Methodological aspects of quantitative cardiac molecular imaging

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The objective of this research was to facilitate the use of quantitative cardiac molecular imaging by developing and validating methods and applications. More specifically:

we determined the optimal tracer kinetic model for analysis of $^{11}$C-PIB and evaluated the performance of two simpler measures, retention index (RI) and standardized uptake value (SUV), in the quantification of cardiac $^{11}$C-PIB uptake in amyloidosis. An irreversible two-tissue (2Tirr) model best described the $^{11}$C-PIB uptake in cardiac amyloidosis. RI and SUV showed high correlation with quantitative results from this kinetic model and also a better discrimination between amyloidosis patients and controls than a 2Tirr model with population averaged metabolite correction. RI and SUV are furthermore more feasible for use in clinical routine and therefore the preferred measure to use in PET diagnosis of cardiac amyloidosis.

We also tested the feasibility of a semiautomatic software to analyze RI and visualize cardiac uptake of $^{11}$C-PIB in amyloidosis. The RI values were comparable with RI based on manual segmentation, showing significantly higher $^{11}$C-PIB RI in amyloidosis patients than in healthy volunteers. A fast and accurate semiautomatic analysis process is thus feasible to use for PET in cardiac amyloidosis instead of the laborious manual analyses that were used so far.

Furthermore, we assessed the quantitative accuracy of cardiac perfusion measurements with $^{15}$O-water PET in a digital time-of-flight PET-MR scanner. A high correlation and agreement between PET-MR based and PET-CT based MBF was found; cardiac perfusion measurements with $^{15}$O-water can therefore be performed accurately with the fully integrated Signa PET-MR scanner.

Finally, we assessed the quantitative accuracy of cardiac perfusion measurements using dynamic contrast-enhanced MRI with simultaneous $^{15}$O-water PET as reference at rest and during adenosine-induced hyperemia with a fully integrated PET-MR scanner. The correlations between global and regional MRI- and PET-based MBF values were good and the biases were negligible for both global and regional MBF comparisons, but the limits of agreement were wide for both global and regional MBF, with larger variability for high MBF-values indicating that MRI-based quantitative MBF measurement based on widely available acquisition protocols is not yet ready for clinical introduction.

Keywords: PET, cardiac amyloidosis, $^{11}$C-PIB, retention index, standardized uptake value, PET-MR, MRI, myocardial blood flow, $^{15}$O-water, quantification, quantitative modeling

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


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Abbreviations

AIC: Akaike information criterion
AL: Immunoglobulin light-chain amyloidosis
ATTR: Transthyretin-related amyloidosis
BGO: Bismuth germanium oxide
BP: Binding potential
$^{11}$C: Carbon-11
$C_A(t)$: Radioactivity concentration in arterial blood
CAD: Coronary artery disease
CFR: Coronary flow reserve
CMA: Central molecular imaging array
CMR: Cardiac magnetic resonance
CT: Computed tomography
$C_{PET}(t)$: Radioactivity concentration as measured in a voxel by PET
$C_{RV}(t)$: Radioactivity concentrations in the right ventricular cavity
DCE MRI: Dynamic contrast-enhanced magnetic resonance imaging
DTPA: Diethylenetriamine penta-acetic acid
EF: Ejection fraction
ESC: European Society of Cardiology
$^{18}$F: Fluorine-18
FDG: Fluorodeoxyglucose
FIESTA: Fast Imaging Steady-state Acquisition
FGRE: Fast gradient echo sequence
FOV: Field of view
FUR: Fractional uptake rate
Gd: Gadolinium
ICC: Intraclass correlation coefficient
$K_i$: Net influx rate; rate of irreversible binding
LGE: Late gadolinium enhancement
LYSO: Lutetium yttrium orthosilicate
MBF: Myocardial blood flow
MPR: Myocardial perfusion reserve
MR: Magnetic resonance
MRAC: Magnetic resonance attenuation correction
MRI: Magnetic resonance imaging
$^{13}$N: Nitrogen-13
NEMA: National Electrical Manufacturers Association
\(^{15}\text{O}\) Oxygen-15
OSEM Ordered subsets expectation maximisation
PET Positron emission tomography
PTF Perfusable tissue fraction
PIB Pittburg compound B
PMT Photomultiplier tube
PS Permeability-surface area product
RI Retention index
\(^{82}\text{Rb}\) Rubidium-82
ROI Region of interest
RPP Rate pressure product
SiPM Silicon photomultiplier
SD Standard deviation
SPECT Single photon emission computed tomography
SSFP Steady-state free precession
SUV Standardized uptake value
1T Single-tissue compartment model
1TCM Single-tissue compartment model
2Tirr Irreversible two-tissue model
2Trev Reversible two-tissue compartment model
TAC Time activity curve
TBR Target to background ratio
TE Echo time
TIC Time intensity curve
TOF Time of flight
TR Repetition time
UAA Upper anterior array
\(V_{LV}\) Left ventricular spillover fraction
\(V_T\) Distribution volume
\(V_{RV}\) Right ventricular spillover fraction
VOI Volume of interest
Introduction

Molecular imaging is defined as the ability to visualize and quantitatively measure the function of biological and cellular processes in vivo (1). Positron emission tomography (PET), single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) are examples of currently used clinical molecular imaging methods.

PET is a non-invasive imaging technique based on the detection of annihilation photons from a substance labelled with a positron emitting radionuclide. The radionuclides are used for labelling substances of biological interest and this allows the imaging and quantification of various physiologic processes; blood flow, substrate metabolism, receptor function etc. PET is, on the contrary to other techniques like SPECT and MRI, inherently quantitative, i.e. the PET signal is proportional to the PET tracer concentration. Furthermore, it is a tracer method, i.e. the PET substance is injected in so small quantities that it does not affect biology.

PET has from the very beginning of the PET era been used in studies of cardiac physiology and pathophysiology. Due to limited availability, technical complexity and high cost PET has until now been mainly a research tool in the cardiovascular field. This is now changing as PET is increasingly being used in oncology and more PET scanners are installed together with improved radiotracer availability. In recent years PET is also increasingly being used in cardiology as a diagnostic tool in clinical routine, mainly in perfusion imaging. Other clinical applications in cardiac PET are also emerging; PET as a diagnostic tool in cardiac amyloidosis is a new and relatively unexplored field.

MRI is a non-invasive imaging technique that is based on measuring the response of the atomic nuclei of body tissues to high-frequency radio waves when placed in a strong magnetic field, and is not associated with radiation exposure. Cardiac magnetic resonance (CMR) is widely used for cardiac morphology, function and viability assessment in daily clinical work. CMR is also increasingly used for myocardial perfusion imaging. CMR perfusion images are mostly interpreted visually and semi-quantitatively but myocardial perfusion can also be quantified with dynamic contrast-enhanced MRI although it is technically demanding.

Hybrid imaging devices, like SPECT/CT and PET/CT are increasingly being used in clinical routine, mainly in the oncologic field, and are also now emerging in cardiac imaging. Recently, integrated PET-MRI systems have become available, which allow for imaging with PET and MRI simultaneously.
However, the experience in simultaneous cardiac PET and MR imaging is limited and there are several technical challenges why applications need to be validated.

Quantitative molecular imaging is promising but until now relatively new in clinical cardiac imaging. There is need of methodological advances with development and validation of new methods to further increase the use of quantitative molecular imaging, both as a research tool, and in daily clinical work, in cardiovascular medicine.

$^{15}$O-water PET in myocardial perfusion imaging

Early detection of coronary artery disease (CAD) and in particular of myocardial ischemia remains a major challenge even with the advent of novel non-invasive imaging techniques and further development of existing modalities. Several imaging modalities are being used in assessment of myocardial perfusion and in detection of coronary artery disease (CAD). According to ESC Guidelines, non-invasive imaging modalities like PET, SPECT, stress-echo-cardiography and CMR should be used to evaluate patients with stable symptoms and known or suspected CAD with intermediate pre-test probability of obstructive coronary disease (2). Compared with SPECT myocardial perfusion imaging, which is a widely used gammacamera-based nuclear medicine imaging technique, PET perfusion imaging has a higher diagnostic accuracy (3-5), although most of the previous studies were based on qualitative PET imaging using $^{82}$Rb and $^{13}$NH$_3$. Unlike SPECT and other modalities, PET has the ability to measure myocardial blood flow (MBF) in absolute terms. The added value of quantitative MBF over qualitative myocardial perfusion imaging has been pointed out in several studies (6-10) showing that quantitative assessment of perfusion is especially valuable in patients with multivessel disease, microvascular dysfunction and for detecting early changes in MBF. However, although studies have been published evaluating the diagnostic performance of qualitative cardiac PET imaging (7, 11, 12), there has been a paucity of data on the diagnostic accuracy of quantitative cardiac PET imaging. In a recent collaboration study including patients evaluated for CAD from Amsterdam, Turku and Uppsala the optimal cut-off values of quantitative myocardial perfusion were determined and in addition, the diagnostic accuracy of quantitative $^{15}$O-water cardiac PET on a per-patient and per-vessel level was determined (13). The optimal cut-off value of quantitative $^{15}$O-water PET MPI for the detection of hemodynamic significant CAD was ≤2.3 mL·min$^{-1}$·g$^{-1}$ for hyperemic MBF and 2.5 for the CFR. The PET perfusion results indicated that the diagnostic performance of absolute hyperemic MBF was greater than the flow reserve. Quantitative $^{15}$O-water PET MPI provided an accuracy of 85% for the detection of flow-limiting CAD as defined by abnormal FFR.
There are several PET-tracers, with different properties, available for the assessment of myocardial perfusion. An ideal perfusion tracer is extracted from the blood to the myocardium proportionally linear to perfusion, even at high flow rates and irrespectively of metabolic state (14), which is true only for $^{15}$O-water. $^{15}$O-water PET perfusion measurements show close agreement with the invasive reference standard, microsphere flow in animal experiments, and has good test-retest variability, and is considered to be the current gold standard for non-invasive quantitative measurements of MBF (15-17). $^{15}$O-water is freely diffusible and is not, like other PET or SPECT perfusion tracers, trapped in the myocardium, thus no late-uptake images for visual assessment of myocardial perfusion is possible. In recent years, however, software packages have been developed, that generate parametric images of myocardial perfusion, i.e. graphical presentations of absolute MBF values, making visual assessment of quantitative perfusion possible (18, 19).

![Figure 1: $^{15}$O-water PET images; rest and stress perfusion. Reduced stress perfusion is seen in the left ventricular anterior, septal and apical wall. Images from Cardiac VUer software.](image)

The physical half-life of $^{15}$O-water is only 122 s, and thus challenging for logistics, requiring a cyclotron in close proximity of the PET unit and injection of the tracer when the patients is lying in the PET scanner. $^{15}$O-water can therefore not be used for perfusion studies during physical exercise but is used in protocols with pharmacological stressors. On the other hand, the short physical half-life of $^{15}$O-water makes short imaging protocols possible and gives a low radiation burden. The imaging protocol for $^{15}$O-water myocardial perfusion at Uppsala PET-centre, shown in Fig 2, takes about 30 min and the radiation dose from rest and stress PET perfusion is 0.88 mSv.
Recently, integrated PET-MRI systems have become available, which allow for measurements with PET and cardiac MRI simultaneously. Cardiac MRI has become the gold standard in assessment of myocardial volumes, myocardial mass and ventricular function and is also used for tissue characterization and vascular flow measurements. Cardiac PET-MRI can give improved functional and morphological information (size, regional and global cardiac function, ejection fraction, stroke volume, intravascular flow measurements, tissue characterisation, etc.) compared to PET-CT or PET alone. Although myocardial perfusion can be quantified with MRI, it is technically demanding and correlates poorly with PET-based MBF according to earlier studies (20). Combining MBF quantified with PET and functional and morphological information obtained with MRI is promising and will allow for a more comprehensive assessment in cardiac disease in a single patient visit. In addition, radiation doses can be reduced because no CT is needed for attenuation correction of PET data.

However, dynamic scans with short-lived tracers such as $^{15}$O-water are among the biggest challenges to PET systems, because of the combination of very high countrates immediately after injection when all of the injected radioactivity is inside the field of view (FOV) of the scanner, and very low count rates at the end of the scan because of the near homogeneous distribution in the body and the passing of three radioactive half-lives. In addition, the larger axial FOV and smaller detector ring diameter compared to PET-CT result in a higher sensitivity, and hence higher count rates which presents a challenge for countrate linearity. These also result in a larger fraction of scattered radiation, which is further amplified by the presence of coils inside the FOV. Furthermore, attenuation correction based on MRI (MRAC) is still challenging (21, 22), and little is known on the quantitative accuracy of cardiac perfusion PET imaging with a PET-MR scanner. Hence, the performance of the PET systems in the new PET-MR scanners in relation to the measurement of MBF needs to be validated.
CMR in myocardial perfusion imaging

\(^{15}\)O-water PET is considered to be the gold standard for non-invasive quantitative measurements of myocardial blood flow (MBF)\(^{(15, 16)}\). However, PET is associated with radiation exposure and is still not widely available. Furthermore, \(^{15}\)O-water is freely diffusible and is not, like other PET or SPECT perfusion tracers, trapped in the myocardium, thus making assessment of left ventricular volumes and function technically very challenging. On the other hand, cardiac magnetic resonance (CMR) is more widely available, does not require ionizing radiation and can be used for morphology, function and viability assessment. CMR is also increasingly used for myocardial perfusion imaging and has an excellent diagnostic performance in the detection of obstructive CAD\(^{(3, 5, 23, 24)}\). Although there are different approaches in perfusion assessment with MRI, myocardial blood flow is usually assessed or measured from the contrast enhancement observed during the first pass of a contrast agent bolus. In a clinical setting the CMR perfusion images are mostly interpreted visually or semi-quantitatively. Improvements in acquisition and post-processing methods have paved way for CMR quantification of MBF and calculation of myocardial perfusion reserve (MPR), a ratio of stress and rest perfusion values. MPR is a measure commonly used in the diagnosis of CAD, however, several studies have shown that absolute MBF at stress is superior to perfusion reserve in the detection of haemodynamically significant CAD\(^{(8, 11, 13, 25)}\). Quantitative perfusion imaging with CMR has been validated against microspheres in animals\(^{(26-28)}\) and also been compared to PET in a few sequential studies with findings that MPR correlates well between PET and CMR, but that the absolute MBF values correlate relatively poorly, possibly suggesting that the errors in quantification have a similar influence on both rest and stress perfusion values and are cancelled by the calculation of MPR\(^{(20, 29-31)}\). These errors might be due to either physiological or methodological differences.

