Non-invasive in vivo coronary artery thrombus imaging

ORIGINAL ARTICLE

Non-invasive *in vivo* coronary artery thrombus imaging

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ABSTRACT

Background
The diagnosis and management of myocardial infarction is increasingly complex and establishing the presence of intracoronary thrombosis has major implications for both the classification and treatment of myocardial infarction.

Objectives
To investigate whether positron emission tomography (PET) and computed tomography (CT) could non-invasively detect *in vivo* thrombus formation in human coronary arteries using a novel glycoprotein IIb/IIIa receptor antagonist-based radiotracer, ^{18}F-GP1.

Methods
In a single centre observational case-control study, patients with or without acute myocardial infarction underwent coronary ^{18}F-GP1 PET-CT angiography. Coronary artery ^{18}F-GP1 uptake was assessed visually and quantified using maximum target-to-background ratios.

Results
^{18}F-GP1 PET-CT angiography was performed in 49 patients with, and 50 patients without, acute myocardial infarction (61±9 years, 75% male). Coronary ^{18}F-GP1 uptake was apparent in 39 (80%) of the 49 culprit lesions in patients with acute myocardial infarction. False negative scans appeared to relate to time delays to scan conduct and low thrombus burden in small calibre distal arteries. On multivariable regression analysis, culprit vessel status was the only independent variable associated with higher ^{18}F-GP1 uptake. Extra-coronary cardiac ^{18}F-GP1 findings included a high frequency of infarct-related intramyocardial uptake (35%) as well as left ventricular (8%) or left atrial (2%) thrombus.

Conclusions
Coronary ^{18}F-GP1 PET-CT angiography is the first non-invasive selective technique to identify *in vivo* coronary thrombosis in patients with acute myocardial infarction. This novel approach can further define the role and location of thrombosis within the heart and has the potential to inform the diagnosis, management, and treatment of patients with acute myocardial infarction.

Keywords: Myocardial infarction, Intracoronary thrombosis, Positron emission tomography, Computed tomography

Abbreviations

CCTA	Coronary computed tomography angiography
LAD	Left anterior descending
LCx	Left circumflex artery
PET	Positron emission tomography
RCA	Right coronary artery
SUV_{max}	Maximum standardized uptake values
TBR_{max}	Maximum target to background ratio

Condensed Abstract

^{18}F-GP1 positron emission tomography can identify *in vivo* intracoronary thrombus in patients with acute myocardial infarction. This technique appears to hold major promise as a method of determining the role and origin of thrombosis in acute myocardial infarction, especially in cases of thromboembolism or myocardial infarction with non-obstructive coronary arteries. It could also assist in cases of multivessel coronary artery disease where the localisation of the culprit lesion or presence of thrombus may influence patient management. We also demonstrated a high frequency of extra-coronary findings including unrecognised left ventricular and atrial thrombus as well as infarct-related intramyocardial ^{18}F-GP1 uptake.

Introduction

High-sensitivity cardiac troponin testing has transformed cardiological practice and has ensured that at-risk patients and those with myocardial infarction can be effectively identified.(1-3) However, this greater sensitivity has come at the cost of identifying many more patients with acute myocardial injury who may or may not have myocardial infarction. There has also been the increasing complexity of the diagnosis of myocardial infarction typified by type 2 myocardial infarction and myocardial infarction with non-obstructive coronary arteries.(4,5) Clinicians are often left with the challenge of determining whether an atherothrombotic event has occurred and establishing the underlying cause.(6) This is critically important if patients are to be correctly diagnosed and treated.

At present, invasive coronary angiography is the primary modality used to detect the presence of intracoronary thrombus in patients with acute myocardial infarction. However, it lacks sensitivity and may fail to identify, or misidentify, the culprit artery in over a third of patients with non-ST elevation myocardial infarction.(6) Adjunctive invasive techniques, such as intravascular ultrasound and optical coherence tomography, are valuable tools to clarify angiographic ambiguity.(7,8) However, these catheter-based techniques require instrumentation of the coronary artery and are limited in their ability to assess smaller or stenosed vessels due to the size and physical limitations of the intravascular probes. Like other non-invasive imaging approaches, intravascular imaging also only provides circumstantial evidence of the presence of thrombus based on the characteristic appearances of the images.(9)

