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Imaging Biological Pathways in Abdominal Aortic Aneurysms Using Positron Emission Tomography

Michael Bell MSc[1], Richa Gandhi PhD[1], Heba Shawer (شاور هبة) MSc[1], Charalampos Tsoumpas (Χαράλαμπος Τσούμπας) PhD[1], Marc A Bailey MB ChB PhD[1*]

[1] Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Clarendon Way, Leeds, LS2 9NL, United Kingdom

*Corresponding author: Dr Marc A Bailey

7.26 LIGHT Laboratories, Clarendon Way, Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Leeds, LS2 9NL, United Kingdom

Tel: +44 (0) 113 343 1050

Email: M.A.Bailey@leeds.ac.uk

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Abstract

Abdominal aortic aneurysm (AAA) is a focal dilation of the aorta associated with high mortality through rupture. Most of our understanding of the biology that drives AAA progression originates from surgical samples acquired in cases of elective open repair. These markers, which include macrophage infiltration and angiogenesis have led to the exploration of novel radiopharmaceuticals to study AAA in preclinical models and human patients. Current clinical practice to detect AAA involves ultrasound based screening and surveillance. Although ultrasound is cheap and without radiation risk, aortic diameter does not predict the heterogenous growth of AAA between patients. Positron Emission Tomography (PET) takes advantage of novel radiolabelled markers of disease to track biological changes. In human trials, the role of 2-[^{18}F]-FDG in detecting aneurysm growth and outcome is still debated, whereas Na[^{18}F]F (microcalcification) has been shown to predict AAA growth and clinical outcome. Murine studies have been used to assess the suitability of radiotracers detecting inflammation, angiogenesis and proliferation. However, in the absence of human data, the clinical suitability and applicability of these tracers remains speculative. This review examines how markers of AAA change over time and the ability of PET to track these changes and discusses the radiopharmaceuticals that could have an application in stratifying AAA subjects.

Abbreviations

2-[^{18}F]-FDG	2-[^{18}F]-Fluorodeoxyglucose
3-[^{18}F]-FLT	3-[^{18}F]-Fluorothymidine
AAA	Abdominal Aortic Aneurysm
AngII	Angiotensin II
AOD	Occlusive Arterial Disease
ApoE $^{-/-}$	Apolipoprotein E deficient background
ARIC	Atherosclerosis Risk in Communities
BMP	Bone Morphogenic Protein
CCR2	C-C Chemokine Receptor 2
CD31	Cluster of Differentiation 31
CNT	Concentrative nucleoside transporter
CT	Computed Tomography
ENT	Equilibrative nucleoside transporter
EVAR	Endovascular Aneurysm Repair
FAP	Fibroblast Activation Protein
GLUT4	Glucose transporter type 4
ILT	Intraluminal thrombus
KLF4	Kruppel-like factor 4
mRNA	Messenger ribonucleic acid
MASS	Multicentre aneurysm screening study
MCP-1	Monocyte chemoattractant protein 1
MI	Myocardial Infarction
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stem cell
NAAASP	NHS AAA screening program
Na[^{18}F]-F	Sodium Fluoride
NHS	National Health Service
OSR	Open Surgical Repair
OPG	Osteoprotegerin
PAH	Peripheral arterial hypertension
PCR	Polymerase Chain Reaction

PET	Positron Emission Tomography
PTA	Percutaneous transluminal angioplasty
RunX2	Runt related transcription factor 2
SUV	Standard Uptake Value
TGF-B1	Transforming growth factor beta 1
TK-1	Thymidine Kinase 1
USS	Ultrasound scan
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cell

Introduction

An abdominal aortic aneurysm (AAA) is an important vascular disorder associated with high mortality despite a relatively low incidence rate. Analysis of the first five years of the National Health Service (NHS) AAA Screening Programme (NAAASP) in the UK showed the prevalence of AAA in 65 year old men was 1.34%.[1] By definition, an AAA is a focal dilation of the abdominal aorta greater than 3 cm in maximal diameter. The risk of rupture increases as the diameter enlarges. At a diameter above 5.5 cm, the risk of rupture is typically quoted to be around 2% per year.[2, 3] Analysis of data acquired by NAAASP between 2009 and 2017 however suggests the risk of rupture is only 0.4% per year.[4] In the UK, AAA rupture accounts for about 4,000 deaths per annum.[5] Male gender, hypertension, smoking, age and a family history are the most important clinical risk factors with clear evidence of diabetes being protective against aneurysm formation.[3, 6, 7] Aortic aneurysms can be caused by single gene defects such as *FBN-1* in Marfan's Syndrome or large vessel vasculitis such as Takayasu's Arteritis.[8, 9] There is also a complex polygenic predisposition for degenerative aneurysms such as AAA that only now are being unveiled through Genome Wide Association Studies.[10] Once the intervention threshold of 5.5 cm is reached, patients are considered for interventional treatment of the aneurysm by endovascular repair (EVAR) or open surgical repair (OSR).[11] Based on data from the MASS trial, patients with a confirmed diagnosis of AAA enter an ultrasound based surveillance programme (annual scans which indicate that the diameter of the aneurysm is larger than 3.0-4.5 cm; or 3 monthly scans showing a diameter larger than 4.5-5.5 cm).[12] Typical growth rates are around 2 mm per year but there is significant heterogeneity among patients and the aneurysm growth is not necessarily linear.[13, 14]

