Imaging cellular activity and proliferation in the aortic wall

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BACKGROUND

Abdominal aortic aneurysms are characterised by abnormal dilation of the infrarenal aorta. The disease process is silent and consequently diagnosed incidentally or as part of a screening programme. As aortic size increases so does the risk of aortic rupture.[1] This is catastrophic. Despite modern resuscitation techniques and emergency surgery, the mortality from ruptured abdominal aortic aneurysms exceeds 80%.[2] Predicting aortic rupture or dissection is a significant challenge. While aortic size is typically used to estimate the risk of these catastrophic events, many individuals experience aortic complications below the conventional thresholds used to trigger surgery. On the other hand, such prophylactic surgery is invasive and carries a burden of morbidity. There is therefore a pressing need to stratify the risk of aortic rupture better, with markers of disease activity demonstrating early promise in this regard.

The aorta is biologically active. Environmental exposure to vascular irritants, such as cigarette smoking and hypertension, exacerbate an underlying genetic predisposition to developing aneurysmal disease. Study of the cellular aneurysmal architecture reveals diffuse medial atrophy and extracellular matrix degeneration, with upregulation of matrix metalloproteinases.[3] This is accompanied by inflammatory infiltration of the aortic wall by helper-T cells and macrophages. Cytokines released within the aortic media cause vascular smooth muscle cells to undergo phenotypical change,[4] losing their ability to replenish the extracellular matrix and instead exhibiting a pro-calcific phenotype.[5] Vascular injury therefore results in a self-propagating microenvironment where calcium and phosphate precipitate to form hydroxyapatite crystals and foci of micro- and then macro-scopic calcium deposits.[4]

Advances in biological radiotracer development and positron emission tomography (PET) potentially allow us to study these pathological processes in the aortic wall (Table 1). The most widely used cardiovascular PET radiotracer is ^{18}F-Fluorodeoxyglucose (^{18}F-FDG), although longitudinal clinical studies have failed to consistently demonstrate the predictive value of this tracer in abdominal aortic aneurysm disease.[6–8] Inflammation within aortic aneurysms may be insufficient for a generalised radiotracer such as ^{18}F-FDG to detect reproducibly.^{18}F-Sodium fluoride detects developing microcalcification and serves as a marker of vascular injury and disease activity across a range of cardiovascular conditions.[9–13] In a recent small prospective study patients with increased AAA ^{18}F-fluoride activity demonstrated faster aneurysm growth, and an increased probability of requiring aortic repair or encountering aortic rupture, independent of aortic size.[8] This has stimulated further interest in the development of novel molecular imaging techniques to assess AAA disease activity and to improve clinical assessment and outcomes.

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Table 1. Emerging radiotracers, their targets and stage of research development

Targeting agent	Target	Imaging modality	Stage of development	
			Abdominal aortic aneurysms	Oncology
^{18}F-Fluorodeoxyglucose (^{18}F-FDG)	Glycolysis	PET	In clinical use	In clinical use
^{18}F-Sodium fluoride (^{18}F-NaF)	Microcalcification	PET	Clinical research	In clinical use
^{18}F-Fluorothymidine (^{18}F-FLT)	Cellular proliferation	PET	Pre-clinical research	Clinical research
Ultrasmall paramagnetic particles of iron oxide (USPIO)	Macrophages	MRI	Clinical research	Clinical research
^{18}F-DOTATATE	Macrophages	PET	Clinical research	Clinical research
Vascular cell adhesion molecule-1 (VCAM-1)	Macrophages	PET	Pre-clinical research	Pre-clinical research
^{11}C-Choline	Macrophages	PET	Pre-clinical research	In clinical use
^{11}C-PK11195	Macrophages	PET	Pre-clinical research	Clinical research
^{18}F-Fluoromisonidazole (^{18}F-MISO)	Hypoxia	PET	None	Clinical research
^{18}F-Galacto-RGD	Angiogenesis	PET	Pre-clinical research	Clinical research
$\alpha_v\beta_3$ targeted paramagnetic particles	Angiogenesis	MRI	Pre-clinical research	Clinical research

PET, Positron emission tomography; *MRI* Magnetic resonance imaging

WHAT THIS STUDY ADDS

In this issue of the journal Gandhi et al. investigate ^{18}F-fluorothymidine (^{18}F-FLT) PET/CT in abdominal aortic aneurysms, providing an important step forward in characterising cellular activity throughout the aneurysm lifecycle.[14] ^{18}F-Fluorothymidine becomes trapped within cells that are in the S-phase of their cell cycle.[15] This phase is typically characterised by DNA replication prior to cell division. ^{18}F-FLT is retained within the cell following phosphorylation by thymidine-kinase-1 (TK-1), but is not incorporated within the DNA. This makes ^{18}F-FLT an ideal radiotracer to detect cellular proliferation – a property already exploited in oncology.[16]