We have previously described the use of ^{18}F-sodium fluoride positron emission tomography and computed tomography (PET-CT) imaging in patients with acute myocardial infarction and stable coronary artery disease.(10-14) We have shown that increased coronary ^{18}F-sodium

fluoride uptake correlates with high-risk plaque characteristics, disease progression and subsequent myocardial infarction. However, assessments of plaque characteristics or disease activity do not directly inform about the presence of plaque rupture and active atherothrombosis. Prior radiotracer imaging studies have investigated thrombus imaging in the heart but have been limited to the assessment of atrial or ventricular thrombus rather than coronary thrombosis.(15-17) ^{18}F-GP1 is a novel and selective elarofiban-derived radiotracer that binds with high specificity to the glycoprotein IIb/IIIa receptor on activated platelets.(18) Previous phase 1 *in vivo* studies (19,20) and case reports (21,22) have demonstrated that ^{18}F-GP1 PET-CT can detect *in vivo* venous and arterial thrombi. We hypothesized that ^{18}F-GP1 PET-CT could provide the first non-invasive technique to identify *in vivo* coronary thrombosis in patients presenting with acute myocardial infarction.

Methods

^{18}F-GP1 Binding to Human Thrombus

Fresh human thrombus formation was generated using an *ex vivo* model of deep arterial injury as described previously (23,24) and performed during infusion of ^{18}F-GP1 in the presence or absence of tirofiban co-infusion. ^{18}F-GP1 autoradiography was performed in the presence or absence of tirofiban on thrombectomy specimens obtained from patients undergoing percutaneous coronary intervention for acute myocardial infarction. Further details of the methodologies are provided in the Supplementary Material.

Study Design and Study Population

This was a single centre cross-sectional case-control study (In-vivo Thrombus Imaging With ^{18}F-GP1, a Novel Platelet PET Radiotracer (iThrombus); NCT03943966). We recruited patients over 40 years of age who presented with type 1 myocardial infarction defined by the fourth universal definition of myocardial infarction (4) and cardiac troponin concentration greater than 50 times the upper reference limit within seven days of presentation to the Edinburgh Heart Centre. All patients underwent invasive coronary angiography. Control subjects consisted of patients with stable coronary artery disease and previous aortic valve replacement who underwent ^{18}F-GP1 cardiac PET-CT as part of a concurrent study (NCT04073875).(25) These patients had no angina or recent myocardial infarction (Supplementary Figure i). Exclusion criteria included inability to provide informed consent, pregnancy or breastfeeding, contraindications to iodinated contrast, use of anticoagulant therapies, estimated glomerular filtration rate <30 mL/min/1.73 m^2, metastatic malignancy and an inability to tolerate the supine position.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Southeast Scotland Regional Ethics Committee (18/SS/0163). Written informed consent was obtained from all subjects.

Cardiac Imaging and analysis

^{18}F-GP1 cardiac PET-CT was performed on a 128-multislice scanner (Biograph mCT, Siemens, Germany) with prospective electrocardiogram-gating. PET list mode acquisition was performed 60 min after intravenous injection of 250 MBq ^{18}F-GP1 with a single bed position centred on the heart, preceded by an attenuation correction CT scan. Further details of the cardiac imaging and analysis are provided in the Supplementary Material.

Definition of Culprit Lesion

The clinical team ascribed the location of the culprit lesion at the time of invasive angiography independent of the research team or knowledge of the ^{18}F-GP1 PET-CT findings. The culprit lesion was defined as the lesion that led to the index admission and upon which the interventional procedure was performed. Further details of the culprit assessment are provided in the Supplementary Material.

^{18}F-GP1 uptake assessment

Qualitative and quantitative ^{18}F-GP1 assessment was performed on the co-registered PET and CT coronary angiogram images. ^{18}F-GP1 PET uptake was assessed in the left anterior descending artery, left circumflex and right coronary arteries where the vessel diameter was ≥2 mm. Further details of the tracer uptake are provided in the Supplementary Material.

