

# CHAPTER 1

Introduction

## INTRODUCTION

### Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease that causes proliferation of synovial tissue, and subsequent destruction of cartilage and bone if not treated adequately.

Characteristic for RA is the inflammation of synovial tissue in multiple joints predominantly symmetrically in small hand and feet joints, which results in joint pain, joint swelling and functional disability. In addition, in approximately 40% of patients, also non-articular tissues and organs are involved in the disease process (1).

In this thesis, the potential of macrophage-targeted positron emission tomography (PET) in early diagnostics and treatment monitoring of RA is investigated.

### Early diagnosis of RA

The diagnosis of RA is based on clinical expert opinion. Over time, various definitions have been used for scientific purposes. The most recent 2010 ACR/EULAR classification criteria for RA (2) – comprising the number and site of involved joints, serological status, acute phase response, and duration of symptoms – have been developed in response to the call for more stringent criteria to identify RA patients early in the course of their disease. Early diagnosis is mandatory, since joint damage may occur already in the earliest phases of the disease. Timely start of treatment can prevent long-term joint damage, functional disability and loss of quality of life (3). This concept of a therapeutic ‘window of opportunity’ has resulted in early and aggressive treatment of RA patients with intensive disease-modifying anti-rheumatic drug (DMARD) regimens promptly after diagnosis, preferably in outpatient clinics specifically arranged for (early) RA patients (4).

Studies have shown, however, that the pathogenetic processes underlying the development of RA may start long before patients develop clinically manifest arthritis (5). This preclinical phase is characterized by the formation of auto-antibodies such as rheumatoid factor and anti-citrullinated protein antibody (ACPA) (5;6). In addition, patients can experience joint pain (arthralgia) without clinical evidence of arthritis (7). The existence of a preclinical phase creates opportunities for very early intervention, although, of all seropositive arthralgia patients, only ~35% will eventually develop the typical clinical signs of RA. Therefore, additional tools are needed for optimal identification of individuals at risk.

Advanced imaging could address this need by assessment of subclinical synovitis (defined as synovitis that cannot be detected by clinical examination) in the preclinical phase (8). So far, power Doppler ultrasonography (PDUS) and magnetic resonance imaging (MRI) have shown promise in the detection of subclinical synovitis in ACPA-positive arthralgia patients (9;10). However, the clinical

relevance of ultrasonography (US)- and MRI-detected abnormalities at a patient level is still unclear (9;10). This leaves room for other imaging techniques such as PET.

#### Monitoring of RA disease activity

Once a patient has been diagnosed with RA, treatment is aimed at optimal suppression of disease activity because curative therapies are not available. With the current (combined) classic and biologic DMARD treatment regimens, increasing numbers of patients achieve a state of clinical remission or, more often, minimal disease activity (MDA). This is of clinical importance since these patients will show less radiological deterioration and impairment of physical function (11;12).

However, the concept of clinical remission is complex, which is reflected by the wide range of definitions developed over the years (13). Moreover, ongoing structural progression was demonstrated even despite fulfilment of remission criteria (14). Recently, the 2011 ACR/EULAR remission criteria were developed to comply with the demand for sensitive, uniform measures to define true clinical remission (15). Still, none of the available criteria addresses the presence of subclinical synovitis as assessed by imaging, which could be an important cause of proceeding joint damage despite clinical remission (16).

In contrast to conventional radiography, which is the gold standard of RA imaging, advanced imaging techniques can depict inflammation. MRI-detected synovitis (i.e. synovial thickening and/or hyperperfusion) and bone marrow edema were present in 96% and 52% of patients in clinical remission/MDA, respectively (17;18). Furthermore, US-detected synovial hypertrophy and power Doppler (PD) activity were present in 50-95% and 15-62% of these patients (19). Moreover, studies have shown that the presence of bone marrow edema is predictive for the development of joint erosions in early RA patients (20-22). Similarly, the presence of baseline MRI synovitis and US abnormalities (synovitis and PD activity) were predictive for erosive progression and/or flaring in RA patients in clinical remission/MDA (16;23;25-27).