Perfusion imaging uses dynamic T1-sensitive sequences during the first-pass of a bolus of gadolinium-based contrast agent, typically covering 3-5 short-axis slices through the left ventricle, with images every cardiac cycle during the passage of the contrast agent bolus. Regional delivery of gadolinium with the blood increase the signal intensity on T1-weighted images. Time signal-intensity curves (TICs) for myocardial tissue regions and for blood-pool are generated after tracing the myocardial borders and placing a ROI in the left ventricular blood. Mathematical models are then used for calculation of myocardial blood flow with parameters from the signal-intensity curves. The different quantification approaches can broadly be divided in model-based (compartment models) and model independent/deconvolution-based methods\(^{(32, 33)}\). Different compartment models are also frequently being used in quantitative PET and they are described more in detail below in the
section “Quantification in PET”. Briefly, compartment models divide the myocardial tissue and blood vessels into distinct spaces – compartments; for example, intravascular, intracellular and interstitial compartments. The gadolinium concentration varies in these compartments as gadolinium molecules move from one compartment to another. Rate constants describe the movement between the compartments. Gadolinium contrast agents are extracted from the intravascular compartment into the interstitial compartment but do not enter the intracellular compartment, why a 2-compartment model has been used for quantification of myocardial blood flow. The transfer of gadolinium from the intravascular compartment to the interstitial compartment is denoted $K_1$, and is equal to the product of myocardial blood flow (MBF) and the extraction fraction (E) of gadolinium:

$$K_1 = MBF \times E$$

An ideal perfusion tracer is extracted from the blood to the myocardial tissue proportionally linear to perfusion, even at high flow rates, which is true only for $^{15}$O-water (14). For many other perfusion tracers, as well as contrast agents such as gadolinium (34), the extraction fraction decreases with increasing flow rate. This non-linear extraction has to be corrected for in the modeling and calculation process in order to accurately quantify perfusion. The extraction of a tracer depends on the permeability of the capillary wall and on the capillary surface area. The Renkin-Crone model (35, 36) describes the relationship between the transfer of gadolinium to the interstitial compartment ($K_1$) and myocardial blood flow (MBF), capillary permeability (P) and capillary surface area (S):

$$K_1 = MBF (1 - e^{-PS/MBF})$$

The model independent/deconvolution-based quantitative methods can briefly be described as: the output from a system (the myocardial TIC) is equal to the input to the system (the blood pool TIC) merged with a transfer function by convolution. Convolution is mathematical operation on two functions to produce a third function that expresses how the shape of one is modified by the other (a mathematical process to fold two curves together). A commonly used transfer function in myocardial perfusion quantification with MRI is the Fermi-function (37). The amplitude of the transfer function equals the myocardial blood flow. To obtain the transfer function, the input and output curves undergoes a reverse process – deconvolution, a mathematical operation that reverses the effect of convolution on data.

Accurate quantification further requires that the MRI signal intensity is proportional to the contrast agent concentration. However, for gadolinium MR-contrast agents this is true only up to a certain concentration limit (38) and
non-linearity between the signal intensity and gadolinium-concentration is a major challenge for perfusion quantification with MRI, especially for assessment of the arterial input function (AIF) from the contrast enhancement in the blood-pool; underestimation of the Gd-concentration in the blood-pool will then overestimate tissue perfusion. Low injected contrast doses, dual-bolus techniques (26, 39), dual-sequence techniques (40) or retrospective correction by the use of calibration curves or modeling (41-43) can be used to overcome this challenge.

Reproducibility of quantitative cardiac perfusion measurements with MRI have been reported to be good or at least moderate (44-48). Direct comparison with the reproducibility of PET based myocardial perfusion measurements is hampered by different measures of repeatability used in different studies, wide time-ranges between the repeated measures in some repeatability studies and different methods used for the perfusion quantification. However, the repeatability coefficients reported in some MRI studies (45, 48) are somewhat higher and the ICC somewhat lower (45, 47) than PET-studies (17, 49), suggesting that the reproducibility of MRI based cardiac MBF measurements so far has been inferior to that of $^{15}$O-water PET.

A recent study comparing quantitative myocardial perfusion with sequential MRI and PET however showed promising results with reasonable agreement between MBF values from the two modalities (50) and the repeatability was reported to be good (51). The MRI method that was used was recently developed and optimized for quantification of MBF by the use of several integrated corrections (52).

In the last few years integrated PET-MRI systems have become available, which allow for MBF measurements with CMR and PET simultaneously during the same physiological condition. In a recent study simultaneous MRI and PET perfusion measurements using a cardiac phantom showed similar first-pass dynamics for the PET radiotracer $^{18}$F-fluoride and MRI gadolinium contrast agent and a linear relationship between absolute PET perfusion and relative MRI perfusion parameters (53). To our knowledge, there are no previously published studies on simultaneous $^{15}$O-water PET and MRI myocardial perfusion quantification in humans. Using PET-MR this validation of CMR perfusion and correlation to PET can be done. If a good agreement between PET and MRI can be shown, using a clinically feasible MRI method, this would greatly enhance the availability of quantitative MBF imaging in hospitals where PET with short-lived tracers is unavailable.
Cardiac amyloidosis

Amyloidosis is a disease in which different types of insoluble proteins, amyloid fibrils, are deposited extracellularly in various tissues, leading to progressive organ dysfunction (54). Cardiac amyloidosis is the result of the deposition of amyloid-type fibrils in the myocardium. This might either be due to the expansion of a plasma cell clone in the bone marrow producing the immunoglobulin light chains of the fibrillary deposits (acquired monoclonal immunoglobulin light-chain amyloidosis, AL), or due to deposition of misfolded transthyretin (TTR), a transport protein synthetized mainly in the liver. TTR amyloidosis is either hereditary or due to misfolding of wild-type TTR (55).

Cardiac involvement in amyloidosis is associated with high morbidity and mortality due to arrhythmia, ischemia and progressive heart failure (56). Treatment of amyloidosis is targeting the plasma cell clone that is producing the immunoglobulin light-chains in AL-amyloidosis. For TTR-amyloidoses several treatments are under investigation in ongoing clinical trials (57, 58). Reliable early diagnosis is therefore important, as well as quantification of the amyloid load, for appropriate management and for assessment of disease burden, progression and response to treatment.

The gold standard for diagnosis of cardiac amyloidosis is based on histological analysis on tissue obtained by endomyocardial biopsy, but this gives limited information of amyloid load or distribution. Several non-invasive imaging modalities, including echocardiography, magnetic resonance imaging (MRI) and various nuclear imaging methods have been used in the assessment of cardiac amyloidosis, but until now, none of these methods have been able to directly visualize and quantify the amyloid deposits in the heart (59-72).

Echocardiography is usually the initial modality used in cardiac amyloidosis patients. Cardiac amyloidosis is often suspected in a patient with heart failure symptoms and increased left ventricular wall thickness on echocardiography together with a QRS low voltage pattern on ECG. Other ECG findings in cardiac amyloidosis can be pseudo-infarct patterns, arrhythmias, conduction defects and axis deviation. Echocardiography can besides increased wall thickness also show a restrictive LV filling pattern, decreased LV end-diastolic volumes, RV wall thickening and typically a preserved or mildly reduced LVEF. A sparkling appearance of the LV myocardium on echocardiography is often reported in amyloidosis patients but is not specific (73). Although echocardiography is a cornerstone in imaging in cardiac amyloidosis, it can still be difficult to differentiate between amyloidosis and other types of LV hypertrophy. New echocardiographic techniques are increasingly being used and might improve accuracy (66).

Cardiac MRI gives excellent images of cardiac morphology and function and can be used to measure volumes, mass and assess ventricular function to evaluate the typical morphological and functional changes in cardiac amylo-
dosis. The added value of CMR lies in tissue characterization showing characteristic LGE patterns, prolonged T1 times (native or post gadolinium contrast) and increased extracellular volume in the myocardium in cardiac amyloidosis patients, but although being sensitive markers, they are still not specific for amyloidosis on the molecular level (73).

Several radionuclide imaging methods for amyloidosis are available. $^{123}$I-serum amyloid P binds to amyloid fibrils and can visualize the distribution of amyloid in the body, but the heart is however not visualized by this method (64). Bone imaging scintigraphy agents, such as $^{99m}$Tc-DPD are taken up in the heart in patients with TTR-amyloidosis, probably by a non-specific calcium-mediated process, but these agents are not specific to amyloid and not useful in patients with AL-type of amyloidosis (67, 74). $^{123}$I-MIBG scintigraphy is used to image cardiac sympathetic denervation, which can be an early sign of cardiac amyloidosis (75-77), but can also be found in other types of cardiomyopathies, and is thus not specific for amyloidosis. $^{99m}$Tc-aprotinin, has in a few studies shown low uptake in the heart of patients with amyloidosis, probably by binding to antiproteases in amyloid deposits. (59, 62).

None of these gammacamera-based nuclear medicine imaging methods can quantify the amyloid deposits in tissue. With PET however, it is possible to obtain quantitative measures of tracer uptake in tissue and thus to quantify the amyloid load.

PET in cardiac amyloidosis

The $^{11}$C-labeled PET tracer Pittsburgh compound B ($^{11}$C-PIB), was developed for visualization and quantification of amyloid in the brain in Alzheimer’s disease (78). This tracer is a derivate of thioflavin-T, which is a dye that binds specifically to amyloid fibrils and is used in histology for identification of amyloid deposits (79). $^{11}$C-PIB was shown to be able to visualize amyloid deposits in the heart in patients with both immunoglobulin light-chain (AL) and transthyretin-related (ATTR) amyloidosis (80) and another study showed similar results for another PET-tracer; $^{18}$F-florbetapir PET (81). A recent study using $^{11}$C-PIB confirmed its ability to detect cardiac amyloidosis in patients with AL-amyloidosis (82). Another amyloid-specific PET-tracer, $^{11}$C-BF-227, also has shown cardiac uptake in one patient with ATTR amyloidosis (83). PET is thus a promising non-invasive tool for direct imaging of the amyloid fibrils and for quantification of the amyloid burden allowing specific diagnosis and follow-up after treatment in patients with cardiac amyloidosis.

In clinical routine the PET analysis process needs to be fast and simple to perform and yield accurate and reliable results. The earlier PET studies of cardiac amyloidosis (80, 81) relied on manually defined regions and volumes of interest (ROIs and VOIs), which is both time consuming and difficult to reproduce, while an automatic or semiautomatic segmentation and ROI/VOI
definition would be preferred. The cardiac amyloid deposits are often diffuse and homogenous, but the distribution can also be heterogeneous, so both global and regional analysis of the amyloid deposits are needed for comprehensive assessment. Parametric images facilitate the visual assessment of the amyloid distribution in the heart and are thus desirable. Finally, any analytic tool needs to proof its performance also when there is low or no amyloid deposition in the heart. This is challenging for accurate delineation of the myocardial wall, even for manual ROI definition, and of major concern for automatic segmentation tools.

Retention index (RI) is a simple analysis method, which seems to perform well with amyloid-specific PET tracers as a diagnostic tool for cardiac amyloidosis (80, 84). Standardized uptake value (SUV), SUV ratios and target to background ratio (TBR) are other simplified analysis methods that have been used and all of these measures showed higher values in amyloidosis patients than in controls (81, 82). However, these measures cannot differentiate between amyloid-specific binding and non-specific tracer uptake, tracer in the blood-pool, spill-in from surrounding tissues, or radioactive metabolites, which probably explain why also the healthy volunteers have had non-zero values. Detecting early amyloidosis and small changes in the amyloid load after therapy might therefore be challenging for RI and other simple analysis models. RI and SUV have yet not been validated against full compartment modeling and metabolite analysis in cardiac amyloidosis.

Quantification with PET

PET enables imaging and quantitative measurements of physiological processes in the body. Positron-emitting isotopes are incorporated or bound to different physiological compounds that are administered in small quantities, tracer amounts, intravenously. With the PET scanner we detect the annihilation radiation of the isotopes and after the data is reconstructed and corrected for attenuation, scatter, randoms etc. we get quantitative images of the concentration of radioactivity, representing regional tracer tissue concentration (Bq/mL). Various methods can be used for quantification in PET.

ROIs/VOIs, TACs

In order to be able to calculate physiological parameters from the PET data, like for instance blood flow in tissue, we need to know what happens to the radioactivity concentration over time. PET data is therefore obtained as a dynamic scan in separate frames over time. From the dynamic PET images we can now obtain information of the radioactivity concentration over time in regions of interest (ROIs) or volumes of interest (VOIs) and we can present the data in time-activity curves (TACs), as shown in Figure 3.
Compartment models

For calculations of physiological parameters from the PET data we can use mathematical models based on the knowledge of the biochemical and physiological behavior of the radiotracer in tissue. One way to do this is to describe the process by spaces or compartments, which are interconnected through the exchange of substance (85). The simplest compartment model is the one-tissue compartment model (or two-compartment model) shown in Fig. 4. This model can be used to calculate the myocardial blood flow with freely diffusible PET tracers, like $^{15}\text{O}$-water.

\[ C_a \xrightarrow{K_1} C_t \xrightarrow{k_2} \]

Figure 4: $C_a$ defines the concentration of substance in arterial blood, $K_1$ the rate of change of substance from blood to the tissue-compartment, defined by $C_t$, the concentration of substance in tissue, and $k_2$ is the efflux rate of substance back to blood.

In the figure, the tissue concentration is affected by a rate of movement of substance from blood into tissue and by a rate of loss of substance from the tissue. Changes in the tracer tissue concentration $C_t$ can be described in terms of the tracer blood concentration $C_a$ and the two unidirectional rate constants.
K₁ and k₂. By expressing the values of these parameters mathematically, the values of these parameters can be computed from the measured PET data.

A two-tissue compartment model (or three-compartment model) as shown in Fig 5. is for example used for measurement of glucose transport and phosphorylation rate using ¹⁸F-FDG.

![Figure 5: Two-tissue compartment model for the concentrations of arterial Cₐ, free C₉ and bound substance Cₜ.](image)

Outcome measures

For models where there is no trapping of tracer within a compartment (reversible models), the volume of distribution, Vₚ, can be calculated as an outcome measure. The volume of distribution is equal to the equilibrium ratio of tracer concentration in tissue and in plasma. For the one-tissue model: \( V_T = \frac{K_1}{k_2} \) and for the two-tissue model: \( V_T = \frac{K_1}{k_2} (1+k_3/k_4) \).

Binding potential, BP, is another commonly used PET-measure, and expresses the total density/concentration of receptors or binding sites for the tracer in a tissue and equals the ratio of specifically bound tracer to free tracer concentration. \( BP_{ND} = \frac{k_3}{k_4} \), where \( BP_{ND} \) expresses the non-displaceable tracer uptake.

If tracer uptake is irreversible, the \( k_4=0 \). For irreversible tracers the net influx rate or rate of irreversible binding, \( K_i \), can be calculated:

\[
K_i = \frac{K_1 k_3}{k_2 + k_3}
\]
Input function

There is a constant influx of tracer from blood to the tissue. Some tracers are metabolized already during the PET scan and also bind to proteins and blood cells in the blood. Only the concentration of the tracer that is unchanged (not metabolized) and free in arterial plasma is in most cases considered as influx to the tissue and is called the input-function for the model.

Calculation of a correct input-function is a complex process in several steps needing arterial cannulation and several arterial blood samples during the PET scan for:

- measurement of radioactivity in arterial blood over time for a precise TAC for the input function.
- measurement of radioactivity in plasma and in whole-blood to be able to calculate the fraction of tracer that is free in plasma.
- measurement of metabolized and non-metabolized fraction of tracer.

With these measurements a metabolite-corrected plasma input function can be calculated.

The arterial blood activity can also be measured from the heart cavities and aorta in the PET images for calculation of an image-derived input function (IDIF). This is for example done for quantification of myocardial blood flow using $^{15}$O-water. For many tracers, except $^{15}$O-water, the IDIF still needs to be corrected for metabolites and converted to a plasma curve.

Ideally, fractions of plasma metabolites should be measured for each individual in a PET study. However, this is expensive, time consuming and complicated and can also cause analytical errors in the data. Another alternative is to calculate a population averaged metabolite corrected plasma curve from a limited group of individuals.

