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Abdominal Aortic Aneurysm

Molecular Imaging Studies of Pathophysiology

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ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2013

ISSN 1651-6206
ISBN 978-91-554-8656-3
urn:nbn:se:uu:diva-194663

Dissertation presented at Uppsala University to be publicly examined in Auditorium Minus, Museum Gustavianum, Akademigatan 3, Uppsala, Friday, May 31, 2013 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Tegler, G. 2013. Abdominal Aortic Aneurysm: *Molecular Imaging Studies of Pathophysiology*. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 899. 111 pp. Uppsala. ISBN 978-91-554-8656-3.

The pathological process behind abdominal aortic aneurysm (AAA) formation is poorly understood and difficult to study. The aim of the thesis was to study the pathophysiology of AAA formation with positron emission tomography (PET) technology, a molecular imaging technique, allowing *in vivo* studies of pathophysiological changes.

In **study I** ^{18}F-FDG, a glucose analogue, was tested. It had previously been reported as a useful tracer studying inflammation in AAAs. These studies included, however, foremost large, symptomatic, and inflammatory AAAs. In the present study on five small and seven large asymptomatic AAAs, no increase in ^{18}F-FDG uptake could be revealed *in vivo*.

In **study II** ^{11}C-PK11195, a macrophage tracer, and ^{11}C-D-deprenyl, an unspecific inflammatory tracer, previously never tested on asymptomatic AAAs, were tested *in vivo* on five and 10 AAA-patients respectively, without signs of increased levels of inflammatory activity in the aorta.

In **study III** several tracers were screened *in vitro* through autoradiography on AAA tissue. [^{18}F]fluciclatide, targeting the integrin $\alpha_V\beta_3$ receptor upregulated in angiogenesis, was the only tracer with an increased uptake.

In **study IV** [^{18}F]fluciclatide-autoradiography was performed on AAA tissue from five patients and non-aneurysmal aortic tissue obtained from five age and sex matched organ donors. The study showed a 56% increased specific uptake in AAA, although not significant (P=0.136). Immunohistochemical revealed inflammatory cell foci in close relation to the vessels.

In conclusion, PET has potential to elucidate the pathophysiology of AAA formation. For the large group of small asymptomatic AAAs, ^{18}F-FDG is not suitable, as the chronic inflammation in asymptomatic AAA is not sufficiently metabolically active. Furthermore, ^{11}C-PK11195 and ^{11}C-D-deprenyl were unable to show the chronic inflammation seen in asymptomatic AAA.

The interesting finding with uptake of [^{18}F]fluciclatide showed that angiogenesis may be imaged in large asymptomatic AAAs *in vitro,* through the integrin $\alpha_V\beta_3$ receptor. Thus, it is likely that future studies of the role of angiogenesis in AAA formation *in vivo*, in small AAAs, could use this target site. The development of an integrin $\alpha_V\beta_3$ receptor tracer, preferably with higher affinity, is in progress for further *in vitro* and *in vivo* studies.

Keywords: Abdominal aortic aneurysm, AAA, Positron emission tomography, PET, Molecular Imaging, Pathophysiology, Autoradiography, Angiogenesis, integrin alphaVbeta3, FDG, 18F-FDG, 11C-PK11195, 11C-D-deprenyl, [18F]fluciclatide, Fluciclatide

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ISSN 1651-6206
ISBN 978-91-554-8656-3
urn:nbn:se:uu:diva-194663 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-194663)

He who neglects to drink from the spring of experience is likely to die of thirst in the desert of ignorance.

Ling Po

To
Lise-Lotte
Henrietta and Carolina
My very own LHC

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Tegler G, Ericson K, Sörensen J, Björck M, and Wanhainen A. Inflammation in the walls of asymptomatic abdominal aortic aneurysms is not associated with increased metabolic activity detectable by 18-fluorodeoxglucose positron-emission tomography. *J Vasc Surg*, *2012; 56(3): 802–807*

II Tegler G, Sörensen J, Ericson K, Björck M, and Wanhainen A. 4D-PET/CT with [^{11}C]-PK11195 and [^{11}C]-D-deprenyl does not identify the chronic inflammation in asymptomatic abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg 2013; 45(4): 351-356*

III Tegler G, Estrada S, Hall H, Wanhainen A, Björck M, Sörensen J, and Antoni G. Autoradiography screening of potential positron emission tomography tracers for Asymptomatic Abdominal Aortic Aneurysms *(manuscript)*

IV Tegler G, Wallgren AC, Estrada S, Wanhainen A, Björck M, Sörensen J, and Antoni G. [^{18}F]fluciclatide - Autoradiography study of angiogenesis in abdominal aortic aneurysm *(manuscript)*

Cover illustration:

The wooden sculpture of an infrarenal abdominal aortic aneurysm was carved by my talented tutor Dr Anders Wanhainen and photographed by Matti Rantatalo.

The canvas displaces, from upper left, clockwise: 3D rendering of the molecule [^{18}F]fluciclatide, (ChemAxon's MarvinSketch Drawing Applet); the basic principle of position annihilation; an ^{18}F-FDG PET/CT scan of a postoperative AAA; and last but not least, an important molecule in this thesis, the molecule structure of caffeine.

Contents

Abbreviations

AA	Abdominal aorta
AAA	Abdominal aortic aneurysm(s)
rAAA	ruptured Abdominal Aortic Aneurysm
ACE	Angiotensin converting enzyme
ARG-GLY-ASP	Arginine-glycine-aspartic tripeptide amino acid sequence, also called (RGD)
ASA	Acetylsalicylic acid
Avogadro´s constant, N_A, mole	$6{,}02214 \times 10^{23}$ 602,214,141,070,409,084,099,072 (Fox 2007)
BGO	Bismuth germinate
BSA	Body surface area
Bq	Becquerel
CASS	Collaborative Aneurysm Screening Study group
CD3	Cluster of differentiation 3 = T cell co-receptor is a protein complex on the cell surface involved in activating the T lymphocytes
CD4	Cluster of differentiation 4 = a glycoprotein found on the surface of T-helper cells, monocytes, macrophages, and dendritic cells
CD8	Cluster of differentiation 8 = a co-receptor on the surface of cytotoxic T-lymphocytes (killer cells), natural killer cells, thymocytes, and dendritic cells activated by the T-helper cells
CD20	Also called B-lymphocyte antigen is a glycoprotein found on the surface of all B-cells enabling optimal B-cell immune response, specifically against T-independent antigens
CD31	The antibody labels endothelial cells and is valuable for the demonstration of capillaries and neoplasms arising from endothelial cells.
CD68	Cluster of differentiation 68 = a glycoprotein found on macrophages, monocytes, and histiocytes.
CD138	Cluster of differentiation 138 is expressed on plasma cells (white blood cells with large amounts of antibod-

	ies)
CEA	Carcinoembryonic antigen
CI	Confidence Interval
CRP	C-reactive protein
CRPB	CRP-binding polypetide
CT	Computed Tomography
DAA	N-(2-phenoxy-5-fluorophenyl)-N-(2,5-dimethoxy-benzyl)acetamide
Dako	Dako Denmark A/S, supplier of diagnostic reagents
DED	Deuterium-L-deprenyl
e^-	electron
e^+	positron
EANM	European Association of Nuclear Medicine
ECG	Electrocardiography
EVAR	Endovascular aneurysm repair
FAN	Fluciclatide
^{18}F-FDG	fluoro-deoxy-glucose (=FDG)
fmol	Femtomole, A billionth of a millionth (10^{-15}) of a mole
FOV	Field Of View
FOVZ	18F-vorozole, 6-[(4-chlorophenyl)(1,2,4-triazol-1-yl)methyl]-1-methylbenzotriazole
γ-rays	Gamma rays, equivalent to photons
Ga	Gallium
Ge	Germanium
GLUT	Glucose transporters
hsCRP	High sensitive C-reactive protein
HE	hematoxylin and eosin
HIF-1	Hypoxia-inducible factor-1
HLA	Human Leukocyte Antigen
IAEA	International Atomic Energy Agency
IgG4	Antibodies found in various autoimmune diseases
IH	Immunohistochemical
iNOS inhibitors	Calcium insensitive Nitric Oxide Synthase inhibitor
IUPAC	International Union of Pure and Applied Chemistry
K_d	Dissociation constant. The equilibrium constant that measures the tendency of a larger object to separate (dissociate) reversibly into smaller components

keV	Kilo electron volt
LRP1	Lipoprotein receptor-related protein 1
LSO	Lutetium oxyorthosilicate
LYSO	Lutetium yttrium oxyorthosilicate
MAC387	Monoclonal antibody against granulocytes, monocytes, and tissue macrophages
MAO-B	Monoamine oxidase B
MASS	The Multicentre Aneurysm Screening Study
MeV	Mega electron volt
µm	Micro meter, 10^{-9} m
min	Minute(s) or minimum
MLU	Medial Laminar Unit(s)
MMP	Matrix metalloproteinases
MR	Magnetic Resonance
MRS	Magnetic Resonance Spectroscopy
MTO	Metomidate
NSAID	Non-steroidal anti-inflammatory drugs
OR	Odds Ratio
PASW	Predictive Analytics SoftWare; PASW Statistics 18 (formerly SPSS Statistics)
PBR	Peripheral Benzodiazepine Receptors
PET	Positron Emission Tomography
PIB	Pittsburgh compound B
RGD	Sequence of the tripeptide amino acids Arginine-glycine-aspartic, also called Arg-Gly-Asp
RGD-receptor	Receptor targeting the tripeptide amino acids Arginine-glycine-aspartic,
RR	Relative Risk
SD	Standard Deviation
SEP	Serum Elastin Peptides
SLE	Systemic Lupus Erythematosus
SMC	Smooth Muscle Cell(s)
SPECT	Single Photon Emission Tomography
[ ], squared brackets	In chemistry, squared brackets are used to indicate condensed chains. They may also signify a concentration. In radiolabeling nomenclature, the isotope is commonly written between the squared brackets. These have been

	intentionally omitted in most places in this thesis to enable easier reading
SUV	Standardized Uptake Values
TIMP	Tissue inhibitor of metalloproteinases
tPA	Tissue plasminogen activator
t½, T1/2	Half-life
TSPO	Translocator protein (18kDa)
TUT	Tracer Uptake Time
UK	United Kingdom
ve	electron neutrino
VEGF	Vascular Endothelial Growth Factor
VOI	Volumes Of Interest
Voxel	Volumetric Pixel or Volumetric Picture Element (it has a 3D dimension and is analogous to a pixel, which represents a 2D image)
WAD	Whiplash Associated Disorder

SOME DEFINITIONS and CLARIFICATIONS

Annihilation	From Latin *nihil* meaning "nothing", total destruction or complete obliteration of something
Isotope	Variants of a particular chemical element. While all isotopes of a given element share the same number of protons, each isotope differs from the others in its number of neutrons.
Tracer	Something that can be followed or detected; radioactive tracer — a radioactive isotope connected to the compound; histochemical tracer – compound used in histopathological studies of cells and tissues
et al	From Latin: *et* (and), *alii* (others); and co-writers; and co-workers
About the references	The references in the text are displayed as (Surname of the first author plus year), whereas when part of a sentence in the text they are displayed as Surname of the first author *et al*. Some of the references have the same author and year. These references are separated with an a), b), or c) after the year in the text, and lastly after the reference in the reference list.

Introduction

Aneurysm, which in Greek means widening, refers to a permanent localized dilation of an artery or a vein. The most frequently affected vessel is the infrarenal abdominal aorta, and as long as the vessel wall is intact, most patients with abdominal aortic aneurysm (AAA) do not present any symptoms.

The natural course for an aneurysm is to gradually expand and sometimes rupture (rAAA), for rAAA, the overall mortality rate is up to 90% (Bengtsson 1993). As rAAA has a poor outcome, screening programs for AAA has been introduced in several countries to identify patients at an early stage of the disease in order to treat in an elective setting, which has a much lower mortality rate (Svensjö 2011).

Due to screening programs for AAA, many patients with small aneurysms are identified and followed through surveillance programs to the time when surgical therapy is indicated, usually when the aortic diameter is more than 55 mm (Brown 1999). This renders large cohorts of patients with small AAA with only the actual size of the aneurysm, or an increased growth rate, indicating the need for surgery.

Thus, a key limitation of contemporary treatment strategies is the lack of therapy directed at reducing expansion. Hence, increased knowledge of the pathophysiological processes behind aneurysm formation, expansion, and rupture ought to lead to the development of more active therapeutic options for small AAA, including medical treatment.

Molecular imaging techniques, such as positron emission tomography (PET) might be useful tools in bringing valuable knowledge to the understanding of these early processes.

The Normal Aorta

The diameter of the normal abdominal aorta (AA) is 16.8 ± 2.9 mm (mean ± SD) for men over 50 years of age and 14.6 ± 1.9 mm for women (Pedersen 1993). The diameter of the aorta is related to the size of the specimen, e.g. the mouse aorta is about 1.2 mm (Wolinsky 1967) and the blue whale thoracic aorta is 230 mm (Caspar 2013). In humans, this relationship was studied by Pearce *et al*, who related aorta diameter to age, gender, and body surface area (BSA) though the Du Bois and Du Bois formula for calculating BSA. (Equation 1) (Du Bois 1916 ; Pearce 1993).

$$BSA\ (cm^2) = 71.84 \times Height\ (cm)^{0.725} \times Weight\ (kg)^{0.425}$$

Equation 1. *The Du Bois and Du Bois formula for calculating body surface. Variations on Du Bois formula do exist as well as other equations published by Boyd (1935), Gehan and George (1970), Haycock (1978), and Mosteller (1987) (see http://en.wikipedia.org/wiki/Body_surface_area).*

Pearce *et al* measured the diameter of the aorta with computed tomography scans (CT) and correlated this with BSA: the different diameters are presented in Table 1.

Table 1. *Expected diameter of the normal infrarenal aorta between the ages of 50 and 85, according to Pearce et al.*

BSA (m^2)	Approximate height and weight	Diameter (mm)	
		Male	Female
1.3	140 cm, 45 kg	15.9 to 18.0	13.3 to 15.4
1.9	180 cm, 70 kg	18.1 to 20.2	15.5 to 17.6
2.5	190 cm, 125 kg	20.2 to 22.3	17.7 to 19.8

Sonesson *et al* found a correlation between sex, age, BSA, and expected normal infrarenal aortic diameter and illustrated this in a nomogram for men and women (Sonesson 1994). With Du Bois's formula and Sonesson's nomogram, the author's aorta would be approximately 18 mm, which could be confirmed by ultrasound (Figure 1).

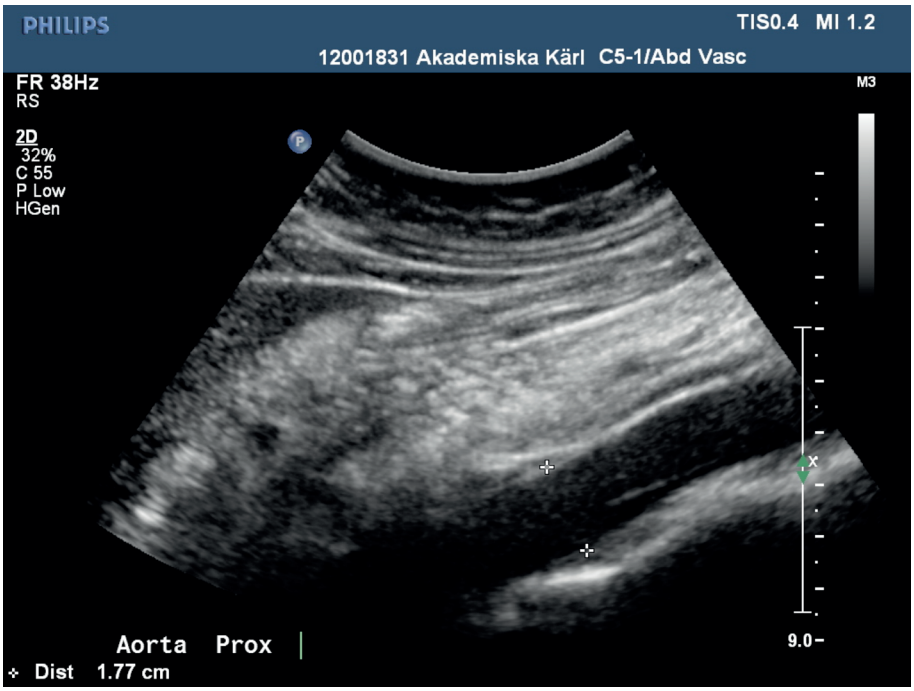


Figure 1. *The author's aorta viewed on a longitudinal axis, with a proximal diameter of 18 mm.*

The wall of the aorta includes three layers, the tunica intima, the tunica media with muscular-elastic characteristics, and the collagen rich tunica adventitia. Within the tunica media, the elastic fibres, together with smooth muscle cells (SMC), form medial lamellar units (MLU). The number of aortic MLU in humans is highest in the thoracic aorta and lowest in the abdominal aorta (Wolinsky 1967, 1969). The wall thickness of the human aorta corresponds to the diameter of the vessel: however, the number of MLU in the AA is less than expected in relation to its wall thickness or diameter (Wolinsky 1967, 1969). Although the number of MLU in the human AA was calculated to be approximately 35 units, Wolinsky *el al* found only about 28 units, which results in a higher average tension per lamellar unit than in the thoracic segment. Similar to other media with less than 29 lamellar units, but unlike other media with a thickness of more than 0.5 mm, the inferior abdominal aorta has no medial *vasa vasorum*, the vessels that nourishing the aortic wall. In the normal infrarenal aorta the *vasa vasorum* is mainly distributed in the tunica adventitia, and it has been suggested the tunica media is avascular (Wolinsky 1969). However in aorta of neonatal, infants, and children studied through X-ray microscopy, the *vasa vasorum* of the AA appears to increase in density with age and reach through the tunica adventitia to the outer 3rd of the tunica media (Clarke 1965). In a study of the *vasa vasorum's* eventual role in the failure of homologous vessel grafts, disruption of the *vasa vaso-*

rum is suggested to be the cause of aneurysm formation (Benjamin 1960). In addition, changes in the *vasa vasorum* may be an important factor in the formation of another pathology of the aorta, namely aorta dissection (McCloskey 1951).

Abdominal Aortic Aneurysm (AAA)

Various definitions of AAA exist. The most frequently used definition is the one by McGregor *et al* who defined AAA as an infrarenal aortic diameter of 30 mm or larger (McGregor 1975). This definition is based on work by Steinberg and Stein who examined 838 intravenous aortographs (Steinberg 1966) and Leopold's ultrasonography exams (Leopold 1970). Before Steinberg *et al´s* work, AAA were merely defined as small (< 40 mm), large (40 - 60 mm), or huge (> 60 mm). The ratio between the infrarenal aortic diameter and the suprarenal aortic diameter is the most important factor for judging expansion and the risk of rupture: Sterpetti *et al* defined AAA as having a ratio of 1.5 or more (Sterpetti 1987). Collin *et al* also refer to the relationship between supra- and infrarenal aortic diameter and define AAA as a maximum infrarenal aortic diameter of 40 mm or more, and/or an infrarenal diameter larger than the suprarenal aortic diameter by at least 5 mm (Collin 1988). The International Society for Cardiovascular Surgery (ISCVS) suggest a definition of an aneurysm as a 50% permanent dilatation of the normal artery adjusted for gender and radiological modality (Johnston 1991) (Table 2). In a population-based MRI study the dividing-line between normal aorta and aneurysm in 70-years old men and women was evaluated (Wanhainen 2008). To define a suitable dividing-line between normal aorta and aneurysm a threshold-diameter (mean diameter + 2 SD) as well as a threshold-ratio (mean ratio + 2 SD) between the infrarenal- and the suprarenal aortic diameter was calculated, below which 97.5% of the measurements would be expected to be. For men the suggested dividing-line (diameter and/or ratio) was 30 mm and/or 1.1. The corresponding dividing-line for women was 27 mm and/or 1.0.

Table 2. *Different definitions of AAA*

Definition	**Criteria**
McGregor, 1975	Infrarenal aorta ≥ 30 mm
Sterpetti, 1987	Infra/suprarenal aorta ratio ≥ 1.5 times
Collin, 1988	Infrarenal aorta ≥ 40 mm and/or infrarenal aorta ≥ 5 mm larger than suprarenal aorta
International Society for Cardiovascular Surgery 1991	Permanent dilatation ≥ 50% of normal artery diameter
Wanhainen 2008	Men: ≥ 30 mm and/or ≥ 1.1 times suprarenal aortic diameter Women: ≥ 27 mm and/or ≥ 1.0 times suprarenal aortic diameter

Historical Aspects of AAA

Andreas Vesalius (1514-1664) presented one of the first descriptions of AAA in a letter to his colleague Gasser (Osler 1905). In the early 1900s, there was an overwhelming predominance of thoracic aneurysms, which may be explained by a more common infectious aetiology at that time, mainly syphilis.

On the evening the 25th April 1817, Sir Astley Cooper performed the first operation for rAAA through ligation of the proximal neck of the AAA, on a 38-year-old man. The operation is thoroughly described with the digital dissection of the proximal neck and careful supervision for not getting the intestines within the ligation. Even so, the man died 40 h after surgery (Cooper 1839).

The first attempts to treat an aneurysm with electrothermal coagulation was performed by Moore and Murchison in 1864 (Moore 1864). In 1938, Blakemore and King reported work on 11 patients, nine thoracic and two abdominal aneurysms, treated with electrothermic coagulation of large symptomatic aneurysms, one also ruptured (Blakemore 1938): the aneurysms were both sacculare and fusiform. Up to 60 meters of thin wire made of an alloy of silver and copper was inserted into the aneurysm and heated up to 80° Celsius in order to slow the flow of the blood and induce clotting. The results of wiring abdominal aneurysms with Colts apparatus were reported by Power 1927, and provides an insight into medical treatment in the early twentieth century, with a 45% mortality rate within a week and almost two-thirds of the 11 patients dying within 90 days, whereas four patients lived between one to 11 years (Power 1927).