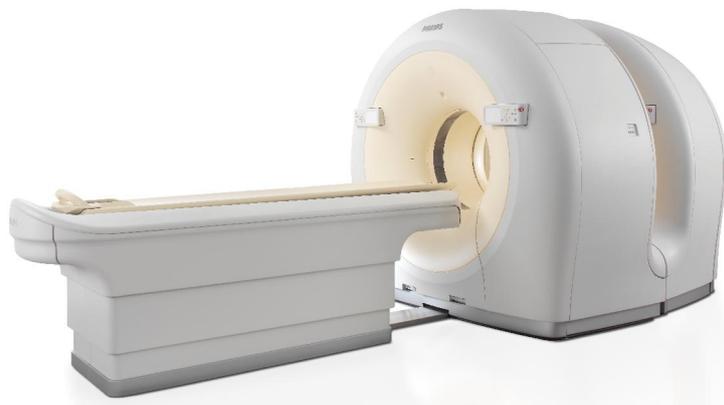
These results underline that advanced imaging may be a useful tool in the monitoring of treatment in RA. However, the number of patients in clinical remission/MDA that exhibits MRI/US-detected inflammation largely exceeds that of patients who will eventually experience a flare of disease activity (20-50%) or progression of radiological joint damage (15%) (14, 48, 49). This indicates that not all MRI/US-detected abnormalities may be clinically relevant. Previous studies have proposed a threshold level of MRI synovitis that differentiates between patients with and without a risk of structural progression (24;28). But also other imaging techniques, such as PET, could further contribute to specificity by identification of biologically relevant disease.

## Positron Emission Tomography (PET)

### Principles and applications of PET

PET is a nuclear imaging technique that can be used for *in vivo* non-invasive detection of functional processes. In contrast to other imaging techniques such as MRI and US that visualize principally anatomical and functional changes, PET provides molecular data. PET is a highly sensitive imaging technique, which is illustrated by the findings of Nahrendorf *et al.*, who showed a 20-fold higher sensitivity of PET compared to MRI in the detection of a nanoparticle that was designed for multimodality diagnostic imaging of macrophages (29). Furthermore, PET is highly specific due to the use of radiopharmaceuticals (i.e. PET tracers) that are composed of a positron-emitting radionuclide coupled to a biologically active molecule that is specifically targeted to the cell or molecule of interest. In general, radionuclides with a short half-life ( $T_{1/2}$ ), such as carbon-11 ( $^{11}\text{C}$ ,  $T_{1/2}$ : 20 min) or fluorine-18 ( $^{18}\text{F}$ ,  $T_{1/2}$ : 109 min) are most frequently used. These radionuclides are produced by a particle accelerator (cyclotron).

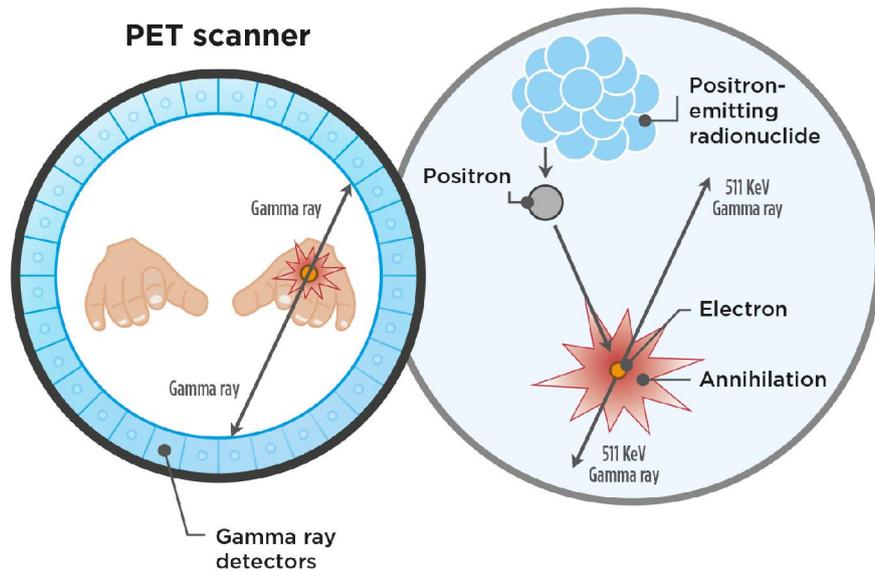
The PET imaging procedure starts with the positioning of the patient in the PET scanner. For the studies described in this thesis, patients were lying in prone position in the PET scanner (Figure 1), with their arms extended above their heads and their hands fixed to prevent movement artefacts.



**Figure 1.** Example of a PET scanner (kindly provided by Philips Healthcare)

Subsequently, a PET tracer is injected intravenously. The tracer accumulates in the tissue of interest, where positrons are emitted during positive beta decay of the radionuclide (30). A positron can only travel for a short distance (a few millimeters) in tissue, before meeting a tissue-residing electron. This encounter results in an annihilation reaction that produces two 511 keV photons emitted at an angle

of 180 degrees. Coincident detection of a pair of 511 keV photons by opposite detectors of the PET scanner allows assessment of the exact site of positron emission (Figure 2). Measured PET data are corrected and reconstructed into a three-dimensional image, from which PET tracer uptake in tissues can be localized and quantified.