Choosing the best model - comparison of fits

There are several different tests that can be used for statistical comparison of fits, that is how well a model fits the PET data. Akaike information criterion and Akaike weights are commonly used methods for model comparison. The Akaike information criterion (AIC) (86) is given by:

$$AIC = N \times \ln(WSSE) + 2 \times p$$

in which $N$ is the number of frames, $WSSE$ is the weighted squared sum of residual fit errors and $p$ is the total number of parameters for each model. The fit with the lowest AIC is considered to be the “best” fit. The Akaike weights (87) can be interpreted as probabilities; the probability that the given model is
the best model. If we were to go back and obtain more data from the population and refit the same models again, then the Akaike weight gives the probability that a given model would be judged the best model on repeated sampling.

Simplified PET measures

Standardized uptake value (SUV) is a widely used simple PET measure, calculated as a ratio of tissue radioactivity concentration at a certain time and administered dose at the time of injection divided by body weight. SUV calculation does not require dynamic imaging or blood sampling.

\[
SUV_{BW} = \frac{C_{PET}(T)}{Dose/Weight}
\]

Retention index (RI) or Fractional uptake rate (FUR) is calculated as a ratio of tissue activity \(C_{PET}(T)\) at a certain time \(T\) and the integral of blood activity from time 0 to \(T\) \((88, 89)\).

\[
FUR = \frac{C_{PET}(T)}{\int_0^T c_{blood}(t)dt}
\]

\(RI/FUR\) can be calculated from a single late PET scan if blood sampling from the injection to the scan time has been done, alternatively the integral of blood activity is calculated from the PET images if a dynamic scan was performed.

Target to background ratio (TBR) is calculated as tracer uptake in tissue divided by tracer uptake in reference or background tissue. In cardiac studies the blood pool in left ventricular cavity can be used as background/reference. Neither blood sampling or dynamic scanning is required for calculation of TBR.
The overall aim of this work was to facilitate the use of quantitative molecular imaging in the cardiac field by developing and validating methods and applications in quantitative cardiac PET and MRI. More specifically, the aims of the studies were:

**in paper I:** to determine the optimal tracer kinetic model for analysis of $^{11}$C-PIB data and to evaluate the performance of two simplified methods, retention index (RI) and standardized uptake value (SUV), in the quantification of cardiac $^{11}$C-PIB uptake in amyloidosis. Finally, all methods were applied to a previously acquired dataset including both amyloidosis patients and healthy controls to address the ability of each method to discriminate between patients and controls.

**in paper II:** to evaluate the feasibility of a semiautomatic analysis process to analyze and visualize the left ventricular retention index (RI) of $^{11}$C-PIB in cardiac amyloidosis. Additionally, RI of two different time intervals were compared, to evaluate if the difference in mean RI between patients and healthy volunteers varies with different time frames.

**in paper III:** to validate a silicon photomultiplier (SiPM)-based time-of-flight (TOF) capable PET-MR scanner for quantitative cardiac PET imaging using $^{15}$O-water, by comparison to routine clinical PET-CT data. In addition to the cardiac PET-MR reconstruction protocol, as recommended by the scanner manufacturer, comparisons were made using a PET-CT resolution-matched reconstruction protocol both without and with TOF to assess the effect of time-of-flight and reconstruction parameters on quantitative MBF values.

**in paper IV:** to assess the quantitative accuracy of cardiac perfusion measurements using dynamic contrast-enhanced MRI with simultaneous $^{15}$O-water PET as reference at rest and during adenosine-induced hyperemia with a fully integrated PET-MR scanner in patients with known or suspected CAD.
Materials and Methods

Study population

The subjects described in this thesis were patients with systemic amyloidosis and heart involvement, healthy volunteers and patients with known or suspected CAD referred for a cardiac PET study for evaluation of MBF.

For paper I nine patients with systemic amyloidosis and heart involvement were included. All patients had immunohistochemistry-confirmed amyloid disease of AL- or ATTR-type, and heart involvement was diagnosed by echocardiography (n=6) or by myocardial biopsy (n=2), according to the criteria published by Gertz et al (90), or by cardiac MRI (n=1). One patient died before performing the PET scan. Eight patients (mean age 68 years, range 54 to 78; 6 males) completed the study. Data from one subject however was excluded due to errors in the metabolite analysis. Also, the same retrospective data as in paper II, was used in paper I.

In paper II data from ten patients (mean age 66 y; range 48-77 y) with systemic amyloidosis and heart involvement and 5 healthy, age matched, volunteers (mean age 64 y; range 54-75 y) were analyzed retrospectively. In brief, all patients had immunohistochemistry-confirmed amyloid disease of AL- or ATTR-type, and heart involvement was diagnosed by myocardial biopsy or echocardiography. The subjects have been described in detail elsewhere (80). The healthy volunteers had no symptoms or history of cardiac disease.

The study population of paper III consisted of eleven patients (9 male; mean age 59 y; range 46-74 y). The patients had known or suspected CAD with intermediate pre-test probability of obstructive coronary disease (20-84% clinical pre-test probability) according to ESC Guidelines (2), and were referred for a $^{15}$O-water PET-CT study for evaluation of MBF according to clinical routine. MBF data from one patient was excluded because of movement during the PET-CT scan.

15 patients were included in paper IV (9 male; mean age 66 y; range 51-75 y). They had known (n=7) or suspected CAD (n=8; 20-68% clinical pre-test probability according to ESC Guidelines) (2) and were referred for a $^{15}$O-water PET study for evaluation of MBF according to clinical routine. The study protocol was completed in 12 patients (one subject was excluded due to contrast injector failure and two subjects were excluded due to ECG-gating failure during the MR-perfusion scan), and further analysis relates to these patients.
PET-CT and PET-MR scan procedures

In paper I the subjects underwent a 35-min dynamic emission scan of the heart, which was started simultaneously with intravenous bolus injection of $^{11}$C-PIB (5 MBq/kg) on a Discovery ST PET/CT (subject nr 1-6) or Discovery MI scanner (subject nr 7-8) (GE Healthcare). Recovery was matched in the two scanners based on previous measurements with a NEMA image quality phantom. Imaging was performed in 3D-mode. All appropriate corrections for normalization, dead time, decay, scatter, randoms, and attenuation were applied. Images were reconstructed into 31 frames (12x5, 6x10, 4x30, 2x60, 2x120 and 5x300 s) using ordered subset expectation maximization (OSEM) with 2 iterations and 21 subsets (Discovery ST) or time-of-flight OSEM with 3 iteration and 16 subsets (Discovery MI) and a 5 mm gaussian post-filter. Images consisted of 128 x 128 voxels, with dimensions of 2.34 x 2.34 x 3.27 mm (Discovery ST) and 2.34 x 2.34 x 2.79 mm (Discovery MI), and a spatial resolution of approximately 7 mm.

In paper II the patients and healthy volunteers underwent a 25-min dynamic emission scan of the heart, which was started simultaneously with an intravenous bolus injection of $^{11}$C-PIB (6 MBq/kg) on a Discovery ST PET/CT scanner (GE Healthcare). Imaging was performed in 2-dimensional mode. A low-dose CT scan was performed for attenuation-correction. All appropriate corrections for normalization, dead time, decay, scatter, randoms, and attenuation were applied. Images were reconstructed into 29 frames (12x5, 6x10, 4x30, 2x60, 2x120 and 3x300 s) using ordered subset expectation maximization (2 iterations, 30 subsets), with the application of a 5-mm Gaussian filter. Images consisted of 128 x 128 voxels, with dimensions of 2.34 x 2.34 x 3.27 mm, and a spatial resolution of approximately 7 mm.

In paper III the subjects underwent $^{15}$O-water PET scans at rest and during adenosine-induced hyperaemia on both a GE Discovery ST PET-CT and a GE Signa PET-MR scanner on the same day (9 subjects) or within four days (2 subjects). All the subjects were instructed to abstain from caffeine for 24 hours before imaging.

PET-CT: A 6-min dynamic PET perfusion scan during rest was started simultaneously with the administration of 400 MBq of $^{15}$O-water. After a 20-30 min delay to allow for decay of the remaining activity following the first injection, an identical PET scan was performed during adenosine-induced hyperaemia. Adenosine infusion $140 \mu g \times kg^{-1} \times min^{-1}$ was started 2 min prior to the stress scan and continued during the 6-min scan time. To correct for photon attenuation, a single low-dose respiration-averaged CT scan during normal breathing was acquired before the resting PET scan (140 kV, 10 mAs, rotation time 1 sec, pitch 0.562). PET-CT images were reconstructed using OSEM (2 iterations, 21 subsets), applying all appropriate corrections such as for random coincidences, dead time, normalisation, scatter, etc., using a transaxial FOV of 50 cm and a 128x128 image matrix.
PET-MR: A 6-min dynamic PET perfusion scan during rest was started simultaneously with the administration of 400 MBq of $^{15}$O-water. After a 20-30 min delay following the first injection, an identical PET scan was performed during adenosine-induced hyperaemia as described above. Functional MR-imaging was obtained between the rest and stress PET-scans with a FIESTA cine sequence covering the left ventricular myocardium from apex to base in 8-mm thick short-axis slices with 2.0 mm gap. To correct for photon attenuation, a two-point Dixon sequence during breath-hold was acquired during the resting PET scan and during the hyperaemic PET scan. This sequence enables segmentation of fat and water tissue, lungs and air, which form the basis for creation of the MR-based attenuation map. The arms, which are not included in the MR images, are added to the attenuation map from non-attenuation corrected TOF-PET data (91). PET-MR images were reconstructed using OSEM into 128x128 pixel images and a FOV of 53.4 cm, using the cardiac protocol as recommended by the manufacturer (from here on referred to as std). To assess the effect of TOF and reconstruction settings on MBF values, PET-MR data were also reconstructed using the PET-CT resolution-matched protocol based on the phantom study, both without and with TOF. Reconstruction parameters are summarised in Table 1. All appropriate corrections such as for random coincidences, dead time, normalisation etc. were applied in all reconstructions.

In paper IV all subjects underwent simultaneous $^{15}$O-water PET and gadolinium-DTPA-perfusion MR scans at rest and during adenosine-induced hyperaemia. All the subjects were instructed to abstain from caffeine for 24 hours before imaging.

A 6-min dynamic PET perfusion scan during rest was started simultaneously with the administration of a bolus of 400 MBq of $^{15}$O-water. MR perfusion imaging was performed during the PET scan; a single bolus of gadolinium-DTPA contrast agent (Dotarem 0.1 ml/kg) was injected 3 minutes after the start of the PET scan at 5 ml/s by a power injector followed by a bolus of saline (25 ml NaCl at 5 ml/s). An ultrafast gradient echo sequence (FGRE Time Course) was used for MR perfusion imaging with the following imaging parameters: TR 3.4 msec, TE 1.4 msec, flip angle 20°, 380 x 304 mm field of view, slice thickness 8 mm, 128x102 matrix, prepulse delay 120 msec. The perfusion images were acquired for 65 heartbeats during breath-hold and consisted of 3 short-axis slices (basal, midventricular and apical).

Functional MR-imaging was obtained between the rest and stress PET-scans with a FIESTA cine sequence covering the left ventricular myocardium from apex to base in 8-mm thick short-axis slices with 2.0 mm gap.

After the functional MR-images a dynamic PET scan and a MR perfusion scan as described previously were performed during adenosine-induced hyperaemia. Adenosine infusion $140 \mu g \times kg^{-1} \times min^{-1}$ was started 2 min prior to the stress scan and continued during the PET and MR scan time.
To correct for photon attenuation, the same technique was used as in paper III. PET- images were reconstructed using OSEM into 128x128 pixel images and a FOV of 53.4 cm, using the cardiac protocol as recommended by the scanner manufacturer.

**Blood sampling and definition of input functions for $^{11}$C-PIB**

In paper I all subjects received a radial artery catheter for arterial blood sampling during the dynamic PET-scan. Discrete blood samples (5 mL) were drawn manually at circa 2.5, 5, 10, 15, 20, 25 and 35 min post injection. For each sample, activity concentrations in whole blood and plasma were determined. The percentage of intact $^{11}$C-PIB in plasma was determined by HPLC analysis using UV- and radio detection: an 1.8 mL sample was injected onto a semi-preparative HPLC column (Genesis C18, 7 µm, 250x10 mm, Phenomenex) equipped with a guard column (C18 SecurityGuard, 10x10 mm, Phenomenex). The column was eluted at a flow rate of 6 mL/min with acetonitrile-50 mM ammonium acetate pH 5.3 (55:45, v/v). The outlet from the detector was connected to a switching valve on the arm of the liquid handler to enable automatic fraction collection. Three fractions were collected, the first two containing the metabolites and the third containing the unmetabolized parent compound, and the radioactivity in each fraction was measured by a well-type scintillation counter.

Regions of interest were placed in the left ventricular cavity in 5 consecutive transaxial planes and then combined into a volume of interest (VOI). A second VOI was placed over the right ventricular cavity. These VOIs were transferred to the dynamic image sequence to obtain the left and right ventricular time-activity curves (TACs). Input functions were calculated by multiplication of the left-ventricular TAC with a single exponential fit to the measured plasma - whole blood ratios and a sigmoid fit to the fraction of unmetabolized $^{11}$C-PIB in plasma.

**Data analysis**

**Paper I**

**Volumes of interest**

The dynamic $^{11}$C-PIB scan was analyzed using Carimas software (version 2.63) developed at Turku PET Centre in Finland (www.turkupetcentre.fi/carimas/). 17 myocardial segment VOIs were semiautomatically drawn over the
left ventricle according to the 17-segment model of the American Heart Association (92) and segmental TACs were extracted.

**Tracer kinetic modelling**

Whole-myocardium and segment TACs were fitted to a single-tissue compartment model (1T), an irreversible two-tissue compartment model (2Tirr), as well as a reversible two-tissue compartment model (2Trev) and two variations of this model where the non-specific distribution volume $K_1/k_2$ or both $K_1/k_2$ and $k_4$ were fixed to their whole-myocardium values, respectively. In addition, a dual-input single-tissue model (1T-1T), with parallel compartments for $^{11}$C-PIB and radioactive metabolites, was evaluated, accounting for the possibility that radioactive metabolites of $^{11}$C-PIB enter myocardial tissue. Fitted corrections for spill-over from left and right ventricular cavities were included in all models and fits were performed using non-linear regression in in-house developed software in Matlab. Outcome measure for the 1T model was the volume of distribution $V_T = K_1/k_2$, for the 2T models $V_T = K_1/k_2 (1+k_3/k_4)$ and the binding potential $BP_{ND}$ were evaluated, whereas for the irreversible models the net influx rate $K_i = (K_1k_3/(k_2+k_3))$ was used.

To exclude unreliable fits, fits with outcome parameters with standard errors larger than 25% were discarded. The best fit was determined using the Akaike information criterion (AIC) (86) and Akaike weights (87).

**Simplified methods**

$RI_{15-25}$ was calculated as the mean $^{11}$C-PIB radioactivity concentration between 15 and 25 min after injection divided by the integral of the arterial whole blood TAC between 0 and 20 min, as described in detail previously (93). $SUV_{15-25}$ was calculated as the mean $^{11}$C-PIB radioactivity concentration between 15 and 25 min after injection normalized to the injected dose divided by patient weight. This time-frame was chosen as it was used when calculating the $^{11}$C-PIB RI in our previous work (80). Correlations between RI, SUV and the outcome parameter of the preferred model were assessed using linear regression. In addition, RI and SUV were calculated between 10 and 20, 20 and 30, 25 and 35 and between 10 and 30 min post injection to assess time-dependent variations in correlations with fully quantitative data.