Gandhi et al. demonstrate the application of ^{18}F-FLT PET/CT in a pre-clinical apolipoprotein-E knock-out ($ApoE^{-/-}$) mouse-model and the first study to comprehensively characterise ^{18}F-FLT uptake in abdominal aortic aneurysm disease. An angiotensin-II (AngII) infusion was used to induce abdominal aortic aneurysms, while control mice received saline infusions at the same rate. The authors studied ^{18}F-FLT PET/CT in the aneurysms at 14- and 28-days following commencement of the infusion in both groups. Histological validation was also performed, with Ki67 antibody staining for foci of cellular proliferation, and western blotting techniques to quantify concentrations of TK-1 and protein markers of cellular proliferation: equilibrative nucleoside transporter (ENT) -1 and -2, and concentrative nucleoside transporter (CNT) -1 and -3.

The authors demonstrate that the ^{18}F-FLT PET signal is increased in mice that underwent AngII infusion compared to healthy saline-infused controls. This was true at both, 14- and 28-day time points (SUV_{max} control 0.007 ± 0.002 vs. 14-day 0.31 ± 0.03 vs. 28-day 0.20 ± 0.05, $p < 0.001$). Within groups, Gandhi et al. found that the ^{18}F-FLT signal intensity peaks early in the disease cycle at 14-days ($p < 0.001$) and co-localised to hypertrophied segments of the diseased aortic wall.

Histological interrogation of diseased aortic tissue confirmed that the AngII infusion group of mice had both, increased aortic diameter and increased proportion

of Ki67-stained nuclei. Aortic diameter and the proportion of Ki67-stained nuclei were strongly correlated ($r = 0.81$). Both time points demonstrated increased TK-1 expression compared to healthy controls. The pattern of TK-1 ENT-1 and -2, and CNT-1 expression mirrored PET findings: expression being highest at 14-days followed by a fall at 28-days ($p < 0.001$ for all).

STRENGTHS AND LIMITATIONS OF STUDY

This is the first study to confirm that cellular proliferation is indeed elevated within the aorta of AngII-infused ApoE$^{-/-}$ mice and that ^{18}F-FLT can non-invasively quantify this process. Moreover, the timing of ^{18}F-FLT uptake closely mirrored histological and proteomic analysis of aneurysmal tissue, with cellular proliferation appearing to be higher in the early stages of aneurysm development before later tailing off. This study has therefore provided novel insight into the cellular mechanisms observed in maturing aneurysmal tissue, and demonstrated ^{18}F-FLT PET as a useful technique to detect these cellular changes.

The Angiotensin-II infusion in ApoE$^{-/-}$ mouse provides a model of abdominal aortic aneurysm disease exhibiting the major pathological components of aneurysm development, including medial degeneration, inflammation, thrombus formation and atherosclerotic disease.[17] This model, however, causes widespread aneurysm formation that often affects the supra-renal aorta and aortic arch.[18] Abdominal aortic aneurysm disease in humans overwhelmingly favours an infrarenal morphology, suggesting differences in the mechanisms underlying disease development in the model versus clinical disease. Moreover a direct comparison of in vivo ^{18}F-FLT PET activity with ex vivo Ki67-staining and protein expression was lacking in this study, which would have further strengthened the association between the non-invasive PET signal and histological markers of cellular proliferation.

NEXT STEPS

The next step is to translate this promising imaging technique into humans. ^{18}F-FLT PET has already been used to investigate cellular proliferation in patients with cancer, facilitating early translation. Such studies are likely to provide important insights into the pathology underlying human aneurysm disease and may also hold value in predicting aneurysm growth and refining patient risk stratification. A further potential application of ^{18}F-FLT PET/CT is in novel drug discovery. As Gandhi et al. point out, blocking aortic cellular proliferation using microRNA-21 and Kruppel-like factor 4 prevents aneurysm formation in the angiotensin-II ApoE$^{-/-}$ AAA mouse model. Pharmaceutical agents that manipulate this pathway are yet to be developed for human use, but ^{18}F-FLT PET/CT might potentially provide an imaging technique to assess the efficacy of future therapies on this pathway.

CONCLUSION

Gandhi et al. have characterised, for the first time, ^{18}F-FLT PET/CT in abdominal aortic aneurysms of ApoE-/- knockout mice exposed to Angiotensin-II. They have demonstrated increased radiotracer uptake in aneurysms compared to control tissue and an association between histological markers of cellular proliferation and the ^{18}F-FLT signal. This study provides a foundation to build upon our clinical understanding of aneurysm biology, with further research now required.

Disclosure

The authors have indicated that they have no financial conflict of interest.

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