In the early 1900s, Osler did not consider there was hope for the treatment of aneurysms, even though nature and a few physicians did cure aneurysms, but Osler himself had never seen any such case (Osler 1905).

Despite several attempts to ligate aneurysms, it was not until April 1923 that the first successful ligation proximal of an aortoiliacal aneurysm was performed by Rudolph Matas on a 28-year old woman who, died of pulmonary haemorrhage just over 17 months after the operation on the aorta (Matas 1925).

Other methods have been tried to treat aneurysm. In December 1948, Rudolph Nissen treated Albert Einstein, perhaps the most famous person treated for AAA (Cohen 1990). The top modern surgical procedure at that time involved wrapping the wall of the aneurysm with cellophane to induce a fibrotic process and decrease the expansion rate (Cohen 1990). Six years later, Einstein complained of severe abdominal pain. Initially, this was diagnosed as cholecystitis, however later, the correct diagnosis was presented, and acute surgery with ligation of the ruptured AAA was suggested to Einstein, who declined further procedures with the words:

"I want to go when I want. It is tasteless to prolong life artificially. I have done my share, it is time to go. I will do it elegantly." *(Surg Gynecol Obstet, 1990; 170(5): 457-458)*

And so he did, 76 years 6 month and 4 days old on April 18th 1955.

The initial symptoms of upper right quadrant pains that Einstein presented when he was admitted to the hospital, suggesting the diagnosis of acute cholecystitis, has been referred to as "The Einstein Sign" – a sign of rAAA (Chandler 1984).

Rudolph Matas suggested as early as 1902 the aneurysmorhaphy technique in the treatment of AAA (Matas 1903), which has been recommended as a surgical technique for renal aneurysm (Creech 1966).

However, techniques where the aneurysm is occluded, wired, and/or ligated present the risk of ischemia of the lower extremities and to overcome this, vascular graft for bypassing the circulation to the legs is often needed.

In February 1951, the first successful resection of an AAA with a vein graft was reported by Freeman and Leeds. The common iliac vein was used for the bypass, and the wrapped aneurysm sac was filled with whole blood up to half the patients systolic blood pressure (Freeman 1951). In March the same year, Dubost *et al* reported a similar bypass technique that used a thoracic aortic homograft from a 20-year-old female, when resecting a large AAA on a 50-year-old man (Dubost 1951, 1952).

The need for artificial grafts was addressed: the Vinyon, the "N" cloth was tested with promising results on dogs (Voorhees 1952) and Schumacker and King used synthetic prosthetic tubes in humans (Shumacker 1954), which is still the most frequently used technique for open repair and resection of AAA.

With the introduction of the endovascular technique for repairing an AAA, the Ukrainian surgeon Volodos revolutionized the treatment of AAA, as it could be performed under local anesthetic (Volodos 1986). However, it was not until Parodi, five years later, presented the technique in an English language journal that the knowledge of endovascular repair of aneurysm (EVAR) was made more widely spread and the development took off (Parodi 1991).

AAA can also be treated through laparoscopic surgery: the first laparoscopic assisted resection of an AAA was by Chen *et al* (Chen 1995 ; Chen 1996). This technique is advocated foremost by French centers, where total resections of AAA are with the laparoscope (Alimi 2000 ; Coggia 2002).

Etiology

Initially, atherosclerosis was considered the leading cause of AAA: however, this has been revised due to the epidemiological and histological differences to atherosclerosis. Aneurysm is considered to have its own unique patho-

physiology, yet no specific cause of AAA has been identified. Several known risk factors are similar to those for atherosclerosis, such as high age, male gender, smoking, hypertension, while other differ, such as for diabetes. In addition a positive family history of aneurysmal disease is a strong risk factor suggesting a genetic cause (Lederle 1997 ; Blanchard 1999 ; Wanhainen 2005, a)).

The histological findings from AAA show a degeneration of the connective tissue, with degeneration of elastin and a proliferation of collagen fibres in the early stage (Dobrin 1984 ; Campa 1987). As the disease progresses and the aneurysm increases in size, the collagen fibres also degenerate, and in end-stage larger AAA, there is an infiltration of inflammatory cells, B- and T-cell lymphocytes, and macrophages (Koch 1990 ; Brophy 1991 ; Paik 2004 ; Choke 2005).

Myofibroblasts and smooth muscle cells (SMC) are found within the tunica media in the normal aorta and appear to have the ability to transdifferentiate back and forth (Kalluri 2003) and are able to synthesize protein such as elastin, collagen, gelatin, laminin, and proteoglycan, important elements of the extracellular matrix of the media (Rasmussen 1995 ; Forte 2010). In AAA, SMC within the tunica media decrease through apoptosis (López-Candales 1997 ; Henderson 1999): animal studies suggest this apoptosis is genetically linked and caused by reduction of the tumour suppressor gene Cdkn2b (Leeper 2013).

The importance of inflammation in the development of AAA has been addressed in studies of biomarkers in peripheral blood. Elevated concentrations of C-reactive protein (CRP) is found in large but not in small AAA (Norman 2004), and are associated with developing AAA in a longitudinal study with high sensitive CRP (hsCRP) (Wanhainen 2005, a)).

Matrix metalloproteinases, especially MMP-2 (formerly known as gelatinase-A and 72 kDa gelatinase), MMP-9 (known as gelatinase-B or 92 kDa gelatinase), and MMP12 are expressed in AAA tissue and are suggested to be of importance in the degenerative process (Freestone 1995 ; Thompson 1995 ; Thompson 1996 ; Tamarina 1997 ; Wassef 2001).

Hypoxia may also play a role in the development of AAA. *In vitro* experiments on ruptured AAA demonstrate increased concentrations of hypoxia-inducible factor-1 (HIF-1), Ets-1 transcription factor, and vascular endothelial growth factor (VEGF) (Erdozain 2011). The increased neovascularization in aneurysmal tunica media correlates with signs of chronic inflammation and the destruction of elastin (Holmes 1995). There is also a connection between hypoxia and angiogenesis, as hypoxia stimulates recruitment of novel vessels (Pugh 2003): in rAAA, a marker of hypoxia HIF-1-α is responsible (Choke 2006, a); Choke 2006, b)). Neovascularization is a process activated in normal development, wound healing, and inflammation and is an important characteristic seen in e.g. malignant tumours (Carmeliet 2003). Integrins are a group of transmembrane glycoprotein receptors involved in

cell-to-cell and cell-to-intracellular matrix communications, and consist of two subunits with an extracellular and a cytoplasmic segment. Known are 18 α-units and eight β-units in mammals; the combinations of these subunits give the different integrin-receptors their characteristics. Several extracellular matrix protein e.g vitronectin, fibrogen and fibronectin interact with integrins through the exposed tri-peptide sequence arginine-glycine-aspartic, also recognized as RDG in the single letter code (Ruoslahti 1987), and radiolabelled linear and cyclic RDG peptide antagonists have been evaluated in the development for a $\alpha_V\beta_3$ integrin tracer (Liu 2006).

Some parts of the vascular tree are affected by aneurysmatic dilatation more often than others, for example the segment of the external iliac artery does not appear to develop aneurysms, whereas, the infrarenal aorta and the common iliac artery are often affected (Norman 2003).

Patients with deficiency and weaknesses in the connective tissue, such as in Marfan's syndrome, are more afflicted by aortic dissection with later dilatation of the infrarenal aorta and the aortic root and aorta ascendance, than by true degenerative AAA (Takayama 2009). However, the average age of these patients (mean 45 years) is lower when they present their AAA than those with normal AAA. Other known (and perhaps also unknown) connective tissue deficiency diseases have an increased incidence of AAA. A common disease suggested to be caused by collagen alteration is inguinal hernia (Friedman 1993 ; Pans 2001 ; Szczesny 2006) and patients with this disease have a higher degree of AAA as well (Lehnert 1992 ; Pleumeekers 1999): this correlation is stronger in smokers. Antoniou *et al* suggest that men over 55 years of age with inguinal hernia should undergo ultrasound check of the aorta (Antoniou 2011).

The pathogenesis of AAA is hypothesized to be autoimmune in character by some authors (Gregory 1996 ; Tilson 1996 ; Hirose 1998 ; Jagadesham 2008). Gregory *et al* found that the concentrations of immunoglobulin G (IgG) was significantly higher in AAA tissue (79% of the 14 studied patients), than in normal aortic tissue, (11% of the nine studied patients), (Gregory 1996). In a Japanese study, Hirose *et al* found that the HLR-DR2(15)-gen was associated with higher frequency of AAA (Hirose 1998). However, AAA has a predominance in men, in contrast to other autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus (SLE) being overrepresented in females (Jagadesham 2008).Furthermore, Badger *et al* could not confirm a correlation between human leukocyte antigen (HLA) genes and AAA in 241 Irish AAA-patients, and the conclusions by Hirose *et al* on 50 cases have been questioned (Badger 2007).

Several microorganisms are considered involved in the process of AAA development. *Chlamydophila pneumoniae*, previously named TWAR (Taiwan Acute Respiratory) is more frequent in the aneurysm wall than in controls (Karlsson 2000 ; Falkensammer 2007). In a randomized controlled

double-blind trial with more than 200 patients with small AAA, azithromycin was administered and the expansion rate was studied (Karlsson 2009). Under ultrasound surveillance, no effect on the expansion rate on the small AAA was detected but there was a decrease in AAA volume in patients treated with statins and ASA (Karlsson 2009).

Borrelia is suggested to be a plausible agent in developing AAA. Elevated serum concentrations of *Borrelia burgdorferi senus lato* IgG antibodies was found in patients with AAA, compared to sex and age matched controls with peripheral arteriosclerotic disease (Hinterseher 2012). The effect of *B. burgdorferi sl* on the formation of AAA may be similar to that of *Treponema pallidum,* in that some proteins of the microorganism can induce an autoimmune reaction by mimicking the aortic wall proteins wall (Hinterseher 2012). DNA for *Borrelia burgdorferi* was, however, not detected in aneurysmal tissue by polymerase chain reaction (Falkensammer 2007), nor has Borrelia DNA been detected in the AAA wall (Hinterseher 2012).

Increased levels of angiotensin II-forming enzymes, chymase, and angiotensin converting enzymes (ACE) have been identified in AAA compared to normal aortic tissue, suggesting they may play a role in AAA formation, even though the normal tissue in this study was from the ascending aorta (Nishimoto 2002). Chymase-positive mast cells release various inflammatory mediators. The chymase activates pro-matrix metalloproteinase to matrix metalloproteinase (Saarinen 1994), and may inactivate tissue inhibitor of metalloproteinases (TIMP) and reactivate inactive MMP-TIMP complex into active MMP (Frank 2001) and an increase of ACE has been seen during monocyte/macrophage differentiation (Okamura 1999).

The first report of a family trend in AAA was by Clifton in 1977 where he described three brothers, all undergone repair for aneurysm (Clifton 1977). With 2700 subjects from over 300 pedigrees, Verloes *et al* calculated an overall relative risk (RR) of 17.9 (95% confidence interval 12.9 to 22.9) for men to develop AAA if they had brothers with AAA: in the age of 50 – 59 years the RR was as high as 94.3 (95% CI 0-425) (Verloes 1995), strongly suggesting a genetic factor involved in the pathogenesis of AAA. In a Canadian study, siblings to AAA patients had eight times higher prevalence (19.2%) than their spouse (2.3%) to develop AAA (Ogata 2005). A large Swedish twin study by Walhgren *et al* provided strong evidence for hereditary factors involved in the pathogenesis of AAA. They calculated a 24% probability for a monozygotic twin of an AAA person to have the disease, and the risk of AAA for a twin of a monozygotic AAA twin was 71 times that of a monozygotic twin of a person without AAA (Wahlgren 2010). Several genome-wide association studies (Elmore 2009 ; Gretarsdottir 2010 ; Bown 2011) have tried to identify the gene responsible for AAA, Elmore *et al* found a candidate gene on chromosome 3p12.3, which had an even higher correlation with the subgroup of smokers (Elmore 2009). The genome association study of the top candidate genes on 3267 patients with AAA and

7451 control patients found the A allele of rs7025486 on 9q33 to be associate with AAA, with an odds ratio (OR) of 1.21 ($p = 4.6 \times 10^{-10}$) (Gretarsdottir 2010). A variant of low-density lipoprotein receptor-related protein 1 (*LRP1*) is associated with AAA (Bown 2011), with the pathway of *LRP1* playing a role in MMP9 regulations. MMP9 is suggested to play a crucial role in formation of abdominal aortic aneurysms in at least in two ways: in response to tPA binding to the *LRP1* receptor and by directly regulating the intracellular processing (Hahn-Dantona 2001 ; Wang, X. 2003). Over weight, as a risk factor, is suggested to be associated with AAA formation. Not BMI, but rather the waist circumference, > 100 cm for men and >88 for women, is associated with a 30 % increased risk of treatment of AAA (Stackelberg 2013).

Epidemiology

As AAA is four to six times more frequent in men than in women, most screening studies focus on men. The four large randomized screening studies in Chichester, UK, in Viborg, Denmark, in Western Australia, and in MASS (from the four centres in Portsmouth, Southampton, Winchester, and Oxford, UK) found a prevalence between 4.0 and 7.6% among men aged 64-83 years (Scott 1995 ; Lindholt, Jes S. 1998 ; Jamrozik 2000 ; Ashton 2002). The prevalence among 50-80 years old women is reported to be 1.0-1.3% (Lederle 2001 ; Scott 2002). A Swedish study the estimated total prevalence of AAA in women is reported to 0.5%: the correlation to smoking is strong as the prevalence for current smokers is reported to 2.1%, for former smokers it is 0.4%, whereas the prevalence for never smokers is reported to as low as 0.03% (Svensjo 2013).

In several contemporary screening studies a decreased AAA-prevalence has been observed. In a Swedish population of 65-year old men the prevalence is 2.2% (Svensjö 2011): 1.7% were newly detected at screening and 0.5% had a known disease. A significant decreased cigarette consumption in the population is suggested a major contributing factor to the decreasing prevalence of the disease (Lederle 2011 ; Norman 2011 ; Sandiford 2011 ; Svensjö 2011).

Screening programs for AAA have been initiated for identifying the aneurysm at an early stage before rupture. The vast majority of aneurysms detected are small, more than 70% of AAA detected through screening are < 40 mm, (Svensjö 2011) and only10-20% are ≥ 50 mm at detection and 7-12% ≥ 55 mm (Group CASS, Collaborative Aneurysm Screening Study Group 2001).

Most screening studies are on Caucasians, whereas, other ethnic groups are not well explored. In the screening program in United Kingdom, 65-year-old men of Asian origin had a lower prevalence (0.45%) compared to Caucasians (4.7%) (Ishikawa 2001 ; Salem 2009). In a Chinese cohort of men with

severe coronary artery disease, mean age 63 years, the prevalence is 1.8% (Poon 2010). Other studies report a two to three times higher AAA-mortality ratio in white American men than in black men (Maxwell 1994 ; Gillum 1995 ; LaMorte 1995 ; Blanchard 1999), and the ADAM study found a 1.79 times higher prevalence of AAA in white men than in black men (Lederle 1997).

Management of AAA

Surgical Treatment

Although there are different definitions of an AAA (Table 2), in the clinical setting, the most frequently used is McGregor's definition, where the cut off value for an AAA is 30 mm. As the aneurysm increases in size and diameter the risk of rupture increases approximately according to the LaPlace's law, which states that the tension on the wall is proportional to the product of the pressure and the radius (Dobrin 1988). The rupture risk of small aneurysms is low, Brown *et al* determined a rupture risk per 100 patient-years of 0.3 for small AAA less than 40 mm, the corresponding risk for AAA between 40 and 49 mm is 1.5, and for AAA 50 to 59 mm it is 6.5 (Brown 1999). Lederle *et al* determined the rupture risk in the first year for an aneurysm between 55 and 59 mm to be 9.4% and 19% for an aneurysm 65-69 mm. With a diameter of more than 80 mm the annual rupture risk was as high as 36% (Lederle 2002).

Small asymptomatic AAA has often been diagnosed as an *en passent* finding in patients investigated with ultrasound or computed tomography (CT) for reasons other than suspected aortic disease. Although large aneurysms can sometimes be detected by palpation, the sensitivity and specificity are low: in 2955 patients the sensitivity was 39% for AAA $\geq$ 3.0 cm; 29% for AAA 3.0-3.9 cm, 50% for 4.0-4.9 cm, and 76% for AAA $\geq$ 5.0 cm (Lederle 1999). A positive predictive value of 43% makes palpation a rather crude diagnostic tool.

The classic presentation of a rAAA is the triad: 1) a palpable pulsating abdominal mass, 2) abdominal or back pain, 3) and hypotension or a patient history of fainting.

In screening programs, patients with an AAA of 30-55 mm are offered participation in surveillance programs, usually with annual ultrasound, until it is time for elective open or endovascular repair. Based on the UK small aneurysm trail, elective surgical therapy for intact AAA is usually recommended when the aneurysm reaches a size of 55 mm (Brown 1999). In a meta-analysis, the growth rate between the studies investigated was diverse and ranged from 1.95 to 2.70 mm/year, and it was estimated that it would take 6.2 years for a 35 mm AAA to reach 55 mm, and only 2.3 years for a 45 mm AAA to reach 55 mm (Powell 2011).

The ability to determine whether a patient's aneurysm has an increased risk of rupture depends primarily on the diameter of the aneurysm. The presence of irregularities, such as bulges and "blebs", on ultrasound or CT scans may also indicate a greater risk of rupture. Several biomarkers such as Cystatin C (Lindholt, J. S. 2001, a)), interleukin 6 and 8 (IL-6 and IL-8) (Treska 2000), serum elastin peptides (SEP) (Lindholt, J. S. 2001, b); Wilson 2001), plasma elastase (Lindholt, J. S. 2003), and tissue plasminogen activator (tPA) (Lindholt, J. S. 2003), among others, have been investigated in the quest to identify a biomarker that can assist in surgical decision-making (Urbonavicius 2008). Currently no biomarker has presented clinical potential, but further knowledge and additional biomarker information would be useful.

Although the natural course of AAA is well characterized, several important aspects need further research, the most important being the pathophysiological processes behind expansion and rupture, and increased knowledge of these factors, would increase the availability of therapeutic options for small AAA.

Some Costs

The prevailing therapies for AAA are surveillance of small AAA and open resection or endovascular stent grafting of large AAA. The scope of this thesis is not the economics of AAA management, but costs always need to be considered. In 2010, the overall cost for treatment of an AAA in Sweden was approximately €26 000 for endovascular EVAR and €30 000 for open resection (Mani 2008 ; Mani 2010). In 2005, the cost for ultrasonic scanning of an AAA was roughly €80-185 (Wanhainen 2005, b)). For patients under surveillance, there is a degree of anxiety for both the patients and their relatives, which cannot be ignored.

Medical Treatment

Medical treatment aiming to reduce the expansion rate of small AAA will reduce the need for surgical repair and rupture. Several medications, such as beta-blockers, angiotensin converting enzyme (ACE) inhibitors, statins, acetylsalicylic acid (ASA), and different antibiotics have been studied.

In a randomized double-blinded trail from Viborg, Denmark, the effect of propranolol was evaluated in 122 patients with small AAA (Lindholt, J. S. 1999). However, the study was terminated after two years, as there was a high frequency of dropouts in the propranolol treated group due to side effects, mainly dyspnoea. The propranolol treated group scored lower on quality of life, pulmonary function, and ankle brachial index (ABI) (Lindholt, J. S. 1999), and 95% of the aorta measurements were within the 2 mm variation, rendering the analysis of the expansion rate uncertain.

The Propranolol Aneurysm Trial Investigators in Canada concluded in a randomized double-blinded study that propranolol did not decrease the en-

largement rate of small AAA and the medication not well tolerated by the patients. The propranolol group had lower quality of life, with decreased physical functioning, physical role, and vitality dimensions (Laupacis 2002).

Moran *et al* found a correlation between osteoprotegerin and aneurysmal growth, and a reduction of secreted osteoprotegerin during treatment with angiotensin II blocker, suggesting angiotensin converting enzyme (ACE) inhibitor is a potential pharmaceutical treatment for AAA (Moran 2005). However, in a cohort study by Sweeting *et al* on patients enrolled in the UK small aneurysm trail, the expansion rate was faster in patients taking ACE inhibitors and the authors urged for randomized studies to evaluate whether ACE inhibitors are harmful or beneficial for AAA-patients (Sweeting 2010).

Several groups suggest the use of statins and have shown positive effects in reducing the enlargement rate of small AAA (Schouten 2006 ; Mosorin 2008 ; Karrowni 2011). However, the Cochrane review, "Medical treatment for small abdominal aortic aneurysms", 2012 did not include statins due to major bias in previous studies, all retrospective (Rughani 2012). The value of a well-designed randomized clinical trial, addressing this particular question, has been raised several times. However, it is difficult to obtain a sufficiently large population in a randomized trial to answer this question, as many patients already are prescribed statins due to coronary and atherosclerotic disorders.

A meta-analysis by Twine and Williams on the effects of statins on the expansion rate concludes that most studies have low quality evidence and found no proof that statin reduce AAA expansion rate. However, statins improved the all-cause survival after elective AAA (Twine 2011).

As the pathogenesis of AAA could be due to an infection, antibiotics have been used in an attempt to decrease the enlargement rate. Both Mosorin *et al* and Vammen *et al* found a reduced enlargement rate in their double-blinded randomized trails with doxycycline or roxithromycin (Mosorin 2001 ; Vammen 2001). In Viborg, Høgh *et al* suggested an intermittent antibiotic treatment, four weeks annually, with roxithromycin against Chlamydia pneumoniae and could show a reduction rate of 36% in a group of 84 randomized AAA patients (Høgh 2009). However, Karlsson *et al* were unable to confirm these results in a similar randomized trail on a larger group of totally 247 patients (Karlsson 2009). Calls for additional well-designed randomized controlled studies, addressing antimicrobial therapy for reduction of AAA expansion, as well as the potential role of the intraluminal thrombus in AAA expansion rate along with the potentials of ASA therapy have been made (Bergqvist 2012 ; Lindholt, J. S. 2012)

Other medicines have been tested on animal models, for example NSAID, MMP inhibitors, iNOS inhibitors, vitamin E, and tamoxifen, to mention a few (Assar 2009).