**Population-averaged metabolite correction**

A population-averaged correction for plasma/whole blood ratios and parent fractions was calculated using the data from all six subjects. Tracer kinetic modelling was repeated using input functions based on this correction and correlation between outcome measures were assessed using regression analysis. In addition, correlation between RI, SUV and outcome parameters of tracer kinetic analysis based on population-averaged blood data was calculated.
Retrospective data

Furthermore, retrospective data from 10 amyloidosis patients with heart involvement and 5 healthy controls that has been described in detail previously (80), was analysed using the population-averaged metabolite correction and the optimal tracer kinetic model. RI and SUV between 15 and 25 min were also calculated for this retrospective dataset. Differences in tracer kinetic model outcome, SUV and RI between amyloidosis patients and healthy controls were assessed using Mann-Whitney U test and Cohen’s d.

Paper II

In paper II the dynamic transaxial $^{11}$C-PIB PET images were analyzed with Carimas software (version 2.63) developed at Turku PET Centre in Finland (www.turkupetcentre.fi/carimas/). Two independent observers, one nuclear medicine physician and one PET-technologist, both with long experience in cardiac imaging, performed the analysis.

The first step in the analysis process is to select an image delineating the myocardial wall well for subsequent automatic segmentation. Using a difference image, subtracting early frames (blood-pool images) from later frames, the myocardium could be well delineated in the $^{11}$C-PIB images, even in the healthy volunteers and in the patients with low or no myocardial $^{11}$C-PIB uptake. The base and the apex of the left ventricle are manually identified, as well as the right and left ventricular cavities, and then VOIs are automatically created in the left ventricular cavity and over the entire left ventricular wall. The VOIs can be modified if needed. The left ventricle is further automatically segmented into 17 standardized segments, according to the definition of the American Heart Association (92). An arterial plasma time-activity curve is calculated from the VOI in the left ventricular cavity, and time-activity curves of the left-ventricular tissue are calculated from the left ventricular wall VOI. The FUR (Fractional Uptake Rate) analysis model was chosen to calculate the $^{11}$C- retention index (RI). The RI was calculated as a ratio of tissue activity at 15-25 min and integral of plasma activity from time 0 to 20 min. Additionally, the RI of $^{11}$C-PIB was also calculated also at 10-20 min. In all, the analysis process could be performed in less than five minutes. The earlier, manual analysis method performed by Antoni et al. has been described in detail elsewhere (80), but in brief, an arterial time-activity-curve was calculated after drawing regions of interest in the ascending aorta in the $^{11}$C-PIB images. The left ventricular wall was difficult to delineate in the $^{11}$C-PIB images of the healthy volunteers and the patients with low cardiac retention of PIB, and thus the left ventricular ROIs were defined in co-registered $^{11}$C-acetate images from a dynamic $^{11}$C-acetate PET scan that was performed the same day. The ROIs were then transferred to the $^{11}$C-PIB images. The mean $^{11}$C-PIB RI was then calculated as the mean $^{11}$C-PIB radioactivity concentration between 15 and 25 min.
after injection divided by the integral of the arterial time-activity curve between 0 and 20 min.

Paper III

In paper III the PET data was analysed semi-automatically using Cardiac VUer software, resulting in both parametric MBF images and segment-based MBF values for the entire left ventricle and for three regions corresponding to the coronary artery territories (18). Coronary flow reserve (CFR) was defined as stress perfusion divided by rest perfusion as was calculated for each segment. The calculation of MBF was based on a one-tissue compartment model with an input function from arterial cluster analysis comprising left atrial and ventricular cavities and ascending aorta and with correction for spillover from left and right ventricular cavities into the myocardium (18):

\[
C_{\text{PET}}(t) = PTF \cdot MBF \cdot C_A(t) \otimes e^{\frac{-MBF}{V_T} \cdot t} + V_{LV}C_A(t) + V_{RV}C_{RV}(t)
\]

Here, \(C_{\text{PET}}(t)\) is the radioactivity concentration as measured in a voxel or region by PET, \(PTF\) is the perfusable tissue fraction, \(V_T\) is the distribution volume of water, here fixed to 0.91 mL/g. \(C_A(t)\) and \(C_{RV}(t)\) are the radioactivity concentrations in arterial blood and in the right ventricular cavity, respectively, and \(V_{LV}\) and \(V_{RV}\) are the left- and right-ventricular spillover fractions. Parametric images were computed using a basis-function implementation of this model (18), whereas regional values were calculated using non-linear regression of equation 1. For PET-MR data, the cluster analysis and parametric image construction in Cardiac VUer were only performed for the standard clinical reconstruction protocol. For assessment of the regional MBF values for the other reconstruction methods, the blood vessel and regional myocardial VOIs resulting from the standard clinical analysis were projected onto the resolution-matched images both without and with TOF. Parametric MBF images from PET-CT and from PET-MR were compared visually.

To verify the count rate linearity of PET-MR during the first pass of the radioactivity through the PET FOV, the area under the time-activity curves from the arterial input functions during the first minute of the scans was compared for PET-CT and PET-MR. For this comparison, the arterial time-activity curves were normalised to their mean radioactivity concentrations during the last 4 minutes of the scan to account for possible small differences in amount of injected \(^{15}\)O-water.
The MRI and the PET images were analysed and segments were defined separately. The right ventricular insertion was used as reference point. Myocardial segment VOIs were drawn over the left ventricle based on the 17-segment model of the American Heart Association (92) and segmental signal and activity vs. time curves were extracted and myocardial blood flow was calculated for the entire left ventricle and for three regions corresponding to the coronary artery territories.

Regions of interest were defined in the MRI images using the software package Segment (Medviso, Lund Sweden)(94). The left ventricular endo- and epicardial borders were manually delineated for both rest and stress data. and signal vs. time curves were then generated for myocardial tissue and for ventricular blood. The PET data was analysed semi-automatically using Cardiac VUer software (18), generating MBF-values for the entire left ventricle and in three regions corresponding to the coronary artery territories.

**Calculation of Gd concentrations**

To convert the MRI signal to Gd concentrations, the following steps were taken. The signal intensity for a spoiled gradient echo sequence can be described by:

\[ S = k \frac{(1 - e^{-T_R/T_1})\sin\alpha}{1 - (\cos\alpha)e^{-T_R/T_1}} e^{-T_E/T_2^*} \]

Here, \( T_R \) is the repetition time (3.4 ms), \( \alpha \) is the flip angle (20°) and \( k \) is an arbitrary constant. As long as \( T_E<<T_2^* \), the exponential term containing \( T_2^* \) can be neglected. The constant \( k \) was determined for each individual VOI by dividing the baseline signal (before contrast arrival) during the rest scan by the remainder of the right-hand side of the equation evaluated for a \( T_{10} \) (baseline \( T_1 \) ) of 1052 ms in myocardial tissue. For blood, a haematocrit-dependent \( T_{10} \) was used: \( T_{10}=1000/(0.52\times HCT+0.38) \) (95). Then, for each myocardial segment and for blood the signal curve \( S(t) \) was converted to a \( T_1 \) curve \( T_1(t) \) by interpolating the \( T_1 \) versus \( S \) curve. Gd-DOTA concentrations were subsequently calculated as:

\[ C_{Gd}(t) = \frac{1}{r} \left( \frac{1}{T_1(t)} - \frac{1}{T_{10}} \right) \]

where \( r \) is the relaxivity of Gd-DOTAREM: 2.8 s\(^{-1}\)mM\(^{-1}\) at 3 T (96). Gd-DOTA concentrations during stress were corrected for Gd-DOTA remaining after the rest scan by subtracting the Gd-DOTA concentration prior to contrast arrival.
Tracer kinetic model

Both PET and DCE-MRI data were analysed using the standard single-tissue compartment model (1TCM) with fitted blood volume:

\[ C_T(t) = (1 - V_A)K_1C_A(t) \otimes e^{-k_2 t} + V_A C_A(t) \]

For PET, the arterial input curve \( C_A(t) \) was equal to the whole blood curve because of the free diffusibility of \(^{15}\)O-water whereas for DCE-MRI, \( C_A(t) \) was the Gd-DOTA concentration in plasma, obtained using each individual’s hematocrite. For DCE-MRI, an additional parameter was added to account for the delay between the left ventricle and the arrival of the bolus in each myocardial segment. For \(^{15}\)O-water, an extra term \( V_{RV}C_{RV}(t) \) to account for spill-over from the right ventricular cavity was added, and \( V_A \) is rather a spill-over term than a blood volume term, which is why \((1-V_A)\) was omitted in the first term of the equation. For both tracers, MBF was assumed equal to \( K_1 \), and can then be interpreted as the transmural blood flow. Two different approaches to account for the limited extraction of Gd-DOTA were implemented. Firstly, a relation between Gd-DOTA \( K_1 \) values and \(^{15}\)O-water MBF values by fitting the Renkin-Crone model to the measured data:

\[ K_1 = MBF(1 - e^{-PS/MBF}) \]

where PS is the permeability surface area product, and \( K_1 \) values were converted to Gd DOTA MBF values by interpolation of this function. Secondly, the single-tissue compartment model was also implemented using permeability-surface area product PS as a fourth parameter (1TCM-PS):

\[ C_T(t) = (1 - V_A)MBF(1 - e^{-PS/MBF}) \otimes C_A(t) \otimes e^{-k_2 t} + V_A C_A(t) \]

Since PS and F cannot be determined independently using a single fit, PS was determined using a coupled fit of rest and stress whole-myocardium Gd-DOTA concentration curves, assuming identical PS values during rest and stress scans, and then used as a fixed parameter for each individual segment.

For \(^{15}\)O-water, the model was fitted to the full 6 min of data, whereas for Gd-DOTA, data between the arrival of the bolus in the left-ventricular cavity and either the peak of the second passage of the bolus or the time at which the patient started breathing was used.

Analysis of functional MR images in paper III and IV was performed on a GE AW workstation using commercially available software (CardiacVX). The endocardial contour was semi-automatically traced and manually adjusted when needed. The ejection fraction was calculated with the software using Simpson’s rule.
Statistics

Statistical analyses were performed using the IBM SPSS Statistics (versions 21.0 and 25.0 for Macintosh, SPSS, Chicago, Illinois) and GraphPad Prism (version 6 for Windows, GraphPad Software, La Jolla California). A two-sided p-value of less than 0.05 was considered significant. Measurements are presented as mean and range (paper I and II) or as mean values ± standard deviations (SD) (paper III and IV).

In paper I Akaike information criterion (AIC) (86) and Akaike weights (86) were used for statistical comparison of fits in order to choose the best model to describe the dynamic $^{11}$C-PIB PET data.

Shapiro-Wilks test was used to check if data was normally distributed. Levene’s test was used to assess the equality of variances for variables for groups. Comparisons between two groups were assessed with Mann-Whitney U test (papers I and II) and for repeated measurements with Wilcoxon Signed Rank test (papers II and III) or paired T-test (paper IV).

Linear regression (paper I and II) and Deming regression (paper III) were used to investigate the relationship between variables. The degree of association between two variables was measured using Spearman’s rank correlation coefficient (papers II and IV).

Agreement was assessed with intra-class correlation coefficient (ICC) (papers I, II and III) and Bland-Altman analysis (papers I, III and IV).

In paper I and II effect size was estimated and instead of using Cohen’s d, which measures the difference between the means of two groups in terms of their pooled SD, we measured the discriminative power as the difference between the lowest value of the parameter in patients and the mean value of the parameter in controls in terms of the SD of the control group. This method was chosen because of the very skewed, non-normal, distribution of and large spread in values in the patients.
Results

Paper I:
The analysis of the percentage of intact $^{11}$C-PIB in plasma failed in one patient and the data from this patient was excluded from further analysis. An example of a myocardial TAC from a typical patient together with corresponding fits is shown in Figure 6.

Figure 6: $^{11}$C-PIB time-activity curve of a myocardial segment in a patient with cardiac amyloidosis. Black, red, green and blue lines represent best fits according to single-tissue (1T), irreversible two-tissue (2T irr) and to two different reversible two-tissue compartment models (2T rev model and 2T rev model with fixed $V_{NS}$ and fixed $k_4$). 2T irr model fit is superimposed over 2T rev model fit.
Tracer kinetic modelling
Based on AIC, the 2Trev model was preferred in 45 out of 119 VOIs followed by the 2Tirr model (38/119) and the 2Trev model with fixed K_i/k_2 and fixed k_4 (28/119). The 2Trev model with fixed K_i/k_2 was preferred in only 7 out of 119 VOIs, the 1T-1T model in 1/119 and 1T-model in 0/119. Mean Akaike weights for the three preferred models were 0.41, 0.22 and 0.14, respectively. However, the 2Trev model was unable to provide robust estimates of either V_T or BP_ND, with standard errors frequently larger than the parameters themselves. To a lesser extent, this was also the case for the 2Trev models with fixed K_i/k_2 or fixed K_i/k_2 and k_4. The 2Tirr model, however, provided robust parameter estimates in all VOIs and was therefore chosen as the preferable model. When omitting the 2Trev model from the Akaike analysis, the 2Tirr model was preferred in 50 out of 119 VOIs (followed by the 2Trev models with fixed K_i/k_2 and fixed k_4 (31/119) and fixed K_i/k_2 (23/119)) and the Akaike weight increased to 0.39 for the 2Tirr model. The global mean value of the total net influx rate, K_i, using the 2Tirr model, was 0.043 (range 0.014-0.125) mL/cm^3/min.

Simplified methods
Global mean RI_{15-25} was 0.042 (range 0.027-0.096) min\(^{-1}\) and global mean SUV_{15-25} was 1.6 (range 1.0-3.8). Figure 7 shows parametric SUV/RI- and K_i-images from one patient.

Figure 7: Cardiac short axis \(^{11}\)C-PIB images from a patient with AL-amyloidosis. Left: SUV/RI image. Right: Net influx rate K_i image calculated using a basis function implementation of the 2Tirr model.
Figure 8 shows the relationships of global and segmental RI\textsubscript{15-25} respective SUV\textsubscript{15-25} with the net accumulation rate (K\textsubscript{i}) from the 2Tirr model. There was a clear correlation of global and segmental RI\textsubscript{15-25} with K\textsubscript{i} (r\textsuperscript{2}=0.99 and r\textsuperscript{2}=0.95) and of global and segmental SUV\textsubscript{15-25} with K\textsubscript{i} (r\textsuperscript{2}=0.97 and r\textsuperscript{2}=0.94).

Figure 8: Global and segmental RI\textsubscript{15-25} (a and b) respective SUV\textsubscript{15-25} (c and d) as a function of K\textsubscript{i} from 2Tirr model.
However, it was also clear that the relationships of $RI_{15-25}$ and $SUV_{15-25}$ with $K_i$ varied between the subjects as shown in Table 1. Furthermore, correlations between $RI$, $SUV$ and $K_i$ varied with time, as shown in Table 2.

Table 1: Parameters of correlation between segmental $K_i$, $RT_{irr}$ and $RI_{15-25}$ and $SUV_{15-25}$ for each subject, mean values of all individual parameters (mean) and parameters of correlation for the whole dataset (total).

