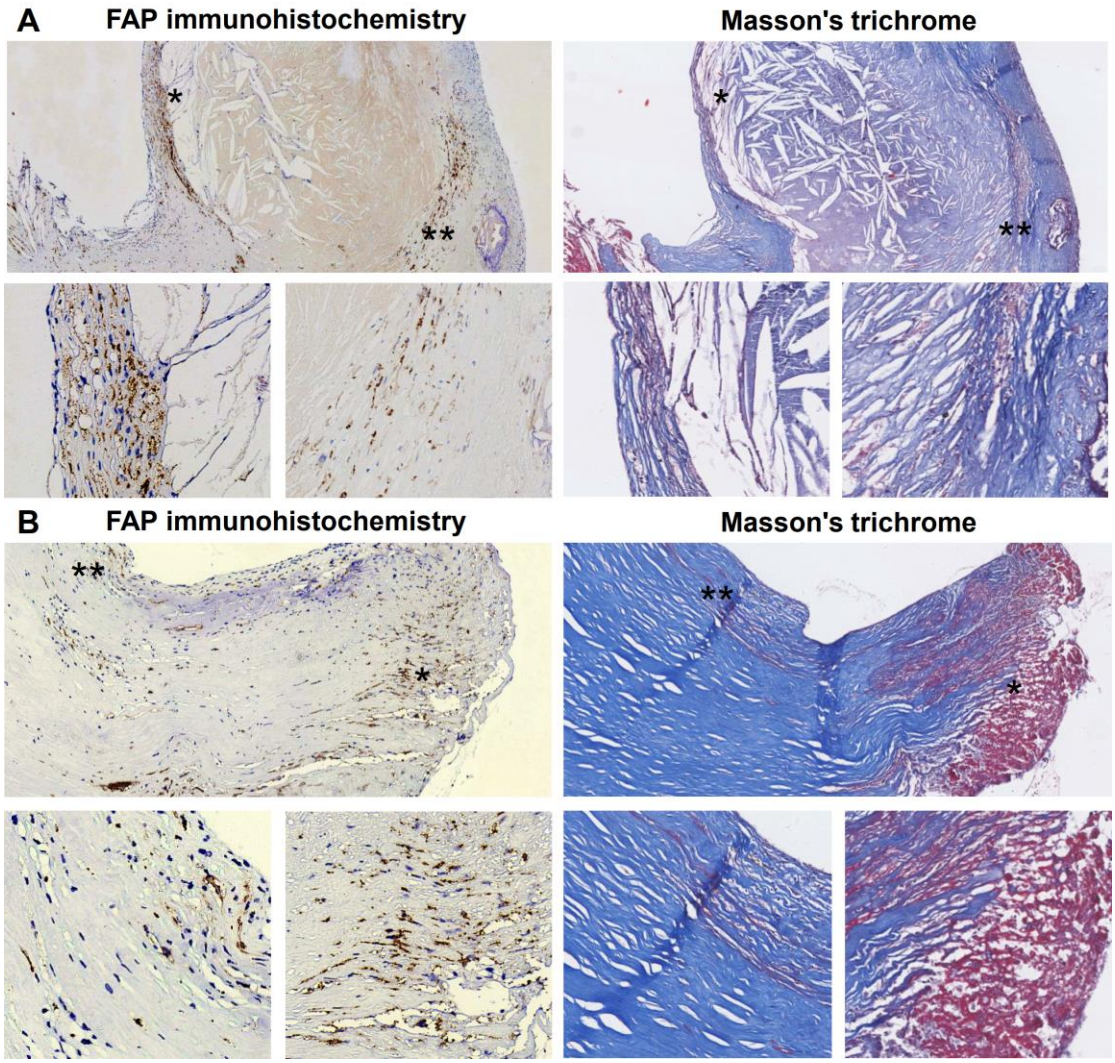

FIGURE 4. FAP expression in thin-capped (A) and thick-capped (B) human carotid atherosclerotic plaque lesions. Masson staining shows collagen-rich thin and thick fibrous caps. Plaque A exhibited with thin fibrous cap with major FAP expression (*). The fibrosis-rich region in the intima also showed moderate FAP expression (**). Plaque B exhibited with thick fibrous cap with sparse FAP expression overall and FAP expression only in denatured collagen fibers (* and **).

255 **TABLE 1.** Patient characteristics.

Baseline patients characteristics (n=41)	
Age, mean±SD	59±11
Sex ratio (female: male)	1: 3.1 (10: 31)
Suspicious hepatic lesion for malignancy, n(%)	27(66%)
IgG4-related disease, n(%)	14(34%)
Body mass index(kg/m2), mean±SD	23±3
Risk factors, n(%)	
Hypertension	12(30%)
Diabetes mellitus	10(24%)
Dyslipidemia	7(17%)
Smoking	21(51%)
History of a cardiovascular event	4(10%)

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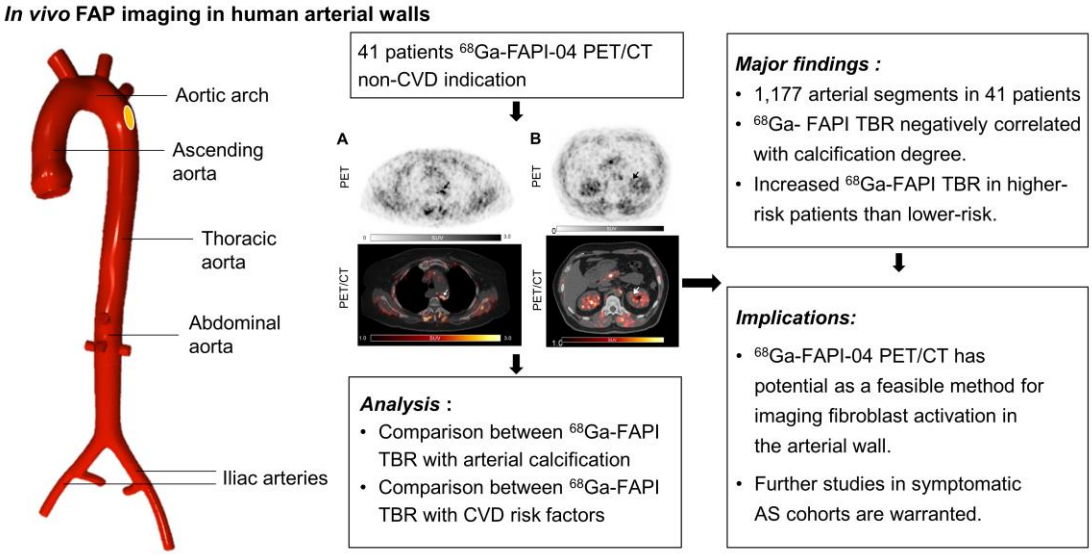
257 Graphic Abstract



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