Molecular Imaging

The main purpose of molecular imaging is to study biological processes *in vivo* through visualization, characterization, and quantification at the cellular and molecular level, without interfering with the biological process (Weissleder 2001 ; Massoud 2003). Several different techniques are used, for example Single Photon Emission Tomography (SPECT), Magnetic Resonance Spectroscopy (MRS) and Positron Emission Tomography (PET).

Autoradiography

Autoradiography, an *in vitro* and *ex vivo* molecular imaging technique, is useful for developing new tracers. With the *in vitro* method, sections of tissue (≈ 20 μm thick) are incubated in a buffer solution together with a radio labelled tracer. In the ex vivo method, the tracer is administered to the subject, that is subsequently euthanatized, and thin whole-body tissue samples are taken and placed on a fluorescent plate for recording radioactivity for later analysis. When the isotope decays and targets the fluorescent plate, the positrons generate an image. This in contrast to PET where the γ-rays detected generates an image. The auto-radiographic images become sharper at higher resolution, and for determining a suitable tracer, it is possible to block the tracer in order to calculate the specificity: the blocking substance is the same ligand, but without the radioactive isotope (Figure 2).

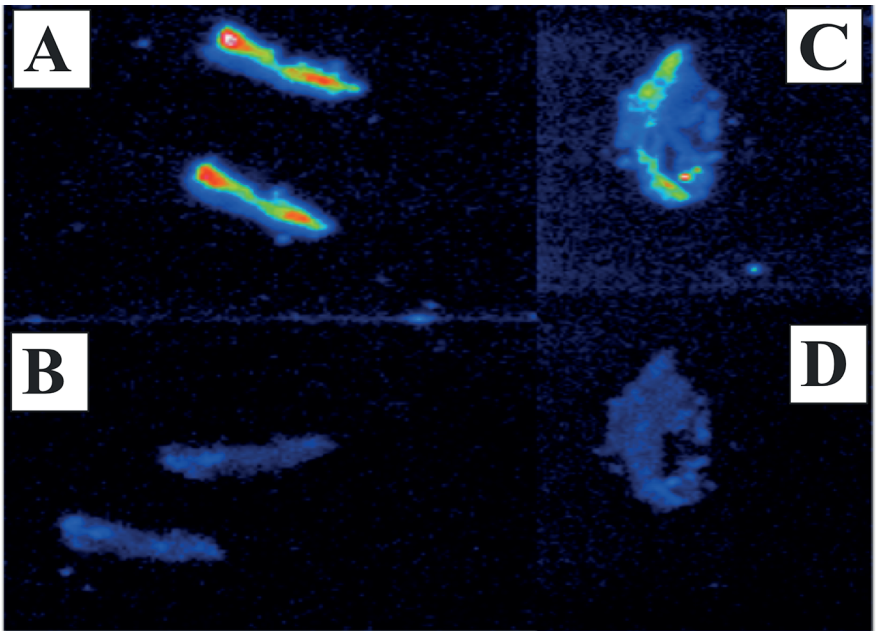


Figure 2. *Example of autoradiography from two patients: Patient 1: A and B sequential sectioned slices of AAA wall tissue incubated in radioactive isotope, B with additional blocking agent. Patient 2: C and D sequential sectioned slices of AAA wall tissue, D with blocking agent.*

Positron Emission Tomography – PET

Positron emission tomography is an advanced molecular imaging technique that uses γ-rays produced when the positrons annihilates with electrons: the γ-rays then generate an image. When the radioactive isotope linked to the tracer decays, it emits a positron, which when it annihilates with an electron produces two photons of 511 keV emitted in opposite directions 180° ± 0.25°.

History

Tracer

In 1934, Frederic Joliot and Irène Curie discovered artificial radioactivity by creating positron emitters of two "natural" elements, phosphorus-30 and nitrogen-13 (Joloit and Curie 1934): this lead to the rapid discovery of a number of nuclides of many elements, among them phopsphorus-32, ^{32}P.

György Hevesy, later Georg Charles de Hevesy or Georg Karl von Hevesy (1885 –1966), who is often called the father of nuclear medicine, recognized the same year the potential of isotopes in the study of natural metabolic processes. Together with Ole Chiewitz, Hevesy published the first paper

on the study of biological processes in life sciences with a man-made radionuclides, ^{32}P “Radioactive indicators in the Study of Phosphorus Metabolism in Rats” (Chiewitz and Havesy 1935). Hevesy was rewarded the 1943 Nobel Prize "for his work on the use of isotopes as tracers in the study of chemical processes" (Myers 1979) and "George de Hevesy – Biography" (Nobelprize.org 2013, a)).

PET camera development

The medical use of positron emission technology was developed in the 1950s and 60s by Brownell *et al* with scanners that initially produced planar images. In the late 1960s transaxial emission tomography images could be generated, and the Mark II scanner was developed by Kuhl and Edwards (Kuhl 1968 ; Schlyer 2004). In 1973 Phelps *et al* designed the first modern positron emission tomography with circular detectors (Nutt 2002).

In the 1970s, advanced computer tomography scans (CT) were fused with the PET scanners, and the first combined PET/CT scanner was introduced for clinical use in 1998 at the University of Pittsburgh, USA (Beyer 2000 ; Townsend 2001).

The PET Camera

PET technology is based on the imaging of the radiation from solid scintillation detectors. Various materials are used in the scintillation detector, of which the most commonly used are LYSO (lutetium yttrium oxyorthosilicate), LSO (lutetium oxyorthosilicate), and BGO (bismuth germinate), but they have different scintillation decay times, photon yields, linear attenuation coefficients, and energy resolutions. The detectors are arranged in photomultiplier tubes where the photons are converted to an electrical signal, and the tubes are arranged in multiple rings with a diameter of 80-90 cm (Saha 2010). This enables the PET camera to simultaneously investigate a larger area (Yamamoto 1984).

In order for a system to recognize annihilation (Figure 3) as a true signal, two detectors, positioned opposite to each other, have to record the event within a set time e.g. 12 nanoseconds. This is to be considered a true coincidence event (Kuncic 2011). The other types of coincidence are:

- Scattered coincidence, where one of the photons somehow interacts with the body before its detection;
- Spurious true coincidence, where a single photon is detected at the same time as the annihilation photon, both emitted from the same decay event;
- Random coincidence, where two annihilation photons emitted from separate decay events are detected by chance within the set coincidence time;

- Attenuation, when one or both photons are lost due to absorption or scattering inside the body (Figure 3) (Verel 2005).

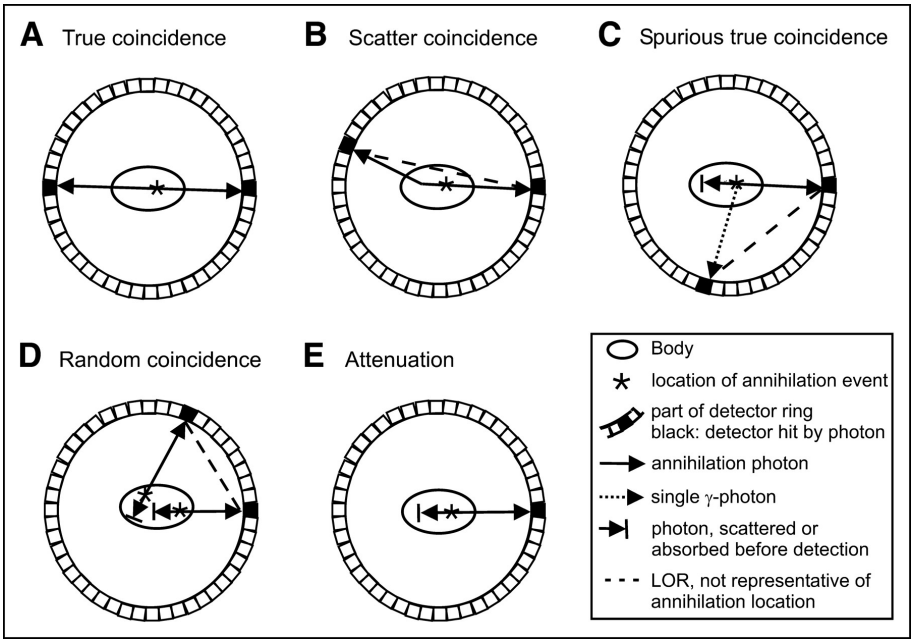


Figure 3. *Different types of coincidence of detected photon emissions (Verel 2005)*

The spatial resolution of current PET scans is limited for several reasons: the intrinsic resolution is set by the positron range i.e. the distance the positron travels before annihilation, which depends on the energy of the positron: the higher energy a positron has, the lower its resolution will be. The angle of the γ-ray, 180° ± 0.25°, will also have a negative effect on resolution due to the high speed that the positron and electron are moving in relation to the stationary detectors. Detector size is another limitation, as a smaller detector has better resolution (Yamamoto 1984).

Cyclotron

In the process of creating radioactive isotopes for PET tracers, there is a need for a particle accelerator, which can create a beam of charged particles towards a target i.e. the tracer. The cyclotron was first patented by Lawrence in 1934 (Lawrence 1934) and as the name indicates, the cyclotron, in contrast to linear particle accelerators, is designed as a spiral, which accelerates the particles from the centre outwards in order to obtain sufficient energy when colliding with the target. In order to accelerate the particles in a cyclotron, a high frequency altering voltage is applied between two "C"-shaped electrodes.

A complement, or an alternative to, a cyclotron for producing radioactive isotopes, is a $^{68}Ge/^{68}Ga$ radioisotope generator. In this case, a radioactive isotope with a long half-life ($t_{1/2}$), i.e. germanium, ^{68}Ge ($t_{1/2}$ 271 days), will decay to its short-live radioactive daughter gallium, ^{68}Ga ($t_{1/2}$ 68 min) via $^{68}Ge(p,2n)^{68}Ga$, and subsequently, to stable ^{68}Zn (Zhernosekov 2007 ; Palige 2011).

Tracers

The characteristics of radioactive tracers can be used for studying molecular and biological processes *in vivo*, without any disturbance them. The development of these tracers is an advanced, time consuming, costly, and multidisciplinary process, in which production chemists, analytical chemists, cyclotron engineers, physicists, biologists, computer experts, physicians, and others, work in collaboration.

Isotopes

There are several different suitable isotopes for molecular imaging techniques. Depending on the physiological event being investigated, an appropriate tracer and radioactive isotope has to be found. For instance, if rapid events such as the blood flow, blood volume, or the oxygen consumption are to be explored, an isotope with a short half-life is required. The oxygen isotope ^{15}O, with a $t_{1/2}$ of just two minutes is suitable for creating short-lived tracers of $H_2{}^{15}O$, $C^{15}O_2$, $C^{15}O$ and $^{15}O_2$ (Clark 1975 ; Hermansen 1998 ; Schlyer 2004). If the biological and physiological process is slower, a tracer with an isotope with longer half-life is required. Besides ^{15}O, the most frequently used radioactive isotopes for PET tracers are 13-nitrogen (^{13}N), 11-carbon (^{11}C), 18-flourine (^{18}F), 68-gallium (^{68}Ga), 124-iodine (^{124}I), and 76-Bromium (^{76}Br).

In both clinical medicine and medical research, the ^{18}F and ^{11}C isotopes are the two most frequently used isotopes for PET tracers.

Table 3. *Some properties of a few commonly used isotopes in PET (http://www.pet.ubc.ca/radioisotopes. url at University of British Columbia 2010)*

Isotope	Half-life (min)	Maximum energy (MeV)	Range in water (mm)
^{18}F	109.7	0.635	2.39
^{11}C	20.4	0.96	4.11
^{13}N	9.96	1.19	5.39
^{15}O	2.07	1.72	8.2

Flourine-18 (^{18}F)

The most common way of producing the ^{18}F isotope is to bombard enriched ^{18}O-water ($H_2{}^{18}O$), which naturally is only 0.2% in water, with protons from

a cyclotron; the yield is ^{18}F and a neutron. The nuclear reaction is expressed as ^{18}O(p,n)^{18}F (Cogneau 1999 ; Hess 2001).

The half-life of ^{18}F is 109,77 minutes and the decay is by β^+ mode (97%), which means the nucleus converts to its next-lower neighbour on the periodic chart, while emitting one positron (e^+) and one electron neutrino (v_e) (3%), a subatomic lepton elementary particle with no net electric charge (Formula. 1). 18-fluorine converts to stable 18-oxygen.

$$^{18}F \rightarrow {}^{18}O + e^+ + v_e + 0.69\ MeV$$

Formula 1. *The decay reaction of 18-flourine*

The positron moves a few mm (2.39 mm in water (Table 3) (Sossi 2010)), depending on the low decay energy (with lower energy, the distance is shorter), before it collides with an electron (e^-) and annihilates. When annihilation takes place, the rest energy of the positron and the electron, each 0.511 mega electron volt (MeV) is converted into two gamma rays (γ) moving in opposite directions with the same energy, 0.511 MeV each (Formula 2, Figure 4).

$$e^+ + e^- = \gamma + \gamma$$

Formula 2. *Two photons are emitted when a positron and an electron annihilates to produce two gamma rays.*

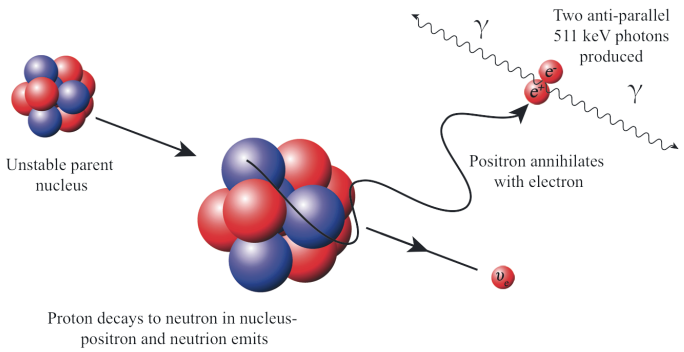


Figure 4. *Schematic illustration of β^+ decay and annihilation of the positron*

Carbon-11 (^{11}C)

Although carbon-11 can be produced in various ways, the most common method in medical settings is by proton bombardment of natural nitrogen through the ^{14}N(p,α)^{11}C nuclear reaction (Perris 1974 ; Christman 1975 ; IAEA 2001). With a target gas mixture of 2% oxygen in nitrogen, the proton bombardment will produce radioactive carbon dioxide, $^{11}CO_2$, and with a gas mixture of 5% hydrogen in nitrogen it will produce methane, $^{11}CH_4$. Carbon monoxide, ^{11}CO, can be produced through reduction of $^{11}CO_2$ on activated charcoal at 900°C.

^{11}C is a commonly used isotope in PET and has a half-life of 20.38 minutes, and a clean decay mode with a high proportion of β^+: the decay in β^+ mode is up to 99.79%, and the rest, 0.21%, is in electron capture (K-capture) (Campbell 1967). The radioactive isotope carbon-11 converts into the stable isotope boron-11 (Formula 3).

$$^{11}C \rightarrow {}^{11}B + e^+ + v_e + 0.96\ MeV$$

Formula 3. *The decay reaction of 11-carbon.*

The decay energy (0.96 MeV) is low: even though ^{18}F is lower (0.64 MeV). This means the collision between the positron and the electron is near the actual receptor, which gives acceptable distortion in the images. The range in water for the positron from ^{11}C is 4.11 mm (Table 3) (Sossi 2010).

The use of ^{11}C as a radioactive element in positron emission tracers can be advantageous. For example, carbon is common in biological molecules, therefore, a large variety of substances are available when designing tracers, and different characteristics of the tracer can be obtained, depending on which natural carbon atom is replaced by a radioactive one.

In clinical research, one disadvantage of ^{11}C is the short half-life of 20 minutes, which means the tracer has to be produced at the same facility that the PET camera is situated. In contrast, ^{18}F labelled tracers (t½ 110 min) can be produced at one site and then distributed by car or by plane to other regions, or even countries, to PET institutions without their own cyclotron. The number of investigations that can be done with one batch of tracer also differs: two or three patients may be examined with one batch ^{11}C labelled tracer, whereas, more than 10 investigations may be performed with an ^{18}F tracer batch.

Half-life

Radioactive half-life, defined by International Union of Pure and Applied Chemistry (IUPAC), is the time required for a single radioactive decay process to decrease the activity to half of its activity by that process (Figure 5) (de Bruin 1982). Thus half-life is expressed as the time by which the activity has decreased to half (Equation 2). The reason for defining half-life is because it is constant for a certain radioactive substance, independent of time and amount of the substance.

$$N(T) = N(0) \times 2^{-T/t_{1/2}}$$

Equation 2. *The activity of the particles (N) at the time (T), where: $t_{1/2}$ is half-life, and N(0) the activity (roughly = the number) of the particles at the beginning of the study.*

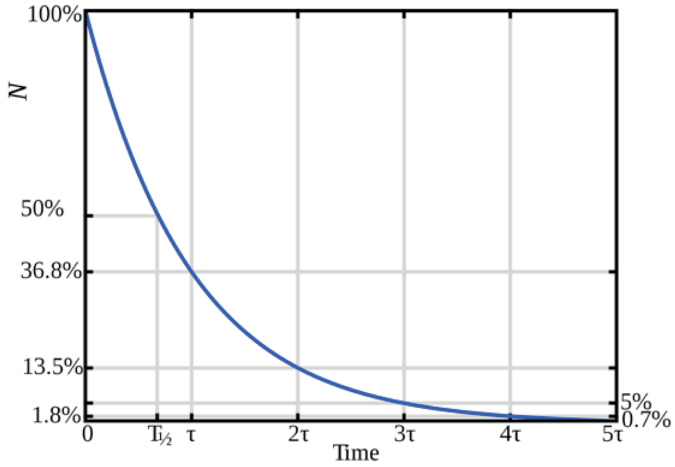


Figure 5. *The radioactivity as a function over time. $t_{½}$ 1/2= ln(2) * τ.*
τ is the mean life i.e. the average lifetime of a radioactive nuclide (de Bruin 1982).
N is the remaining radioactivity in % of the initial value, 100%.

Thus, after one $t_{1/2}$, only half of the initial activity remains, after two $t_{1/2}$, 25% of the activity remains, and after three $t_{1/2}$, 12.5% of the activity is still remains (Figure 6). For example, ^{11}C has a half-life of 20 min, so after 60 min only 12.5% of the activity remains, whereas, ^{18}F with $t_{1/2}$ ≈110, min as much as 70.5% of the activity remains after one hour (Equation 3).

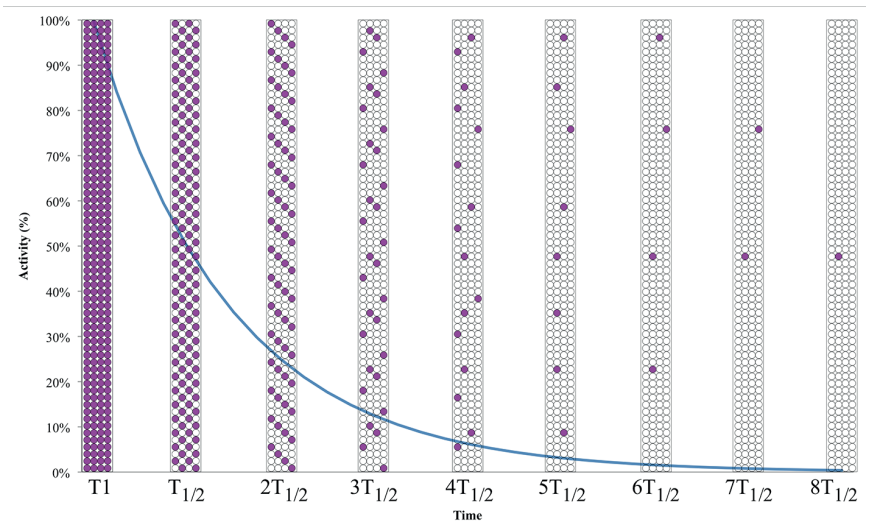


Figure 6. *A simplified illustration of half-life over time, starting with 256 dots.*

$$RF = e^{(-0.693/t_{1/2}) \times time}$$

Equation 3. *How to calculate the remaining fraction (RF) of activity after a certain time.*

Idea and Inspiration for this Thesis

The inspiration for this thesis included the promising study on AAA with FDG-PET in 2002 by Dr Sakalihasan and colleagues, the fact that we knew of the chronic inflammation in the aneurysms, and the interesting findings by in 2005 by Dr Wanhainen and colleagues of high sensitivity CRP being identified as a risk factor.

In the initial planning stage of the thesis, it was intended to include magnetic resonances (MR) as an additional modality for investigating the inflammation in AAA. The two first patients underwent both PET/CT and MR, however, difficulties with the MR protocol and lack of uptake meant the use of MR had to be abandoned, and the focus was directed at PET technology.

Aims of this Thesis

The overall aim of this thesis was to investigate the pathophysiology of asymptomatic small and large AAA by means of molecular imaging.

The specific aims were:

1) To determine whether an *in vivo* increased glucose metabolism is detectable with ^{18}F-FDG-PET/CT, in small and large asymptomatic AAA, and whether this correlates with histological findings (Paper I).

2) To evaluate the *in vivo* uptake of PET/CT radio ligand ^{11}C-PK11195 (suggested signalling for inflammation) in the wall of small and large asymptomatic AAA, and how this correlates with histological findings (Paper II).

3) To evaluate the *in vivo* uptake of PET/CT radio ligand ^{11}C-D-deprenyl (suggested signalling for inflammation) in the wall of small and large asymptomatic AAA, and how this correlates with histological findings (Paper II).

4) To evaluate potentially useful PET tracers for the study of asymptomatic AAAs. A number of tracers, originally intended for use in other disorders, were evaluated in vitro by means of autoradiography (Paper III).

5) To study the relevance of angiogenesis in the pathogenesis of AAA *in vitro* with ^{18}F-Fluciclatide autoradiography and immunohistochemistry targeting the $\alpha_V\beta_3$ integrin-receptor (Paper IV).

