CHAPTER 3

Macrophage positron emission tomography imaging as a biomarker for preclinical rheumatoid arthritis: findings of a prospective pilot study

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ABSTRACT

Objective: To conduct a prospective pilot study to determine whether macrophage targeting by ^{11}C-(*R*)-PK11195 positron emission tomography (PET) can visualize subclinical synovitis in arthralgia patients who have anti-citrullinated protein antibodies (ACPAs).

Methods: Twenty-nine arthralgia patients who were positive for ACPAs but did not have clinical arthritis were studied. High (spatial)-resolution ^{11}C-(*R*)-PK11195 PET scans of the hands and wrists were performed. For all metacarpophalangeal, proximal interphalangeal, and wrist joints (i.e., 22 joints per patient), tracer uptake was scored semiquantitatively (0-3 scale) by 2 observers who were blinded with regard to the clinical data. Patients were followed up prospectively for 24 months to investigate the development of clinical arthritis.

Results: Overall agreement and kappa values for the readings of the 2 observers were, respectively, 97% and 0.91 (95% confidence interval [95% CI] 0.74-1) at the patient level and 99% and 0.81 (95% CI 0.65-0.96) at the joint level. In 4 patients, at least 1 and as many as 5 PET-positive joints (score >1) were found at baseline. Within 2 years of followup, 9 patients had developed clinical arthritis. This included all 4 patients with positive findings on the ^{11}C-(*R*)-PK11195 scan, who developed clinical arthritis in the hand/wrist region, as identified on PET scans. Of the 5 remaining arthritis patients with negative findings on PET scans, 2 developed arthritis in the hand joints and 3 developed arthritis at locations outside the field of view of the PET scanner.

Conclusion: Subclinical arthritis in ACPA-positive arthralgia patients could be visualized by ^{11}C-(*R*)-PK11195 PET scanning and was associated with development of arthritis within 2 years of followup. This indicates that ^{11}C-(*R*)-PK11195 PET may be useful in determining arthritis activity in the preclinical phase of RA.

INTRODUCTION

Timely recognition of rheumatoid arthritis (RA) is highly relevant, as early treatment may slow down disease progression and improve long-term clinical outcome (1). There is evidence that RA is preceded by a preclinical phase that is characterized by the development of autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) (2-4). The presence of arthralgia may even further increase the risk of developing arthritis in ACPA-positive individuals (4). However, it was shown that 73% of 95 ACPA-positive patients that were included in an arthralgia study cohort (n=147) did not develop clinical arthritis after a median followup of 28 months (interquartile range [IQR] 19-39) (4).

Development of sensitive methods for detecting subclinical arthritis could offer a window of opportunity for early detection of disease and, hence, initiation of treatment at a very early stage. There are indications that subclinical arthritis can be detected by advanced imaging techniques (5-7). Advanced imaging may therefore be useful for providing additional predictive information on the development of RA.

Positron emission tomography (PET) is a novel imaging technique for the investigation of subclinical arthritis in preclinical RA. Previous studies have shown the value of ^{11}C-(*R*)-PK11195 (1-[2-chlorophenyl]-*N*-methyl-*N*-[1-methylpropyl]-3-isoquinoline carboxamide) and ^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) in visualizing active arthritis in established RA (8,9). ^{11}C-(*R*)-PK11195 specifically targets the 18-kd translocator protein, which was previously known as peripheral benzodiazepine receptor, a mitochondrial membrane protein that is up-regulated in activated macrophages (10). Macrophages infiltrate synovial tissue during the early development of RA (11). Therefore, PET using ^{11}C-(*R*)-PK11195, along with the determination of ACPA levels, may be valuable in the early detection of arthritis.

The possibility of detecting subclinical arthritis by PET scanning was previously demonstrated in studies showing uptake of ^{11}C-(*R*)-PK11195 in clinically uninflamed joints of patients with RA (8). The purpose of the present prospective pilot study was to determine whether ^{11}C-(*R*)-PK11195 PET scanning is useful for the detection of subclinical arthritis during the preclinical phase of RA.