Statistical analysis

We assessed the distribution of data with the Shapiro-Wilk test and quantile-quantile plots. Categorial variables were reported as frequencies (percentages). Continuous data are presented as mean ± standard deviation or median [interquartile interval]. Statistical significance was assessed using chi-squared, Wilcoxon, Kruskal–Wallis, or Mann–Whitney *U* tests as appropriate. Correlations were assessed using Spearman coefficient. A multivariable linear regression model was constructed with $\log_2$-transformed TBR_{max} as the independent variable and age, sex, time after myocardial infarction, presence of culprit lesion and presence of ST elevation myocardial infarction as the independent variables. Model residuals were checked against fitted values and distributions confirmed with quantile-quantile plots. Two-sided p-values <0.05 were considered statistically significant. Analysis was performed using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

^{18}F-GP1 Binding to Human Thrombus

^{18}F-GP1 uptake occurred in the presence of freshly forming human thrombus in the *ex vivo* model of deep arterial injury (Figure 1).(23) This uptake was inhibited by co-administration of tirofiban, a glycoprotein IIb/IIIa receptor antagonist. In thrombectomy specimens obtained from patients undergoing percutaneous coronary intervention for acute myocardial infarction, ^{18}F-GP1 autoradiography demonstrated uptake that co-localized to thrombus on histology and platelets on CD41 immunohistochemistry (Figure 1).

Study Populations

Fifty-nine patients with recent myocardial infarction (time from myocardial infarction onset to scan: 9 [5 to 21] days) were recruited. Eight patients either withdrew or were unable to attend due to government public health restrictions, and two patients were excluded because they were subsequently diagnosed with takotsubo syndrome (Supplementary Figure i). Forty-nine patients (mean age 61±9 years and 75% male) underwent ^{18}F-GP1 PET-CT and were included in the analysis (Table 1). There were 35 patients with ST-segment elevation myocardial infarction and 14 with non-ST-segment elevation myocardial infarction. Of the latter, 10 had a culprit lesion identified angiographically by the clinical team. All patients presenting with ST-segment elevation myocardial infarction received primary percutaneous coronary intervention within 4 hours of symptom onset and all patients with non-ST-segment elevation myocardial infarction underwent invasive coronary angiography within 48 hours of symptom onset. Ten patients were managed with guideline-directed medical therapy only and thirty-nine patients underwent percutaneous coronary intervention. In the control group, we included 50 patients

without recent myocardial infarction (Table 1) who had undergone ^{18}F-GP1 PET-CT as described previously.(25)

Invasive Coronary Angiography

Most patients with myocardial infarction (28/49, 57%; all presenting with ST-segment elevation) had complete vessel occlusion (TIMI 0 flow) at the time of invasive coronary angiography with the remainder having TIMI 2 or 3 flow. Sixteen patients received an intravenous tirofiban infusion for 12 hours after the index event. In the absence of percutaneous coronary intervention, intracoronary thrombus was visible as a filling defect in four cases (8%) which was visible on subsequent computed tomography angiography performed at 5 and 7 days (Supplementary Figure ii).

Qualitative Coronary ^{18}F-GP1 Uptake in the Culprit Plaque

Most patients with myocardial infarction (39/49, 80%) had focal coronary ^{18}F-GP1 uptake corresponding to the culprit vessel (Table 2). There was no qualitative coronary ^{18}F-GP1 uptake in the non-culprit vessels of patients with myocardial infarction or in any vessel of patients without myocardial infarction. Coronary ^{18}F-GP1 uptake was present in 30 (86%) patients with ST-segment elevation myocardial infarction, all of which co-localized to the culprit lesion (Figure 2). Coronary ^{18}F-GP1 uptake was present in 9 out of 14 (64%) patients with non-ST-segment elevation myocardial infarction, and this co-localized to the culprit lesion in each case (Supplementary Figure iii). Detection of intracoronary thrombus formation appeared to be less frequent in patients with non-ST-segment elevation myocardial infarction compared to those with ST-segment elevation myocardial infarction, but this was not statistically significant (64% vs 86% respectively, p=0.197).

Overall, the identification of intracoronary thrombus in the culprit artery with ^{18}F-GP1 uptake had a sensitivity of 80%, specificity of 100%, positive predictive value of 100%, negative predictive value 92% and accuracy of 96% in this population.

Patients with myocardial infarction and demonstrable coronary ^{18}F-GP1 uptake were scanned earlier (8 [6-9] versus 13 [10 to 22] days from myocardial infarction to scan, p=0.001; Table 2) and showed higher peak cardiac troponin concentrations (25,225 [4,111 to 50,000] versus 6,239 [868 to 11,000] ng/L, p=0.036; Table 2) than patients with no focal coronary uptake (10 out of 49; 20%). Most cases without focal uptake (n=6) involved culprit lesions located in smaller distal vessels (Table 2). There were no other differences between cases with or without coronary ^{18}F-GP1 uptake including coronary revascularization rates, TIMI flow or use of tirofiban.