Classical investigations of the mechanisms underpinning aneurysm formation relied on tissue harvested at the time of open surgery, which represents the end stage of aneurysm disease and does not necessarily represent events earlier in AAA development. Consequently, murine models are often used to understand the biological processes involved in aneurysm development and growth alongside human data. Notwithstanding, histological staining of end-stage human disease revealed infiltration of inflammatory cells (macrophages and T-cells), breakdown of elastic lamellae, apoptosis of vascular smooth muscle cells (VSMC) and increased formation of neo-vessels.[15, 16] Murine models have shown influx of macrophages as an early

event in AAA formation, which suggests an immune mediated destruction of the aortic wall.[17, 18] As a result of the breakdown of the aortic wall, extensive remodelling occurs including the formation of intraluminal thrombus (ILT).[19, 20] Although there is evidence of ILT being protective of AAA rupture by reducing wall stress, there are confounding data suggesting the presence of ILT in small aneurysms is associated with rupture at low wall stress.[21] Biomechanical assessment, such as finite element analysis, allows gross estimation of the wall stress as a result of environmental changes in the vessel.[22] Aneurysm formation has also been thought of as a cellular response to atherosclerosis. The remodelling of the native VSMC can release metalloproteinases that break down the extracellular matrix.[23] The atherosclerosis risk in communities (ARIC) study concluded that subjects with atherosclerosis have a 1.31-fold increased risk of clinically presenting an AAA.[24] However, other studies have reported that atherosclerosis is not a requirement for the development of AAA.[25, 26]

Currently, AAA can be detected incidentally in medical images acquired for other purposes via clinical examination or else through a dedicated, ultrasound-based screening programme. A maximal anterior-posterior (AP) diameter of 3 cm on ultrasound scan (USS) is diagnostic for AAA. Once diagnosed with a AAA, patients are enrolled into a surveillance programme (based on the MASS trial, when AAA diameter is between 3-4.5 cm are rescanned annually, when AAA diameter is between 4.5-5.5 cm are rescanned at 3-month intervals; and when the aortic diameter becomes larger than 5.5 cm, subjects are referred for consideration of elective surgical repair).[12, 27] Screening programmes typically implement ultrasound as a diagnostic tool because it is an inexpensive diagnostic procedure, non-ionising, and a high throughput of subjects can be achieved. The RESCAN trial, however, showed that an aneurysm can take up to 7-13 years to reach a clinically relevant endpoint (5.5 cm).[28] Although the authors argue that increasing the follow-up period for subjects with an aneurysm of 3 cm would be safe, this has not been translated into clinical practice and furthermore the aortic size may not be the best predictor of aortic growth and outcome.[28] Therefore, a substantial scan burden is placed on patients and more than that, individual differences in aortic growth patterns and rupture rate are not considered in routine AAA surveillance.[13] This leaves a burden on health services and patients, with no other clinically relevant imaging or serum biomarkers predicting aneurysm growth implemented clinically. An imaging stratification biomarker to accurately predict the

growth and outcome of AAA would be advantageous to personalise and rationalise AAA surveillance strategies and/or intervention thresholds.

Positron Emission Tomography (PET), normally coupled with computed tomography (PET/CT) or less commonly magnetic resonance imaging (PET/MRI) is a very sensitive molecular imaging technique that can assess biological function *in vivo*. A molecular compound radiolabelled with a positron emitting radioactive probe, termed a radiotracer, is injected into a subject (patient or pre-clinical disease model) and has the affinity to accumulate in regions of disease. Each radiotracer is designed for a particular biological process, for example 2-[^{18}F]-Fluorodeoxyglucose (2-[^{18}F]-FDG) is a partial analogue of glucose biochemical pathways and accumulates in regions with high glucose metabolic activity, which are often associated with infiltration of highly metabolically active immune cells. Localisation of the radiotracer uptake is determined through coincidence detection of back to back gamma rays by photomultiplier detectors in the PET gantry.