<table>
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<th>$K_i$ $2T_{irr}$ vs. $RI$</th>
<th>$K_i$ $2T_{irr}$ vs. $SUV$</th>
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<tbody>
<tr>
<td></td>
<td>$r^2$ Slope (95% CI)</td>
<td>$r^2$ Slope (95% CI)</td>
</tr>
<tr>
<td>1</td>
<td>0.93 0.77 (0.65-0.88)</td>
<td>0.93 31.82 (27.12-36.53)</td>
</tr>
<tr>
<td>2</td>
<td>0.90 0.85 (0.69-1.00)</td>
<td>0.90 35.77 (29.23-42.30)</td>
</tr>
<tr>
<td>3</td>
<td>0.71 0.66 (0.43-0.89)</td>
<td>0.71 24.22 (15.72-32.72)</td>
</tr>
<tr>
<td>4</td>
<td>0.63 0.64 (0.37-0.92)</td>
<td>0.63 25.15 (14.43-35.88)</td>
</tr>
<tr>
<td>5</td>
<td>0.94 0.74 (0.64-0.84)</td>
<td>0.94 25.32 (21.85-28.79)</td>
</tr>
<tr>
<td>7</td>
<td>0.40 0.70 (0.23-1.18)</td>
<td>0.40 27.96 (9.16-46.77)</td>
</tr>
<tr>
<td>8</td>
<td>0.61 0.83 (0.47-1.19)</td>
<td>0.61 32.92 (18.54-47.29)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.73 0.74 (0.47-1.19)</td>
<td>0.73 29.02</td>
</tr>
<tr>
<td>Total</td>
<td>0.95 0.66 (0.64-0.69)</td>
<td>0.94 26.58 (25.32-27.89)</td>
</tr>
</tbody>
</table>

Table 2: Correlation between segmental $K_i$, $RT_{irr}$ and $RI$ and $SUV$ calculated from different time frames.

<table>
<thead>
<tr>
<th></th>
<th>10-20 min</th>
<th>15-25 min</th>
<th>20-30 min</th>
<th>25-35 min</th>
<th>10-30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r^2$ RI vs. $K_i$</td>
<td>0.94</td>
<td>0.95</td>
<td>0.97</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>$r^2$ SUV vs. $K_i$</td>
<td>0.94</td>
<td>0.94</td>
<td>0.95</td>
<td>0.95</td>
<td>0.81</td>
</tr>
</tbody>
</table>
**Population-averaged metabolite correction**

There was a rapid metabolism of $^{11}$C-PIB resulting in a large fraction of labelled metabolites towards the end of the scan (over 80% of the measured radioactivity at 35 min), with a substantial variation between subjects. Global mean $K_i$ was 0.038 (range 0.018-0.097) ml/cm$^3$/min using population-averaged metabolite corrections. Figure 9 shows a scatter-plot of global mean $K_i$ calculated with individual and with population-averaged metabolite corrections. Correlation ($r^2=0.99$) and agreement (ICC=0.97) were high, although for two patients $K_i$ based on population averaged metabolite correction resulted in lower values than $K_i$ based on individual metabolite correction.

Figure 9: Correlation (a) and Bland-Alman plot (b) of global mean $K_i$ calculated using individual metabolite corrections (horizontal axis) and $K_i$ calculated using population-averaged metabolite correction (vertical axis). Line of identity is shown as a solid line and regression line as a dashed line (a). The solid line in b indicates the mean difference (bias), whereas the dashed lines show the limits of agreement. Bias (limits of agreement) are -0.004 (-0.029 - 0.021).
**Retrospective data**

When retrospective data from 10 amyloidosis patients and 5 healthy controls were analyzed with the population-averaged metabolite correction and the 2Tirr model, the global mean $K_i$ in amyloidosis patients was 0.053 (range 0.016-0.179) ml/cm$^3$/min, compared with 0.015 (range 0.015-0.017) ml/cm$^3$/min in healthy controls. The correlations of global mean $RI_{15-25}$ and $SUV_{15-25}$ with $K_i$ were high ($r^2=0.98$ and $r^2=0.96$, respectively). There was a significant difference in $K_i$ between amyloidosis patients and healthy controls ($p=0.001$), although there was an overlap between the lowest $K_i$ in amyloidosis patients and the highest $K_i$ in controls (Figure 10).

![Figure 10](image-url)

Figure 10: Scatter dot plot diagrams of the myocardial global mean $K_i$ (a), $RI_{15-25}$ (b) and $SUV_{15-25}$ (c) in Figure 11.amyloidosis patients and in healthy controls. Lines indicates median values.
For comparison, global mean $RI_{15-25}$ was 0.056 (range 0.029-0.158) min$^{-1}$ in amyloidosis patients and 0.024 (range 0.022-0.026) min$^{-1}$ in controls ($p=0.001$) and global mean $SUV_{15-25}$ was 2.7 (range 1.6-8.0) in amyloidosis patients and 1.0 (range 0.9-1.2) in controls ($p=0.001$). Using a modified effect size measure the difference between patients and healthy volunteers was greater for $RI$ and $SUV$ than for $K_i$ (2.99 SD between lowest $RI$ in amyloidosis patients and mean $RI$ in controls, whereas the respective effect size measures for $SUV$ and $K_i$ were 2.54 SD and 1.11 SD).

**Paper II**

The mean $RI$ at 15-25 min from the semiautomatic analysis was compared with $RI$ based on manual analysis and showed comparable values (0.056 min$^{-1}$ vs. 0.054 min$^{-1}$ for amyloidosis patients and 0.024 min$^{-1}$ vs. 0.025 min$^{-1}$ in healthy controls; $p=0.78$). The correlation between $RI$ from semiautomatic analysis and $RI$ from manual analysis was excellent for global mean values (Spearman’s rho correlation coefficient, $r=0.97$, $p<0.001$) and good for regional values (Spearman’s rho correlation coefficient, $r=0.93$, $p<0.001$), as shown in Figure 11.

![Figure 11: Linear regression of the $^{11}$C-PIB $RI$ at 15-25 min; semiautomatic analysis vs manual analysis. a: global $RI$ values and b: regional $RI$ values.](image)

Inter-reader reproducibility also was excellent (intraclass correlation coefficient, ICC>0.98). Parametric polarmaps and histograms made visual separation of amyloidosis patients and healthy controls fast and simple. Figure 12 shows the analysis results from one healthy volunteer and four subjects with different levels and patterns of amyloid deposition in the heart.
Figure 12: Analysis results of $^{11}$C-PIB with semiautomatic software at 10-20 min; images are modified from Carimas showing polarplots and histograms for five different subjects: a: healthy volunteer, b: TTR amyloidosis, c: AL kappa amyloidosis, d: AL lambda amyloidosis, e: AL lambda amyloidosis. The histogram y-axis shows frequency (relative number of pixels) and the x-axis shows RI value.
The mean RI was higher in amyloidosis patients than in controls at both time frames (p=0.001), although the difference in RI between patients and controls was greater at the earlier time frame (4.4 SD between lowest RI in amyloidosis patients and mean RI in controls at the earlier time frame vs. 2.9 SD at the later time frame).

Paper III
Global mean (± SD) MBF values at rest and stress were 0.92 ± 0.12 and 2.74 ± 1.37 mL/g/min for PET-CT and 0.90 ± 0.23 and 2.65 ± 1.15 mL/g/min for PET-MR, respectively (p=0.33 and p=0.74). Global mean (± SD) CFR values were 2.97± 1.31 for PET-CT and 3.05± 1.23 for PET-MR (p=0.65). The relations between PET-MR-based and PET-CT-based regional MBF and CFR are shown in Figure 13. Intra-class correlation coefficients (ICC) between PET-CT and PET-MR regional MBF and CFR were 0.98 and 0.89.
Figure 13: Correlation (a, c) and Bland-Altman plots (b, d) of PET-MR based regional MBF std (clinical protocol) versus PET-CT based regional MBF (a,b) and regional CFR (c, d). Rest and hyperemic stress values are plotted for MBF. The solid lines in a and c are lines of identity. The solid lines in b and d indicate the mean difference (bias), whereas the dashed lines show the limits of agreement. The regression slopes are 0.91 (0.85-0.98) and 0.98 (0.64-1.32) in a and c, respectively. Bias (limits of agreement) are -0.04 (-0.73-0.65) and 0.11 (-1.56-1.78) in b and d, respectively.
Image quality was improved with PET-MR as compared to PET-CT. ICC between PET-MR-based MBF with and without TOF and using different filter and reconstruction settings was 1.00. The agreement between resolution-matched PET-MR-based MBF with and without TOF (FX and HD) and with standard reconstruction (PET-MR std) is shown in Figure 14.

Figure 14: Resolution matched PET-MR based MBF without TOF (HD) versus PET-CT based MBF (a), resolution-matched PET-MR based MBF without TOF (HD) vs resolution-matched PET-MR based MBF with TOF (FX) (b), and PET-MR FX versus clinical protocol (std) (c). Solid lines are lines of identity. Regression slopes are 0.90 (0.83-0.96), 0.98 (0.96-1.02) and 1.01 (1.00-1.01) in a, b and c, respectively.
Paper IV

An example of a myocardial time activity curve (TAC) from a typical patient together with corresponding fits and input curves from MRI and PET perfusion analysis is shown in Figure 15.

Segmental MBF and MPR values were excluded in 6 regional segments due to unreliable fits of the 1TCM+PS model during analysis of MRI perfusion data.

Using a 1TCM for MRI and PET perfusion analysis the relationship between MRI-based perfusion related parameter, $K_1$, and PET-based MBF, is shown in Fig 16a; the MR-based $K_1$ values underestimated perfusion above approximately MBF > 1 ml/min/g, as compared to PET MBF. Fig 16b shows the relationship between MRI based $K_1$ and PET based MBF when an extraction fraction correction has been applied to the MRI based $K_1$-values. The permeability surface area product of Gd-DOTA was estimated to be 2.6 mL/g/min. Although perfusion values >1 mL/min/g were no longer systematically underestimated after this correction, the extraction fraction-corrected $K_1$ values correlated poorly with PET MBF at high values.
When using an MRI-analysis model that included the permeability surface area product (PS) in order to correct for the low extraction of Gd-DOTA, global mean (± SD) MBF values at rest and stress were 0.97 ± 0.27 and 3.19 ± 0.70 mL/g/min for MRI and 1.02 ± 0.28 and 3.13 ± 1.16 mL/g/min for PET (p=0.66 and p=0.81). Mean PS was 2.91 ± 0.37 mL/g/min. The relationships between MRI-based and PET-based global and regional MBF values are shown in Figures 17 and 18.

Figure 16: Correlation of MR 1TCM based global K₁ versus PET based MBF (a). Correlation of MR 1TCM based global K₁ with correction for extraction fraction (EF) versus PET based MBF (b).
Figure 17: Correlation (a) and Bland-Altman plots (b) of MR based global MBF versus PET based global MBF at rest and stress. The solid line in a is line of identity. The solid lines in b indicate the mean difference (bias), whereas the dashed lines show the limits of agreement. Bias (limits of agreement) in b are 0.01 (-1.24 – 1.25).

Figure 18: Correlation (a) and Bland-Altman plots (b) of MR based regional MBF versus PET based regional MBF at rest and stress. The solid line in a is line of identity. The solid line in b indicate the mean difference (bias), whereas the dashed lines show the limits of agreement. Bias (limits of agreement) in b are 0.00 (-2.17 – 2.17).

The correlations between global and regional MRI- and PET-based MBF values were strong (r=0.86 and r=0.75, p<0.0005 for both). The biases were negligible for both global and regional MBF comparisons (0.01 and 0.00 mL/min/g, respectively), but the limits of agreement were wide for both global and regional MBF (-1.24 – 1.25 and -2.17 – 2.17), with larger variability for higher MBF-values.
The relationships between MR-based and PET-based global MBF values at rest and at stress compared separately are shown in Figure 19.

Figure 19: Correlation and Bland-Altman plots of MR based global MBF versus PET based global MBF at rest (a, b) and at stress (c, d). The solid lines in a and c are lines of identity. The solid lines in b and d indicate the mean differences (bias), whereas the dashed lines show the limits of agreement. Bias (limits of agreement) in b are -0.05 (-0.76 – 0.67) and in d 0.06 (-1.58 – 1.71).

The MBF values at rest did not correlate between MRI and PET (r=0.21, p=0.51) while the correlation was moderate for stress MBF values (r=0.69, p=0.013). Biases were negligible for both rest and stress MBF comparisons (0.06 and -0.05) but the limits of agreement were wide for stress MBF values (-1.58 – 1.71).

Global mean (± SD) MPR values were 3.44 ± 0.97 for MRI and 3.05 ± 0.76 for PET (p=0.83).
The relations between MRI-based and PET-based global and regional MPR are shown in Figure 20. There was no significant correlation between MRI- and PET-based MPR ($r=0.08$, $p=0.80$).

Figure 20: Correlation (a) and agreement (b) of MR based global MPR versus PET based global MPR. The solid line in a is line of identity. The solid line in b indicate the mean difference (bias), whereas the dashed lines show the limits of agreement. Bias (limits of agreement) in b are 0.39 (-1.94 – 2.73).
Discussion

The overall aim of this work was to facilitate the use of quantitative molecular imaging in the cardiac field by developing and validating methods and applications in quantitative cardiac PET and MRI.

$^{11}$C-PIB in cardiac amyloidosis

Recent studies have shown promise for PET as a non-invasive diagnostic and quantitative tool in amyloidosis, but PET in cardiac amyloidosis is a new and relatively unexplored tool. The $^{11}$C-labeled PET tracer Pittsburg compound B ($^{11}$C-PIB), was developed for visualization and quantification of amyloid in the brain in Alzheimer’s disease (78) and was recently shown to be able to visualize amyloid deposits in the heart in patients with cardiac amyloidosis (80). The optimal analysis method of $^{11}$C-PIB in cardiac amyloidosis has until now not been determined.

In paper I we therefore determined the optimal tracer model for kinetic analysis of $^{11}$C-PIB and evaluated the performance of two previously used, simpler measures, RI and SUV, in the quantification of cardiac $^{11}$C-PIB uptake in amyloidosis. An irreversible two-tissue (2Tirr) model was found to best describe the $^{11}$C-PIB uptake in cardiac amyloidosis. RI and SUV showed high correlation with quantitative results from this kinetic model.

We also applied all methods to a previously acquired dataset including both amyloidosis patients and healthy volunteers to address the ability of each method to discriminate between patients and controls. RI and SUV showed better discrimination between amyloidosis patients and controls than $K_i$ and are also more feasible for use in clinical routine. Therefore, RI and SUV are preferred in clinical diagnosis of cardiac amyloidosis.

Previous PET studies on cardiac amyloidosis have relied on manual analysis, which is laborious and subject to errors when tracer uptake is low or heterogeneous. In clinical routine work the analysis process needs to be both fast and simple to perform and yield accurate and reproducible results. In paper II we therefore tested the feasibility of a semiautomatic software to analyze and visualize cardiac uptake of $^{11}$C-PIB in amyloidosis. The semiautomatic analysis process was fast and easy to perform and the software performed well even in the subjects with no or low tracer uptake in the heart. The semiautomatic RI values were comparable with the RI based on manual segmentation.
and ROI tracing, showing significantly higher $^{11}$C-PIB RI in amyloidosis patients than in healthy volunteers. Parametric polarplots presented by the semiautomatic software made visual assessment simple, making it easy to separate healthy subjects from patients with cardiac amyloidosis and to assess regional differences in cardiac amyloid deposits. Furthermore, the inter-reader reproducibility was excellent with the semiautomatic method. A fast and accurate semiautomatic analysis process is thus feasible to use for PET in cardiac amyloidosis instead of the laborious manual analyses that has been used so far.