Quantitative Coronary ^{18}F-GP1 Uptake in the Culprit Vessel

Per-vessel coronary ^{18}F-GP1 uptake (SUV_{max} and TBR_{max}) was higher in culprit vessels compared to non-culprit vessels as well as all coronary arteries in control subjects without myocardial infarction (Figure 3 and Supplementary Table i). Linear regression models demonstrated univariable associations between coronary ^{18}F-GP1 TBR_{max} and the timing of the scan, as well as culprit vessel status. On multivariable analysis, only culprit vessel status was associated with TBR_{max} (r^2=0.246, p=0.006; Table 3). Based on the Youden's index, TBR_{max} >1.16 was the optimal threshold for identifying the culprit artery with high sensitivity (83%), specificity (86%) and accuracy (84%).

Sub-types of Myocardial Infarction

One patient had an apparent spontaneous coronary artery dissection involving the distal left anterior descending artery and demonstrated focal ^{18}F-GP1 uptake at the site of the dissection (Supplementary Figure iv). Another patient presented in sinus rhythm with an infero-posterior ST-segment elevation myocardial infarction. They had diffuse coronary atherosclerosis and a heavy thrombus burden in the culprit artery on invasive coronary angiography and underwent primary percutaneous coronary intervention to the left circumflex artery. The patient had one brief episode of paroxysmal atrial fibrillation on day 2. Focal ^{18}F-GP1 uptake was seem both in the left circumflex artery and the left atrial appendage suggesting a thromboembolic event and reclassification from type 1 to a type 2 myocardial infarction (Supplementary Figure v).(21)

Identification of the Culprit Lesion

Coronary ^{18}F-GP1 uptake identified the culprit lesion (25/27; 93%) in patients with multivessel disease (Figure 4). In a patient with triple vessel coronary artery disease and bypass grafting, ^{18}F-GP1 uptake only occurred in the culprit lesion of a degenerated saphenous vein bypass graft and no uptake was seen in the other severely diseased native vessels, bypass grafts or indeed the prior stented segment of the bypass graft upstream of the culprit lesion (Supplementary Figure vi). In all five patients with triple vessel disease undergoing multivessel percutaneous coronary intervention, coronary ^{18}F-GP1 uptake was only seen within the stented segment of the culprit lesion but not the stented segments of non-culprit lesions. Bystander disease was treated at the index procedure, but in each of these cases, the non-culprit stented vessel demonstrated no ^{18}F-GP1 uptake (Supplementary Figure vii).

Intraventricular and myocardial ^{18}F-GP1 uptake

Seventeen (35%) patients demonstrated focal intramyocardial ^{18}F-GP1 uptake (TBR_{max} 2.2 [1.45-3.6]) at the site of myocardial infarction suggestive of microvascular obstruction or intramyocardial haemorrhage: 7 with anterior wall or apical uptake, 5 with inferior wall uptake and 5 with lateral wall uptake (Figure 5). Infarct-related intramyocardial ^{18}F-GP1 uptake was similar for patients presenting with ST-segment elevation (34%) or non-ST segment elevation (36%) myocardial infarction. Intraventricular ^{18}F-GP1 uptake was seen in four cases indicative of left ventricular thrombus, with only 2 cases having been detected on echocardiography (Figure 5).

Discussion

This is the first demonstration that non-invasive imaging can identify *in vivo* intracoronary thrombus in patients with acute myocardial infarction. We have confirmed the high selectivity and specificity of ^{18}F-GP1 binding to activated platelets within fresh human thrombus and coronary thrombectomy specimens. We have applied this tracer to populations of patients with and without acute myocardial infarction and have observed ^{18}F-GP1 uptake only occurs within the culprit coronary arteries of those with acute myocardial infarction. We also demonstrate a high frequency of extra-coronary thrombosis including unrecognized left ventricular and atrial thrombus as well as infarct-related intramyocardial ^{18}F-GP1 uptake. This technique appears to hold major promise as a method of determining the role and origin of thrombosis in acute myocardial infarction, especially in cases of thromboembolism or myocardial infarction with non-obstructive coronary arteries. It could also assist in cases of multivessel disease where the localization of the culprit lesion or presence of thrombus may influence patient management.