Materials and Methods

Patients and Tissues

Papers I and II

All patients were recruited from the AAA surveillance program at the Uppsala University Hospital, Sweden, between January 2006 and December 2009. The inclusion and exclusion criteria are listed in Table 4. None of the patients had increased growth rate of the AAA (> 0.5 mm/6 month or > 10 mm/1 year).

Table 4. *Inclusion and exclusion criteria.*

Inclusion criteria	**Exclusion criteria**
Male gender	Female gender
Age 51 to 75	Age ≤ 50 or > 75
Suitable for open repair	Dementia
AAA ≥ 35 mm	Malignant disease
An infrarenal aortic diameter >50% than suprarenal aortic diameter at ultrasound	rAAA, symptomatic, inflammatory or mycotic AAA
	Thoracoabdominal AA
	Severe heart and/or pulmonary disease
	Severe renal failure
	Systemic inflammatory disease e.g. rheumatoid arthritis
	On-going treatment with corticosteroids or immunosuppression
	Insulin dependent diabetes (Type I) *(Paper I only)*

Paper I

Twelve men with asymptomatic AAA were included. Seven patients had large aneurysms (52-66 mm) scheduled for open repair, and five patients had small aneurysms (34-40 mm) under surveillance. A control group of 13 age-matched men without aneurysms were identified, after been clinically examined with FDG-PET/CT for other diseases with an identical protocol as that used for this study. The baseline characteristics in the AAA groups, (small and large), are presented in Table 5.

Table 5. *Baseline characteristics of the AAA patient groups scanned with ^{18}F-FDG. *median (range).*

	Small AAA n=5	**Large AAA n=7**
Age (*years*)*	65 (65-66)	67 (65-75)
Aneurysms size (*mm)**	36 (34-40)	58 (52-66)
Smoking habits (*n*), former/current	2/3	4/3
Hypertension	3	5
Treatment with ASA/Statins	1/0	4/1
Days from PET to surgery*		43 (21-56)

Paper II

A total of fifteen men were included in the study between November 2006 and December 2009. Five were scanned with ^{11}C-PK11195 PET/CT: four with large asymptomatic AAA (range 58-66 mm) scheduled for surgical repair and one with a small (35 mm) AAA under surveillance. Ten were investigated with ^{11}C-D-deprenyl PET/CT: five with large AAA (range 54-64 mm) and five with small AAA (range 35-44 mm) (Table 6).

Table 6. *Baseline characteristics of the AAA patient groups scanned with ^{11}C-PK11195 and ^{11}C-D-deprenyl. *median (range).*

	^{11}C-PK11195		**^{11}C-D-deprenyl**	
	Small AAA n=1	Large AAA n=4	Small AAA n=5	Large AAA n=5
Age (*years*)*	65	66 (65-75)	65 (60-65)	66 (65-71)
Aneurysms size (*mm)**	35	61 (58-66)	41 (35-44)	56 (54-64)
Smoking habits (*n*), non/former/current	0/1/0	0/2/2	1/1/3	3/1/1
Hypertension	1	4	5	3
Treatment with ASA/Statins/ASA & Statin	0/0/1	1/1/0	1/1/2	0/1/2
Days from PET to surgery*	-	46 (21-56)	-	5 (1-56)

Paper III

The AAA tissues investigated with autoradiography were obtained from three male patients (age 65-74 years) who underwent open surgery for asymptomatic AAA (diameter 52-66 mm). One patient was a former smoker and two were current smokers. The tissue was covered with Tissue-Tek® OCT™ in a small tube and quickly frozen in a mixture of isopentane and dry ice and stored at –70°C.

Paper IV

The AAA tissue was obtained from five male patients, (age 65-73 years) who had open surgery for asymptomatic AAA. The normal, non-aneurysmatic infrarenal aorta was obtained from five male organ donors (age

49-69 years). Due to patient confidentiality, no further information about the patients was available. The tissue samples were transported dry on ice to the Department of Pathology, where they were covered with Tissue-Tek® OCT™, fresh frozen in a mixture of isopentane and dry ice, and stored at –70°C.

Comments

During enrolment of patients in Paper I and II, a parallel study was running at the Vascular Department (investigation on postoperative myocardial infarction in AAA patients undergoing open repair), which meant only every second suitable patient was enrolled.

For the AAA samples in Paper IV, the maximum time until delivery at the pathology laboratory was a few hours. For the normal aortic tissue, the time until arrival at the Department of Pathology for most cases was 3-6 hours, however, in some instances, it was up to a of 60 hours, if the transplant surgical team operated on Friday night, as the Department of Pathology is only open during office hours. For the AAA samples, the maximum time until delivery at the pathology laboratory was a few hours. For the normal aortic tissue, the time until arrival at the Department of Pathology for most cases was 3-6 hours, however, in some instances, it was up to a of 60 hours, if the transplant surgical team operated on Friday night, as the Department of Pathology is only open during office hours.

PET/CT Protocol

Papers I and II

The scanner used was a GE Discovery ST16 (General Electric, Waukesha, WI, USA). A low-dose CT scan without contrast media enhancement was acquired before the PET scan and used for anatomic correlation and attenuation correction.

Before the tracer injection, the patients fasted 6 h (Paper I) and 4 h (Paper II). Static PET images were acquired 1 h (Paper I) and 30 min (Paper II) after an intravenous injection of 5.0 MBq/kg body weight of the tracers ^{18}F-FDG (Paper I), or ^{11}C-PK11195, or ^{11}C-D-deprenyl (Paper II). For the ^{18}F-FDG scan, this was the recommended dose for whole-body studies for the scanner type and reconstruction protocol issued by the European Association of Nuclear Medicine (EANM). The scan covered the entire extent of the aorta.

From 0-25 minutes after the tracer injection, additional dynamic scans covering the AAA areas were taken on three patients studied with ^{11}C-PK11195 and all ten patients investigated with ^{11}C-D-deprenyl (Paper II).

Autoradiography protocol

Paper III

The autoradiography setting are displayed in table I in Paper III

Paper IV

Sections of AAA tissue, 20µm thick, were incubation bath containing 13 nM [^{18}F]fluciclatide, in the absence or presence of 0.56 µM of unlabeled fluciclatide for the assessment of non-specific binding. The samples were exposed to phosphor imaging plates for 18 hrs. An aliquot, 20 µl, of the incubate was pipetted onto filter paper and exposed together with the slides for quantification purposes. The plates were scanned in a Phosphor Imager Model 400S using 100 µm pixel width (Molecular Dynamics, USA) and the digital images analysed using software ImageQuant 5.1 (Molecular Dynamics). Regions of Interest (ROIs) were drawn manually on the digital images, outlining the aortic tissue and average pixel values were calculated, which then were transformed to molar amounts of bound [^{18}F]fluciclatide to the aortic sections.

PET Tracers

The various radioactive tracers used in the four papers are listed below:

^{18}F-FDG – Paper I

In clinical PET, ^{18}F-2-deoxy-2-fluoro-D-glucose (^{18}F-FDG) is the most widely used tracer, the real workhorse. ^{18}F-FDG is a glucose molecule labelled with a fluorine isotope, ^{18}F, which is placed on the second position in the glucose molecule and substitutes for a hydroxyl group (Figure 7).

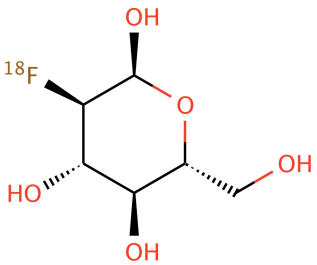


Figure 7. *The 2-deoxy-2-(^{18}F)-fluoro-D-glucose molecule, ^{18}F-FDG.*

The ^{18}F-FDG molecules enter cells by facilitated diffusion through the glucose transporter proteins, GLUT. Inside the cells, the ^{18}F-FDG is phosphorylated by hexokinases to ^{18}F-FDG-6-phosphate, which prevents it from diffus-

ing out of the cell. Normally, glucose-6-phosphate is glycolised into pyruvate but with the flourine-18 isotope located on the ´2 carbon atom this is not possible. Thus the ^{18}F-FDG-6-phosphate formed when ^{18}F-FDG enters the cell cannot enter the citric acid cycle and move out of the cell before radioactive decay. The ^{18}F decays to $^{18}O^-$ that picks up a proton and transforms the radioactive ^{18}F-FDG-6-phosphate into normal glucose-6-phosphate.

The true nature of ^{18}F-FDG as a ligand could be argued, as it does not actually target a specific receptor, but enters the cells through the membrane proteins GLUT. Therefore, ^{18}F-FDG is not particularly suitable for *in vitro* experiments such as autoradiography, even though it may be used (Marini 2012).

The ^{18}F-FDG tracer can be used to show the glucose metabolism, which makes it suitable for imaging malignant lesions due to the high glucose consumption in these cells. In oncology, ^{18}F-FDG has become a routine diagnostic tool (Tyler 2000). The body recognizes ^{18}F-FDG as normal glucose, thus inflammatory processes, which also have a high glucose turnover, can be detected with ^{18}F-FDG (Love 2004 ; Keidar 2008 ; Kumar 2008 ; Makis 2009). In addition, ^{18}FDG PET is recognized as a valuable tool in the diagnosis and follow-up of the challenging, and often life threatening, problems with vascular graft infections (Krupnick 2003 ; Fukuchi 2005 ; Balink 2007 ; Tegler 2007 ; van Assen 2007 ; Choi 2008 ; Wassélius 2008 ; Spacek 2009 ; Bruggink 2010 ; Kragsterman 2011).

The first study on ^{18}FDG PET and AAA, in a mixed group of 26 patients with large, inflammatory aneurysm, aneurysms with rapid expansion rate, or symptomatic aneurysm was published by Sakalihasan *et al* published in 2002 (Sakalihasan 2002). Later studies have detected increased ^{18}F-FDG uptake in similar aneurysms (Wilkinson 2003 ; Sakalihasan 2004 ; Defawe 2005 ; Reeps 2008 ; Truijers 2008 ; Kotze 2009 ; Kotze 2011 ; Sarda-Mantel 2012). However, a study from Italy failed to detect increased ^{18}F-FDG uptake among 40 patients with asymptomatic large (48-54 mm) AAA (Palombo 2012).

^{11}C-PK11195 – Paper II

The ligand PK11195 was developed in the early 1980s, and Le Fur *et al* have showed that it had binding sites in the heart, kidney, adrenals, platelets, and brain in rat (Le Fur 1983). Originally, PK11195 was called peripheral benzodiazepine receptor (PBR), as well as the ω_3 receptor.

Further research has shown that the name peripheral benzodiazepine receptor is misleading for several reasons; PBR is not only located peripherally, but also in the central nervous system (CNS), the term benzodiazepine is imprecise as other ligands bind to PBR, the term binding sites is too general, and finally, the term receptor may be misleading, as the current understanding of its physiological nature is not fully understood. Thus, Papadopoulos *et*

al suggested the use of a more exact term, translocator protein (18kDa), (TSPO) (Papadopoulos 2006); however, there are several PBR with various affinities, for example PBR06, PBR28, PBR111.

Macrophages have been shown to express TSPO-receptor (Zavala 1984 ; Zavala 1987). Vasculitis in large vessels was visualised using ^{11}C-PK11195 (Figure 8) (Pugliese 2010) as well as active knee rheumatoid synovitis (van der Laken 2008); both inflammatory conditions where activated macrophages and T lymphocytes play a key role.

Bird *et al* showed high uptake of [^{3}H](R)-PK11195 in autoradiography *in vitro* experiments on human carotid artery plaques, suggesting an enhanced regulation of the TSPO (Bird 2010).

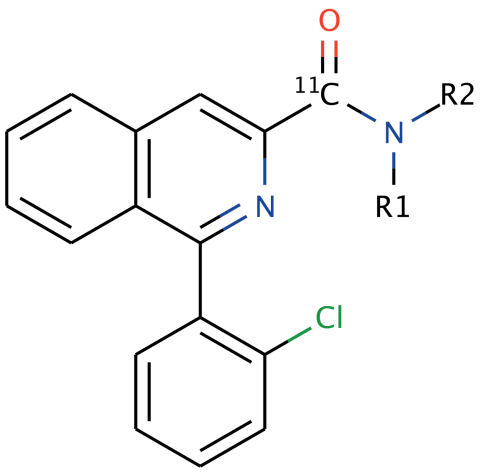


Figure 8. *The molecular structure of ^{11}C-PK11195.*
R1 = sec-butyl, isopropyl, propyl, allyl or phenyl; R2 = methyl, H, propyl or allyl.

^{11}C-D-deprenyl – Papers II and III

Deprenyl, also known as selegilin, was first introduced in the 1970s and enhanced the effects of levodopa in the treatment of advanced Parkinson's disease (Lees 1977 ; Csanda 1978). Deprenyl, a monoamine oxidase B (MAO-B) inhibitor, decreases the degradation of dopamine and reduces the reuptake of dopamine.

The exact binding mechanisms of the [N-methyl-^{11}C]-D-deprenyl tracer are unknown and to study the exact targeting of the deprenyl molecule, it needs to be radiolabelled as MacGregor did, while investigating mice and Freedman later in man (MacGregor 1985 ; Freedman 2005). However, in these studies, the L-isomer of the deprenyl was used. L-deprenyl deactivates the enzyme when binding irreversibly to the MAO A and B, MacGregor *et al* labelled its enantiomer (Figure 9) the D-deprenyl with ^{11}C to produce a better tracer accepting lower biological activity (MacGregor 1988). Thus the

D-deprenyl has been labelled with ^{11}C for PET studies, primarily of the brain (Fowler 1987). A comparison of brain uptake and retention of ^{11}C-labeled inactive (D-) and active (L-) enantiomers of deprenyl indicated rapid clearance of the inactive enantiomer and retention of the active enantiomer within MAO B-rich brain structures, which is agreement with the known stereoselectivity of MAO B for the L-isomer, L-deprenyl.

In clinical studies, the deprenyl tracer may have an ability to disclose inflammatory conditions (Danfors 1997 ; Linnman 2011). In the inflammatory activity in rheumatoid arthritis, there is a significant uptake of ^{11}C-D-deprenyl in patients with untreated inflammation in the knee joint, which decreases after intra-articular glucocorticoid treatment (Danfors 1997).

An increased uptake has also been observed in the neck of patients with chronic whiplash associated disorder (WAD), especially in the area around the spinous process of the second vertebra (Linnman 2011).

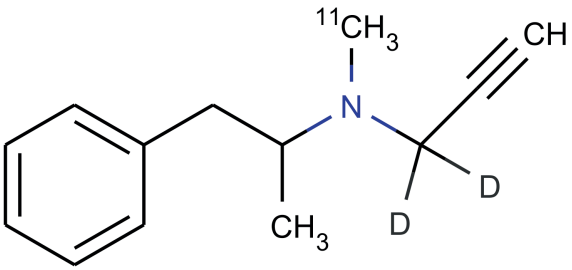


Figure 9. *The chemical structure of D-deprenyl.*

^{68}Ga-CRP-binder — CRPB – Paper III

CRPB, ^{68}Ga-CRP-binder, is a polypeptide with a phosphocholine group that has moderate affinity to C-reactive protein (CRP) (K_D = 5µM) (Christopeit 2009). CRP is a protein that can be detected in plasma as a response to inflammation and infections (Black 2004). With a radio labelled polypeptide targeting CRP, it is hypothesized that the tissue responsible for the inflammation will be visualized *in vivo*.

^{11}C-DAA1106 — DAA – Paper III

DAA, ^{11}C-DAA1106 (Figure 10), is a ligand that targets the translocator protein (18kDa), TSPO, which is expressed on macrophages (Zavala 1984 ; Vowinckel 1997), and in the brain in a variety of conditions, such as after stroke, (Pappata 2000) in multiple sclerotic plaques (Vowinckel 1997 ; Banati 2000), in dementia (Cagnin 2001), and in refractory epilepsy (Goerres 2001). DAA has higher affinity to the TSPO than the commonly used tracer ^{11}C-PK11195 (Maeda 2004 ; Pugliese 2010).

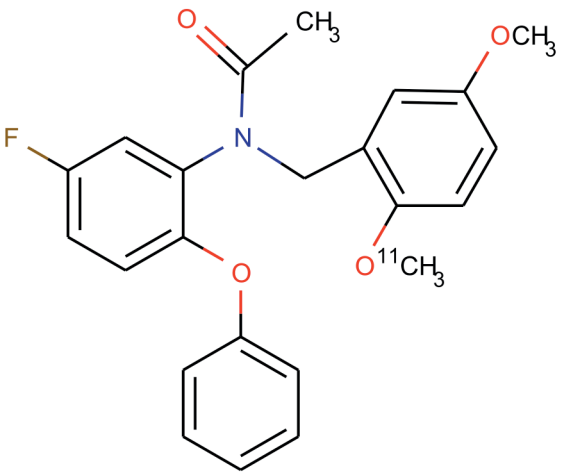


Figure 10. *Chemical structure of the TSPO tracer ^{11}C-DAA1106.*

^{11}C-Di-deuterium-L-deprenyl — DED – Paper III

DED, ^{11}C-di-deuterium-L-deprenyl, (Figure 11) is based on the monoamine oxidase type B (MAO-B) inhibitor L-deprenyl, and has a high affinity and specificity for the enzyme. Thus, selectively and irreversibly inhibiting MAO-B (Fowler 1987 ; Fowler 2005). The tracer has been di-deuteriated in order to minimize the metabolism of the compound (MacGregor 1988 ; Fowler 1995). As the ligand targets astrocytes, it has been used in the diagnosis of epilepsy (Kumlien 1995 ; Kumlien 2001), and in additional investigations of astrocytic invasion (Carter 2012) in patients with Alzheimer's disease, along with the amyloid-β specific tracer ^{11}C-Pittsburgh compound B — ^{11}C-PIB (Klunk 2004).

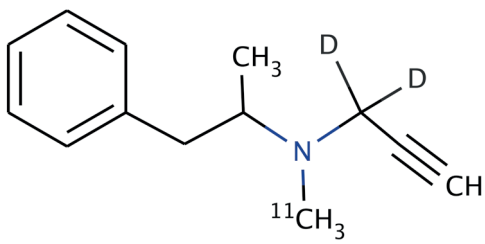


Figure 11. *The chemical structure of ^{11}C-deuterium-L-deprenyl.*

^{68}Ga-IMP461 and bispecific antibody TF2 052107 — TF2-IMP – Paper III

TF2-IMP is a bispecific antibody. TF2 052107 is a combined pre-targeting antibody for carcinoembryonic antigen (CEA), and has a specific target site for the PET tracer called ^{68}Ga-IMP461 (Figure 12) (Hall 2012, a)). The hapten IMP461 (NOTA-D-Ala-D-Lys(HSG)-D-Tyr-D-Lys (HSG)-NH2) was obtained from Immunomedics Inc., Morris Plains, NJ, USA.

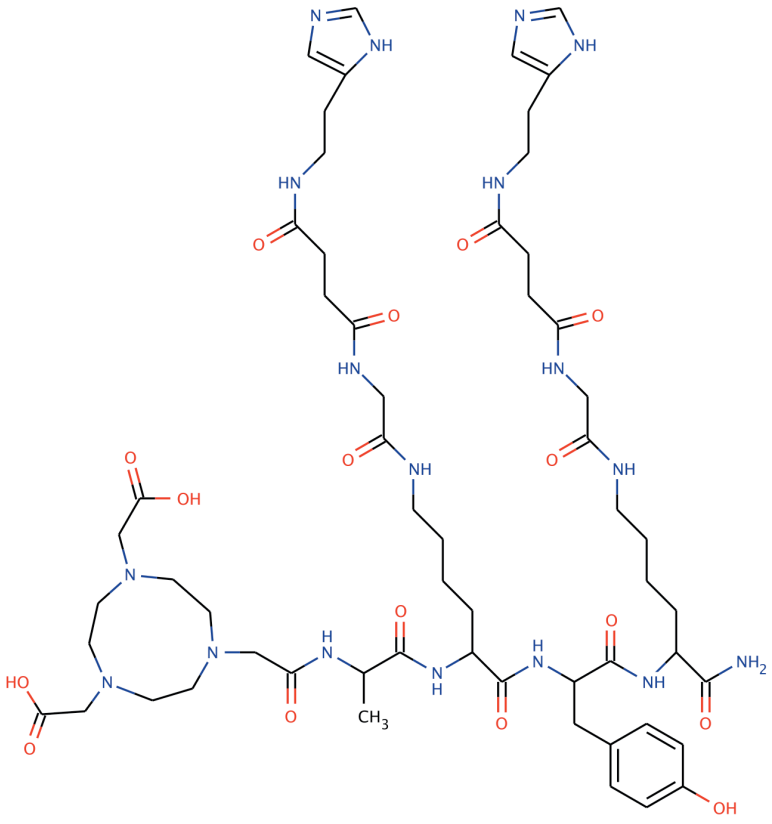


Figure 12. *The chemical structure of IMP461.*

[^{18}F]F-metomidate — FMTO – Paper III

FMTO, [^{18}F]F-Metomidate, (Figure 13) is an analogue to the more commonly used ^{11}C-metomidate. FMTO is useful in the visualization of the adrenal glands and their tumours such as incidentalomas, adenomas, and primary and metastatic cortical carcinomas (Bergström 1998 ; Ettlinger 2006 ; Erlandsson 2009).

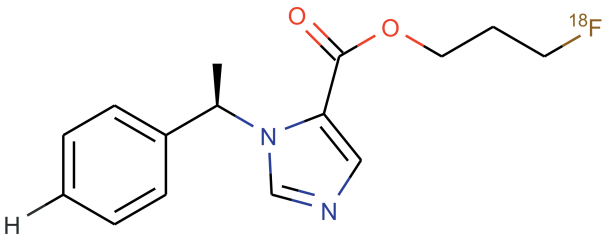


Figure 13. *Chemical structure of [^{18}F]F-Metomidate.*

^{18}F-vorozole — FVOZ – Paper III

FVOZ, ^{18}F-vorozole, (Figure 14) 6-[(4-chlorophenyl)(1,2,4-triazol-1-yl)meth- yl]-1-methylbenzotriazole, is a selective and potent non-steroidal aromatase inhibitor (Wouters 1993). Aromatase is an enzyme that converts androgen to estrogens. Aromatase inhibitors are used for treating postmenopausal women with early-stage or advanced, hormone-sensitive breast cancer. It has previous been labelled with the radionuclide ^{11}C (Lidström 1998) and used in the visualization of the aromatase distribution in the brain, especially the amygdala (Takahashi 2006). To be able to provide PET centres without on-site cyclotron capacity, ^{18}F-vorozole, FVOZ, has been synthesized (Hall 2012, b)).