**Tracer kinetic modelling**

The exact mechanism and kinetics of $^{11}$C-PIB binding to amyloid are not known. For fully quantitative brain studies using compartment modelling, reversible two-tissue models best described the $^{11}$C-PIB kinetics (97, 98), although there is some discussion on whether an irreversible model is also appropriate for the scan durations typically used in PET studies (98). In paper I the reversible two-tissue models were unable to provide robust estimates of the outcome parameters, which the irreversible two-tissue model did. Omitting the 2Trev-model Akaike criteria and Akaike weights indicated that the 2Tirr model was the preferred model to describe myocardial $^{11}$C-PIB kinetics. A longer scan-time, as was used in the kinetic brain-studies, could hypothetically provide more robust fits for reversible models also in cardiac studies. On the other hand, the reversible two-tissue models are more complex as they contain more parameters that have to be estimated, introducing more uncertainty. Moreover, towards the end of the dynamic $^{11}$C-PIB scans the activity in the myocardium was very low, which is why increasing the scan time probably would not yield different modelling results in cardiac studies.

**Simplified methods**

Since fully quantitative studies with arterial blood sampling and metabolite analysis are not feasible in routine clinical practice, simplified analysis methods are needed. The first studies on $^{11}$C-PIB-imaging of brain β-amyloid in Alzheimer’s disease used SUV as a measure of $^{11}$C-PIB uptake (78) and subsequent brain studies have used simplified reference tissue models and a target-to-reference ratio in a late time interval (99-101). Due to the propensity of amyloidosis affecting multiple organs, reference tissue models are less suitable for quantification in cardiac amyloidosis. The simplified measures RI and SUV seem to perform well with amyloid-specific PET tracers in cardiac amyloidosis studies (80, 81). SUV ratios and target to background ratio (TBR) are other simple analysis models that have been used in cardiac amyloidosis studies (81, 82), but were not evaluated in our study.

Paper I showed a high correlation of RI and SUV with the total net accumulation rate, $K_i$, using the 2Tirr model ($r^2=0.99$ and $r^2=0.97$ for global values and $r^2=0.95$ and $r^2=0.94$ for segmental values respectively), with lower within-patient correlation for segmental values in most patients and with substantial
variation between individuals as shown in Table 1. This can most probably be explained by variations in the metabolism of $^{11}$C-PIB between patients, although technical challenges in metabolite analysis may also influence the results. Furthermore, the correlations between $K_i$ and the simplified measures varied when RI and SUV were calculated from different time frames. In the present study, RI and SUV were based on uptake between 15 and 25 min post injection. In an earlier study we showed that the difference in mean RI between amyloidosis patients and healthy subjects was greater at an early time frame (10-20 min) compared to a late time frame (15-25 min) (93). However, when RI and SUV were calculated using uptake from 10 to 20 min post injection in the present study, the correlations with $K_i$ were slightly lower ($r^2=0.94$ and $r^2=0.92$, respectively). Both simplified measures correlated better with $K_i$ when calculated at later time frames, as shown in Table 2.

**Population averaged metabolite correction**

In agreement with $^{11}$C-PIB brain studies (97, 98), the fraction of labelled metabolites was large towards the end of the scan (Figure 21B) and therefore a metabolite correction is needed for accurate quantification of $^{11}$C-PIB.

![Figure 21](image)

Figure 21: Plasma/whole blood concentration ratio (a) and parent fraction in arterial plasma (b) as a function of time. Whiskers show min and max values.

A population-averaged metabolite correction could make quantification of $^{11}$C-PIB possible without arterial blood sampling. $K_i$ was not significantly different when using the population averaged metabolite correction (global mean $K_i$ 0.038 (range 0.018- 0.097) ml/cm$^3$/min vs. 0.043 (range 0.014-0.0125) ml/cm$^3$/min; $p=0.92$) and correlation ($r^2=0.99$) and agreement (ICC=0.97) were high between $K_i$ based on population average metabolite data and $K_i$ based on individual metabolite data. However, for two patients with faster metabolism, $K_i$ resulted in lower values when using the population-averaged metabolite correction, clearly demonstrated in Figure 9. There was a substantial variation in the fraction of labelled metabolites of $^{11}$C-PIB between subjects, as shown in Figure 21b and a population-averaged metabolite correction could therefore result in inaccurate quantitative results for some subjects.
When assessing changes within a single patient, for instance before and after treatment, quantification using population-averaged metabolite corrections may be considered, assuming that the intervention does not change the metabolism of PIB. However, this assumption should also be tested as both disease progression and intervention may affect organ function and thus metabolism and confounders.

**Retrospective data**

In paper I, using retrospective data from 10 amyloidosis patients and 5 healthy controls, the 2Tirr model with population-averaged metabolite correction resulted in a significant difference in $K_i$ between patients and controls ($p=0.001$), although there was an overlap between the lowest $K_i$ in amyloidosis patients and the highest $K_i$ in controls. As has been shown before, both RI and SUV values were also significantly higher in patients than in controls (80, 81). Furthermore, in our retrospective data, there was no overlap in RI or SUV values between patients and controls and using a modified effect size measure the difference between patients and healthy volunteers was greater for RI and SUV than for $K_i$, suggesting that both simplified measures discriminated better between cardiac amyloidosis patients and healthy subjects than the 2Tirr model when based on population-averaged metabolite corrections. Individual metabolite corrections could maybe give other results with better separation of $K_i$ between patients and controls, but this would not be feasible to use in clinical routine.

**Semiautomatic analysis**

In our previous work the $^{11}$C-PIB RI was calculated by manual ROI/VOI definition over the left ventricle and in the aorta, which is a time-consuming process (80). Manually defined ROIs and VOIs depending on subjective decisions could also be difficult to reproduce; however, no reproducibility measurements were reported in that study. Furthermore, the analysis process was performed in two separate software packages and depended on transfer of ROIs from one scan to another, further increasing the risk of error. In contrast, the semiautomatic analysis process in paper II was fast and easy to perform; a few initial steps to orient the heart and to find a good image for segmentation were needed, whereas the ROI/VOI definition was automatic. By choosing an appropriate analysis model and the time frame, the software then calculated the RI. In all, the entire analysis process could be performed within five minutes. Furthermore, the inter-reader reproducibility for the semiautomatic analysis of RI was excellent (ICC>0.98).

Instead of using Cohen’s $d$, which measures the difference between the means of two groups in terms of their pooled SD, we measured the discriminative power as the difference between the lowest RI in patients and the mean RI in controls in terms of the SD of the control group. This method was chosen
because of the very skewed, non-normal, distribution of and large spread in values in the patients. The difference in mean RI between patients and volunteers was greater with the semiautomatic than with the manual analysis (2.9 SD between lowest RI in amyloidosis patients and mean RI in controls for semiautomatic analysis vs. 1.7 SD for manual analysis). This could probably be explained by the higher standard deviation and larger spread of the mean RI values with the manual analysis in healthy controls (SD 0.0045 for mean RI in healthy volunteers with manual analysis vs. SD 0.0017 with semiautomatic analysis).

In our previous work (80) and in the study by Dorbala et al. (81), the RI values were only presented as a global mean of the entire left ventricle. In most cases the amyloid is deposited diffusely in the myocardial tissue and the PET tracer uptake is homogenous, and a global mean value is representative for the cardiac amyloid deposition. However, some subjects with amyloidosis have heterogeneous PET tracer uptake, as shown in Figure 9. In these cases, a global mean RI value alone is probably not a good measure of cardiac amyloid deposition. In subjects with small areas of focal amyloid deposits a global mean RI could be low and could lead to a missed diagnosis. The semiautomatic software that was used in paper II presents the RI values both as a global mean for the entire left ventricle and as regional RI values in 17 standardized segments. The semiautomatic software had excellent agreement with manual analysis in global RI values and good agreement also in regional RI values. Two patients though (one with moderate and one with very high RI) had higher regional values with the semiautomatic software than with manual analysis. This can be explained by different ROI sizes; the semiautomatic ROIs were generally smaller than the manual ROIs, and smaller ROIs give higher RI values specifically in patients with high uptake because of the limited resolution recovery of the PET scanner.

The RI values are also presented in a parametric polarplot and as a histogram, which make it easy to visually assess both the global level of and the regional differences in amyloid deposition. In paper II all amyloidosis patients had higher global mean RI than the healthy controls, also the subjects with heterogeneous amyloid deposition. One subject had high $^{11}$C-PIB uptake in the septal regions and no or low uptake in apical regions; the RI values at 15-25 min varied from 0.023 min$^{-1}$ to 0.093 min$^{-1}$, with a global mean RI of 0.047 min$^{-1}$. One other subject with amyloidosis had one small area of low $^{11}$C-PIB retention with homogenous tracer uptake in the rest of the left ventricle, one subject had slightly higher uptake in the septal than in the lateral regions and seven subjects had fairly homogeneous uptake.

Subjects with low or no amyloid uptake present a challenge in analyzing the PET images. With low or no uptake, the left ventricle is difficult to delinate and the segmentation and ROI and VOI tracing becomes challenging. In our previous work we used $^{11}$C-acetate images for ROI tracing and copied the ROIs and VOIs to the co-registered $^{11}$C-PIB images. This approach requires
an additional $^{11}$C-acetate PET scan, which is not practical in clinical routine, increases radiation burden to the patient and adds a risk of misalignment. In the semiautomatic software that we tested in paper II, one of the first steps is to select an image that delineates the left ventricle well to allow for subsequent automatic segmentation. By using a differential image, subtracting early frames (blood-pool images) from later frames, the myocardium could be well delineated in the $^{11}$C-PIB images, even in the healthy volunteers and in patients with low myocardial $^{11}$C-PIB uptake, and the automatic segmentation and ROI/VOI definition performed well in all subjects. If needed, the ROIs/VOIs could be manually adjusted. In the semiautomatic software there is also an option to create a summed image to use for subsequent segmentation. This works well if the myocardial retention of the tracer is high, but for patients with low amyloid load or for healthy subjects, the summed image is not a good option.

The optimal time frame for calculating the RI of amyloid-binding tracers in cardiac amyloidosis has not been established. Dorbala et al. calculated the RI of $^{18}$F-florbetapir at 10-30 min (81). We previously analyzed the $^{11}$C-PIB mean RI at 15-25 min post injection, but we also showed that the difference in myocardial mean standardized uptake value (SUV) between amyloidosis patients and healthy volunteers was greater at earlier time points (80). Therefore, in paper II we calculated the mean RI at two different time frames; at 10-20 min and 15-25 min post injection. The difference in mean RI between patients and controls indeed was greater at the earlier time frame (4.4 SD between lowest RI in amyloidosis patients and mean RI in volunteers at the earlier time frame vs. 2.9 SD at the later time frame). The mean RI values were higher at the earlier time frame, both for amyloidosis patients and for healthy volunteers. The normal range and cut-off values for RI therefore are depending on the time frame for analysis.

Limitations

Fully quantitative PET studies with arterial sampling and metabolite analysis are technically demanding and prone to errors. Due to the technically demanding and costly procedure the sample size in paper I was relatively small. Metabolite analysis of $^{11}$C-PIB is challenging and the substantial variation in the fraction of labelled metabolites of $^{11}$C-PIB between subjects could therefore be a result of either technical difficulties in the metabolite analysis or true individual variations in metabolism. However, extensive quality control was applied during the metabolite analysis, measuring recovery in each step, and no systematic errors were found. In one subject, however, the metabolite analysis failed due to technical reasons and the data from this subject was excluded.

Due to the small sample size comparison of metabolism of $^{11}$C-PIB and $K_i$ values between subjects with AL- and ATTR type of amyloidosis could not
be done. Furthermore, no healthy volunteers participated in the study, and hence it is not certain that population average metabolite corrections based on patients with amyloidosis can be used for subjects without amyloidosis.

In paper I heart involvement of amyloidosis was diagnosed by myocardial biopsy in two subjects only and with echocardiography (n=1) in the others. For the retrospective data, cardiac involvement of amyloidosis was based on endomyocardial biopsy in 5 subjects, whereas echocardiographic criteria were used for the remaining 5 patients. The controls were considered healthy based on medical history. In the retrospective analysis one patient had lower \( K_i \) than the highest \( K_i \) in controls; this subject had TTR-type of amyloidosis and cardiac involvement was confirmed by endomyocardial biopsy.

The number of subjects in the study presented in paper II also was small and future studies including more subjects with various tracer uptake patterns and levels are needed to further validate the performance of the semiautomatic software. Subjects with high tracer uptake close to the myocardium could pose a problem for automatic segmentation, especially if the segmentation is performed on uptake images (summing frames with high uptake). Automatically created ROIs could then erroneously include adjacent extracardiac activity and the calculated myocardial RI could be falsely too high. The number of subjects in our study with high tracer uptake in the liver or in the lung was very small, but the software performed well also in these cases, possibly because the segmentation was done on difference images. Difference images could also be problematic if the close-by organs or tissues would have blood-flow similar to the myocardium, and thus affect the difference image and subsequent automatic segmentation. To avoid false analysis results caused by ROIs including extracardiac activity the images and ROIs have to be visually inspected and adjusted if needed. Indeed, in a small number of cases we needed to adjust the ROIs manually, but this was fairly simple and fast.

Quantitative myocardial perfusion imaging with PET-MRI

In paper III we assessed the quantitative accuracy of cardiac perfusion measurements with \(^{15}\)O-water PET in the Signa PET-MR scanner. A high correlation and agreement between PET-MR based and PET-CT based MBF was found; cardiac perfusion measurements with \(^{15}\)O-water can therefore be performed accurately with the fully integrated Signa PET-MR scanner. The previously established cut-off value for MBF with \(^{15}\)O-water in PET-CT is therefore applicable in PET-MR studies (13). We also found that TOF and reconstruction settings had little impact on MBF values.
In paper IV we assessed the quantitative accuracy of cardiac perfusion measurements using dynamic contrast-enhanced MRI with simultaneous $^{15}$O-water PET as reference at rest and during adenosine-induced hyperemia with a fully integrated PET-MR scanner. Although a good correlation and a negligible bias between MRI based and PET based MBF was found, the agreement was only moderate, with a large variability, especially for the higher MBF values.

Quantitative accuracy of cardiac perfusion measurements with $^{15}$O-water PET-MR

There was a high correlation and agreement between PET-MR based and PET-CT based MBF. The small differences between the MBF measurements in the PET-CT and in the PET-MR can likely be attributed to physiologic variations of myocardial blood flow and lie well within the variability of repeated measurements of $^{15}$O-water myocardial perfusion at rest and during adenosine hyperaemia as reported by Kaufman et al (17). The repeatability coefficients for MBF (calculated as 1.96 x SD of the differences) they reported were 0.17, 0.28 and 0.90 for global rest, global corrected rest and global adenosine stress respectively and the repeatability coefficients for regional MBF were 0.20-0.46 at rest and 0.41-0.59 at adenosine stress. The repeatability coefficients for MBF measured in the PET-CT and in the PET-MR in our study, as shown in Table 3 below are comparable to those reported by Kaufman et al.

Table 3: MBF and repeatability coefficients. Repeatability coefficient = 1.96 x SD of differences.