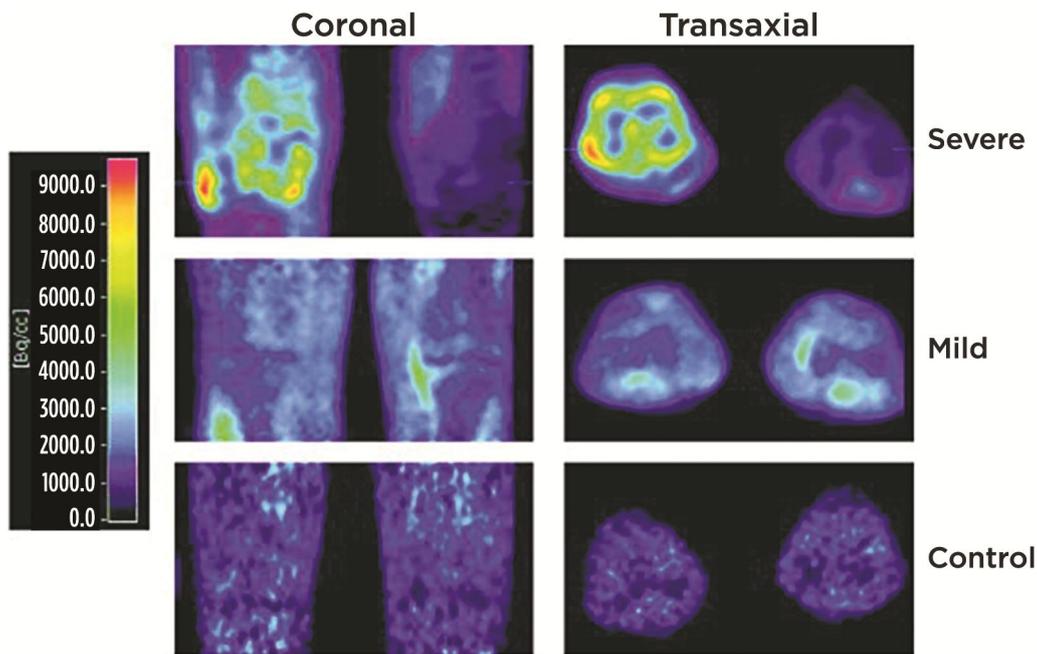
**Figure 2.** Schematic illustration of positron emission tomography. As shown on the right, a positron is emitted from an unstable radionuclide. The collision of a positron with a nearby tissue-residing electron results in the coincident emission of two 511 keV gamma rays in opposite directions. These gamma rays are detected within a ring of scintillation detectors as shown on the left.

Of all PET tracers, [ $^{18}\text{F}$ ]fluorodeoxyglucose (FDG) is the most widely used (31). FDG is a glucose analogue, which is taken up by metabolically active cells such as tumor cells and inflammatory cells. After internalization, FDG is phosphorylated, which prevents efflux from the cell. Hence, the tracer concentrates at the site of enhanced metabolic activity. In oncology, PET-Computed Tomography (CT) with [ $^{18}\text{F}$ ]FDG has been widely integrated into routine clinical practice and is used for detection, staging and therapy evaluation of amongst others malignant lymphoma and cancers of the lung, breast, gastrointestinal tract and head and neck (30).

(Macrophage-targeted) PET in RA

In RA, several [<sup>18</sup>F]FDG PET studies proved the feasibility of PET imaging of clinically active arthritis (33;35). Advanced imaging is, however, not required for the detection of clinically active arthritis, since (standardized) physical examination of the joints is sufficient to determine the level of disease activity. But, PET imaging of inflammation might prove useful for the assessment of subclinical disease activity in patients at risk of developing RA and RA patients who are in clinical remission/MDA, as well as for the monitoring of therapy response in RA (32;34). Though, to this end, other PET tracers than [<sup>18</sup>F]FDG may be needed, because FDG has limited specificity. For example, [<sup>18</sup>F]FDG uptake in joints does not allow distinction between locally active osteoarthritis and RA (36). More selective imaging of inflammation could be achieved by targeting of synovial macrophages. It is known that macrophages infiltrate the synovia of affected RA joints in the earliest stages and are still involved in established disease (8;37). Therefore, macrophage-targeted PET could be valuable in early diagnostics as well as monitoring of disease.