PATIENTS AND METHODS

Patients

The study protocol was approved by the local Medical Ethics Review Committee, and informed consent was given by all patients and healthy volunteers prior to study inclusion.

Twenty-nine patients presenting with arthralgia were consecutively recruited after referral by a general practitioner. Inclusion criteria were the presence of ACPAs (regardless of RF status) and arthralgia, as defined by joint pain that was not secondary to trauma. Exclusion criteria were the presence of arthritis or tenosynovitis (as revealed by systematic chart review and physical examination of 44 joints (12) by 2 independent physicians), the absence of ACPAs on a second analysis performed at least 4 weeks after the first, pregnancy, previous corticosteroid injection in the hands or wrists, trauma of the hands or wrists in the 6 months before inclusion, and use of a benzodiazepine agonist within 10 days prior to PET scanning.

Following inclusion into the study, high (spatial)-resolution ^{11}C-(*R*)-PK11195 PET scans of the hands and wrists were performed. Clinical examination was repeated at least 12 months and 24 months after inclusion. If patients presented with symptoms of arthritis, they were examined during an additional visit by 2 independent investigators who were blinded with regard to the PET results.

Controls

^{11}C-(*R*)-PK11195 PET scans were also acquired in 6 age- and sex-matched healthy volunteers who had no symptoms of arthritis, as demonstrated by clinical examination (negative control group), as well as in 3 patients with established RA who had clinically inflamed hand and wrist joints (positive control group).

Scanning protocol

Metacarpophalangeal (MCP), proximal interphalangeal (PIP), and wrist joints were selected for examination by ^{11}C-(*R*)-PK11195 PET because they are often involved in early RA, and a total of 22 joints can be evaluated in a single scan session. Scans were performed using a double-layer ECAT high-resolution research tomograph (CTI/Siemens), a small animal and human brain 3-dimensional (3-D) scanner with high spatial resolution (2.3-3.4 mm full width at half maximum) and high sensitivity (13). Each subject's hands and wrists were positioned in the scanner and fixed in place to prevent movement artifacts. Ten minutes after intravenous injection of ^{11}C-(*R*)-PK11195 (mean ± SD

dose 457 ± 51 MBq), a static emission scan of 20 minutes' duration was performed, followed by a transmission scan of 7 minutes' duration. The measured PET data were normalized and corrected for scatter, randoms, attenuation, decay, and dead time. Data were reconstructed using an iterative 3-D ordinary Poisson ordered-subsets expectation-maximization (OSEM) algorithm (14) with 8 iterations and 16 subsets.

Data analysis

Data were transferred to a Sun Microsystems workstation for analysis. Coronal and, if necessary, sagittal and transverse images of the hand and wrist joints were scored semiquantitatively for joint and background uptake of ^{11}C-(*R*)-PK11195. Both joint uptake and background uptake in periarticular soft tissue and bone marrow were scored on a scale of 0-3, where 0 = absent, 1 = faint, 2 = moderate, and 3 = intense. Final scores were calculated by subtracting the background scores from the joint scores. Joints were considered positive if the score was ≥1. Scans, including scans of controls, were presented in random order to 2 independent observers (CJvdL and OSH) who were blinded with regard to the clinical data. In case of discrepancies, scoring was repeated in a combined session to reach consensus.

Laboratory tests

At baseline, ACPA and IgM-RF levels were determined in a second-generation anti-cyclic citrullinated protein enzyme-linked immunosorbent assay (ELISA; Axis-Shield) and in an in-house ELISA, respectively, as described previously (2). Cutoff levels for ACPA and RF positivity were 50 arbitrary units/ml (AU · ml^{-1}) and 30 international units/ml (IU · ml^{-1}) respectively.