PET/CT is an attractive option for assessing the growth potential of aneurysms as a supplementary technique to ultrasound, but due to the relatively high procedural cost and the involved radiation dose to a subject is not so widespread. Due to the availability of a wide range of radiotracers, uptake of an appropriate PET probe may be correlated to patient progression and clinical outcome. The same strategy could be used to rationalise ultrasound screening frequency, personalisation of the intervention threshold or subject response to novel medical treatment. Markers of inflammation, calcification, angiogenesis, proliferation and chemokine receptors have been studied previously in AAA (Figure 1). The radiotracers studied and the biological model used are summarised in Table 1. However, only 2-[^{18}F]-FDG (inflammation, metabolic activity) and Na[^{18}F]F(microcalcification) have been evaluated in AAA patients to date with Na[^{18}F]F uptake showing promising results in the prediction of patient endpoint and aneurysm growth.[29] This review looks at the current progress of PET/CT in tracing biological remodelling of AAA and the potential tracers that could longitudinally track aneurysm growth. The future goal would be to implement PET/CT into a clinical setting and complement ultrasound measurements from screening programs to offer tailored pathways for AAA patients also referred to as precision medicine.

Macrophages and Metabolism

2-[^{18}F]-FDG is the most common and widely used radiotracer available to clinical PET/CT centres and is an obvious candidate in assessing the molecular pathway of many diseases. 2-[^{18}F]-FDG is a partial analogue of glucose which is transported into cells through the GLUT4 transporter. Differently to glucose, after phosphorylation by hexokinase, 2-[^{18}F]-FDG remains trapped within the cell. Each 2-[^{18}F]-FDG molecule decays producing two gamma rays that can be detected using PET. To mitigate any dietary effects of glucose, subjects fast for 6 hours before a scan. Cardiac 2-[^{18}F]-FDG imaging is challenging due to the accumulation of radiotracer in healthy myocardium. Employment of a low carbohydrate meal followed by twelve hour fast in subjects can mitigate these effects.[30] The success of implementing 2-[^{18}F]-FDG in glucose imaging is well documented clinically in oncology and neurology with some emerging possible applications in cardiovascular disease.[31-33] For example, 2-[^{18}F]-FDG has been used to detect areas of inflammation in atherosclerosis.[34] In a group of subjects with carotid stenosis, 2-[^{18}F]-FDG uptake correlated with macrophage infiltration suggesting that it can underline inflammation in atherosclerotic plaques. In addition, 2-[^{18}F]-FDG uptake has been shown to be 93% specific and 76% sensitive in detecting large vessel vasculitis.[35] It should be noted that in AAA, 2-[^{18}F]-FDG has been used as a presumed marker of inflammation but is not specific to this purpose and could equally represent high uptake due to metabolic activity in other remodelling cells of the aortic wall such as the VSMCs.[36]

Macrophages are phagocytic cells that can differentiate to perform a variety of functions, including inflammation and the release of metalloproteinases.[37, 38] Inflammatory macrophages downregulate inflammatory mediators and upregulate anti-inflammatory mediators in responses to inflammation. Macrophages are activated by metabolic pathways. Naïve and immunomodulatory macrophages, termed M_0 and M_2, are activated by oxidative phosphorylation metabolic pathways. M_1, or proinflammatory macrophages, are activated by the glycolysis pathway. Macrophages are thought to drive aneurysm formation through an increase in the release of matrix metalloproteinases (MMPs), promoting elastin destruction, and a heightened response to inflammatory markers.[19]

Substantially increased presence of macrophages in human and animal AAA studies have been widely published.[39, 40] However, the origin of the location of macrophages in AAA is debatable. The density of M_2 cells in the aortic wall is higher than M_1.[41] In

contrast, M_1 cells are located in the adventitia whilst the density of cells in the ILT corresponds to M_2 cells. Flow cytometry analysis of the adventitial layer of AAA human tissue demonstrated an upregulation of M_2 markers and M_2 cells compared to control aortic tissue with a decrease in M_1 macrophages.[42] A possible discrepancy between the results could be due to the analysis of tissue originating from different stages of the aneurysm development.

A single study has shown that a PET positive scan for 2-[^{18}F]-FDG uptake in AAA subjects corresponded with increased macrophage infiltration from histological analysis.[43] There is, however, confounding evidence in the literature suggesting that uptake does not necessarily correlate with increased macrophage activity. None to low 2-[^{18}F]-FDG uptake in PET/CT and *ex vivo* autoradiography suggests little macrophage infiltration.[44] In contrast, histological analysis of AAA samples has shown upregulation of macrophage markers and inflammatory markers in none to low 2-[^{18}F]-FDG uptake studies.[45] Similarly, low 2-[^{18}F]-FDG uptake correlated with an increase in macrophage infiltration in another study.[46] It is not clear at what stage of aneurysm progression patients were scanned at, or when a biopsy was taken; however, evidence from the literature shows a possible lack of understanding of the exact biological processes involved with 2-[^{18}F]-FDG uptake in AAA. There is ongoing research in the preferential uptake of 2-[^{18}F]-FDG in M_1 or M_2 macrophages to identify the main differentiated macrophage involved using vascular PET/CT imaging.[47, 48] Confounding evidence in AAA studies could be a result of non-specific uptake of 2-[^{18}F]-FDG into other cell types that are metabolically active in the aortic wall, such as remodelling VSMC, endothelial cells or fibroblasts. The signal could also be due to relative hypoxic stimulation of macrophages, increasing glucose uptake and perturbing the measured signal.[36]