^{18}F-GP1 is a radiolabelled analogue of elarofiban, a glycoprotein IIb/IIIa receptor antagonist. Unlike other drugs in this class, elarofiban only binds to platelets with activated glycoprotein IIb/IIIa receptors, rendering it a very specific marker of fresh platelet-containing thrombus. Indeed, we have previously demonstrated that ^{18}F-GP1 binding is markedly increased when platelets are activated.(25) In the current study, we have further assessed this tracer for the coronary circulation. First, we used an *ex vivo* model of deep arterial injury analogous to coronary plaque rupture.(23,26,27) We demonstrated selective binding of ^{18}F-GP1 to freshly forming human thrombus which could be inhibited by the glycoprotein IIb/IIIa receptor antagonist, tirofiban. We then undertook autoradiography and histology of human coronary

thrombi extracted at the time of acute myocardial infarction and confirmed that ^{18}F-GP1 does indeed bind to activated platelets within human coronary thrombus.

Qualitative assessments of ^{18}F-GP1 uptake in patients with acute myocardial infarction demonstrated the accurate localization of thrombus formation within the coronary arteries. Neither the non-culprit arteries of patients with myocardial infarction nor any coronary arteries in patients without myocardial infarction demonstrated coronary ^{18}F-GP1 uptake. However, some patients with acute myocardial infarction did not have demonstrable ^{18}F-GP1 uptake in the culprit coronary artery. This imperfect sensitivity appeared to relate to three main factors: delays in performing the scans, thrombosis of small calibre coronary arteries and overspill from hepatic ^{18}F-GP1 uptake. All false negative cases were scanned more than 8 days after myocardial infarction which perhaps reflects the prompt resolution of intracoronary thrombus with pharmacotherapy. Indeed, in the Clopidogrel as Adjunctive ReperfusIon TherapY (CLARITY) TIMI 28 study, dual antiplatelet therapy was associated with resolution of angiographic evidence of intracoronary thrombus in over a half of patients by a median of 8 days.(28) Previous cases series suggest that up to 92% of patients with ST-segment elevation myocardial infarction have evidence of intracoronary thrombus with 16% presenting with a large thrombus burden.(29,30) In these series, a large thrombus burden was linked to larger vessels (>2 mm in diameter) and led to worse outcomes including distal embolization and microvascular obstruction with no reflow. We found evidence of a large thrombus burden in a similar proportion of patients with ST-segment elevation myocardial infarction on invasive angiography. For those without obvious thrombus on invasive or CT coronary angiography, intense coronary ^{18}F-GP1 uptake was seen in majority of cases. Cases with no obvious coronary ^{18}F-GP1 uptake involved culprit lesions in smaller distal vessels (<2 mm) leading to more modest elevations in cardiac troponin indicative of a lower thrombus burden.(31,32)

^{18}F-GP1 offers thrombus detection beyond the resolution of CT. Even though most culprit arteries were stented, there was demonstrable ^{18}F-GP1 uptake in most of these segments, consistent with a degree of abluminal thrombus trapped behind the stent which is not visible on invasive or computed tomography angiography. This is supported by cases where severe non-culprit disease was concurrently stented during the index procedure, but only the stented culprit segment demonstrated ^{18}F-GP1 uptake. The fact that later scans show no thrombus may be a testament to the efficacy of contemporary antiplatelet regimes. Although there are no prospective studies investigating the time course of intracoronary thrombus resolution after myocardial infarction, case reports of serial coronary angiography confirm that the thrombus burden resolves over a few days (2 to 7 days) with administration of modern antiplatelet therapies including glycoprotein IIb/IIIa receptor antagonists.(33,34) In our study, imaging was performed between 5 and 31 days post myocardial infarction and delays in imaging beyond one week from the index event appears to have reduced the sensitivity of GP1 to identify intracoronary thrombus. Therefore, for diagnostic purposes, it should be performed within the first 7-10 days of acute myocardial infarction.