(*R*)-[<sup>11</sup>C]PK11195 is an established tracer for imaging of macrophage activity. It targets the translocator protein (TSPO, formerly known as the peripheral benzodiazepine receptor), which is upregulated in activated microglia and macrophages (38). (*R*)-[<sup>11</sup>C]PK11195 PET has been widely applied in the imaging of neuro-inflammation (e.g. multiple sclerosis, Alzheimer's disease, cerebral vasculitis). In a previous study of our group in RA patients, (*R*)-[<sup>11</sup>C]PK11195 PET showed clear tracer accumulation in arthritic knees (Figure 3) (39). This uptake correlated significantly with clinically observed joint swelling and presence of macrophages in synovial tissue as detected with immunohistochemistry. Also (*R*)-[<sup>11</sup>C]PK11195 uptake in clinically non-inflamed knees of RA patients exceeded that in knees of healthy controls. This suggests that (*R*)-[<sup>11</sup>C]PK11195 PET could detect subclinical inflammation in RA; an interesting finding that is further explored in the studies presented in the first part of this thesis.



**Figure 3.** (*R*)-[<sup>11</sup>C]PK11195 PET images in coronal and transaxial directions. Top: images of severe clinical inflammation of the right knee (depicted at the left in both images) and no clinical inflammation of the left knee in an RA patient. Middle: images of mild inflammation of both knee joints in an RA patient. Bottom: images of knees without joint disease in a control subject. The different levels of tracer uptake correspond to the colors in the color bar at the left. From: van der Laken *et al.* (39) (with permission).

In the second part of this thesis, three alternative macrophage-targeting PET tracers, [<sup>11</sup>C]DPA-713, [<sup>18</sup>F]DPA-714 and [<sup>18</sup>F]fluoro-PEG-folate, were compared to the established macrophage tracer (*R*)-[<sup>11</sup>C]PK11195 in an experimental rat model of arthritis. Despite the promising results that were achieved with (*R*)-[<sup>11</sup>C]PK11195 in the detection of (neuro-)inflammation, a search for novel TSPO radioligands was triggered by the relatively low specific-to-nonspecific binding ratios found for (*R*)-[<sup>11</sup>C]PK11195 (38;40). Also, considerable (*R*)-[<sup>11</sup>C]PK11195 background uptake was found in the region of the bone/bone marrow of RA patients (see chapters 3,5,6) (41). New generation TSPO tracers, such as [<sup>11</sup>C]DPA-713 and [<sup>18</sup>F]DPA-714 exhibited better specific-to-nonspecific binding ratios compared with (*R*)-[<sup>11</sup>C]PK11195 in neurological disease models (40;42-44), but had not yet been applied in RA.

In addition, other molecular targets present on macrophages, such as the folate receptor (FR) have been explored. Xia *et al.* showed that the beta isoform of FR (FR $\beta$ ) is upregulated on activated macrophages in an inflammatory environment (45). In RA, enhanced expression of FR $\beta$  was found in synovial tissue of patients with clinically active disease (46). Moreover, studies with a <sup>99m</sup>Tc-labeled

folate agent demonstrated that FR could be used for specific targeting of imaging agents to arthritic joints in RA (47). Since PET provides higher sensitivity and spatial resolution compared to scintigraphy, FR-targeted PET imaging could be particularly interesting for detection of subclinical arthritis in RA.

### **Outline of the thesis**

In this thesis, the ability of macrophage-targeted PET to visualize arthritis activity in the preclinical and remission phase of RA is explored. As an introduction to these studies, we reported on the current status of PET as a diagnostic and monitoring tool in peripheral inflammatory arthritis in a systematic review (**chapter 2**).

From there, the thesis comprises two experimental parts. In the first part, our aim was to investigate the presence of subclinical synovitis in RA by macrophage-targeted PET, and to determine the relation between PET abnormalities and clinical outcome. We studied this in various patient groups. In **chapter 3**, we investigated (*R*)-[<sup>11</sup>C]PK11195 PET imaging in ACPA-positive arthralgia patients (early diagnostics) and the results of contrast-enhanced MRI scanning performed in this group are presented in **chapter 4**. Next, we assessed the feasibility of (*R*)-[<sup>11</sup>C]PK11195 PET and contrast-enhanced MRI to detect residual subclinical synovitis in RA patients in clinical remission or with MDA, and the relation with clinical outcome (**chapter 5**: RA patients with long-standing disease, and **chapter 6**: early RA patients).

The second part of this thesis describes preclinical work on the development and validation of three alternative macrophage-targeted PET tracers in an arthritis model in rats. In **chapter 7**, we report on the potential of two new generation TSPO tracers, [<sup>11</sup>C]DPA-713 and [<sup>18</sup>F]DPA-714, for arthritis imaging. In **chapter 8**, the synthesis of the novel PET tracer [<sup>18</sup>F]fluoro-PEG-folate is described, and the results of PET imaging with this novel tracer in experimental arthritis are shown.

In **chapter 9 and 10**, the most important findings that were presented in this thesis are summarized and discussed.

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