Independent of the lack of understanding of the underlying biological signature associated with the 2-[^{18}F]-FDG signal, there has been some interest in determining its potential in predicting AAA growth. However, the evidence in the ability to accurately detect and track AAA growth with 2-[^{18}F]-FDG is at best contradictory.[43, 49-51] Although one small *in vivo* study showed 2-[^{18}F]-FDG uptake could discriminate between symptomatic and asymptomatic AAA subjects,[52] heterogeneous 2-[^{18}F]-FDG uptake has been seen across multiple studies. Low maximum standardised uptake value (SUV_{max}) at baseline linked to subjects with greatest aneurysm growth over a

nine month period.[53] Both positive and negative correlations between measured 2-[^{18}F]-FDG uptake and measured aneurysm diameter have been reported. Although variable uptake has been reported, uptake of 2-[^{18}F]-FDG has been suggested to correlate with aneurysm wall remodelling and calculated wall stress from finite element simulation of CT data.[43, 51, 54, 55] In contrast, there is evidence that 2-[^{18}F]-FDG could be a useful tool in detecting complications after EVAR and aortic arch graft in Marfan Syndrome.[56-58] The results could be confounded for a variety of reasons such as the stage of AAA evaluated (e.g. asymptomatic, symptomatic, end stage), small number of subjects recruited and only a small percentage of subjects with detectable AAA. In addition, only end stage AAA subjects have been studied and the data may suggest that 2-[^{18}F]-FDG cannot predict growth during this period of disease progression. Based on the evidence from clinical studies, the implication of 2-[^{18}F]-FDG as a stratification biomarker of growth may not be useful but further studies in early stage AAA subjects could be beneficial in verifying the role of 2-[^{18}F]-FDG PET in AAA subjects. Studies moving beyond SUV_{max} might be advantageous in this context.

Calcification

A common histological feature of AAA is calcification of the medial layer. Formation of calcified plaques follows a similar process to plaques formed in atherosclerosis. In atherosclerosis, repeated damage to the vessel wall and influx of macrophages promotes secretion of osteogenic factors.[59] This leads to the formation of microcalcification, also defined as calcium hydroxyapatite, a characteristic feature of active vascular remodelling. The presence of microcalcification leads to arterial stiffening and increases the risk of plaque rupture.[60, 61] This in turn increases the stress on the aortic wall, reducing the stability of aneurysms. Consequently, the presence of macrocalcification indicates the stable form of arterial calcification. Although the long-term effect of calcification on patient prognosis is not fully understood, calcium scoring from stand-alone CT scans of AAA subjects showed a correlation with mortality and could predict future cardiac events.[62] Osteogenic remodelling in AAA might be a product of macrophage infiltration and/or VSMC differentiation driving arterial wall remodelling and healing response.[61]

The studies into the biological process underlying the formation of calcified plaques in AAA are still in their infancy. However, the biological process in atherosclerosis

suggests upregulation of bone morphogenetic proteins (BMP) and activation of the SMAD pathway.[63] BMP, in particular BMP-2 and BMP-4, drive osteogenic differentiation in VSMC. The SMAD pathway controls the release of osteogenic transcription factors (RunX-related transcription factor 2 – RunX2) which in turn release osteogenic proteins, such as osteoprotegrin (OPG), that regulate calcium deposits. Western blot analysis of AAA biopsies demonstrated an upregulation of OPG.[61] In addition, the measured concentration of OPG in AAA tissue appeared to be three times higher when compared to biopsies from occlusive arterial disease (AOD). Polymerase Chain Reaction (PCR) analysis of vascular mesenchymal stem cells (MSC) isolated from AAA tissue showed upregulation of OPG and BMP-2.[60] An increase in the expression of BMP-2 was observed when culturing MSC in osteogenic medium. Alternatively, the expression of OPG was lower in AAA studies compared to controls in a human tissue histology study.[64] Generation of a RunX2 knockout ($RunX2^{-/-}$) murine line with an $ApoE^{-/-}$ background demonstrated a lower prevalence of AAA induction using the AngII model. Calcification was 55.6% lower in $RunX2^{-/-}$ compared to $RunX2^{+/+}$ mice.[65]