Infarct-related intramyocardial ^{18}F-GP1 uptake was present in more than a third of cases, and we believe that this likely reflects intramyocardial hemorrhage or thrombotic microvascular obstruction. Intramyocardial hemorrhage occurs because of loss of vascular integrity following infarction and activated platelets will play a role in achieving hemostasis and limiting extravasation of blood. In contrast, microvascular obstruction predominantly occurs due to distal embolization of thrombus with occlusion of the microvasculature leading to no reflow phenomenon and potential expansion of the infarct zone. If confirmed, uptake of ^{18}F-GP1 within regions of microvascular obstruction would reinforce the role of thromboembolism in

the pathogenesis of this condition and provide a biomarker to test the efficacy of potential therapeutic interventions.

There were notable examples of extra-coronary thrombus identified by ^{18}F-GP1 PET-CT that included undiagnosed left ventricular thrombus and pulmonary thromboembolism requiring treatment with oral anticoagulant therapy. Again, this may well have clinical utility in cases where the presence of thrombus is uncertain or suspected. We are currently exploring the incidence and the natural history of left ventricular thrombus as well assessing the treatment efficacy of oral anticoagulant therapy in patients with large myocardial infarctions (NCT04829825).

It is important to consider the clinical utility and potential application of ^{18}F-GP1 PET-CT. In our modest-sized case series, we had examples where ^{18}F-GP1 PET-CT changed both the diagnosis (type 1 reclassified as type 2 myocardial infarction) and the treatment (initiation of anticoagulation) of patients presenting with myocardial infarction. In patients presenting with myocardial infarction and non-obstructive coronary artery disease, ^{18}F-GP1 could potentially identify those with plaque rupture and intracoronary thrombus who would truly benefit from dual antiplatelet therapy. Similarly, thromboembolic causes of type 2 myocardial infarction can be distinguished from those with *in situ* thrombosis typical of type 1 myocardial infarction. We therefore believe that ^{18}F-GP1 PET-CT will have a potential role for patients where the diagnosis is uncertain or where the role or origin of thrombus is in question. However, this needs further prospective evaluation in future studies.

Limitations

We should acknowledge some further limitations of our work. Although these data represent the first description of the application of coronary ^{18}F-GP1 PET-CT, the sample size is modest and further confirmatory studies are required in larger populations. Coronary arteries are small structures, and given the resolution of PET, partial volume effects as well as issues relating to sensitivity in smaller distal or tributary vessels must be considered. The latter may well be overcome by more modern PET-CT scanners which continue to evolve with great detector sensitivity combined with wider field of view scanners that can increase the sensitivity by up to 40-fold.(35,36) We also found that ^{18}F-GP1 liver uptake can preclude analysis of uptake in the distal right coronary artery due to signal overspill. This will be difficult to overcome due to the biliary excretion of ^{18}F-GP1. Although we believe that infarct-related intramyocardial uptake is likely to represent intramyocardial haemorrhage or thrombotic microvascular obstruction, this is currently speculative and further investigation of this interesting observation is warranted and is the subject of ongoing investigation (NCT04829825). Finally, we acknowledge that there may have been some inherent bias in the qualitative assessment of ^{18}F-GP1 uptake given the presence of intracoronary stenting will be readily apparent on review of the PET-CT scans. However, the culprit vessel was correctly identified in those without stent implantation and those with multivessel stenting, and our qualitative findings were consistent with the quantitative analysis which is more objective and independent of observer bias.

In conclusion, coronary ^{18}F-GP1 PET-CT can detect *in vivo* intracoronary thrombus in patients with acute myocardial infarction. This has the potential to help in the diagnosis, management and treatment of patients presenting with suspected or confirmed acute myocardial infarction.

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Disclosures

NK and AS are employees of Life Molecular Imaging, who provided reagents for radiotracer production. MCW received speaker bureau for Canon Medical Systems. The remaining authors have nothing to disclose

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Perspectives

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: Coronary ^{18}F-GP1 PET-CT angiography is the first non-invasive selective technique to identify *in vivo* coronary thrombosis in patients with acute myocardial infarction and can potentially inform the diagnosis, management, and treatment of patients with acute myocardial infarction.

TRANSLATIONAL OUTLOOK: Additional prospective trials are necessary to assess the utility of 18F-GP1 in recognising and treating culprit lesions in patients with non-ST segment elevation myocardial infarction and multivessel disease.