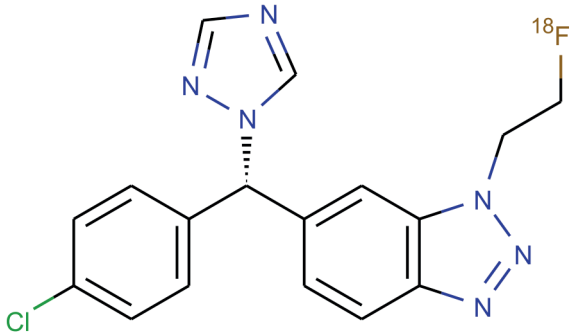


Figure 14. *Chemical structure of ^{18}F-vorozole.*

^{18}F-Fluciclatide — FAN – Papers III and IV

^{18}F-Fluciclatide, formerly named ^{18}F-AH111585 (Figure 15), was developed by GE Healthcare, to target the integrin $\alpha_V\beta_3$ located within many cell adhesion proteins. The integrin class of cell surface adhesion receptors comprises a universal and well-understood adhesion receptor-ligand system (Hynes 1992). Integrin $\alpha_V\beta_3$, also known as the vitronectin receptor, is expressed on macrophages (Bishop 2001), where it appears to be an expression rather than being involved in the activation of the macrophages (Gordon 2003 ; Antonov 2004); platelets (Coller 1991); osteoclasts (Horton 1997 ; Zheleznyak 2012); endothelial cells (Conforti 1992); malignant cells, such as malign melanoma

and breast cancer (Montgomery 1994 ; Kenny 2008) as well as signalling for, and being up regulated in angiogenesis (Brooks 1994 ; Morrison 2009).

However, the role of the integrins in angiogenesis has been questioned, and there is a suggestion they may have an opposite effect (Sheppard 2002). In β_3 and β_5 knockout mice, both "normal" tumour genesis and increased tumour development have been found, with an enhanced angiogenesis in these tumours (Reynolds 2002).

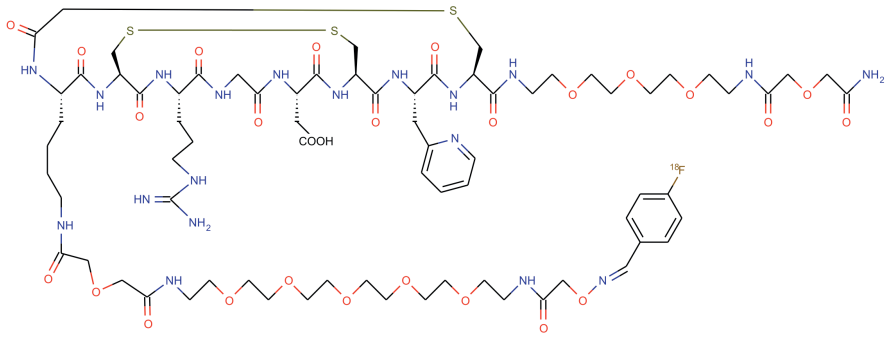


Figure 15. *Chemical structure of ^{18}F-Fluciclatide.*

Standardized Uptake Value – SUV

^{18}FDG PET imaging is used in the exposure of tumours, localized infections and inflammation. The initial step in the diagnosis is visual evaluation of the images. In order to measure and evaluate therapy response, quantitative and semi-quantitative methods are also used: one semi-quantitative technique is Standardized Uptake Value (SUV) (Lucignani 2004).

Papers I and II

Regional activity was measured as SUV normalized for differences in injected dose and body weight. SUV_{mean} and SUV_{max}, the average and maximum pixel activity within the selected area and volume, were calculated with Equation 4. An assumed density of 1 g/mL provides a unitless index of regional activity compared with the average for the whole body.

$$SUV = regional\ activity\ \left(\frac{Bq}{ml}\right) \times \frac{Body\ Weigh\ (g)}{Total\ injected\ dose\ (Bq)} = \frac{g}{ml}$$

Equation 4. *Calculating the SUV, which is unitless as the density is assumed to be 1 g/ml.*

Volumes of interest (VOI) were constructed with the software VOIager, a research tool for PET image analysis developed for internal use at the PET-

Centre, Uppsala, Sweden. In Paper I, SUV data was collected with the software VOIager version 2.0.5 and in Paper II with VOIarger version 4.0.7.

VOI were defined on axial projections of the CT, where VOI of the vessel only contained the vessel wall. The VOI of the aneurysm wall were compared with the VOI of the suprarenal aorta, blood, and liver (Figure 16).

With the VOIager tool, the overlapping of the images from both, the PET scans and the CT scans, can be altered, so that one or the other may be seen in 100% while the other is in the background. As there was no visual uptake on the scans and the structure of interest was the vessel wall, the VOI were constructed on the maximum intensity of the attenuation CT at its maximum intensity. However, the attenuation CT is not as detailed as an ordinary diagnostic CT scan, and the scans were taken without intravenous contrast, which made the plotting of the VOI sometimes rather difficult.

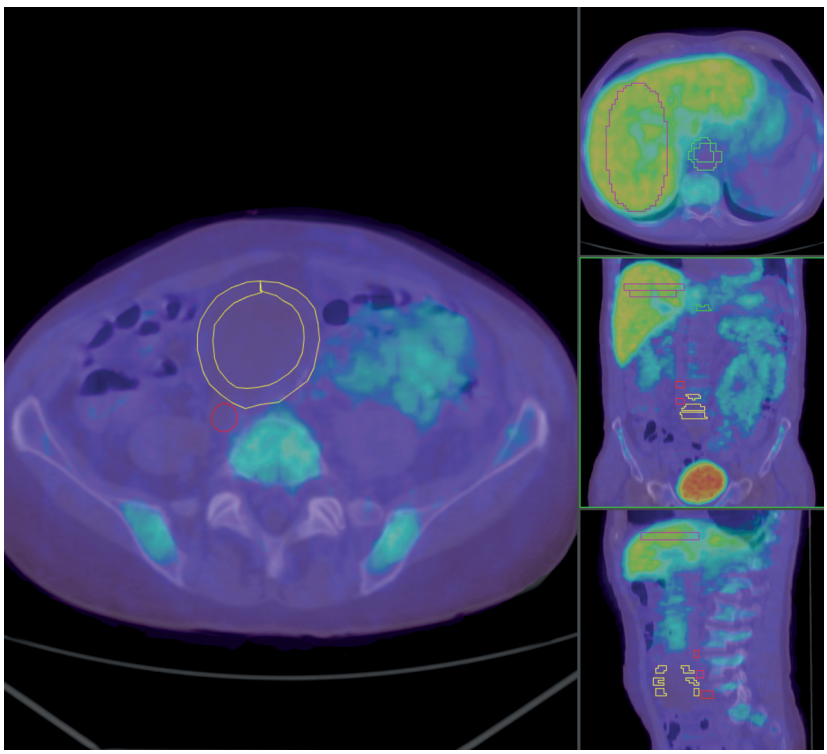


Figure 16. *Example of the placement of the VOI when calculating SUV. VOIarger version 4.0.7.*

Partial Volumes Effect

Due to the limitations in resolution and movements in the body while the scan is done, objects smaller than twice the resolution of the scanner show partial loss of intensity, whereas the activity in a larger object appears

smeared over a larger area. This underestimation of small objects and over-estimation of large objects is termed partial volumes effect (Saha 2010), and has a prominent impact on smaller objects (Figure 17).

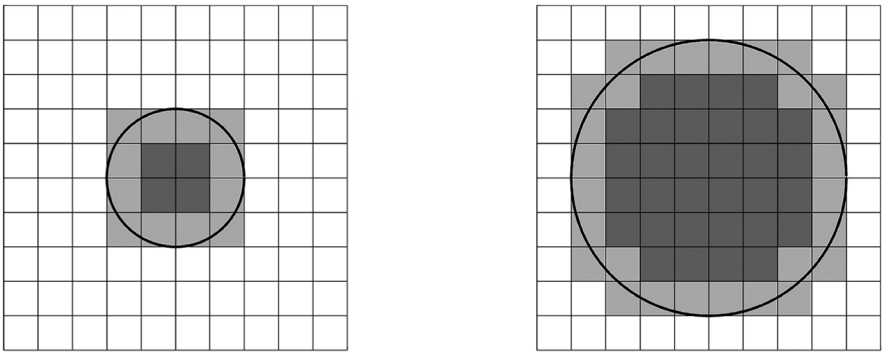


Figure 17. *Schematic presentation of the phenomenon: partial volumes effect. The partial volumes effect illustrated is more pronounced for a smaller object than for a larger object, i.e. the proportion of light areas is much higher for the smaller object, compared to dark areas (Vos 2011).*

The resolution differs between techniques; on CT scans the resolution is set at 512 by 512 pixels, whereas, the resolution on PET scans is set at 128 by 128 pixels. Factors limiting the resolution in PET, and setting the two-point discrimination ability of the scanner (Figure 18), include the geometric size of the detectors; the non-collinearity of annihilation photons (180° ± 0.25°), resulting in a spread ≈ 0.5%, and the β^+ energy of the isotope, which with at higher energy levels results in blurring of the image (Budinger 1998).

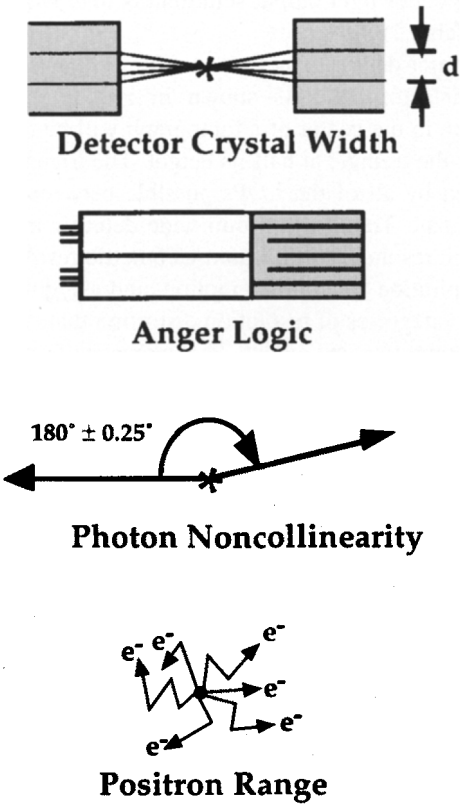


Figure 18. *The four considerations that determine the resolution in PET imaging (Budinger 1998)*

For the whole-body scans performed in this investigation, the field of view (FOV) was set at 50 cm, which with the resolution of 128 by 128 pixels rendered each pixel 3.9 by 3.9 mm, and with the detector width of 47 cm the depth of each voxel was 3.6 mm, resulting in anisometric voxels.

Histological Protocol

Papers I and II

In the study protocol, biopsy specimens were to be taken from areas with positive tracer uptake and/or from the anterior wall of the aneurysm. Samples, including the full thickness of the anterior aneurysm wall, were collected during surgery in the group of patients with large AAA. The samples were immediately fixed in formaldehyde in the operating theatre. Then, processed for histology by being cut in 4 μm thin slices and stained.

The intensity of inflammation was evaluated on routine hematoxylin and eosin (HE) stained slices. For further categorization and quantification of the inflammation, immunohistochemical (IH) staining was used with antibodies against lymphocytes (CD3), T-lymphocytes (CD4 and CD8), B-lymphocytes (CD20), and macrophages and leukocytes (MAC387).

The inflammatory infiltrate was analysed on the entire section and graded according to the Matthias Bechtel score (Matthias Bechtel 2003) (Table 7). The cells positive for MAC387 were calculated with a 10-fold greater magnification within 1 mm^2 and graded according to a modified macrophage and leukocyte infiltration score (Koch 1990) (Table 7).

Table 7. *Histological criteria for grading the inflammatory infiltrate. The overall inflammation is calculated on the entire section. Macrophage/leukocyte infiltration is calculated in 10 powers magnification on a 1 mm^2 square.*

	Overall inflammation
Grade 0	No inflammation
Grade 1	Sparse scattered chronic inflammatory cells or an occasional small focus of inflammatory cells
Grade 2	Multiple small foci of inflammatory cells
Grade 3	Multiple large foci of inflammatory cells or a diffuse, heavy inflammatory cellular infiltrate
	Macrophage/leukocyte infiltration (Macrophage/neutrophils) (PaperII)
Grade 0	No macrophages or leukocytes
Grade 1	0 to 20 cells
Grade 2	21 to 50 cells
Grade 3	>50 cells

Paper III

No histological or immunohistochemical analyses were undertaken on these specimens.

Paper IV

Serial 4 µm cryostat sections were dried for 15 minutes, and then fixed for 5 minutes in neutral buffered formalin. After rinsing in distilled water the sections were stained with hematoxylin-eosin (HE) and van-Gieson (VG) for conventional histopathological examination. Immunohistochemistry was optimized through an antigen-unmasking step with microwave treatment for 30 seconds. Endogenous peroxidase was blocked by incubation with hydrogen peroxide for 5 minutes. Sections were subsequently incubated with the primary antibodies; smooth muscle actin, CD3, CD20, CD31, CD68 (clone PG-M1), CD138, IgG4 (Stone 2012 ; Wang, H. 2012) (from Dako) and anti-integrin $\alpha_V\beta_3$ antibody, clone LM609 (Millipore) for 30 min at room temperature. After washing the sections were incubated with Envision Flex (Dako, K5007) for 30 min at room temperature, followed by incubation with dia-

minobenzamine (Sigma) for 10 minutes. The slides were finally counter-stained with hematoxylin.

Comments

Papers I, II, and IV

After in-depth discussion and further study, the macrophage/leukocyte score in Paper II was additionally clarified in relation to Paper I, in that the antigen detected by antibody MAC387 (Goebeler 1994) was the leukocyte L1 (cystic fibrosis antigen) found on neutrophils, monocytes, and macrophages (Flavell 1987 ; Brandtzaeg 1988). Therefore, the score in Paper II was reformulated to the macrophage/neutrophils score.

In Paper IV, the macrophage antibody was altered from MAC387 to CD68, which also detects monocytes but is considered to be more macrophage specific (Falini 1993 ; Holness 1993).

Several antibodies against integrin $\alpha_V\beta_3$ are available, but Millipore's was chosen because of its good references (Bao 2002 ; Tsopanoglou 2004 ; Chillakuri 2010).

Statistics and Ethics

Statistical evaluation was carried out with PASW 18 software (IBM Corp, Armonk, NY, USA) (Papers I and II). The Wilcoxon signed-rank test was used to compare SUV values and a value of $p < 0.05$ was considered significant. Pearson's correlation coefficient was used to compare the correlation between AAA expansion and SUV-value/retention index. A value < -0.5 or > 0.5 was considered a strong correlation (Paper II).

The software GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used for analyses of quantitative autoradiographic data. Data on group level was reported as means ±SD and differences between groups was assessed by the non-paired student's t-test with a significance level of $p < 0.05$ (Paper IV).

All patients gave their oral and written consent, after they were informed about the study (Paper I-IV) and the organ donors' relatives gave oral and written consent to use the normal aortic tissue in Paper IV. The regional ethics committee of Uppsala/Örebro, Sweden approved the studies (Papers I-IV).

Results

Paper I

Inflammation in the walls of asymptomatic abdominal aortic aneurysms is not associated with increased metabolic activity detectable by 18-fluorodeoxglucose positron-emission tomography.

The visually inspected PET/CT scans did not present any signs of increased ^{18}F-FDG uptake in the AAA wall (Figure 19).

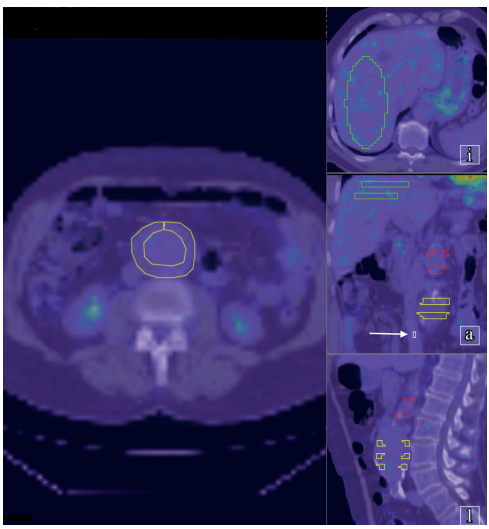


Figure 19. *Different VOI configured on the axial plane. Yellow represents — infrarenal AAA, red — suprarenal aorta, green — liver, and white cube and arrow — blood in vena cava.*

Errata

In the printed and published version of Paper I, Figure 1, (J Vasc Surg 2012; 56:802-807) the description of the colours is incorrect: yellow and red should be transposed.

The SUV was measured as both a mean value and a maximum value, and the different locations were compared between patients and controls (Table 8). No differences in ^{18}F-FDG uptake were observed between small AAA (range, 34-40 mm) and large AAA (range, 52-66 mm; $p = 0.47$). Additional analyses compared the AAA with the other locations: suprarenal aorta, liver and blood (Table 9).

Table 8. *Median SUV-values (min-max) in different locations for AAA-patients and controls. * Mann-Whitney U test.*

		Case (n=12)	**Control (n=13)**	***p****
Blood	SUV_{mean}	1.4 (1.4 – 2.1)	1.3 (0.9 – 1.6)	0.005
	SUV_{max}	2.3 (1.8 – 2.8)	1.6 (1.1 – 2.0)	<0.001
Liver	SUV_{mean}	2.1 (1.8 – 2.5)	2.0 (1.3 – 2.6)	0.27
	SUV_{max}	3.9 (3.3 – 4.5)	3.0 (2.0 – 3.9)	<0.001
Suprarenal aorta	SUV_{mean}	1.6 (1.3 – 2.2)	1.3 (1.0 – 1.6)	0.002
	SUV_{max}	2.8 (2.2 – 3.5)	2.2 (1.8 – 2.7)	0.001
Aneurysmal (infrarenal) aorta	SUV_{mean}	1.3 (1.1 – 1.6)	1.2 (0.8 – 1.4)	0.073
	SUV_{max}	2.7 (2.4 – 3.4)	1.8 (1.2 – 2.3)	<0.001

AAA patients had lower differences between infrarenal aortic wall SUV_{max} and blood or suprarenal aortic SUV_{max} than the controls, which implied increased ^{18}F-FDG uptake in the aneurysmal wall. However, there were no differences between the regions when SUV_{mean} was used.

Table 9. *Median differences in SUV-values between infrarenal aorta and blood, and liver, and suprarenal aorta, in cases and controls. *Mann-Whitney U test.*

Infrarenal aorta compared to		**Case (n=12)**	**Control (n=13)**	***p****
Blood	SUV_{mean}	-0.3 (-0.9 to -0.1)	-0.1 (-0.4 to 0.1)	0.06
	SUV_{max}	0.4 (0.3 to 1.1)	0.3 (-0.4 to 0.5)	0.04
Liver	SUV_{mean}	-0.8 (-1.2 to -0.4)	-0.8 (-1.2 to -0.3)	0.91
	SUV_{max}	-1.2 (-2.1 to -0.6)	-1.2 (-2.0 to -0.7)	0.91
Suprarenal aorta	SUV_{mean}	-0.2 (-0.9 to -0.3)	-0.1 (-0.5 to 0.1)	0.20
	SUV_{max}	0.0 (-0.8 to 0.5)	-0.4 (-0.9 to 0.1)	0.02

Biopsies were taken from the anterior wall of the AAA, as no ^{18}F-FDG uptake was detected in the aneurysm walls. The histological findings revealed a high concentration of inflammatory infiltration (total number of inflammatory cells and number of macrophages and leukocytes) in the aneurysm walls (Table 10).

Table 10. *Immunohistochemical analysis of the aneurysmal wall*

Patient	Total inflammation score[1]	T-/B-lymphocytes (%)[2]	Macrophage/leukocyte infiltrate score[3]
1	Grade 3	50/50	Grade 3
2	Grade 2	75/25	Grade 3
3	Grade 3	75/25	Grade 3
4	Grade 3	50/50	Grade 3
5	Grade 2	50/50	Grade 2
6	Grade 2	50/50	Grade 2
7	Grade 3	50/50	Grade 3

[1] *Evaluated on routine Hematoxylin-Eosin stained slices.*

[2] *Antibodies against T-lymphocytes (CD4 and CD8) and B-lymphocytes (CD20).*

[3] *Antibodies against macrophages/leukocytes (MAC387).*

B-lymphocytes predominantly accumulated within the tunica adventitia, T-lymphocytes in the tunica media and the tunica intima, and macrophages and leukocytes in the tunica media and tunica adventitia (Figure 20).

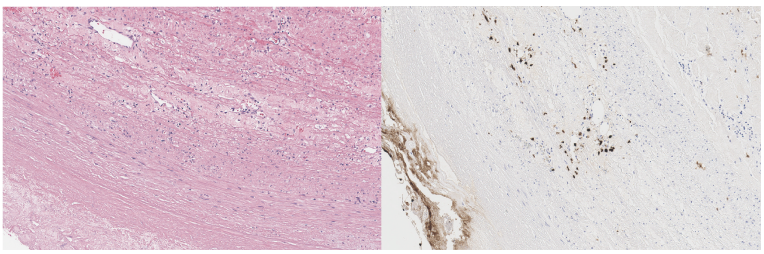


Figure 20. *AAA wall: HE staining exposing the total inflammatory load (left) and IH staining with MAC387 revealing the macrophages and leukocytes (right).*

Comment

In this study, standard protocol for FDG PET, at that time, was used, which meant the tracer uptake time (TUT), time between tracer administrations until scanning was performed, was 60 minutes: in oncology, this is sufficient. Rudd *et al* argued, after some dynamic studies, that for vascular and especially arteriosclerosis FDG PET the optimal TUT should be around 190 min (Rudd 2002). However, the first study that addressed FDG PET in AAA by Sakalihasan *et al* did use the 60 min TUT (Sakalihasan 2002). Subsequent FDG PET-AAA studies use a TUT between 60, 90, and 180 minutes (Reeps 2008 ; Truijers 2008 ; Kotze 2009). In 2009 Menezes *et al* reported an important study performing dynamic FDG PET scans on 17 AAA patients, which could not confirm any advantages of the longer TUT than 60 min (Menezes 2009). This finding is important as longer TUT may cause logistical difficulties at the PET facility, including longer delays and standstills for the patients.