As demonstrated by the recent SoFIA^3 trial, uptake of Na[^{18}F]F in AAA subjects was shown to be a predictor of patient prognosis and growth.[29] *Ex vivo* analysis of AAA tissue demonstrated Na[^{18}F]F uptake was a marker of microcalcification, distinct from CT-defined macrocalcification and tissue disruption. Microcalcification uptake of Na[^{18}F]F was also confirmed in an AngII murine model of AAA.[65] Binding of Na[^{18}F]F to microcalcification occurs through the reversible binding between hydroxyl ions in the calcium hydroxyapatite structure and fluoride ions. Preferential binding to microcalcification structures was found in *ex vivo* atherosclerosis tissue and attributed to microcalcification having a higher surface area compared to macrocalcification.[66] In addition, a human study of atherosclerotic plaque subjects, which CT scan indicated highly dense calcification, showed no Na[^{18}F]F uptake, suggesting that Na[^{18}F]F uptake may reflect microcalcification.[67] The SoFIA^3 trial determined that baseline Na[^{18}F]F activity was a predictor of aneurysm expansion and Na[^{18}F]F uptake predicted the clinical endpoints in 30.6% of the cohort. Importantly, both uptake in relation to aneurysm expansion and clinical endpoint (AAA repair or rupture) were independent of other AAA risk factors including smoking and hypertension. The results of the trial were encouraging as they showed accurate prediction of clinical endpoint

and AAA expansion when compared to ultrasound findings. Furthermore, the potential clinical utility of Na[^{18}F]F has been shown in other vascular diseases: Subjects with known peripheral arterial disease (PAD) were scanned by Na[^{18}F]F PET/CT prior to percutaneous transluminal angioplasty (PTA) intervention.[68] High baseline Na[^{18}F]F uptake in the femoral artery accurately predicted subjects at risk of restenosis after PTA. Positive uptake of Na[^{18}F]F in aortic stenosis subjects correlated with valve severity.[69] Coronary arterial disease subjects, with increased Na[^{18}F]F coronary activity, were at risk of fatal or nonfatal myocardial infarction (MI).[70] Prediction of MI using Na[^{18}F]F coronary activity performed better than classical calcium scoring and was independent of age and risk factors.

Although the SoFIA^{3} trial was conducted in end stage aneurysm subjects, the study hinted that research into the assessment of Na[^{18}F]F as a biomarker for AAA would be beneficial. However, the biological process driving calcification in AAA, the role of calcification in patient outcomes and prognosis as well as the mechanism of Na[^{18}F]F uptake in AAA need to be better understood. In addition, care must be taken when interpreting SUV measured in the aorta due to the anatomical position with respect to the spine, which has substantially higher Na[^{18}F]F uptake. Reconstruction methods have been developed to mask the signal from the bone, marking an important advance in quantitative methods in cardiovascular PET.[71]

Proliferation

VSMCs is the most abundant cell type found in the medial layer of the aorta. In response to vascular injury, including as part of AAA formation, VSMC display remarkable plasticity and can phenotypically switch from a mature contractile to a proliferative and synthetic state. The Kruppel-like factor 4 is an important driver of this process.[72, 73] Limited studies have investigated the overarching mechanism in which proliferation occurs in AAA. Overexpression of microRNA-21, through a lentiviral construct, increased proliferation of VSMC in PPE and AngII induced AAA in murine models.[74] Targeting mircoRNA-21 therapeutically attenuated AAA progression.

3-[^{18}F]Fluorothymidine (3-[^{18}F]-FLT) uptake, a marker of proliferative cells, is increased in ApoE$^{-/-}$ mice, infused with AngII specifically during the active growth phase of the model.[75] 3-[^{18}F]-FLT, a radiolabelled analogue of pyrimidine deoxynucleotide thymidine, is taken into cells by the pyrimidine salvage pathway and

trapped by thymidine kinase 1 (TK1) phosphorylation.[76] Uptake peaked at day 14 post AngII infusion, with SUV_{max} reducing at day 28. The role of proliferative cells in human AAA progression is unknown. Confirmatory evidence in human aortic tissue samples have shown varying levels of Ki-67 and TGF-β1.[77] *In vitro* analysis of human AAA VSMC from end stage disease compared to control VSMC were 40% less proliferative over a 7-day experiment with evidence of replicative senescence.[78] In contrast, in murine studies, an increased number of Ki-67-positive cells were located in the aortic wall and correlated with aortic size.[75] In addition, upregulation of the proliferative substrate TK-1, and transporter proteins ENT-1, ENT-2, CNT-1 and CNT-3 was found in Ang-II induced aneurysms.[75] Differing observations in human and murine samples could be due to differences in disease stages. Thorough investigation is required to assess the role of proliferation in AAA remodelling and how it correlates with the outcome of the disease in patients.[79]