Statistical analysis

Statistical analysis was performed using SPSS version 15.0 software. For continuous data with Gaussian distribution (i.e., age), mean ± SD values were calculated, and for non-normally distributed data (i.e., arthralgia duration, RF concentration, ACPA concentration), median and IQR values were calculated. To determine overall agreement and kappa values, ordinal data were dichotomized. A final joint score of ≥1 was regarded as positive, as described above.

RESULTS

Characteristics of the study subjects

At baseline, the mean ± SD age of the 29 arthralgia patients was 44 ± 12 years. Eighty-three percent of the patients were female. The median duration of arthralgia was 15 months (IQR 12-36). Thirty-eight percent of the arthralgia patients were RF positive. The median RF concentration in the entire group of study patients was 16 IU · ml^{-1} (IQR 1-40) and the median ACPA concentration was 215 AU · ml^{-1} (IQR 114-929). The mean ± SD age of the 3 control patients (1 man and 2 women) with active established RA was 49 ± 10 years, and the mean ± SD age of the 6 healthy control subjects (1 man and 5 women) was 45 ± 18 years.

Interobserver agreement

Overall agreement and kappa values were, respectively, 97% and 0.91 (with an SE of 0.09) at the patient level and 99% and 0.81 (with an SE of 0.08) at the joint level. The trained observers disagreed on the semi-quantitative scores by 1 point for 8 joints and by 2 points for 1 joint. The findings reported in the rest of the Results section below are consensus PET scores.

PET scan findings

At least 1 PET-positive joint (range 1-5 joints) was found in 4 of the 29 patients with preclinical RA, with a maximum of 5 PET-positive joints per patient. Three of the 4 positive PET scans showed moderate-to-high tracer uptake (score ≥2) in the joints. A representative PET scan showing moderate-to-high uptake is shown in Figure 1A. Control patients with positive findings on PET scans had clearly enhanced uptake (score ≥2) in all clinically inflamed joints (range 3-11 joints per patient) (Figure 1B), whereas the 6 healthy volunteers did not show enhanced joint uptake (Figure 1C). ^{11}C-(*R*)-PK11195 images showed background uptake in intrinsic hand muscles, in the region of the bone marrow, and in the soft tissues around the nails.

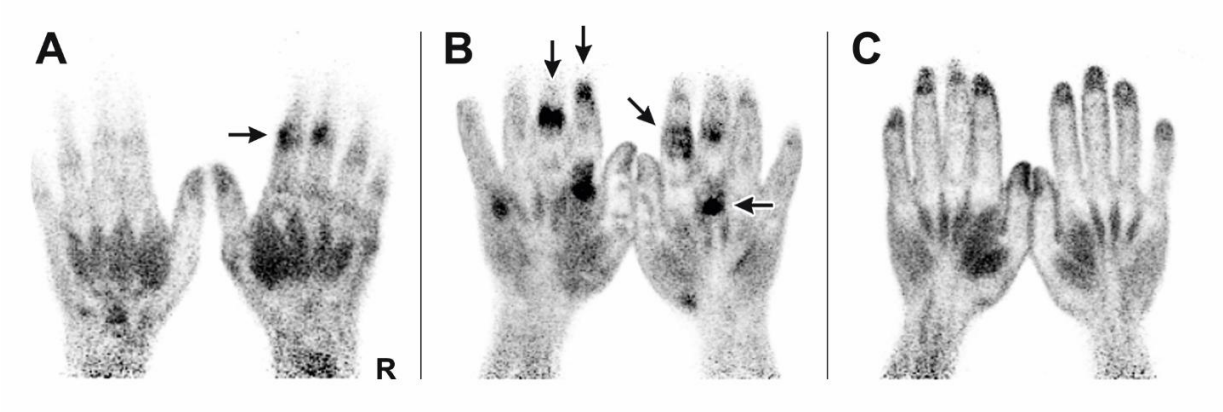

Figure 1. Representative ^{11}C-(*R*)-PK11195-enhanced positron emission tomographic images of the hands and wrists in A, an anti-citrullinated protein antibody-positive patient with arthralgia, B, a rheumatoid arthritis control patient with clinical arthritis, and C, a healthy volunteer. Arrows indicate representative joints with elevated tracer uptake, which is absent in the healthy subject. Note the background uptake of the tracer in intrinsic hand muscles, bone marrow, and soft tissues around the nails in all subjects. R= right.