At the time of the study, the PET centre in Uppsala was a private company, and the focus on time consumption was stronger than it might have been in a dedicated research facility.

Ideally, further optimization of the scanning settings would be beneficial for eliminating potential sources of errors, for example:

- Would resolution increase if FOV were adjusted to show only the aneurysm?
- Would electrocardiography (ECG) triggered scans and breath triggered scans enhance the images by reducing movement distortion?
- Would the VOI be more accurate if diagnostic CT scans (with and without contrast media enhancement) had been used instead of low-resolution attenuation CT scans?

One disadvantage of triggered scans with combined heart and respiratory adjustment is that the patient would have to remain in the scanner for two to three times longer to obtain the same amount of detected photons. This combined with dynamic scanning would compound the disadvantage: one patient occupying the PET facilities would reduce the capacity of the PET investigations, increase inconvenience for the patient, and render unreasonable costs for a single scan. The low resolution of the attenuation CT scan meant the VOI were difficult to draw. At the planning stage of the study, the aspects of performing "quick" attenuation CT scans and diagnostic CT scans were discussed. Although attenuation CT scans are the standard procedure for PET/CT scans, patients with small AAA were included in the study and the increased radiation generated by a diagnostic CT scan may not be justified. If contrast media enhancement CT scans had been used, they would have to have been done after the PET scan, as the contrast may interfere and produce artifacts in the PET images.

The SUV was calculated, initially in the study on the sagittal scan in order to visualize as much of the vessel wall as possible (Figure 21).

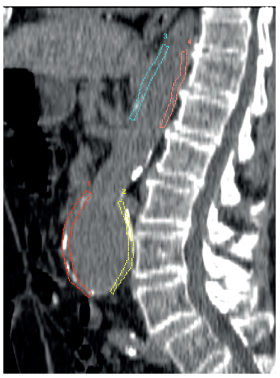


Figure 21. *The initial set-up for obtaining the SUV values from the AAA and the suprarenal aorta.*

This method was revised to obtain measurements from the liver and blood in vena cava, and the VOI were instead defined on an axial plan (Figure 19).

During initial planning, it was intended to collect blood samples from the patients and the controls, both at the time of the PET scan and for surgery. When the study started, the research group did not have sufficient dedicated personnel (e.g. research nurse); therefore, much of the work was conducted from Eksjö, which resulted in too many blood samples, no blood samples, or samples taken at the wrong time. Thus, a correlation analysis between CRP and SUV between different locations was neither feasible nor reliable.

If uptake had been present on the visual inspections, it might have looked similar to in Figure 22, which represents a scan taken on suspected vascular graft infection that was confirmed by FDG PET.

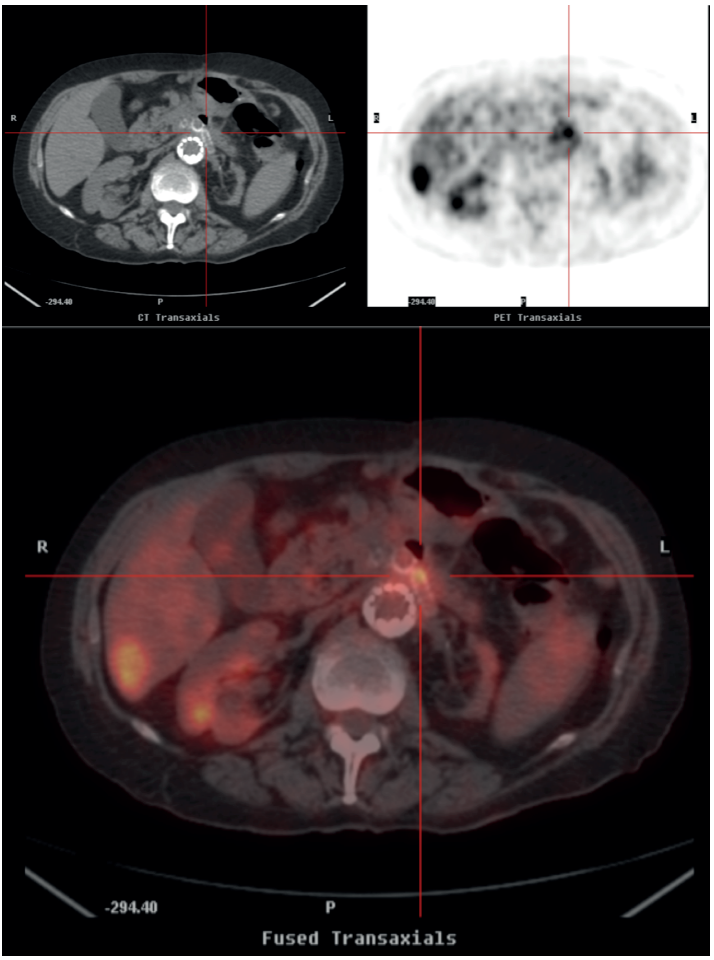


Figure 22. *Example of attenuation CT, PET image, and fused images PET/CT. This examination revealed a vascular graft infection.*

Paper II

4D-PET/CT with [^{11}C]-PK11195 and [^{11}C]-D-deprenyl does not identify the chronic inflammation in Asymptomatic Abdominal Aortic Aneurysms.

The AAA wall was compared with the suprarenal aorta and blood through visual inspection, SUV uptake, and dynamic/retention index. Autoradiography, histology, and IH were conducted on the AAA tissue obtained at surgery.

No visual uptake of the two tracers was observed in the aorta. In the dynamics series and SUV values, there was no accumulation of radioactivity in the aneurysm wall (Paper II: Figure 2 and 3; Figure 23). The non-aneurysmal suprarenal aorta had a slightly higher ^{11}C-D-deprenyl SUV uptake and retention index than the aneurysm wall. No differences in SUV uptake between small and large AAA were seen for ^{11}C-PK11195 or for ^{11}C-D-deprenyl.

The mean expansion rate in the small AAA under surveillance was 0.16 mm per month (range -0.003 – 0.2 mm) with a follow-up time of 36 – 120 months. There was no correlation between expansion rate and SUV uptake was seen.

No uptake of ^{11}C-D-deprenyl in AAA tissue was detected by autoradiography.

Histological examination of the nine aneurysms operated revealed a high degree of inflammatory cells with B- and T-cells lymphocytes as well as macrophages (Paper II: Table 3).

Additional calculations with both AAA SUV_{mean} and AAA SUV_{max} did not reveal any differences when they were compared with uptake in blood, liver, or suprarenal aorta (Tables 11 and 12).

Table 11. *Uptake (SUV) of tracer ^{11}C-PK11195 shown as the difference between AAA and blood, liver, and suprarenal aorta. Values are presented as median (range).* [a]*Mann-Whitney U test.*

Location	SUV	Small n=1	Large n=4	*p* [a]
AAA vs. Blood	Mean	0.16	-0.11 (-0.31 to 0.09)	0.4
	Max	0.60	0.46 (0.33 to 0.58)	0.4
AAA vs. Liver	Mean	-2.59	-2.53 (-2.80 to -2.33)	1.0
	Max	-4.76	-5.36 (-7.18 to -3.84)	0.8
AAA vs. Suprarenal Aorta	Mean	0.03	-0.56 (-0.62 to -0.31)	0.4
	Max	0.14	-1.09 (-2.08 to -0.51)	0.4

Table 12. *Uptake (SUV) of tracer ^{11}C-D-Deprenyl presented as the difference between AAA and blood, liver, and suprarenal aorta. Values are median (range).* [a]*Mann-Whitney U test.*

Location	SUV	Small n=5	Large n=5	*p* [a]
AAA vs. Blood	Mean	-0.16 (-0.4 to -0.02)	-0.06 (-0.18 to 0.25)	0.55
	Max	0.21 (-0.26 to 0.44)	0.48 (0.01 to 0.88)	0.09
AAA vs. Liver	Mean	-4.25 (-4.87 to -3.69)	-3.96 (-4.78 to -3.07)	0.42
	Max	-6.77 (-7.37 to -5.20)	-5.91 (-10.64 to -4.70)	0.84
AAA vs. Suprarenal Aorta	Mean	-0.36 (-0.51 to -0.18)	-0.51 (-0.65 to -0.16)	0.42
	Max	-0.34 (-0.72 to 0.00)	-0.61 (-1.69 to 0.07)	0.84

Inter-individual variations in the blood radioactivity as a cause of SUV variation was eliminated by calculating a retention index for AAA, suprarenal aorta, and liver by assessing the integral of SUV in blood in the dynamic series and comparing this with the SUV obtained from the static series in AAA, suprarenal aorta and liver, respectively.

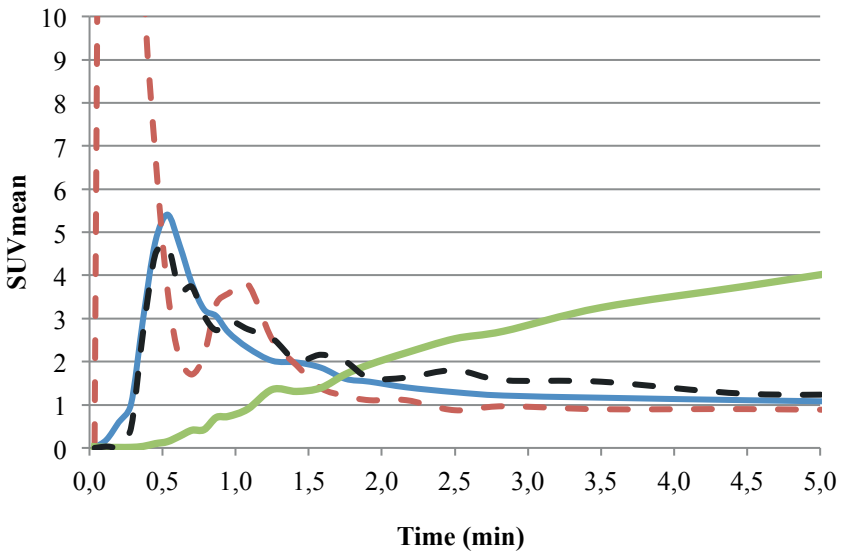


Figure 23. *SUV_{mean} with emphasis on the first 5 minutes and detailed on the lower range of the Y-axis. Note the red dashed line, representing blood, peaks at SUV_{mean} 41 (see Paper II: Figure 3).*

blue continuous line – the AAA vessel wall
red interrupted line – blood
green continuous – liver
black interrupted – suprarenal aortic vessel wall.

Comment

The fourth dimension of time was used. Along with the three other dimensions: length, width or breadth, and height or depth, this is referred to as time

space or 4D and means that the scanning was done over time to give dynamic information on how the tracers were distributed and accumulated in different compartments and tissues of the body. Although it is time consuming and inconvenient for the patient to lie in the scanner for longer, large data files are generates, and the extra information is valuable for investigating new tracers or new uses of existing tracers.

In contrast to Paper I, there were no control patients in Paper II. In addition, ^{11}C-PK11195 has mainly been used in brain studies (Goerres 2001 ; Debruyne 2003 ; Gaemperli 2011), and has not been used in a so many whole body scans at the PET centre in Uppsala. ^{11}C-D-deprenyl has been used in two previous studies, focusing on the knee and neck (Danfors 1997 ; Linnman 2011); thus, no whole body scans were available for analysing the aorta.

As no uptake of the tracers was detected for AAA, the study was not extended to include healthy, non-aneurysmatic controls, which would have been the ideal situation.

As in Paper I, blood sampling generated too many blood samples, no samples, or samples taken at the wrong time. Thus, a correlation analysis between CRP and SUV was neither feasible nor reliable.

Paper III

Autoradiography screening of potential positron emission tomography tracers for asymptomatic abdominal aortic aneurysms.

Only [^{18}F]fluciclatide displayed specific uptake (Figure 24), i.e. 88% of the uptake was blocked by co-incubation with an excess of unlabelled fluciclatide, whereas, there was no uptake displayed for other tracers: CRP-binder, DAA, DDE, DED, TF2-IMP, FMTO, and FVOZ.

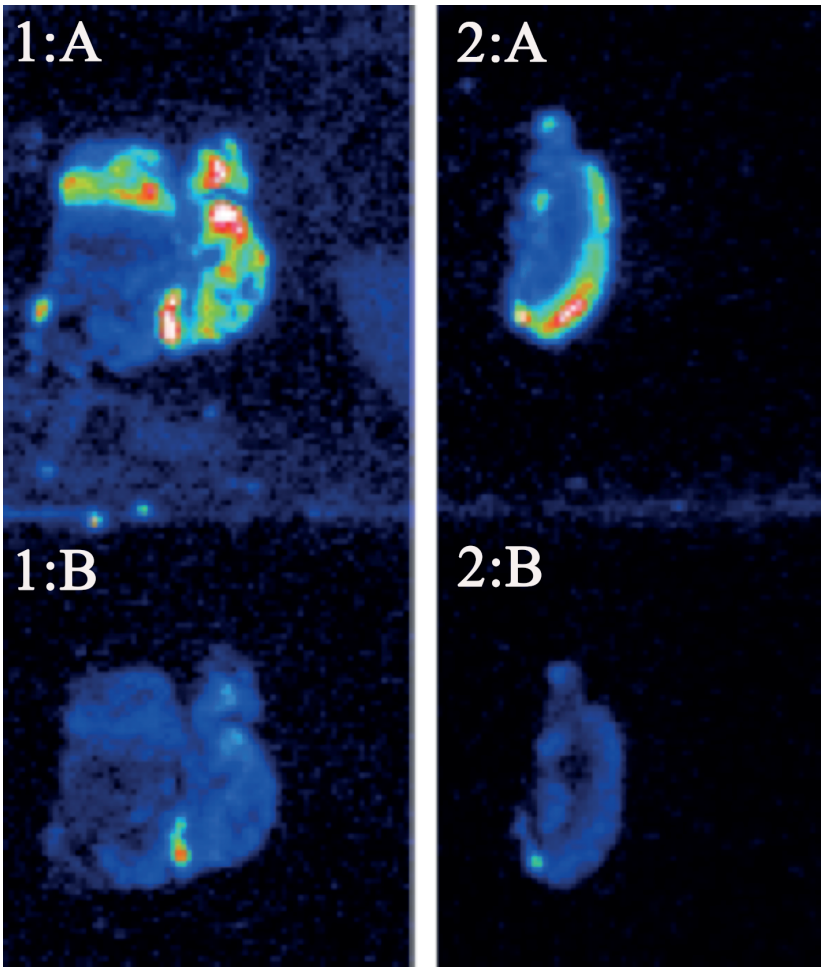


Figure 24. *Autoradiography with [^{18}F]fluciclatide on AAA tissue from two patients. A without additional blocking agent and B with additional blocking agent (unmarked fluciclatide).*

Paper IV

[^{18}F]Fluciclatide - Autoradiography study of angiogenesis in abdominal aortic aneurysm.

Autoradiography revealed specific uptake of [^{18}F]fluciclatide (Figure 25), and accounted for 53% of the signal (Figure 26), as indicated by the blocking obtained through co-incubation with an excess of non-radioactive fluciclatide.

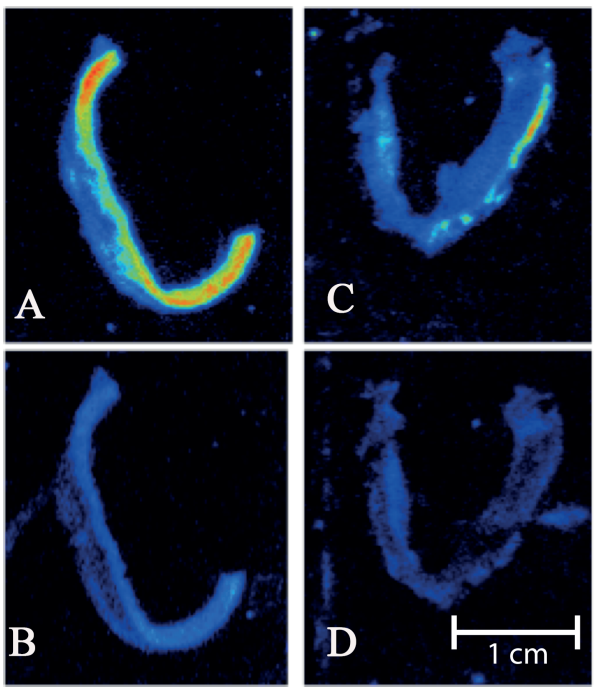


Figure 25. *Autoradiograms showing the uptake of [18F]fluciclatide in sections of AAA tissue from 2 patients (A and B). Controls are normal aortic tissue (C and D). B and D have an excess of unmarked fluciclatide as blocking agent.*

In AAA and in non-aneurysmal aorta the [^{18}F]fluciclatide uptakes were blockable with excess of unlabelled fluciclatide, AAA (p=0.005), control (p =0.0053). There was also an increase [^{18}F]fluciclatide uptake in AAA compared to controls (p = 0.0375), and the uptake was also more blockable in AAA compared to controls (p = 0.0184).There was a 56 % increased specific uptake in aneurysmal AAA tissue, compared with normal aorta; however, this did not reach statistical significance (p=0.136) (Figure 26).

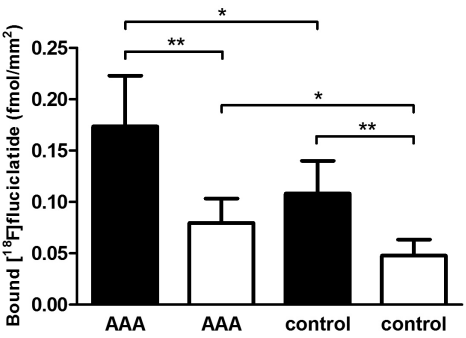


Figure 26. *Mean (± SD) bound [^{18}F]fluciclatide in AAA (n = 5) and controls (n = 5) measured as fmol/mm^2 . Black bars with tracer alone. White bars with tracer and unlabelled fluciclatide as blocking agent. **= significant difference, (p <0.01) *= (p<0.05).*

Histologically, all five aneurysmal aortic tissue samples were characterized by inflammatory cell infiltration. The inflammatory cell infiltrates were found in both the media and the adventitia of the aortic wall, although the amount of inflammation present varied between different tissue samples. In three out of five AAA samples, large and multiple foci of inflammatory cells were detected (Figure 27 A-C), whereas, the other two AAA samples had a more limited and scattered cellular infiltrate. In contrast, non-aneurysmal aortic tissue samples contained no, or very few, infiltrating inflammatory cells (Figure 27 D-F).

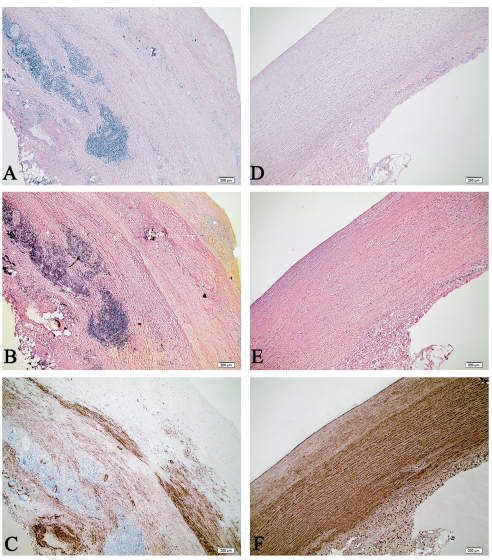


Figure 27. *Histopathology of AAA tissue (A - C) and aortic tissue from controls (D - F) stained with hematoxylin-eosin (A, D), van Gieson (B, E) and smooth muscle actin (C, F). Magnification x40.*

Immunohistochemistry

The predominant cells within larger inflammatory cell foci were CD20-positive B-lymphocytes (Figure 28) that usually formed a central zone surrounded by CD3-positive T-lymphocytes. A limited number of scattered CD68-positive macrophages were identified. A variable number of CD138-positive plasma cells (several of these expressing IgG4) were also found within the inflammatory foci.

In areas with only scattered inflammatory cell infiltration, CD3-positive T-lymphocytes were more frequent than both CD20-positive B-lymphocytes and CD68-positive macrophages. No CD138-positive or IgG4-positive plasma cells were found outside the larger inflammatory cell foci.

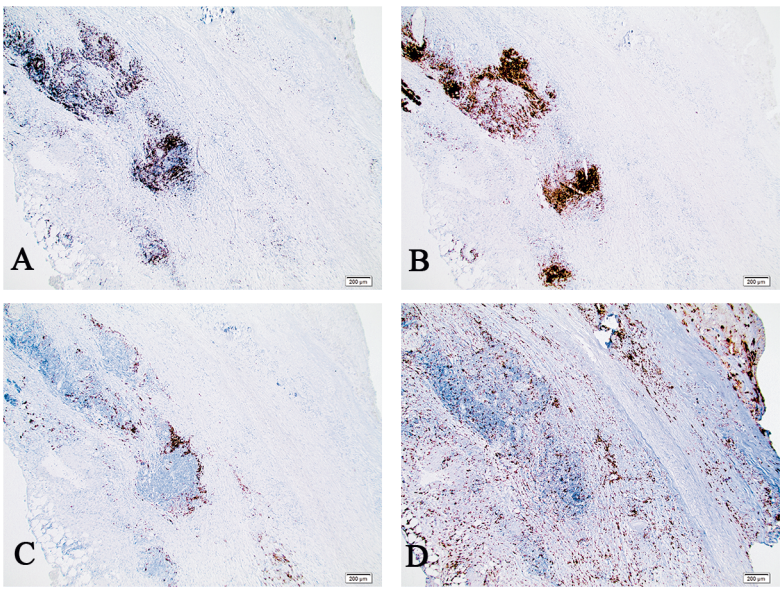


Figure 28. *Immune cell infiltration in AAA tissue from otherwise healthy patients. CD3-positive T-lymphocytes (A), CD20-positive B-lymphocytes (B), CD138-positive plasma cells (C), and CD68-positive macrophages (D). Magnification x40.*

Arterial wall vascularization

In all layers of AAA tissue, the numbers of endothelial cells expressed by the density of the CD31 antibody were increased, whereas mainly adventitial vessels were found in non-aneurysmal aorta tissue (Figure 29). In serially sectioned AAA specimens, co-localization of $\alpha_V\beta_3$-integrin and CD31 antibody was found (Figure 30 A and B), and the vessels expressing $\alpha_V\beta_3$-integrin were mainly found within areas of a dense inflammatory cell infiltration (Figure 30 C and D). Non-aneurysmal specimens with CD31-expressing endothelial cells were all negative for $\alpha_V\beta_3$-integrin, which probably reflected a more mature endothelial cell phenotype (data not shown).