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Figure Legends

Figure 1. **^{18}F-GP-1 binding to human ex vivo thrombus.**

(Top) Micro-positron emission tomography and computed tomography of freshly generated thrombus demonstrating intense uptake of ^{18}F-GP-1 (yellow to red) which is blocked by co-administration of the glycoprotein IIb/IIIa receptor antagonist, tirofiban. Hematoxylin and eosin staining demonstrated thrombus formation on the surface of the porcine aorta which stains heavily for platelets on CD41 immunohistochemistry.

(Bottom) Autoradiography of two coronary thrombectomy specimens showing ^{18}F-GP-1 uptake which is also blocked by 10 μM of unlabelled GP1 and co-localizes with thrombus and activated platelets. Hematoxylin and eosin staining demonstrated thrombus formation which stains heavily for platelets on CD41 immunohistochemistry.

Figure 2. Three exemplar cases of ST segment elevation myocardial infarction.

Anterior (A), lateral (B) and inferior (C) myocardial infarction and corresponding ^{18}F-GP1 uptake from the corresponding culprit artery. Right to left: invasive coronary angiogram, CT angiogram and ^{18}F-GP1 positron emission tomography and CT angiogram. ^{18}F-GP1 uptake noted only in the infarct related artery.

Figure 3. **^{18}F-GP1 uptake in culprit versus non-culprit versus stable vessels.**

18F-GP1 uptake measured as maximum standardized uptake values and maximum tissue to background ratios. (p value was calculated using pairwise Wilcox test)

Figure 4. ^{18}F-GP1 uptake in a patient with triple vessel disease.

Coronary ^{18}F-GP1 uptake was present only in the culprit vessel (right coronary artery; RCA) and there was no uptake in the bystander disease lesions (left anterior descending (LAD) and left circumflex (LCx) coronary arteries).

Figure 5. Left ventricular ^{18}F-GP1 uptake.

(A) Examples of patients with intramyocardial ^{18}F-GP1 uptake on positron emission tomography and CT angiography consistent with microvascular obstruction or intramyocardial haemorrhage.

(B) Examples of patients with apical left ventricular ^{18}F-GP1 uptake on positron emission tomography and CT angiography consistent with intraventricular thrombus following myocardial infarction.

Central Illustration. ^{18}F-GP1 PET-CT for non-invasive coronary artery thrombus imaging.

A) Patient presenting with inferolateral ST-segment elevation myocardial infarction. Invasive coronary angiography showing an occluded left circumflex artery. Patient was treated with primary percutaneous coronary intervention to the left circumflex artery. Nine days later he returned for 18F-GP1 positron emission tomography and computed tomography angiography which showed focal ^{18}F-GP1 uptake was both in the left circumflex artery and the left atrial appendage suggesting a thromboembolic event and reclassification from type 1 to a type 2 myocardial infarction

B) Patient presenting with inferior non-ST-segment elevation myocardial infarction. Invasive coronary angiography showing tandem lesions of the right coronary artery. Patient was treated with percutaneous coronary intervention to the right coronary artery.

Ten days later he returned for 18F-GP1 positron emission tomography and computed tomography angiography which showed focal ^{18}F-GP1 uptake in the right coronary artery. Moreover, there was significant radiotracer uptake noted in the inferolateral wall suggestive of microvascular obstruction.

C) Patient presenting with anterior positron emission tomography and computed tomography angiography. Invasive coronary angiography showing an occluded left anterior descending coronary artery and was treated with primary percutaneous coronary intervention. Twelve days later he returned for 18F-GP1 positron emission tomography and computed tomography angiography which showed focal ^{18}F-GP1 uptake in the left anterior descending coronary artery and left ventricular apex. The left ventricular thrombus had not been identified on echocardiography.

Tables

Table 1. Baseline characteristics of study populations			
	Patients with myocardial infarction (n=49)	**Patients without myocardial infarction (n=50)**	**P value**
Age (years)	61 [52 to 69]	73 [68 to 76]	**<0.001**
Male sex	38 (75%)	38 (76%)	0.456
Body-mass index (kg/m^2)	28 [23 to 32]	29 [24 to 33]	0.654
Past medical history			
Hypertension	13 (25%)	35 (70%)	**<0.001**
Hypercholesterolemia	11 (22%)	37 (74%)	**<0.001**
Diabetes mellitus	8 (16%)	6 (12%)	0.235
Current or ex-smoker	27 (53%)	28 (56%)	0.256
Prior coronary artery disease	7 (14%)	23 (46%)	**<0.001**
Prior myocardial infarction	7 (14%)	6 (12%)	0.345
Stroke/transient ischemic attack	3 (6%)	7 (14%)	**0.02**
Prior procedures			
Coronary artery bypass grafting	1 (2%)	7 (14%)	**<0.001**
Percutaneous coronary intervention	8 (16%)	10 (20%)	0.880
Permanent pacemaker	3 (6%)	3 (7%)	0.870
Antiplatelet medication			
Aspirin	46 (90%)	39 (78%)	**<0.001**
P2Y12 receptor antagonist	43 (84%)	6 (12%)	**<0.001**

n (%); median [interquartile range]