Angiogenesis

The appearance of newly formed vessels in histological examination of tissue samples from human AAA subjects has led to the suggestion that angiogenesis plays an important role in the vascular remodelling associated with aneurysms.[80-82] Angiogenesis is defined as the formation of vessels from the previously existing vasculature. Breakdown of the extracellular matrix and expression of proteinases is required to facilitate the migration of endothelial cells.[82] Regions of extracellular matrix breakdown have been associated with increased vessel distribution along with an increase in vascular endothelial growth factor (VEGF) and CD31 expression.[83, 84] This is accompanied by the overexpression of the integrin heterodimer $\alpha_v\beta_3$.[80]

Histological staining of human aortic tissue samples has shown an increase of vascularisation in AAA samples compared to controls, with increased vascularisation predominately seen in the media and adventitia.[81, 82] Formation of new vessels is accompanied by the overexpression of α_v on mRNA and protein levels as well as VEGF and VE-cadherin protein.[80] High levels of vascularisation, α_v and VEGF were found at the rupture edge compared to the anterior sac of AAA human samples. The role of angiogenesis in longitudinal tracking of aneurysm progression has not been thoroughly studied. Other hallmarks of angiogenesis have been investigated in human AAA surgical samples including upregulation of CD105 (endoglin). CD105 expression

was located in vessels of the inflammatory region of AAA and the inner luminal region of ILT.[85, 86]

Development of novel PET/CT radiopharmaceuticals have focused on targeting the integrin heterodimer $\alpha_v\beta_3$ and CD105. RGD binding radiopharmaceuticals, namely [^{18}F]-FPPRGD2 and [^{18}F]-Fluciclatide for targeting $\alpha_v\beta_3$ have displayed specific uptake in the aneurysmal region. In the AngII murine model, significant uptake of [^{18}F]-FPPRGD2 was seen compared to control models (%ID/g 2.05 vs 0.63).[87] *Ex vivo* autoradiography confirmed uptake of [^{18}F]-FPPRGD2 and correlated with CD31 expression. In an *ex vivo* autoradiography radiopharmaceutical comparison study, [^{18}F]-Fluciclatide displayed specific uptake in human aneurysm tissue when compared to other non-specific AAA ligand tracers.[88]

Targeting CD105 through the development of a novel antibody Fab radiopharmaceutical showed increased uptake in mice with calcium phosphate-induced aneurysms at day 5 and 12 compared to controls (%ID/g 8.8 vs. 6.86 vs. 3.58, respectively).[89] [^{64}Cu]Cu-NOTA-TRC105-Fab demonstrated specific binding in CD105 blocking experiments and *ex vivo* autoradiography. A correlation of [^{64}Cu]Cu-NOTA-TRC105-Fab with CD105 was also confirmed in immunofluorescent staining of murine tissue. Translating [^{64}Cu] labelled radiopharmaceuticals clinically is challenging due to the radionuclide properties of [^{64}Cu] (half-life of 12.7 hours) and the radiation exposure to patients.

The specificity of both RGD and CD105 tracers in murine models has shown potential in the detection of AAA progression. Further preclinical studies would build our understanding of the role of angiogenesis in longitudinal progression of AAA before consideration of human trials using angiogenesis-targeted radiopharmaceuticals.

Chemokine Receptors

Monocyte and leukocyte recruitment to sites of aneurysm induction rely on chemokines activating their receptors.[90, 91] Chemokine receptors, in particular CCR2, are located on endothelial cells, smooth muscle cells and leukocytes. Activation of the CCR2 pathway by MCP-1 has shown recruitment of monocytes, migration of endothelial cells, angiogenesis and proliferation of VSMC. Synthesis of the novel CCR2-specific radiotracer [^{64}Cu]Cu-DOTA-ECL1i showed detection of AAA in rats with PPE-induced aneurysms at day 7, with uptake reported to be double that of sham

models.[92] In addition, high uptake correlated with risk of rupture. *Ex vivo* histology and autoradiography demonstrated high expression of CCR2 and binding of the radiotracer in human samples. Radiotracer binding occurs between ECL1i and extracellular loop 1 on CCR2.[93] Clinical translation of [^{64}Cu] could be problematic due to the 12.1-hour half-life and radiation dose given to a subject. An alternative positron emitting Cu radionuclide with a shorter half-life, such as [^{62}Cu] (9 minutes) or [^{60}Cu] (23 minutes) could overcome this hurdle, though these come with their own limitations. A [^{68}Ga] labelled version of DOTA-ECL1i has been shown to detect CCR2 expressing macrophages in murine heart.[94] There is little known about the role of CCR2 in human AAA progression.