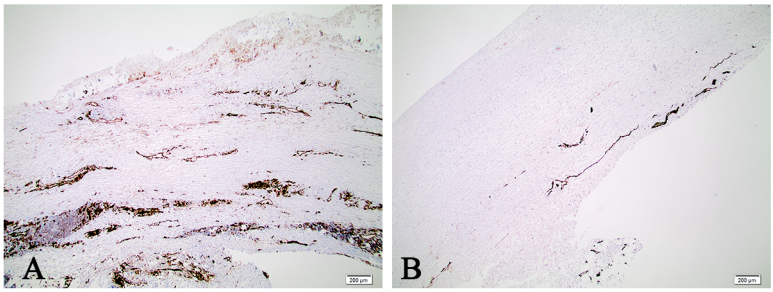


Figure 29. *Greater vascularization of AAA tissue (A) than in normal aortic tissue (B), shown by endothelial CD31-expression. Magnification x40.*

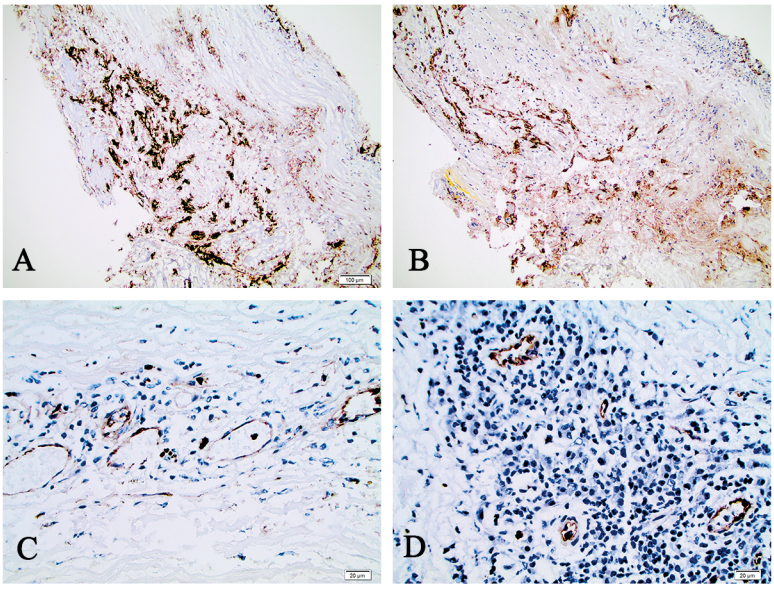


Figure 30. *CD31-expression (A) compared to $\alpha_V\beta_3$-integrin expression (B) in AAA tissue (intima towards left): magnification x100.*
$\alpha_V\beta_3$-integrin expression within areas of cellular infiltration in media (C) and adventitia (D) of AAA tissue: magnification x400.

One non-aneurysmal aorta had an unexpected high inflammatory infiltrate of both lymphocytes and macrophages. This patient had chronic obstructive pulmonary disease (COPD) and had previously undergone pulmonary resection due to aspergillosis.

General Discussion

Abdominal aortic aneurysms have been known for a long time and they are epidemiologically fairly well studied. An elderly Caucasian male smoker, with a father, uncle, or brother who has had an AAA, is at greater risk of developing an AAA, without being aware of it. With time, the AAA will increase in diameter and risk of rupturing, with catastrophic consequences, as the overall mortality rate is up to 90% (Bengtsson 1993).

An aneurysm is characterized by a increase of the vessel diameter of 50% or more than the nearest proximal part of the aorta (Johnston 1991). The abdominal aorta is also defined as having an AAA when the diameter is ≥ 30 mm (McGregor 1975).

Historically AAA has been, and is still by some authors, addressed as arteriosclerotic aneurysms. Even though AAA may have calcifications in the vessel wall, AAA formations must be considered as a separate entity that shares many risk factors, such as age, smoking, and hypertension, with atherosclerotic disease.

Histologically, the final stage of an AAA is characterised by a breakdown of the supporting structures of elastin and collagen, diminish of smooth muscle cells, infiltration of inflammatory cells, such as lymphocytes and macrophages, and neovascularization with *vasa vasorum* (Koch 1990 ; Brophy 1991 ; López-Candales 1997 ; Henderson 1999 ; Kalluri 2003 ; Paik 2004 ; Choke 2005).

Several etiological factors have been studied: infectious agents (Karlsson 2000 ; Falkensammer 2007 ; Hinterseher 2012), genetics (Clifton 1977 ; Verloes 1995 ; Ogata 2005 ; Wahlgren 2010), autoimmunity (Gregory 1996 ; Tilson 1996 ; Hirose 1998 ; Jagadesham 2008), and hypoxia (Holmes 1995 ; Pugh 2003 ; Choke 2006, b)). Some theories have been dismissed and others are foremost based upon the findings from large or even ruptured AAA.

The crucial question is what triggers the process of aneurysm formation, from being an abdominal aorta, AA, with a normal diameter to be an aneurysmal AA i.e.an AAA? From double A to triple A!

A striking thought for a clinician about AAA is that we know quite well how to handle the ruptured AAA. The emergency teams are well drilled and the organizations of the surgical units are well aware of what is expected of them. We also know how to deal with small aneurysm when detected either by screening or *en passent,* by offering follow-up in well-established surveil-

lance programs. But what causes aneurysm formation in the first place, the pathogenesis, we know far too little to be able to offer appropriate advice and medical therapy preventing the aneurysm from continuing to expand.

In order to understand the pathophysiology behind the AAA formation and expansion it seems likely that investigations have to be performed on small aneurysms as well as on pre-aneurysmal dilations of the abdominal aorta, i.e. a diameter of 25-35 mm, and with long-term follow-up data (Wild 2013).

However, the study of small AAA presents a dilemma. To be able to analyse small AAA with histological and immunohistochemical methods, the ethical and practical predicaments to access tissue samples from these aneurysms are quite obvious. It is ethically unacceptable to take biopsies from the abdomen's largest vessel in patients, who are in every other respect, healthy. This means only patients with AAA with a diameter of ≥ 55 mm are available, when the patients most certainly have had their aneurysm for a substantial period of time (Powell 2011) representing the end-stage of the pathological process rather than revealing its true nature.

An emerging additional obstacle to obtain AAA tissue, also from large AAA, is the paradigm shift in the treatment of AAA: from open resection to a minimal invasive endovascular EVAR operation, the later with very limited opportunities for harvesting larger vascular biopsies. The group of patients who still remain and are offered open resection thus becomes selective.

This calls for a different approach, a non-invasive *in vivo* technique in order to study the pathophysiology that may lie behind the formation of AAA, for which molecular imaging with positron emission tomography (PET) would be suitable.

As opposed to previous AAA PET studies, which have focused on large AAA, including rapidly expanding, symptomatic, and even inflammatory AAA (Sakalihasan 2002 ; Reeps 2008 ; Kotze 2009), this thesis had its main focus on small asymptomatic aneurysm, with large AAA used as controls and for obtaining tissue samples for histological and immunohistochemical analysis.

The basic principle of PET is to administer a tracer, usually intravenously; the tracer targets the receptor cells or the receptors that are of interest, and the PET scanner detects the photons that are emitted from the tracer. Principles are, by nature, straightforward, but in practice there are several predicaments to overcome to obtain reliable results:

- What receptors or specific cells are present in the tissue of interest?
- Which tracer molecule has the best characteristic in binding to this receptor?

- Which radioisotope has the best features to label the chosen tracer molecule? Does it demand on site production or may it be transportable to PET clinics without their own cyclotron?
- Does the PET scanner have the optimal features, with the optimal scintillation detector, optimal diameter of the detector ring?
- Must there be synchronization with respiratory movements or heart rate, or even both?

The complex process of development of PET tracers has little in common with vascular surgery, yet the pathophysiology of AAA formation is highly interesting, particularly for a vascular surgeon.

So, what receptors, cells or structures are present in AAA, which could be used as targets? The aorta wall lacks, in contrast to the brain (Gulyas 2012 ; Zimmer 2012), specific receptors. There are, however, various cells and structures in AAA such as lymphocytes, macrophages, smooth muscle cells, collagen, elastin and signs of angiogenesis.

Inspired by the pioneer work by Sakalihasan *et al* we used in Paper I the routine PET tracer ^{18}F-FDG, which signals for increased glucose consumption. Several methodological issues required consideration; one was the use of SUV_{max} or SUV_{mean}. In oncological studies SUV_{max} is frequently used. However, with no visual uptake, i.e. no "hot spots", we measured the SUV at several locations and the mean values of these calculations were used. This is supported by Rudd *et al* and Kai *et al* who argued that SUV_{mean} is a more accurate method in vascular ^{18}F-FDG PET studies (Rudd 2002 ; Kai 2010).

Despite the observed large number of B- and T-lymphocytes along with macrophages in the wall of large AAA, no ^{18}F-FDG uptake was detected. This indicates that the chronic inflammation seen in AAA is not metabolically active. As opposed to other inflammatory conditions, such as arteritis (Blockmans 2011) and vascular graft infections (Wassélius 2008 ; Bruggink 2010), which represent a metabolically active inflammation with high ^{18}F-FDG-uptake. Our findings was confirmed by colleagues in Genoa who also failed to show an ^{18}F-FDG uptake in asymptomatic AAA (Palombo 2012). Thus, it may be concluded that ^{18}F-FDG is not a particularly suitable tracer for studying the pathophysiology of asymptomatic AAA. An intriguing question is therefore raised: What role does the inflammation play in the aneurysm formation, and can other more sensitive or specific PET tracers be developed?

Although the exact target for ^{11}C-D-deprenyl is still to be revealed, the tracer gives a distinct uptake in inflammatory conditions, such as active rheumatoid arthritis, which is reduced after corticosteroid treatment (Danfors 1997), and in the chronic inflammation that is suggested to be involved in whiplash associated disorders (Linnman 2011). Therefore, it was suggested as an in-

teresting tracer for studying the chronic and metabolically inactive inflammation in AAA. Another tracer of interest was the macrophage tracer ^{11}C-PK11195, which has been used to visualize the inflammation in giant cell arteritis and Takayasu´s arteritis (Pugliese 2010).

Even though these two tracers are considered to be rather specific in signalling for inflammation, neither tracer did show any uptake in the studied asymptomatic AAA.

Our findings in Papers I and II, where ^{18}F-FDG, ^{11}C-D-deprenyl, and ^{11}C-PK11195 gave interesting results showing that the inflammation is not sufficiently active to be detected by these tracers in small and large AAA. For ^{18}F-FDG this was somewhat contradictory to previous studies and for ^{11}C-D-deprenyl and ^{11}C-PK11195 this was the first time these tracers were studied on asymptomatic AAA. However, these findings raises questions on the pathophysiological importance of the chronic inflammation seen in large AAA; Are there other mechanisms that play a more important role in the aneurysm formation, and is the inflammation merely a secondary phenomenon present in end-stage aneurysmal disease?

The process of tracer development, as previously mentioned, is complex, demanding, and costly. As the PET centre at Uppsala University has developed several PET tracers we took the opportunity to investigate a few of these on AAA tissue. This was done by means of autoradiography, an *in vitro* technique used for pre-clinical evaluation in tracer development (Paper III). Some of these tracers were designed to detect different aspects of inflammation and another tracer signalling for angiogenesis. Other tracers signalling for pathological processes not associated with AAA, such as astrocytes, carcinoembryonic antigen, mitochondrial cytochrome P-450 enzymes in the adrenal cortex, and the enzyme aromatase.

In their current stage and design we showed that the only tracer that had a specific uptake was the integrin $\alpha_V\beta_3$ tracer [^{18}F]fluciclatide, signalling for angiogenesis. It may seem unorthodox to perform experiments with tracers, which targets receptors that are less likely to be present in the investigated tissue. This is of course quite true from a strictly scientific view. The reason why we despite this went along performing these tests was mainly the fact that the knowledge behind the aneurysm formation still is hidden, and that there might be a small chance of getting some revealing results. Although our experiments were done deliberately, it might be noteworthy that some great discoveries such as the detection of penicillin 1928 and of graphene 2004 were done by mistake or by just fooling around, so to say, and were awarded the Nobel Prize Physiology or Medicine 1943 (Nobelprize.org 2013, b)) and the Nobel Prize in Physics 2010, respectively (Nobelprize.org 2013, c)).

Our *in vivo* experiments were performed on large and small AAA (Papers I and II), but as described earlier, it is difficult to obtain tissue samples from small aneurysmal and normal aorta. To meet this challenge we collected non-aneurysmal infrarenal aortic tissue samples from organ donors. These samples, collected by the colleagues at the section for transplant surgery, were obtained at the final stage of the explant procedure, i.e., the samples were as fresh as possible and obtained as similarly as possible as the AAA tissues had been obtained in Papers I and II.

Paper IV was a continuum of Paper III, showing a potential role for [^{18}F]fluciclatide in our effort to understand the pathophysiology of aneurysm formation. The process of angiogenesis has previously been shown in histological examinations of large AAA (Paik 2004). The main finding of our study was that the [^{18}F]fluciclatide signal was significantly blockable, i.e. it has a specific binding to receptors in the aorta. Furthermore, that the uptake was higher in aneurysmal tissue compared with non-aneurysmal aorta tissue. Although the observed difference of 56% was no significant, ($p = 0.136$), the finding is encouraging. Also, the adjunctive immunohistological analysis showed an interesting co-localisation of neovascularization and inflammatory cell foci.

These *in vitro* findings suggest that [^{18}F]fluciclatide could be a plausible tracer for further *in vivo* studies on asymptomatic small and large AAA that may bring some new light upon the pathophysiology of aneurysm formation. A more specific integrin $\alpha_V\beta_3$ tracer with higher affinity to the receptor is under development and will be used in future *in vivo* studies on small and large asymptomatic AAA.

Angiogenesis as a possible triggering factor for aneurysm formation is a tempting concept. Smoking is the most important risk factor for aneurysm formation, expansion, and rupture. Smoking produces carbon monoxide which induce hypoxia (Lederle 2011 ; Norman 2011 ; Svensjö 2011), which is one driving force of angiogenesis (Pugh 2003). An important issue for further research is to understand the possible association between hypoxia/angiogenesis and the key pathological events in aneurysm formation, the elastin and collagen degradation and the smooth muscle cells apoptosis.

Conclusions

The following conclusions can be drawn from the four papers in this thesis:

- The observed chronic inflammation with lymphocytes and macrophages seen in large asymptomatic AAA does not have sufficient metabolic activity to be detected *in vivo* with ^{18}F-FDG PET/CT (Paper I)

- The chronic inflammation seen in large asymptomatic AAA is not detectable *in vivo* with the tracers ^{11}C-PK11195 and ^{11}C-D-deprenyl (Paper II)

- The seven tracers:
    - ^{68}Ga-CRP-binder,
    - ^{11}C-DAA1106,
    - ^{11}C-D-deprenyl,
    - ^{11}C-deuterium-L-deprenyl,
    - ^{68}Ga-IMP461 with bispecific antibody TF2 052107,
    - [^{18}F]F-metomidate, and
    - ^{18}F-vorozole

  do not reveal any uptake in AAA tissue in by means of autoradiography. Therefore, in their present state, they are not useful for use as tracers for studying asymptomatic AAA with PET (Paper III)

- The $\alpha_V\beta_3$-integrin tracers [^{18}F]fluciclatide show a specific uptake in AAA with autoradiography (Papers III and IV)

- *Vasa vasorum,* positive for $\alpha_V\beta_3$-integrin antibody, are associated with dense inflammatory cell foci in asymptomatic AAA tissue. (Paper IV)

Sammanfattning på svenska (Summary in Swedish)

Abdominellt aortaaneurysm (AAA) är en sjuklig vidgning av stora kroppspulsådern i buken. Ett AAA anses vanligen föreligga när aortadiametern överskrider 30 mm. Naturalförloppet för ett AAA är att successivt öka i storlek för att till slut brista (rupturera). Dödligheten vid ruptурerade AAA är cirka 90 %. I Sverige dör 700 – 1 000 personer om året till följd av ruptурerade AAA. När AAA upptäcks, antingen som ett tillfällighetsfynd eller i samband med screening, är det framförallt diametern på aneurysmet som avgör när en förebyggande operation är aktuell; vanligtvis när diametern är större än 55 mm. I dagsläget saknas tillväxthämmande behandling av små AAA.

För att upptäcka ett aneurysm i aorta tidigt innan det brustit och därigenom kunna operera i en elektiv, planerad situation, har screeningprogram introducerats i flera länder. I och med detta hittas även många mindre aneurysm, som kan följas med ultraljud tills det eventuellt är aktuellt med operation.

AAA är ungefär sex gånger så vanligt hos män som hos kvinnor. Ålder, rökning och hypertoni är andra viktiga riskfaktorer. AAA karakteriseras av en försvagning i kärlväggen, där stödjevävnad såsom elastin och kollagen bryts ner. Histologiskt ses även en minskning av glatta muskelceller genom självdöd, apoptos, och en infiltration av inflammatoriska celler, B- och T-cells lymfocyter tillsammans med makrofager.

Patogenesen, den bakomliggande orsaken till AAA är fortfarande okänd, men det finns flera hypoteser. Vilken roll spelar egentligen den inflammation, som ses i de stora aneurysmen? Spelar hypoxi (lokal syrebrist) och angiogenes (nybildning av små blodkärl i aortaväggen) någon roll för utvecklingen av aneurysm?

För att *in vivo*, dvs. i den levande organismen, studera biologiska processer utan att påverka processen i sig, är molekylär bilddiagnostik mycket användbar. Positron emissions tomografi (PET) är en typ av molekylär bilddiagnostik som har hög känslighet. Principen för PET är att detektera fotoner, som bildas när positronerna annihileras, förintas, i samband med att de sammanfaller med sin antimateria, elektroner. Autoradiografi är en annan molekylärdiagnostik metod, en laboratorieteknik för att studera spårämnen *in vitro*, på vävnader, eller *ex vivo*, på försöksdjur, som först injiceras med spårsubstansen för att sedan undersökas efter det att djuret avlivats. För att kunna studera den substans som man är intresserad av behöver en radioaktiv isotop kopplas till substansen. Substansen innehåller molekyler som binder till specifika receptorer i kroppen och/eller kan tas upp av vissa celler. Vid PET är spårämnet, tracern en radioaktiv substans som har ett mätbart sönderfall med utsläpp av positroner, ex ^{18}F flour-18 eller ^{11}C kol-11.

Syftet med denna avhandling är att i små och stora AAA studera patofysiologin med olika spårämnen.

Delarbete I

Inflammation in the walls of asymptomatic abdominal aortic aneurysms is not associated with increased metabolic activity detectable by 18-fluorodeoxglucose positron-emission tomography.

I delarbete I gjordes PET-undersökningar med den kliniskt mest använda spårsubstansen ^{18}F-FDG, fluorodeoxyglukos, en spårsubstans som består av en glukosmolekyl där en hydroxylgrupp har ersatts av en radioaktiv fluorisotop, ^{18}F, flour-18, som har en halveringstid på knappt 2 timmar. ^{18}F-FDG tas upp av celler som har en förhöjd metabolism, exempelvis maligna cancerceller samt vid infektioner och inflammationer.

Tolv män mellan 65 och 75 år med asymtomatiska AAA inkluderades i studien. Fem hade små AAA (34-40 mm) och sju stora AAA (52-66 mm). Patienterna med stora AAA var planerade för elektiv öppen kirurgi, vilket gav möjlighet att erhålla vävnadsprov från aneurysmväggen. Hälften av patienterna var rökare och hälften var ex-rökare. Kontrollgruppen bestod av 12 män utan AAA vilka av annan anledning genomgick PET med ^{18}F-FDG.

Upptagsmätningar, SUV-mätningar (standardized uptake values, standardiserade upptagsvärden), gjordes på aneurysmväggen, på suprarenal aortavägg, i blod och i lever. Inget upptag av ^{18}F-FDG kunde detekteras i små eller stora asymtomatiska AAA. I vävnadsproverna från de stora aneurysmen fanns det rikligt med inflammatoriska celler, B- och T-lymfocyter samt makrofager.

Delarbete II

4D-PET/CT with ^{11}C-PK11195 and ^{11}C-D-deprenyl does not identify the chronic inflammation in asymptomatic abdominal aortic aneurysms.

I delarbete II studerades 15 män med asymtomatiska AAA med två olika spårsubstanser. Fem patienter undersöktes med spårsubstansen ^{11}C-PK11195, som binder till receptorn TSPO (translocator protein (18kDa)), en receptor som uttrycks av aktiverade makrofager. Tio patienter undersöktes med spårsubstansen ^{11}C-D-deprenyl. Dess exakta receptor är fortfarande okänd, men man har påvisat upptag vid inflammatoriska tillstånd såsom reumatoid artrit och vid kronisk whiplashskada som antas bero på en kronisk

inflammation. Histologiska och immunohistokemiska analyser gjordes på vävnadsprover från aaneurysmväggen.