Table 1. Numbers of patients developing clinical arthritis within 2 years, according to baseline PET score*

Baseline PET score	Clinical arthritis		No arthritis
	In any joint	In the hands and/or wrists	
0	5	2	20
1	1	1	0
2/3	3	3	0

* PET = positron emission tomography

^{11}C-(*R*)-PK11195 uptake and development of clinical arthritis

After 24 months of followup, 9 patients had developed clinical arthritis (Table 1 and Figure 2). At the patient level, all 4 patients with positive PET scan results developed clinical arthritis in ≥1 hand and/or wrist joint within 14 months. Three of these patients were diagnosed as having RA according to the American College of Rheumatology 1987 criteria (15), and the other patient was diagnosed as having oligoarthritis. Five of the remaining 25 patients with negative PET scan findings developed arthritis. Three of these patients presented with arthritis within 12 months after PET scanning, but it was outside the hand/wrist region (i.e., outside the field of view of the PET scanner). Later during followup, these 3

patients did not develop arthritis in the hands or wrists. Of the 2 remaining patients with positive clinical findings on followup but negative PET scan findings, 1 was diagnosed as having very mild arthritis with spontaneous remission in 1 PIP joint at 10 months and the other with oligoarthritis at 2 months.

Of all of the 638 joints evaluated, 14 joints had positive findings on PET scanning, and 12 of them had a score of ≥2. Clinical arthritis developed in 4 PET-positive joints and in 2 PET-negative joints.

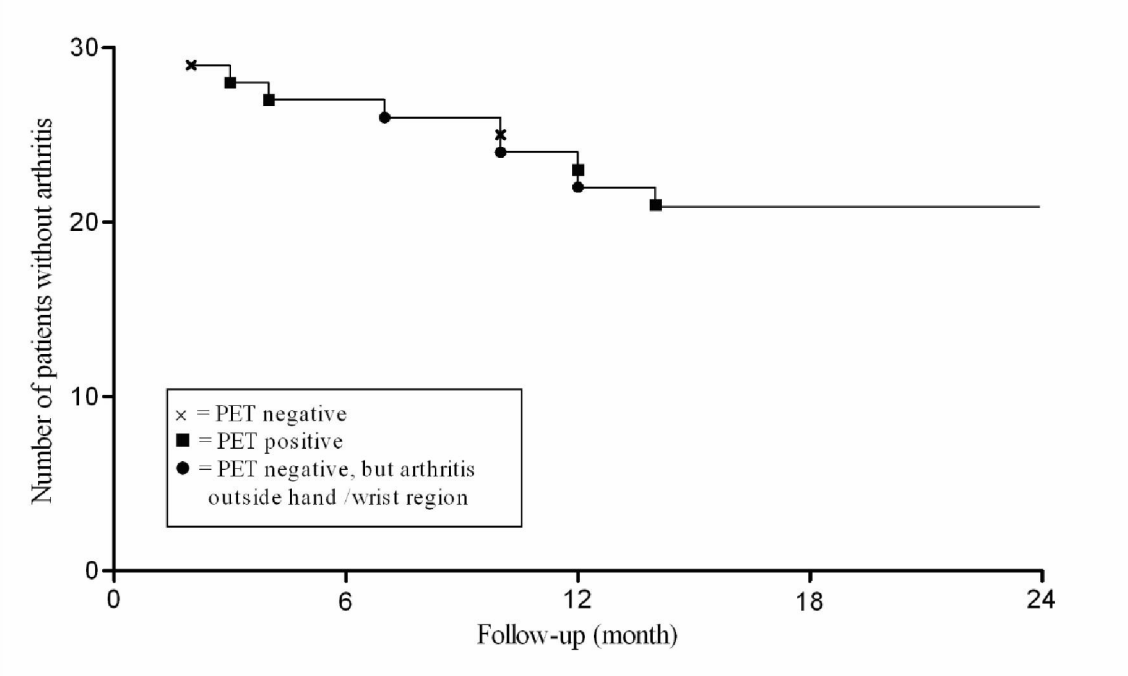

Figure 2. Survival curve showing the probability of not developing arthritis over 24 months of followup among 29 arthralgia patients with anti-citrullinated protein antibodies. PET = positron emission tomography.