Multivariate gene regression analysis of blood taken from end stage aneurysm subjects showed a significant increase in CCR2-V654I VI gene polymorphism compared to matched controls.[95] Studies in murine models have eluded to the role CCR2 could play in aneurysm formation. Deletion of CCR2 in ApoE$^{-/-}$ and wild type mice attenuated AngII and $CaCl_2$ aneurysm formation respectively in the abdominal aorta and aortic root.[90, 91, 96] The measured aneurysm diameter was comparable between surgical models and sham. *Ex vivo* histology demonstrated a preserved elastin structure and reduced levels of MMPs.[91] CCR2 has also been a target for attenuating aneurysm formation using drug therapy techniques such as siRNA constructs and Everolimus.[97, 98] Further understanding into the role of CCR2 with the progression of AAA is necessary to determine whether a CCR2 biomarker is useful clinically.

Outlook

PET/CT is a molecular imaging tool that can probe molecular pathways. Due to the very large range of radiotracers developed, a variety of different biological pathways can be investigated. The current method of monitoring AAA subjects has recently come under scrutiny and a revaluation of how AAA are treated has been proposed.[99] Data from UK NAAASP demonstrated that the observed risk of rupture for AAA between 5.0 and 5.4 cm (i.e. pre-intervention threshold) is only 0.4% per year.[4] As a result, AAA subjects could be undergoing unnecessary interventional surgery. Patients are placed under a significant burden of ultrasound surveillance which is likely more frequent than necessary based on the RESCAN trial.[28] The choice of a relevant PET/CT radiotracer that could stratify AAA subjects and help deliver a personalised

medicine approach to this patient population in terms of ultrasound surveillance frequency and intervention threshold would be advantageous. Currently, markers of metabolism, calcification, angiogenesis, proliferation and chemokine receptors have been studied and reported in murine and human studies.

Our knowledge on the biological mechanisms that drive aneurysm formation have been concluded from human surgical samples collected post-surgery and using antibodies raised against cell identification markers to understand the biology. This approach has limitations. Recently, elegant transgenic murine studies using lineage tracing to track VSMC in atherosclerosis showed early disease formation was a result of VSMC plasticity promoting proliferation and expressing inflammatory markers far more than the expected inflammatory infiltration.[100] Evidence of a similar role for VSMC in driving aneurysm formation has been reported alongside evidence of these cells developing into an osteogenic state.[61, 72] Further investigations linking novel PET radiotracers to fundamental biology in the pre-clinical sphere alongside patient outcomes in the clinical setting are required to move the field forward and translate these early findings into clinical practice.

Initial investigations of the role PET/CT could play in modelling AAA growth implemented 2-[^{18}F]-FDG to indirectly study inflammation. Although the evidence shown from a variety of human studies is inconclusive with quantification via SUV_{max}, a more extensive range of metrics including new artificial intelligence methods may reveal new possibilities for 2-[^{18}F]-FDG PET/CT in this context and there has been some evidence to suggest 2-[^{18}F]-FDG could predict patients at risk of developing endoleaks after EVAR. Tracing inflammation in AAA subjects could involve repurposing a current inflammatory radiotracer used in other cardiac diseases. There have been encouraging studies in using PET/CT to detect inflammation in atherosclerosis subjects. [^{68}Ga]Ga-Pentixafor, a marker of CXC-motif chemokine receptor 4, correlated uptake with cardiovascular risk factors (e.g hypertension), with higher uptake reflective of an increase in risk factors.[101] Uptake of the [^{68}Ga] radiolabelled nanobody, MMR, increased with disease progression and macrophage infiltration in a preclinical model.[102] [^{68}Ga]Ga-DOTATATE has been shown to detect regions of inflammation in atherosclerosis in humans.[103] Alternatively, the classical method of PET/CT image analysis using SUV may be unsuitable for evaluating 2-[^{18}F]-FDG uptake in AAA. Radiomic analysis of 2-[^{18}F]-FDG data could provide a

stable metric that demonstrates the outcome for subjects with positive uptake.[104] In addition, use of deep learning techniques could be employed on 2-[^{18}F]-FDG full body scans from other disease areas (e.g. oncology with presentation of AAA) to study other features of aneurysm growth.[105]