Visuellt kunde inget upptag påvisas vid någon av undersökningarna. Upptagsmätningar, SUV-värden, på aneurysmväggen jämfördes med dem på suprarenala aortaväggen. PET-undersökningarna gjordes dels som en statisk undersökning efter 35 minuter, dels som en dynamisk undersökning över tid från 0 till 25 minuter efter att spårsubstansen administrerats till patienterna. Detta gav en uppfattning om tidsförloppet när spårsubstansen ackumulerades, anrikades, i olika vävnader. I och med detta kunde den samlade mängden radioaktivitet i blod uppmätas och analyseras. Kvoten mellan upptaget i de olika vävnaderna och den totala injicerade radioaktiva mängden uttryckte ett retentionsindex. Detta gav dock inga indikationer på något ökat upptag i aneurysmväggen.

Autoradiografisk undersökning, en laboratoriemetod där vävnad studeras *in vitro,* med ^{11}C-D-deprenyl kunde inte påvisa något upptag. Histologiska analyser av kärlväggen från de nio aneurysm som opererades visade rikligt med B- och T-lymfocyter tillsammans med makrofager.

Delarbete III

Autoradiography screening of potential positron emission tomography tracers for asymptomatic abdominal aortic aneurysms.

I delarbete III gjordes en sammanställning av de olika autoradiografiska screeningundersökningar som genom åren har gjorts på aneurysmatisk aortavävnad i samband med att nya spårämnen har utvecklats vid PET-centrum och vid enheten för Preklinisk plattform för PET vid Akademiska Sjukhuset och Uppsala Universitet.

Av de undersökta ämnena; ^{68}Ga-CRP-binder, ^{11}C-DAA1106, ^{11}C-D-deprenyl, ^{11}C-deuterium-L-deprenyl, ^{18}F-fluciclatide, ^{68}Ga-IMP461 med bispecifik antikropp TF2 052107, [^{18}F]F-metomidate och ^{18}F-vorozole uppvisade ^{18}F-fluciclatide ett blockeringsbart upptag i aneurysmvävnad. De övriga spårämnena gav inget upptag alls.

Delarbete IV

^{18}F-fluciclatide -Autoradiography study of angiogenesis in abdominal aortic aneurysm

För att mer ingående studera angiogenesens betydelse för patofysiologin i AAA användes spårsubstansen ^{18}F-fluciclatide (FAN). Denna binder till RDG-receptorn som uttrycks av integrin $\alpha_V\beta_3$. Detta integrin uttrycks bland annat vid angiogenes, av maligna celler, av osteoclaster och av makrofager.

Vävnad från fem män som opererats elektivt för asymtomatiska AAA jämfördes med icke-aneurysmatisk aortavävnad från fem ålders- och könsmatchade organdonatorer.

Vid autoradiografi *in vitro* inkuberas vävnaden i näringslösning varpå den radioaktiva spårsubstansen tillsätts. Genom att tillsätta ”kall” substans, dvs. molekylen utan någon radioaktiv isotop, kan man beräkna hur specifik inbindningen av spårämnet är.

Standardhistologi jämfördes med immunohistokemi vid analyser med antikroppar mot lymfocyter, makrofager, plasmaceller och integrin $\alpha_V\beta_3$receptorn.

Med autoradiografin sågs ett upptag av FAN i både aneurysmvävnaden och i normal aorta. Upptaget var icke-signifikant högre i den aneurysmatiska vävnaden. Detta upptag korresponderade med de immunohistokemiska analyserna med integrin $\alpha_V\beta_3$-receptorns antikropp LM609. De LM609-positiva kärlen var regelmässigt omgärdade av inflammatoriska cellanhopningar.

Slutsatser

Följande slutsatser kan dras från de fyra delarbeten i avhandlingen:

- Den kroniska inflammationen i stora asymtomatiska AAA har inte tillräckligt hög metabol aktivitet för att kunna detekteras med spårsubstansen ^{18}F-FDG vid PET/CT. (delarbete I)
- Spårämnena ^{11}C-PK11195 och ^{11}C-D-deprenyl kan *in vivo* inte påvisa den kroniska inflammation som ses i asymtomatiska AAA. (delarbete II)
- Spårämnena ^{68}Ga-CRP-binder, ^{11}C-DAA1106, ^{11}C-D-deprenyl, ^{11}C-deuterium-L-deprenyl, ^{68}Ga-IMP461 och bispecifik antikropp TF2 052107, [^{18}F]F-metomidate och ^{18}F-vorozole är inte användbara för att studera asymtomatiska AAA med PET. (delarbete III)
- ^{18}F-fluciclatide är ett potentiellt lovande spårämne att studera asymtomatiska AAA. (delarbete III och IV)
- De inflammatoriska cellanhopningar med CD3-positiva, CD20-positiva och CD68-positiva celler som ses histologiskt i asymto-

matiska AAA finns nära kärl som är identifierade med $\alpha_v\beta_3$-integrin, antikropp LM609. (delarbete IV)

Sammanfattning på enkel svenska för barn (Summary in plain Swedish for children)

Kroppens största blodkärl heter stora kroppspulsådern eller aorta. Den kan bli sjuk. Då kan den börja blåsa upp sig ungefär som en ballong. Precis som en ballong kan spricka och smälla när den blir för stor, kan blodkärlet spricka. Det är vanligast att dom som får den här sjukdomen är gamla farbröder, som tycker om att röka cigaretter.

Varför man kan få den här sjukdomen vet man inte. Man vet inte heller vad själva orsaken är. Men på sjukhuset kan man hjälpa de här patienterna. Då behöver man byta ut den uppblåsta delen av blodkärlet, av aortan.

Det skulle ju vara bäst om doktorn kunde hjälpa patienterna så att dom inte fick den här sjukdomen en överhuvudtaget. Näst bäst skulle det väl vara om det fanns någon medicin som gjorde så att blodkärlet slutade att blåsa upp sig. Då skulle det ju inte spricka.

Man kan se hur det är inne i kroppen och hur allt fungerar. Då kan man använda en speciell undersökning som heter PET. Då får patienten ett speciellt ämne som är radioaktivt. Man säger att ämnet strålar. Den radioaktiva strålningen är inte farlig. Med PET kan man se var i kroppen det samlas extra mycket av det här ämnet. Det strålar extra mycket där. På så sätt kan man forska för att vi ska lära oss hur olika sjukdomar fungerar.

Jag har undersökt patienter med ett par olika sådana lysande ämnen. Ett ämne är vanligt socker som man gjort så att det kan stråla. Socker används ju av alla celler i kroppen. Men en del celler är särskilt hungriga på socker. Då samlas förstås sockret där och det som lyser och strålar mest där. Där det är en sådan där blåsa på aortan finns det alltid en inflammation. Inflammationscellerna kallas för makrofager och dom är alltid sugna på socker. Men när jag undersökte patienterna syntes det inte alls att det strålade särskilt mycket runt stora kroppspulsådern.

Då fick jag i stället operera fram en liten bit av aortan. Det gjorde jag både från sådana som var sjuka och sådana som hade ett friskt blodkärl. De här bitarna undersökte vi på ett laboratorium. Där doppade vi ner den lilla kärlbiten i olika ämnen. Vi ville se om man kunde göra så att det lyste bättre.

Ett nytt ämne som kan visa om det växer nya små kärl inne i kärlväggen verkade bra och lovande.

Så småningom ska jag därför undersöka patienter med det här nya ämnet. Jag ska förstås också fortsätta att undersöka om andra ämnen är ännu bättre för att forska fram orsaken till sjukdomen. Då kan vi ju förstå sjukdomen bättre och hitta bättre behandlingar mot den.

Future Perspectives

With the interesting findings in Paper IV, an additional *in vitro* study on AAA and normal aortic tissue with a new RDG-receptor tracer with higher affinity is planned. Depending on the outcome of that study, an *in vivo* clinical PET/CT study is planned. Also, other novel tracers currently under development in Uppsala will be evaluated.

Our main interest is, of course, to study the pathophysiology of small aneurysms. Currently, the Uppsala AAA biobank contains of tissue from different types of aneurysms and from normal infrarenal aorta from organ donors. The challenge is that tissue samples from small AAA are rare. One source of obtaining tissue from small AAA would be at autopsy. The crucial question is how the tissue demodulates after death.

We therefore plan to compare tissue samples from normal and aneurysmatic aorta obtained at autopsy with tissue samples from our biobank. Standard histology, immunohistochemistry, and autoradiography will be performed in order to determine the possible value of analysing tissue from small AAA obtained at autopsy.

Patients with a sub-aneurysmal aortic dilatation (diameter between 25 to 29 mm) have high risk of developing AAA (Wild 2013). This may indicate a more active pathophysiological stage in the AAA formation. It would therefore be of interest to include this subgroup in future *in vivo* PET studies.

Acknowledgments

The work for this thesis was undertaken at the Department of Surgical Sciences, Vascular Surgery, in close collaboration with the Department of Radiology, Oncology and Radiation Science, Section of Nuclear Medicine and PET; the Department of Medicinal Chemistry, Preclinical PET Platform; and the Department of Immunology, Genetics and Pathology, Molecular and Morphological Pathology, at Uppsala University Hospital, Uppsala Sweden.

I wish to express my gratitude to all enthusiastic friends and colleagues, with whom I have had the privilege of working. Especially, I would like to thank some people of special importance to me:

Associate professor **Anders Wanhainen**, my encouraging tutor, friend and inspiring colleague, for his everlasting enthusiasm and support. Anders has an amazing ability to find the thrilling and extraordinary points of view, even at times when results only seem negative. Sowing a little seed today may yield a large harvest tomorrow. I'm looking forward to our future research together. Thank you!

Professor **Martin Björck**, head of the vascular research team at Uppsala University and my initial tutor, and later co-tutor when Anders assumed this role. Thank you for embracing me through the distance-learning course in vascular surgery in Uppsala 2003, even though at that time I lived and worked in Eksjö, Småland, and for encouraging me and my family to move north to Uppsala in troublesome times. You have my gratitude for the many well-formulated improvement proposals and valuable support as a researcher, colleague, and friend.

Associate professor **Jens Sörensen**, my co-tutor and nuclear imaging virtuosos, with such ease and grace you explained the innermost secrets of PET for three eager vascular surgeons and inspiring discussions on tracer uptake time, VOI, and two point discriminations. Thanks for your valuable comments and advice.

Doctor **Katharina Ericson**, my co-writer and friend at the Department of Pathology, for your valuable contributions and endless positivism when you came to the operation theatre collecting tissue samples and when I have telephoned for delivery of other samples.

Doctor **AnnaCarin Wallgren**, my co-writer who with great enthusiasm took the opportunity to assume Katharina's role when she felt compelled to change research focus from the mysterious aneurysms. I very grateful for your contributions and I hope that we will keep up the great collaboration in the future.

Associated professor **Gunnar Antoni**, co-writer and principal chief at PET centre, with an inspiring enthusiasm for pre-clinical PET research and its implementation in clinical research. You have a new tracer in the sleeve whenever we meet and always look towards new challenges and research ideas. I look forward to future teamwork.

Researcher and PhD **Sergio Estrada**, co-writer at PPP, with the elegantly expressive nametag: "Scientist!" You hosted the PhD course in Molecular Imaging in Biomedical Research with great enthusiasm: a course, which brought the work in this thesis to a higher level. I look forward to future teamwork and autoradiography analysis.

Professor **Håkan Hall**, co-writer at PPP for his valuable help with the screening of tracers on AAA tissue, and elegant phraseology.

Associate professor **Eva Lundgren**, PhD **Staffan Wollert**, and Associate professor **Claes Juhlin**, former heads and present head of the Department of Surgery; along with professor **Olle Nilsson**, head of the Department of Surgical Sciences for providing me the opportunity and means to write this thesis.

Christer Ljungman, head of the vascular department in Uppsala, along with current and former colleagues: **Björn Kragsterman, Annika Boström, Håkan Rudström, Johnny Steuer, Kevin Mani, Nakisa Esfahani** for their encouragement and enthusiasm in the daily clinical practice.

Professor emeritus **David Bergqvist,** for his wisdom and encouragement throughout the work, and with whom I shared his room for the first months at the clinic. Luckily, David is an early bird, so I could use the room and computer evenings and nights.

Co-workers at PET centrum and PPP (preclinical PET platform), **Veronica Asplund,** always with some extra space in the freezer for some tissue samples; **Örjan Lindhe,** with whom I did my first autoradiography test. **Tomas Nyberg**, IT at PET centre, always supportive with logins and malfunctioning passwords. **Lars Lindsjö,** always helpful, and good at finding the correct PET scan in the large archive. **Mimmi Linholm** for help with scheduling of the patients for their scans, and **Johan Doverfjord**, production

chemist at PET centre who showed me the fascinating process of producing [^{18}F]Fluciclatide.

Johan Lilja, the creator of the valuable research tool VOIager, who I met during the PhD course at PET centre, thank you for the much appreciated extra time after class, and for explaining the mystery of pixels, voxels, and partial volumes effect.

Biomedical analyst **Marjam Kerro**, **Simin Tahmesepour** and **colleagues** at the Department of Pathology for their most kindly support and care of the tissue for the AAA bio bank.

Professor **Gunnar Tuvfesson** and **colleagues** at the section for transplantation for their kind support in collecting normal aortic tissue.

Transplant coordinator **Christina Andreasson** and **colleagues** at the section for transplantation for their kind phone calls, particularly late at night or during the weekend, and for informing about the tissue sampling.

Professor **Anders Larsson** at the Department of Surgical Sciences, Hedenstierna laboratory that so kindly helped us with accessing porcine aorta, when we performed the first autoradiography scan with ^{18}F-fluciclatide.

Mats Persborn a dear friend and colleague at Eksjö county hospital, with whom I spent time on the road back and forth to Jönköping for our scientific courses and numerous visits to various electronic stores at A6.

Hans Ravn inspiring colleague and friend at Eksjö county hospital, who was actually the main reason for my initial contact with professors Martin Björck and David Bergqvist when they initiated the first distance learning course in vascular surgery, which eventually led to this thesis.

To my colleagues at Eksjö county hospital: **Karsten Offenbartl, Carsten Berg, Göran Simert, Eibert Einarsson, Johannes Järhult, Per-Olof Nostell, Greger Olsson, Jan-Olof Svärd, David Robinsson and Ludmilla Modig** who in one way or another gave me the opportunity to attend the scientific courses in Jönköping.

Futurm and **Jönköping county council** for initial support with research grants and research time.

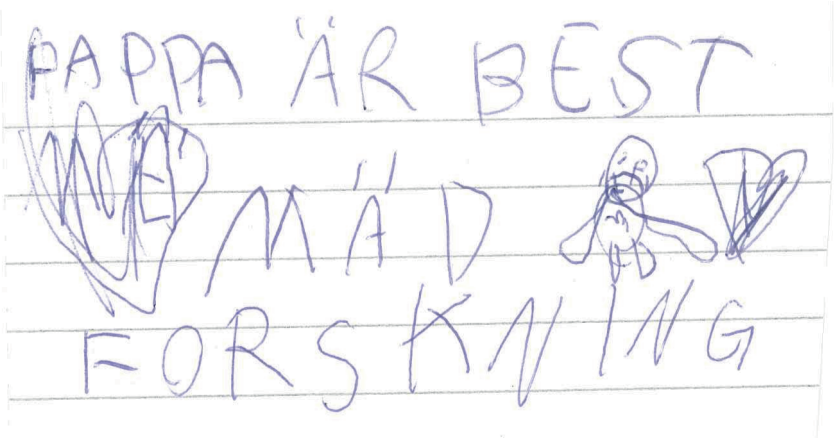
Mona Bergqvist and **Agneta Gustafsson**, secretaries at the Institution of Surgical Sciences for valuable help in getting hold of old and "historical" papers.

The staff at the **vascular ward unit 70B:1, the vascular out-patient unit, the vascular laboratory, wound care centre "Sårcentrum",** and **the new RF venous task force** for support and positive remarks.

Friends and **relatives** who have shown so much interest in my work and given me so much support and encouraging remarks! Thanks!

My father, **Lennart Tegler**, Associated professor and endocrinologist, for warm and encouraging support over the years. Even the smallest detail is important and deserves proper attention! I'm very grateful for your valuable inputs and comments.

To my wonderful and beautiful daughters, **Henrietta** and **Carolina,** for your support and love. A hug (shower hug or not) or a little note where it says :



Love and affection like that means everything and makes it much easier to carry on. Thank you so much. I love you!!

Most of all, I would like to thank my wonderful wife and companion in life, **Lise-Lotte**, "Lotten","LL", who said some nine years ago, after Anders' first question whether I was interested in a research work or not: "Swell, if you want, I'll support you!" Then we went on at trip to NY.

Thanks for all the love, support, and encouragement you give. This thesis would not have been what it became without you by my side! I love you!

ISI Journal Title Abbreviations

This list represents journals with abbreviations in the reference list of this thesis. Journals such as *Lancet* or *Hernia* are not included, because they are not abbreviated.

Am J Hum Genet	American Journal of Human Genetics
Am J Nucl Med Mol Imaging	American Journal of Nuclear Medicine and Molecular Imaging
Am J Pathol	American Journal of Pathology
Am J Roentgenol	American Journal of Roentgenology
AMA Arch Surg	American Medical Association Archives of Surgery
AMA Arch Pathol	American Medical Association Archives of Pathology
Ann Acad Med Singapore	Annals of the Academy of Medicine, Singapore
Ann Biochem	Annals of Biochemistry
Ann Intern Med	Annals of Internal Medicine
Ann Neurol	Annals of Neurology
Ann NY Acad Sci	Annals of the New York Academy of Sciences
Ann Surg	Annals of Surgery
Ann Vasc Surg	Annals of Vascular Surgery
ANZ J Surg	Australian and New Zeeland Journal of Surgery
Arch Int Med	Archives of Internal Medicine
Arch Surg	Archives of Surgery
Arterioscler Thromb Vasc Biol	Arteriosclerosis Thrombosis and Vascular Biology
Arthritis Rheum	Arthritis and Rheumatism
Biochem Pharmacol	Biochemical Pharmacology
Biol Proced Online	Biological Procedures Online
Br J Surg	British Journal of Surgery
Clin Chem Lab Med	Clinical Chemistry and Laboratory Medicine
Clin Nucl Med	Clinical Nuclear Medicine
Circ Res	Circulation Research
Eur Heart J	European Heart Journal
Eur J Clin Microbiol Infect Dis	European journal of Clinical Microbiology & Infectious Diseases
Eur J Neurol	European Journal of Neurology

Eur J Nucl Med Mol Imaging	European Journal of Nuclear Medicine and Molecular Imaging
Eur J Pharmacol	European Journal of Pharmacology
Eur J Vasc Endovasc Surg	European Journal of Endovascular and Vascular Surgery
Exp Mol Med	Experimental & Molecular Medicine
Expert Rev Cardiovasc Ther	Expert Review of Cardiovascular Therapy
FEBS Letters	Federation of European Biochemical Societies Letters
Int Angiol	International Angiology
Int J Appl Radiat Is	International Journal of Applied Radiation and Isotopes
Int J Biochem Cell Biol	The International Journal of Biochemistry & Cell Biology
Interact Cardiovasc Thorac Surg	Interactive Cardiovascular and Thoracic Surgery
Jama	Journal of the American Medical Association
J Am Coll Cardiol	Journal of the American College of Cardiology
J Anat	Journal of Anatomy
J Biol Chem	Journal of Biological Chemistry
J Chem Soc, Perkin Trans	Journal of the Chemical Society
J Clin Invest	The Journal of Clinical Investigation
J Clin Pathol	Journal of Clinical Pathology
J Clin Epidemiol	Journal of Clinical Epidemiology
J Endovasc Ther	Journal of Endovascular Therapy
J Heart Valve Dis	The Journal of Heart Valve Disease
J Histochem Cytochem	The Journal of Histochemistry and Cytochemistry
J Immunol	Journal of Immunology
J Investig Med	Journal of Investigative Medicine
J Label Comp Radiopharm	Journal of Labelled Compounds and Radiopharmaceuticals
J Leuc Biol	Journal of Leukocyte Biology
J Med Screen	Journal of Medical Screening
J Neural Transm	Journal of Neural Transmission
J Neurosci Res	Journal of Neuroscience Research
J Nucl Med	Journal of Nuclear Medicine
J Nucl Med Technol	Journal of Nuclear Medicine Technology
J Steroid Biochem Mol Biol	The Journal of Steroid Biochemistry and Molecular Biology
J Surg Res	The Journal of Surgical Research
J Vasc Res	Journal of Vascular Research
J Vasc Surg	Journal of Vascular Surgery
Life Sci	Life Sciences
Med Chir Trans	Medico-chirurgical Transactions

Med J Aust	The Medical Journal of Australia
Mem Acad Chir	Memoires. Academie de Chirurgie
Microbiol Rev	Microbiological Reviews
Mol Imaging Biol	Molecular Imaging and Biology
N Engl J Med	New England Journal of Medicine
Nat Genet	Nature Genetics
Nat Med	Nature Medicine
Nat Rev Immunol	Nature Reviews. Immunology
Neurosurg Rev	Neurosurgical Review
Nucl. Instr. and Meth. in Phys. Res. A	Nuclear Instruments & Methods in Physics Research Section A- Accelerators Spectrometers Detectors and Associated Equipment
Nucl Med Biol	Nuclear Medicine and Biology
PLoS ONE	Public Library of Science ONE
Proc Natl Acad Sci U S A	Proceedings of the National Academy of Sciences of the United States of America
Pure Appl. Chem	Pure and Applied Chemistry
Scand J Rheumatol	Scandinavian Journal of Rheumatology
Scott Med J	Scottish Medical Journal
Semin Vasc Surg	Seminars in Vascular Surgery
Surg Endosc	Surgical Endoscopy
Surg Gynecol Obstet	Surgery, Gynecology & Obstetrics
Thromb Haemost	Thrombosis and Haemostasis
Vasc Endovascular Surg	Vascular and Endovascular Surgery
Vestn Khir Im I I Grek	Vestnik khirurgii imeni I. I. Grekova (Journal of Surgery named after I. I. Grekov (recipient's translation))

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