<table>
<thead>
<tr>
<th>MBF PET-CT rest</th>
<th>MBF PET-MR rest</th>
<th>Repeatability coefficient</th>
<th>MBF PET-CT stress</th>
<th>MBF PET-MR stress</th>
<th>Repeatability coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.92 ± 0.12</td>
<td>0.90 ± 0.23</td>
<td>0.34 37%</td>
<td>2.74 ± 1.37</td>
<td>2.65 ± 1.15</td>
<td>0.84 31%</td>
</tr>
<tr>
<td>Global corrected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.06 ± 0.27</td>
<td>1.02 ± 0.26</td>
<td>0.20 19%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.91 ± 0.14</td>
<td>0.91 ± 0.23</td>
<td>0.33 36%</td>
<td>2.74 ± 1.33</td>
<td>2.67 ± 1.14</td>
<td>0.92 34%</td>
</tr>
</tbody>
</table>

Abs= Absolute repeatability coefficient, %= repeatability coefficient as % of mean

Although the agreement between PET-CT based and PET-MR based MBF was high, the small differences in MBF values could still result in different clinical decisions for the PET-CT and for the PET-MR based studies. Using the previously established cut-off value of 2.3 mL/g/min to decide between normal and pathological stress MBF (13), on a subject based level, 5 subjects had pathological MBF (at least one segment with MBF <2.3 mL/g/min) in the
PET-CT study and all of these subjects also had pathological MBF in the PET-MR based analysis. 5 subjects had normal MBF in all segments in the PET-CT study; 4 of these subjects also had normal MBF in all segments in the PET-MR study whereas one subject had reduced MBF in all the segments (global MBF was 2.3 mL/g/min with PET-CT and 1.8 mL/g/min with PET-MR). This subject was one of the two subjects that underwent the PET-MR scan on a different day than the PET-CT scan; 3 days later. On a segment-based level 13 out of 30 segments had pathologically reduced MBF in the PET-CT study; 11 of these segments were also pathological with the PET-MR based analysis whereas 2 were normal. 17 segments had normal MBF in the PET-CT study and 13 of these regions were also normal with the PET-MR based analysis, whereas 4 segments were pathological. Altogether, in 24 out of 30 segments the PET-CT and the PET-MR based decisions of normal or reduced MBF agreed and in 6 segments they did not agree. 4 of these 6 segments that did not agree were in the two patients that underwent the PET-CT and PET-MR studies on different days. Patients were requested to not alter any medications between the PET-CT and the PET-MR scans and to withhold from caffeine during 24 hours before both PET-scans, but failure in compliance to this or other physiologic reasons, rather than differences in the scanners, may have influenced the results and we feel confident in trusting the clinical decisions based on the MBF values using the GE Signa PET-MR scanner. Considering the similar ICC values of the present PET-CT – PET-MR comparison and the variability study by Kauffman et al, it is likely that similar differences in clinical diagnoses would have occurred if PET-CT scans had been repeated. When using a fixed cut-off value for pathological MBF, there is always a probability that patients with MBF close to this cut-off value will be diagnosed differently based on different scans even with the relatively high reproducibility of MBF measurements.

CFR is commonly used in the diagnosis of CAD, although several studies have shown that absolute MBF at stress is superior to flow reserve (8, 11, 13, 25). In paper III, MBF values showed better agreement between PET-CT and PET-MR than CFR values, as shown in Figure 13. The same was observed also in paper IV where MBF and CFR was compared between PET and MRI, as shown in Figures 17 and 20.

In $^{15}$O-water cardiac PET scans the count rates are very high in early time frames, which presents a challenge for count rate linearity of the PET scanner and reliable arterial input function definition, which is essential for the calculation of MBF. We recently performed a NEMA count rate linearity test of the PET-MR scanner as part of the scanner’s acceptance procedure, starting at total amount of radioactivity of 950 MBq. This corresponds to a approximately 40 kBq/ml or 340 MBq in the field of view of the scanner, which is similar to the maximum amount encountered during the $^{15}$O-water scans if all the activity would be within the field of view during the first pass of the tracer. The measured radioactivity concentration did not deviate more than 5% from
the true radioactivity concentration at any time during this test, so we are confident that the scanner behaves linearly during the scans and the arterial input function is recovered well. This was further verified by comparing the area under the arterial input function during the first 1 min for PET-CT and PET-MR scans, which did not differ significantly.

As shown in Figure 14A, the relation between the resolution-matched PET-MR data and the PET-CT data was virtually identical to the relation between the clinical standard PET-MR data and PET-CT data depicted in Figure 13. Indeed, as Figure 14B and C show, the standard cardiac PET-MR reconstruction applying 3 iterations, 28 subsets and an 8 mm post-filter produced nearly identical MBF values to the PET-CT-resolution-matched reconstruction with 2 iterations, 28 subsets and a 6 mm filter, both without (HD) and with (FX) TOF. Although this may seem counterintuitive, this is probably due to the fact that for $^{15}$O-water, MBF is based on the clearance rate, i.e. the exponential term in Equation 1, of the tracer instead of the amplitude of the myocardial time-activity curve (TAC). Additional filtering does affect this amplitude, but not the shape of the myocardial TAC, and hence does not affect the clearance rate. This means that for $^{15}$O-water, additional filtering does not decrease MBF, whereas it would for other flow tracers.

In the PET-MR the MRAC is still a matter of concern; the attenuation map does not differentiate between soft tissue and bone, which can result in underestimation of the PET signal (102, 103). A recent study showed comparable relative myocardial FDG uptake in PET-MR and PET-CT images (91) but little is known on the impact of attenuation on the quantitative accuracy of cardiac perfusion in the PET-MR. For PET-CT it has been shown that MBF can be measured accurately with $^{15}$O-water without correcting for attenuation (104). Errors in attenuation correction affect the amplitudes of the time-activity curves but not their shapes, and hence not the measured clearance rates. Indeed, in our study a high agreement was found between PET-MR based and PET-CT based MBF, suggesting that the potential errors in MRAC have little impact on the $^{15}$O-water MBF values. This result cannot readily be extrapolated to other tracer used for measuring MBF such as $^{82}$Rb and $^{15}$N-ammonia, since for those tracers MBF is determined from the uptake instead of the clearance of the tracer.

TOF-PET imaging is an emerging imaging technology both for PET-CT and PET-MR. In a recent study Mehranian et al assessed the impact of TOF image reconstruction on PET quantification errors induced by MR-based attenuation correction in $^{18}$F-FDG and $^{18}$F-choline whole body PET-MR scans (105). They showed that TOF substantially reduced artifacts and significantly improved the quantitative accuracy. In recent cardiac PET-CT studies with $^{15}$N-ammonia and $^{82}$Rubidium, TOF-reconstruction also improved image quality and increased MBF (106, 107). In our study we did not find any significant impact of TOF and filter and reconstruction settings on the quantitative accuracy of cardiac perfusion measurements with $^{15}$O-water in the PET-
MR. However, the parametric PET-MR MBF images with TOF were excellent and in most cases the image quality was visually superior to the PET-CT images. We did not evaluate the effect of TOF, filter and reconstruction settings on parametric image quality, but as well as in PET-CT, (107, 108) TOF is expected to improve image quality and to make it possible to find smaller perfusion defects, which should be evaluated in a further study.

Quantitative CMR perfusion imaging

The integrated PET-MRI system allowed for MBF measurements with CMR and PET simultaneously, during the same physiological condition, ensuring that any differences in MBF values would only be methodological. In a recent study simultaneous PET and MRI perfusion measurements using a cardiac phantom showed similar first-pass dynamics for the PET radiotracer $^{18}$F-fluorine and the MRI gadolinium contrast agent and a linear relationship between absolute PET perfusion and relative MRI perfusion parameters (53). However, the phantom model is an oversimplification of the cardiovascular system and does not represent true myocardial perfusion in the human body, with the main limitation, as pointed out by the authors, that the distribution dynamics of the contrast agent in the phantom does not involve diffusion from the vascular to the interstitial space as occurs in vivo. In a conference report by Zhang et al. from 2014 simultaneous quantitative CMR and $^{13}$N-ammonia PET myocardial perfusion was compared in 10 patients showing a correlation of $r^2 = 0.67$ for rest and stress MBF and $r^2 = 0.48$ for MPR (109), but so far, no clinical studies on simultaneous MRI and $^{15}$O-water-PET myocardial perfusion quantification in clinical studies in humans have been published.

The previously published studies comparing CMR and PET myocardial perfusion in humans were all performed at different time points and in separate MRI and PET scanners. Furthermore, most studies have been either semiquantitative (110, 111) or have included healthy volunteers only (29, 30, 112). Quantitative studies including patients with CAD have all used different MRI and PET methods (20, 31, 113), making direct comparisons difficult. $^{15}$O-water PET, used in the current study, is considered to be the gold standard for non-invasive quantitative measurements of myocardial blood flow (MBF) (15, 16, 114). $^{15}$O-water is metabolically inert and freely diffusible allowing for accurate quantification also at high flow rates, thus being the optimal reference PET-tracer method in comparison studies.

Pärkkä et al. studied 18 healthy males, quantifying MRI and $^{15}$O-water PET myocardial perfusion imaging on separate days. They analyzed the MRI perfusion data using a two-compartment model and calculated a perfusion-related parameter, the unidirectional influx constant ($K_i$), which correlated significantly with PET rest and stress perfusion ($r=0.80$) (30). However, absolute stress values of MRI-based $K_i$ were lower than PET based MBF, which is in line with our results when analyzing our MRI data using the 1TCM, showing
an underestimation of perfusion values >2 mL/min/g, due to the lower extraction fraction of Gd-DOTA as compared to $^{15}$O-water.

Applying a correction for the extraction fraction of Gd-DOTA is technically possible but introduces uncertainty and possible error as the extraction fraction may vary according to the coronary flow (115, 116). Pärkkä et al. considered the extraction of Gd to be constantly 50%, and after correcting for extraction the MRI based stress MBF values became comparable to PET MBF, whereas the resting MBF values became higher with MRI than with PET. Tomiyama et al. also compared quantitative myocardial perfusion between MRI and $^{15}$O-water PET, using a single tissue compartment model to analyze MRI data (113). With the relationship between K1 values from MRI and MBF values from $^{15}$O-water PET they estimated Renkin-Crone parameters and calculated a correction for extraction fraction for Gd-DTPA. When applying this correction to the MRI perfusion analysis, they reported very high correlations between MRI based and PET based perfusion values ($r=0.96$ for global rest and stress MBF) without overt under- or overestimation of absolute perfusion values. When attempting a similar method for extraction fraction correction on our data, the MRI stress perfusion values indeed increased, but the correction algorithm caused a larger variation in stress MBF values (Fig 16b), as high correction factors also multiplies the noise of the measurements leading to larger scatter of the values. However, applying the 1TCM+PS model to our MRI data, which included Renkin-Crone parameters for permeability-surface area, thus correcting for extraction, resulted in comparable global mean MBF values for MRI and PET at both rest and stress ($0.97 \pm 0.27$ vs $1.02 \pm 0.28$ mL/g/min, $p=0.66$) and ($3.19 \pm 0.70$ vs $3.13 \pm 1.16$ mL/g/min, $p=0.81$), with a strong correlation for rest and stress values together ($r=0.86$, $p<0.0005$ for global MBF values), although with large variability, seen as wide limits of agreement (-1.24 – 1.25 mL/min/g) in a Bland Altman comparison, shown in Fig 17b. This cannot be attributed to differences in the physiological state as the PET and MRI perfusion was measured simultaneously, but must then depend on differences between the modalities, tracers and/or analysis methods. The reproducibility of $^{15}$O-water PET myocardial perfusion has been assessed and found to be good (17). Another comparison between sequential $^{15}$O-water PET MBF measurements in a PET-CT and in a PET-MR also yielded high correlation and agreement (ICC=0.98, bias -0.04, limits of agreement -0.73 – 0.65 mL/min/g for rest and stress regional MBF values) (49), although both physiological differences and different scanners might have influenced the measurements. Reproducibility of quantitative cardiac perfusion measurements with MRI have been reported to be good or at least moderate (44-48). Direct comparison with the reproducibility of PET based myocardial perfusion measurements is hampered by different measures of repeatability used in different studies, wide time-ranges between the repeated measures in some repeatability studies and different methods used for the perfusion quantification. However, the repeatability coefficients reported in some
MRI studies (45, 48) are somewhat higher and the ICC somewhat lower (45, 47) than in the previously mentioned PET-studies (17, 49).

Morton et al. studied 38 patients with known or suspected CAD, comparing quantitative myocardial perfusion between MRI and $^{13}$N-ammonia PET (20). They used a Fermi deconvolution method to analyze MRI perfusion data and reported weak correlations for absolute perfusion values when comparing rest and stress perfusion values separately ($r=0.32$ and $r=0.37$ respectively). In our study the MBF values at rest did not correlate between MRI and PET ($r=0.21$, $p=0.51$) while the correlation was moderate for stress MBF values ($r=0.69$, $p=0.013$). Pärkkä and Tomiyama et al. did not analyze rest and stress perfusion separately, however a weak (or no significant) correlation for rest MBF and a moderate correlation for stress MBF values was likely present, as indicated by presented scatterplots (30, 113). From a statistical point of view, analyzing rest and stress values separately is probably more correct.

Morton et al. reported a good correlation for MRI and PET MPR values ($r=0.75$), while the correlation for MPR values was moderate in the study by Pärkkä et al ($r=0.46$) (30). Tomiyama et al. (113) reported a very strong correlation for MPR values ($r=0.93$), but this finding seems to depend on a few extreme MPR values. In our data there was no significant correlation between MRI- and PET-based based MPR values ($r=0.08$, $p=0.80$), which in this case cannot be explained by physiological differences between MRI and PET-scans, but must be solely technical. Myocardial perfusion at rest is highly depending on heart rate and systolic blood pressure, and as MPR values depend on both baseline and hyperemic MBF, in sequential comparison studies a larger variation in values is expected for both rest MBF and MPR, in comparison to stress MBF values. Although MPR is a measure commonly used in the diagnosis of CAD, several PET studies have shown that absolute MBF at stress is superior to perfusion reserve in the detection of hemodynamical significant CAD (8, 11, 13, 25).

In a newly published study by Engblom et al. (50) on 21 patients with stable CAD, quantitative myocardial perfusion with MRI and $^{13}$N-ammonia PET was compared, showing very good correlation and agreement between MBF values from the two modalities ($r=0.92$, $-0.1 \pm 0.6$ mL/min/g for global rest and stress MBF values analyzed together). The MRI method that was used was based on a dual sequence, single-bolus method and MBF was quantified by a recently developed automated perfusion mapping technique based on a distributed blood-tissue exchange model optimized for MRI by the use of several integrated corrections (52). Besides estimation of extraction fraction, the MRI technique and analysis model Engblom et al. used, were also optimized to achieve linearity between the measured blood signal and contrast agent concentration. This new dual sequence approach developed by Kellman et al. (52) addresses several technical challenges and possible causes of error in quantification of myocardial perfusion, further has a good reported repeatability (51), but is currently not available for our MRI-system.
Accurate quantification of MBF with MRI is challenging due to the non-linear relationship between signal intensity and gadolinium contrast agent concentration (32, 38, 42). In order to avoid signal saturation effects low contrast doses can be used. In our study we used 0.05 mmol/kg Gd-DOTA and did not find any evidence of flattening of the bolus peak by saturation effects. The same Gd-dose has been used by others (30), who found that with Gd-bolus doses up to this level, the increase in the peak concentration was proportional to the given dose, suggesting insignificant saturation of signal. If saturation effects exist and are neglected the myocardial perfusion is overestimated, which is also not apparent in our results. A dual bolus technique (39) has also been proposed to avoid saturation of the MRI signal during imaging of the Gd-DOTA contrast bolus for the input function, but this prolongs imaging and the possibility of changes between the two bolus acquisitions, which might affect the results. Other possible sources of bias and errors are patient motion, B1-field variation in combination with saturation fluctuations during the bolus passage and saturation recovery variations due to varying cardiac cycle lengths. T2* decay is another possible source of MRI signal loss that might affect the results but is not expected to be strong in the current experiment setup.