Table 2. Baseline characteristics of patients with and without coronary ^{18}F-GP1 uptake			
	Coronary ^{18}F-GP1 Uptake (n=39)	**No Coronary ^{18}F-GP1 Uptake (n=10)**	**P value**
Age (years)	62 [56 to 71]	60 [52 to 72]	0.607
Male sex	31 (80%)	9 (90%)	0.456
Body-mass index (kg/m^2)	27 [22 to 31]	28 [22 to 32]	0.857
Past medical history			
Hypertension	10 (26%)	3 (30%)	0.975
Hypercholesterolemia	9 (23%)	2 (20%)	0.878
Diabetes mellitus	6 (15%)	2 (20%)	0.335
Current or ex-smoker	22 (53%)	5 (50%)	0.766
Prior coronary artery disease	5 (13%)	2 (20%)	0.345
Prior myocardial infarction	5 (13%)	2 (20%)	0.345
Stroke/transient ischemic attack	3 (8%)	0	0.675
Prior procedures			
Coronary artery bypass grafting	1 (3%)	0	0.985
Percutaneous coronary intervention	6 (15%)	2 (20%)	0.771
Permanent pacemaker	2 (5%)	1 (10%)	0.970
Antiplatelet medication			
Aspirin	37 (95%)	9 (90%)	0.870
P2Y12 receptor antagonist	35 (90%)	8 (80%)	0.770
Culprit vessel			
Left anterior descending	13 (33%)	4 (40%)	0.345

Right coronary artery	7 (18%)	0	**0.035**
Left circumflex artery	18 (46%)	6 (60%)	0.155
Saphenous vein graft	1 (3%)	0	1.000
ST-elevation myocardial infarction	30 (86%)	5 (50%)	0.079
Culprit location			
Proximal vessel	37 (95%)	4 (40%)	**<0.001**
Distal vessel	2 (5%)	6 (60%)	**<0.001**
Culprit artery stenting	30 (82%)	9 (90%)	0.497
Thrombolysis In Myocardial Infarction 0	23 (59%)	5 (50%)	0.479
Tirofiban infusion	18 (46%)	2 (20%)	0.065
Troponin (ng/L)	25,225 [4,111 to 50,000]	6,239 [868 to 11,000]	**0.036**
Time from myocardial infarction to scan (days)	8 [6-9]	13 [10-22]	**0.001**
n (%); median [interquartile range]; mean± standard deviation			

Table 3. Linear regression models for coronary maximum ^{18}F-GP1 target-to-background ratio					
	Univariable		**Multivariable**		
	Coefficient (95% Confidence Interval)	**p value**	**Coefficient (95% Confidence Interval)**	**p value**	**R^2**
Age (per year)	0.004 (-0.006 to 0.016)	0.418	-0.001 (-0.011 to 0.009)	0.828	0.246 **p= 0.006**
Male sex	0.249 (-0.212 to 0.377)	0.577	0.025 (-0.253 to 0.302)	0.857	
ST-elevation myocardial infarction	0.141 (-0.074 to 0.357)	0.194	0.151 (-0.095 to 0.397)	0.222	
Tirofiban infusion	0.194 (-0.021 to 0.409)	0.075	0.205 (-0.010 to 0.419)	0.155	
Time from myocardial infarction to scan (per day)	-0.018 (-0.033 to -0.003)	**0.018**	-0.006 (-0.022 to 0.009)	0.447	
Culprit location (Proximal)	-0.072 (-0.342 to 0.198)	0.548	-0.063 (-0.324 to 0.198)	0.627	
Culprit vessel	0.448 (0.238 to 0.658)	**<0.001**	0.430 (0.180 to 0.681)	**0.001**	

Model for $\log_2$ coronary maximum ^{18}F-GP1 target-to-background ratio