The SoFIA^3 trial showed early promise of the role of Na[^{18}F]F in end stage aneurysm subjects. Na[^{18}F]F uptake could predict patient outcome and hinted at a correlation with aortic expansion.[29] This is the first known trial in humans that has shown detection of biological remodelling in AAA that predicts patient outcome and could offer a personalised medicine approach. Further investigations using Na[^{18}F]F would be a logical next step, for example, in small AAA to predict the rate of growth to clinical endpoint (rupture or repair), in aortic dissection in AAA repair and in the preclinical setting to better understand how the PET/CT signal links to biological events that link to aneurysm progression. Murine studies tracing proliferation in the AngII-induced AAA murine model using 3-[^{18}F]-FLT and detection of angiogenic sprouts using [^{18}F]-FPPRGD$_2$ have shown early promise.[75, 87] A single human study in atherosclerosis demonstrated [^{18}F]-Fluciclatide was able to discriminate regions of myocardial infarction between diseased and healthy subjects.[106] These tracers could be candidates to evaluate in humans to determine their role in AAA growth and outcome. Markers of proliferation and angiogenesis are of interest as they offer the opportunity for therapeutic intervention. In addition, preclinical investigation of other novel tracers, such as [^{68}Ga]Ga-FAPI, are important. [^{68}Ga]Ga-FAPI detects the overexpression of fibroblast activation protein (FAP) and has been shown in atherosclerosis.[107-109] Investigation of these tracers for AAA stratification would be interesting. Caution must also be taken in evaluating the biological mechanism in relation to aortic expansion and rupture because radiotracer uptake may not necessarily relate to aortic size.[49, 87, 92] Future work might show, hypothetically, small aneurysms are driven by proliferation of VSMC and large aneurysms may be unstable due to calcification. It could therefore be seen that more than one radiotracer may be appropriate for PET/CT in order to characterise the stage of the AAA subjects. The future of AAA PET/CT imaging may involve the clinical implementation of these types of molecular markers.

The use of PET/CT has shown the applicability of a wide range of biological probes to detect an aneurysm in a subject or model. Further investigation is needed to determine

if the use of PET/CT would be clinically useful for the management of AAA subjects and modelling AAA growth and patient outcome. PET/CT may provide key information in future pharmaceutical and surgical interventions.

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Highlights

- Current clinical imaging strategies in monitoring AAA growth and rupture could benefit from an additional imaging stratification biomarker to provide personalised care for subjects.
- Radiotracers have been developed and repurposed to image biological remodelling process occurring in AAA, including inflammation, microcalcification, angiogenesis, proliferation and chemokine receptors.
- SoFIA^3 trial showed the most promising advances in the implementation of PET/CT as a stratification biomarker. Uptake of Na[^{18}F]F, a marker of microcalcification, determined patient outcome (rupture) and modelled aneurysm growth independent of classical risk factors.

Figures

Figure 1: Summary of PET radiopharmaceuticals imaging biological remodelling in AAA. (A) macrophage infiltration occurs as part of the inflammatory response due to breakdown of the extracellular matrix in the aortic wall. 2-[^{18}F]-FDG, a marker of glucose metabolism, has been used as a non-specific marker of inflammation in human studies. (B) Endothelial cells, smooth muscle cells and lymphocytes express chemokine receptors, in particular CCR2, in response to injury to recruit monocytes. Binding to Extracellular Loop 1 (ECL1) domain on CCR2 through [^{64}Cu]Cu-DOTA-ECL1i allows visualisation of CCR2 expression. (C) Upregulation of proliferative transporter protein and thymidine kinase 1 (TK-1) has been demonstrated in AngII AAA murine model, allowing influx of 3-[^{18}F]-FLT, indicating a proliferative response in aortic dilation. (D) Extracellular matrix destruction, as a result of elastin loss, promotes vascular remodelling as an attempted healing response. This could promote osteogenic differentiation of vascular smooth muscle cells, resulting in the formation of microcalcification. Na[^{18}F]F detects active remodelling process through exchange of [^{18}F]$^-$ with OH$^-$ ions in microcalcification structure. (E) Angiogenic markers have been detected in *ex vivo* AAA samples, suggesting angiogenesis occurs in vascular remodelling associated with AAA. Overexpression of integrin heterodimer $\alpha_v\beta_3$ provides a target for RGD containing radiotracers (e.g. [^{18}F]-Fluciclatide and [^{18}F]-FPPRGD2). In addition, expression of CD105 in ILT and inflammatory region of AAA is targeted by radiolabelled antibody [^{64}Cu]Cu-NOTA-TRC105-Fab.

Table 1: Summary of AAA PET/CT studies

Biological Remodelling	**Radiotracer**	**Model**	**References**
Inflammation	2-[^{18}F]-FDG	Human	43-46, 49-55
Calcification	Na[^{18}F]F	Human Murine	29, 65
Proliferation	3-[^{18}F]-FLT	Murine	75
Angiogenesis	[^{18}F]-Fluciclatide [^{18}F]-FPPRGD2 [^{64}Cu]Cu-NOTA-TRC-105-Fab	Human (*ex vivo*) Murine	87-89
Chemokine Receptor	[^{64}Cu]Cu-DOTA-ECL1i	Human (*ex vivo*) Murine	92