Our study, as well as a number of other recent studies, shows a good correlation, with negligible bias, between 15O-water PET and CMR perfusion values. However, the limits of agreement between PET- and CMR-based MBF values in Bland-Altman analysis are much wider than those found for test-retest studies with 15O-water. For example, in a recent study with two rest-test protocols with 15O-water on two different scanners, limits of agreement for combined rest and stress MBF values were circa ± 0.7 mL/g/min for regional values, compared to 2.2 mL/g/min in the present work and ± 1.1 mL/g/min in the work by Engblom et al. which was done using an MRI-method which is not available on our scanner. In addition to this, whilst PET MBF analysis can be done very robustly and nearly automatically within minutes using currently widely available acquisition protocols, MRI analysis appears to be much more time-consuming, operator dependent and error-prone. Compared to quantitative perfusion measurements with 15O-water, the quantification analysis with DCE MRI is more complex and needs several corrections in order to quantify perfusion accurately. Correction is needed for the non-linear relationship between gadolinium contrast-agents and the MRI signal. Another correction is needed for the non-linear extraction of gadolinium contrast-agent from blood to tissue, which adds an extra assumption to the model for quantifying perfusion. Absolute myocardial blood flow can be estimated directly with model-free methods where the maximum value of the Fermi function equals blood flow. However, the deconvolution methods are very sensitive to noise (33) and depend most importantly on the assumption of linearity. Other corrections or matters of concern are corrections for hematocrite, T2* decay, effects of water exchange or inflow on the MRI signal (117).
Limitations

The results presented in paper III are depending on the specific technology of the Signa SiPM PET-MRI scanner and reconstruction methods used. Quantitative accuracy of MBF values obtained using other PET-MR scanners and tracers should be validated in a similar manner. However, with $^{15}$O-water offering the largest challenge to PET scans in terms of count rate variations possibly with the exception of $^{82}$Rb, we expect that the results in the present work in terms of PET performance are also valid for dynamic myocardial imaging with other tracers.

The sample sizes in the current studies are small but was considered sufficient for comparison of quantitative perfusion measures. However, the sample sizes were not considered sufficient for comparison of diagnostic accuracy between methods and no comparisons with coronary angiography or fractional flow reserve were performed.

In paper IV no late gadolinium enhancement (LGE) imaging was performed to assess the presence of myocardial scars, which might have influenced myocardial perfusion, however, the patients did not have any known myocardial infarctions and the left ventricular systolic function was normal in all subjects, why at least large infarctions were less likely. For paper IV different acquisition and postprocessing methods inevitably result in differences in the myocardial segmentation between modalities. With PET the whole left ventricle was covered while MRI captured three 8-mm thick short-axis slices in the left ventricle with gaps between the slices.
Conclusions

In paper I we determined the optimal tracer model for kinetic analysis of $^{11}$C-PIB and evaluated the performance of two simpler measures, RI and SUV, in the quantification of cardiac $^{11}$C-PIB uptake in amyloidosis. An irreversible two-tissue (2Tirr) model best described the $^{11}$C-PIB uptake in cardiac amyloidosis. RI and SUV showed high correlation with quantitative results from this kinetic model, using either individual or population average metabolite data. Finally, we applied all methods to a previously acquired dataset including both amyloidosis patients and healthy volunteers to address the ability of each method to discriminate between patients and controls. RI and SUV are more feasible for use in clinical routine and also showed better discrimination between amyloidosis patients and controls than $K_i$ based on population average metabolite correction. Therefore, RI and SUV are preferred in clinical diagnosis of cardiac amyloidosis.

In paper II we tested the feasibility of a semiautomatic software to analyze and visualize cardiac uptake of $^{11}$C-PIB in amyloidosis. The semiautomatic analysis process was fast and easy to perform and the software performed well even in the subjects with no or low tracer uptake in the heart. The RI values were comparable with the RI based on manual segmentation and ROI tracing, showing significantly higher $^{11}$C-PIB RI in amyloidosis patients than in healthy volunteers. Parametric polarplots made visual assessment simple, making it easy to separate healthy subjects from patients with cardiac amyloidosis and to assess regional differences in cardiac amyloid deposits. Furthermore, the inter-reader reproducibility was excellent. A fast and accurate semiautomatic analysis process is thus feasible to use for PET in cardiac amyloidosis instead of the laborious manual analyses that has been used so far.

In paper III we assessed the quantitative accuracy of cardiac perfusion measurements with $^{15}$O-water in the Signa PET-MR scanner. A high correlation and agreement between PET-MR based and PET-CT based MBF was found; cardiac perfusion measurements with $^{15}$O-water can therefore be performed accurately with the fully integrated Signa PET-MR scanner. The previously established cut-off value for MBF with $^{15}$O-water in PET-CT is therefore applicable in PET-MR studies. We also found that TOF and reconstruction settings had little impact on MBF values.

In paper IV we assessed the quantitative accuracy of cardiac perfusion measurements using dynamic contrast-enhanced MRI with simultaneous $^{15}$O-water PET as reference at rest and during adenosine-induced hyperemia with
a fully integrated PET-MR scanner. The correlation between simultaneous quantitative MBF measurements with single bolus DCE MRI and $^{15}$O-water PET measured in an integrated PET-MRI was good but the agreement was only moderate. The variation between the MBF values is due to technical differences between the modalities, tracers and/or analysis methods. Quantification of myocardial perfusion with MRI is technically challenging and depends on several correction algorithms that can lead to large variability of the MBF values. Although MRI analysis likely can be automated in similar ways as PET analysis, the relatively poor agreement with $^{15}$O-water PET shows that MRI-based quantitative MBF measurements based on widely available acquisition protocols are not ready for clinical introduction.
Clinical implications and future perspectives

The findings from the present studies will facilitate the further use of quantitative molecular imaging in cardiac studies by:

- establishing the roles of the simplified measures RI and SUV in the quantification of $^{11}$C-PIB in the diagnosis of cardiac amyloidosis.
- the validation of a semiautomatic analysis method for $^{11}$C-PIB, thus making non-invasive diagnosis of cardiac amyloidosis possible in clinical routine.
- the validation of a silicon photomultiplier (SiPM)-based time-of-flight (TOF) capable PET-MR scanner for quantitative cardiac PET imaging using $^{15}$O-water.
- assessment of the quantitative accuracy of cardiac perfusion measurements using dynamic contrast-enhanced MRI which indicate that MRI-based quantitative MBF measurements based on widely available acquisition protocols is not yet ready for clinical introduction.

There was a substantial variation in the fraction of labelled metabolites of $^{11}$C-PIB between subjects in paper I, and a population-averaged metabolite correction could therefore result in inaccurate quantitative results for some subjects. When assessing changes within a single patient, for instance before and after treatment, quantification using population-averaged metabolite corrections may be considered, assuming that the intervention does not change the metabolism of PIB. However, this assumption should also be tested as both disease progression and intervention may affect organ function and thus metabolism and confounders.

Binding to TTR-amyloidosis (compared to AL) appears to be lower with the amyloid PET tracers. TTR subjects typically do not have multiorgan involvement other than heart failure with liver congestion. We have discussed the possibility to compare the outcome measures from compartment modeling in AL and ATTR patients separately, and also to look into metabolism of PIB separately in these two groups. Unfortunately, our prospective material in paper I (7 patients; 5 ATTR and 2 AL) is too small for such a statistical comparison. However, the highest $K_r$/RI/SUV values were indeed found in the AL
patients. As for the retrospective material, (7 AL, 3 TTR) there was no statistical difference in RI or $K_i$ (based on population average metabolite corrections) between AL and TTR-patients, but again, the sample size was small and a separate analysis of AL and ATTR cases would need a further study with larger sample size.

Our retrospective data in paper I suggested that both simplified measures (RI and SUV) discriminated better between cardiac amyloidosis patients and healthy subjects than the 2Tirr model when based on population-averaged metabolite corrections. No healthy volunteers participated in the prospective study with metabolite corrections, and hence it is not certain that population average metabolite corrections based on patients with amyloidosis can be used for subjects without amyloidosis. Also, individual metabolite corrections could maybe give other results with better separation of $K_i$ between patients and controls, which could be tested in a future study. However, individual metabolite corrections are expensive and technically demanding and would not be feasible to use in clinical routine.

Especially for rare diseases, where individual studies generally consist of small numbers of patients, it is of major importance to achieve a uniform way of reporting data in order to later be able to perform pooled assessments for evidence development. A recent meta-analysis found a low evidence level for using $^{11}$C-PIB in early diagnosis of Alzheimer’s disease, mainly due to a highly variable data presentation (118). Compared to amyloid diseases of the brain there are far less patients with cardiac amyloidosis and a uniform data presentation in coming studies will strongly facilitate subsequent pooled assessments of adequate use of cardiac amyloid imaging for diagnosis, treatment evaluation and possibly drug development. Although further development of processing methods is strongly advocated, the authors suggest future data are additionally reported using a single method. The method presented in paper II uses a freely available software package and provides a method that can easily be used for comparison of data between sites and would as such be suitable as a standardized way of data reporting.

The semiautomatic analysis of RI in paper II was only tested on cardiac $^{11}$C-PIB PET. However, the analysis should perform equally well calculating the RI with $^{18}$F-florbetapir and other amyloid binding PET-tracers, but this needs to be tested.

TOF-PET imaging is an emerging imaging technology both for PET-CT and PET-MR. In paper III we did not find any significant impact of TOF and filter and reconstruction settings on the quantitative accuracy of cardiac perfusion measurements with $^{15}$O-water in the PET-MR as compared to PET-CT. However, the parametric PET-MR MBF images with TOF were excellent and in most cases the image quality was visually superior to the PET-CT images. We did not evaluate the effect of TOF, filter and reconstruction settings on
parametric image quality, but as well as in PET-CT, \(107, 108\) TOF is expected to improve image quality and to make it possible to find smaller perfusion defects, which should be evaluated in a further study.

\(^{15}\)O-water PET is the current gold standard for non-invasive quantitative measurements of myocardial blood flow (MBF). Quantification of myocardial perfusion with MRI is promising but technically challenging and depends on several correction algorithms that can lead to large variability of the MBF values. With technical evolution and further refinement and validation of the MRI methods, MRI analysis can likely be automated in similar ways as PET analysis.

PET-MR is a new promising technique, with several possible applications in cardiac imaging. MBF quantified with PET and functional and morphological information obtained with MRI, is one combination of optimal techniques from the two modalities and could be used for comprehensive assessment in patients with known or suspected CAD.
Sammanfattning på svenska

Med molekylär bilddiagnostik kan biologiska funktioner och cellulära processer avbildas och kvantifieras. Positron emissions tomografi (PET) och magnetisk resonanctomografi (MRT), är exempel på metoder inom molekylär bilddiagnostik, vilka kan användas för att visualisera och mäta t.ex. blodflöde, metabolism och receptorfunktion i hjärtat. Kvantitativ PET och MRT har hittills använts i begränsad omfattning inom klinisk hjärtdiagnostik. Det finns behov av att utveckla och validera metoder för att göra båda metoderna mer tillgängliga för hjärtdiagnostik i klinisk vardag samt inom forskning. Syftet med arbetet som resulterat i denna avhandling var att underlätta användningen av kvantitativ molekylär bilddiagnostik inom det kardiovaskulära området.

Man har nyligen kunnat visa att man med PET spårämnet $^{11}$C-PIB kan påvisa förekomst av amyloid i hjärtat hos patienter med hjärtamyloidos. Vilken analysmetod som bäst kvantifierar $^{11}$C-PIB-upptag i hjärtat är dock ej känt. Syftet med arbete I var därför att bestämma optimal analysmetod för att kvantifiera upptag av $^{11}$C-PIB i hjärtat hos patienter med hjärtamyloidos och att jämföra den optimala analysmetoden med enklare PET-mått på PIB-upptag, såsom retentionsindex (RI) och standardiserat upptags värde (SUV).

Åtta patienter med amyloidos och hjärtengagemang undersöcktes med dynamisk $^{11}$C-PIB PET och olika modeller för kvantifiering testades. Studien visade att en irreversibel tre-kompartment modell bäst beskrev upptaget av $^{11}$C-PIB i hjärtat. RI och SUV korrelerade väl till mätt på inflode av PIB i hjärtat beräknat med den irreversibla tre-kompartmentmodellen. De tre mätmetoderna användes sedan på ett retrospektivt data baserat på patienter med hjärtamyloidos och friska individer och man fann att RI och SUV kunde skilja mellan hjärtamyloidos och friska individer än den irreversibla tre-kompartmentmodellen med populationsmedelvärdeskorrigerat metabolitdata.

De enklare mätmetoderna är dessutom mer användbara i klinisk rutin, och slutsatsen var att RI och SUV är att föredra vid klinisk diagnos av hjärtamyloidos.
I tidigare PET-studier av hjärtamyloidos har analysarbetet varit manuellt och tidskrävande. I arbete II undersökte vi därför huruvida ett halvautomatiskt analysprogram kunde användas för att visualisera och kvantifiera upptag av $^{11}$C-PIB i hjärtat vid amyloidos.

Retrospektiva $^{11}$C-PIB PET undersökningar av 10 patienter med hjärtamyloidosis och av 5 friska frivilliga individer analyserades med halvautomatisk programvara varefter RI värden från den halvautomatiska analysen jämfördes med RI-värden från tidigare manuell analys. Vi fann att den halvautomatiska analysen tog kort tid och var enkel att genomföra, även hos individer med lågt eller inget upptag av PIB i hjärtat. RI-värden beräknade med den halvautomatiska metoden var jämförbara med RI-värden baserade på manuell analys och beräkning, och RI-värdena var signifikant högre hos patienter med hjärtamyloidos än hos friska frivilliga individer. Med parametriska bilder från analysprogrammet var det enkelt att visuellt avgöra huruvida en individ hade hjärtamyloidos eller ej och hur amyloiden fördelade sig i hjärtat. Reproducerbarheten mellan två olika personer som genomförde analyserna var dessutom utmärkt. Studiens slutsats var att det således är möjligt att använda en snabb, enkel och tillförlitlig halvautomatisk metod för att analysera PET-data vid hjärtamyloidos istället för den manuella, tidskrävande process som använts hittills.

Nyligen har integrerade PET och MR kameror utvecklats, vilka medger undersökning av hjärtat med PET och MR vid samma undersökningsställfalle och även simultant. Det var dock okänt huruvida mätning av myokardblodflöde med $^{15}$O-vatten PET var tillförlitligt i sådan integrerad PET-MR kamera. I arbete III ville vi därför validera mätning av hjärtmuskelblodflöde med $^{15}$O-vatten i Signa PET-MR kamera.


I arbete IV var syftet att jämföra blodflödesmätning i hjärtmuskeln mätt med simultan PET och MR teknik i integrerad PET-MR kamera.

Femton patienter med känd eller misstänkt ischemisk hjärtsjukdom undersöktes simultant med $^{15}$O-vatten PET och kontrastförstärkt MR i integrerad PET-MR kamera.

Vi fann att blodflödesvärden mätt med PET och mätt med MR korrelerade väl, men att det var en stor spridning i mätvärden, dvs samstämmighetsintervallet var stort, varför graden av överensstämmelse var måttlig. Studiens slutsats var att mätning av hjärtmuskelnblodflöde med MR-teknik ännu inte är redo att användas som rutinmetod i kliniskt bruk.
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