^{11}C-(*R*)-PK11195 uptake and clinical characteristics

Characteristics of the patients who had positive findings on PET scans were compared with those who had negative findings but still had no arthritis after 2 years. No significant differences in the duration and localization of arthralgia or in the RF status between patients who had positive PET results and those who had negative PET results were observed. Of the 4 patients with positive findings on PET scanning, 2 had an ACPA level >2,500 AU · ml^{-1}, while none of the patients with negative findings on PET scanning

(n = 17) had an ACPA level >1,000 AU · ml^{-1}. The median ACPA levels in patients with positive and those with negative PET scan results were 1,180 (IQR 131-2,473) and 120 AU · ml^{-1} (IQR 76-474), respectively.

DISCUSSION

This prospective pilot study showed that ^{11}C-(*R*)-PK11195 PET is able to detect subclinical arthritis, since all patients with positive findings on PET scanning developed clinical arthritis within 14 months. These results were supported by a lack of ^{11}C-(*R*)-PK11195 uptake in the joints of healthy control subjects and significant uptake in the joints of control patients with clinically active RA.

The majority of arthralgia patients with negative PET findings (20 of 25) did not develop clinical arthritis. Five patients, however, developed arthritis despite negative PET readings. In 3 of these patients, negative PET scan results could be explained by the fact that arthritis did not develop in the hands and/or wrists, but outside the scanning area. The remaining 2 patients developed (subtle) arthritis in their hands, although only transiently in 1 patient. Tracer uptake in these patients may have been minimal relative to the adjacent background activity and therefore below the detection level (see below).

The present study showed good association between macrophage activity on ^{11}C-(*R*)-PK11195 PET scans of the hands and wrists and subsequent development of arthritis in this region. At the individual joint level, however, no clear association was found between the PET results and the clinical findings. This could be due to waxing and waning of arthritis activity in various joints which is often observed clinically in developing RA. A repeat PET scan over time may add additional predictive power at the joint level. For this purpose, carbon-11 is a suitable radionuclide because of its short half-life (20 minutes) and, consequently, its relatively low radiation dose.

Since RA usually develops initially in the small joints, we chose targeted hand/wrist imaging to visualize synovitis in 22 small joints per patient. We used a high (spatial)-resolution PET scanner (with small gantry diameter) to enable the detection of even subtle subclinical synovitis. A limitation of this scanning protocol is that synovitis at sites outside the scanning area could be missed, causing false-negative results. Whole-body imaging, however, might hamper the precise evaluation of uptake in the small joints.

A limitation of ^{11}C-(*R*)-PK11195 as a macrophage tracer is its uptake in hand muscles, soft tissues around the nails, and the bone marrow area. This is likely due to nonspecific binding, as it was also noticed in healthy volunteers, and a relatively high level of nonspecific binding is known to

occur in the brain (16). This background activity did not hamper the detection of obvious joint uptake but may have limited the detection of more-subtle tracer uptake in the joints.

Unfortunately, the study design and the limited number of patients included in the study did not allow investigation of the potential additive value of ^{11}C-(*R*)-PK11195 PET scanning to measurement of ACPA levels. Future studies in a larger arthralgia cohort should address this clinically relevant issue.

In conclusion, this prospective pilot study showed that macrophage targeting using the PET tracer ^{11}C-(*R*)-PK11195 might be a valuable tool for imaging subclinical arthritis in the hands and/or wrists of seropositive arthralgia patients, thereby providing a cue to early intervention in the course of disease.

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