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CONCISE REPORT

Evaluation of synovial angiogenesis in patients with rheumatoid arthritis using ^{68}Ga-PRGD2 PET/CT: a prospective proof-of-concept cohort study

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ABSTRACT

Background The study aimed to evaluate the use of positron emission tomography/computed tomography (PET/CT) with ^{68}Ga-PRGD2 as the tracer for imaging of synovial angiogenesis in patients with rheumatoid arthritis (RA).

Methods Twenty untreated active patients with RA underwent ^{68}Ga-PRGD2 PET/CT and ^{18}F-FDG PET/CT before treatment; two patients with osteoarthritis served as controls. Among the 20 patients with RA, 12 repeated the evaluations after 3-month treatment. The image findings were correlated with core variables of disease activity, including the clinical disease activity index (cDAI).

Results Our findings demonstrated that ^{68}Ga-PRGD2 specifically accumulated in the synovia with active inflammation rich in neovasculature with high-level $\alpha_v\beta_3$-integrin expression, but not in the ^{18}F-FDG-avid inflammatory lymph nodes. In patients with intense ^{18}F-FDG uptake in muscles caused by arthritic pain, we observed that ^{68}Ga-PRGD2 PET/CT was better able to evaluate disease severity than ^{18}F-FDG PET/CT. Both ^{68}Ga-PRGD2 accumulation and ^{18}F-FDG uptake changed in response to therapeutic intervention, whereas the changes of ^{68}Ga-PRGD2, not ^{18}F-FDG, significantly correlated with clinical measures of changes in the form of cDAI.

Conclusions This is the first integrin imaging study conducted in patients with RA that preliminarily indicates the effectiveness of the novel method for evaluating synovial angiogenesis.

Clinical trial registration This study has been registered online at NIH ClinicalTrial.gov (NCT01940926).

INTRODUCTION

Rheumatoid arthritis (RA), one of the most common rheumatic disorders, is characterised by the onset of synovial angiogenesis and inflammation and eventually leads to pannus formation and joint destruction.[1 2] The $\alpha_v\beta_3$-integrin is a transmembrane heterodimeric receptor that mediates cell–cell and cell–extracellular matrix adhesion.[3] The $\alpha_v\beta_3$-integrin plays a pivotal role in promoting and sustaining angiogenesis and has been identified as a biomarker of angiogenesis.[3–5] Cyclic arginine–glycine–aspartic acid (RGD) peptide is the key integrin recognition motif that can strongly bind to the $\alpha_v\beta_3$-integrin and inhibit new blood vessel formation, which make the RGD-based peptides hold a promise for imaging and treatment of diseases characterised with angiogenesis including RA.[6–9]

To date, however, no reports have presented the clinical application of integrin imaging for the evaluation of synovial angiogenesis and pannus formation, which are very important for the histopathological analysis of patients with RA. In this prospective cohort study, we evaluated the ability of RGD positron emission tomography/computed tomography (PET/CT) to assess synovial angiogenesis and monitor response to treatment in patients with RA. The results were compared with those generated by FDG PET/CT through clinical case-by-case evaluations.

PATIENTS AND METHODS

Patients

This study was approved by the Institutional Review Board of Peking Union Medical College Hospital and conducted from February 2012 to December 2013. Written informed consent was obtained from each participating patient. All of the patients with RA recruited for the study met the 1987 revised criteria of the American College of Rheumatology (ACR) for RA.[10]

We recruited 20 patients with RA (n=18 females/2 male; mean age, 49±12 years; disease duration, 36±39 months; the demographic and clinical characteristics of the patients with RA are presented in online supplementary table S1) and two patients with osteoarthritis (OA) as diseased controls. We evaluated all patients using whole-body ^{68}Ga-PRGD2 PET/CT and ^{18}F-FDG PET/CT scans. At the time of enrolment in this study, all patients were assessed for core ACR variables of disease activity, including tender joint count (TJC-28), swollen joint count (SJC-28), pain intensity score (10 cm visual analogue scale (VAS), 0.0 = no pain, 10.0 = very intensive pain), patients' global assessment of overall well-being (PtGA, 10 cm VAS) and physician's global assessment of disease severity (PyGA, 10 cm VAS); a clinical disease activity index (cDAI) was calculated as follows: cDAI=TJC-28+SJC-28+PtGA+PyGA.[11]

Twelve patients repeated the ^{68}Ga-PRGD2 PET/CT and ^{18}F-FDG PET/CT scans and the clinical evaluations 3 months after the treatment. Two patients with RA agreed to undergo synovial biopsy before treatment.

METHODS

Details of ^{68}Ga-PRGD2 PET/CT scanning, ^{18}F-FDG PET/CT scanning, semi-quantitative

analysis, immunohistochemical analysis and statistical analysis are given in online supplementary information.

RESULTS

Comparison of ^{68}Ga-PRGD2 PET/CT with ^{18}F-FDG PET/CT

An intense accumulation of ^{68}Ga-PRGD2 occurred in the primary excretory pathways (including the kidneys and bladder) and moderate uptake occurred in the thyroid, liver, spleen and intestinal tract; the distribution of ^{68}Ga-PRGD2 in other parts of the body was low and stable and therefore able to provide an accurate evaluation of joint inflammation in the patients. The patients with RA experienced high levels of ^{68}Ga-PRGD2 accumulation in the involved joints and tendon sheaths and diffuse distribution in the lining of the synovium (figure 1B); in contrast, the OA controls experienced only slight regional tracer uptake in the affected joints (figure 1H). Moreover, the accumulation of ^{68}Ga-PRGD2 decreased with decreasing disease activity after effective treatment (figure 1D).

In contrast to the results observed in the ^{18}F-FDG PET/CT images, no accumulation of ^{68}Ga-PRGD2 was observed before treatment in the hyperplastic ^{18}F-FDG-avid lymph nodes at the bilateral axillary regions of the patients with RA (figure 1A,C). Moreover, the pain and movement disorder in patients suffering from RA may have caused intense ^{18}F-FDG uptake in the related muscles, which could have significantly influenced the evaluation of disease severity in the joints with^{18}F-FDG PET/CT. However, the distribution of ^{68}Ga-PRGD2 was much less varied in the skeletal muscles, bone marrow and myocardium than that of ^{18}F-FDG; thus,^{68}Ga-PRGD2 introduced less background noise and prevented possible evaluation bias in the assessment of disease severity and treatment response (figure 1E, F).

Correlation of PET/CT images with clinical parameters

The maximum standardised uptake value (SUV_{max}) of ^{68}Ga-PRGD2 was significantly correlated with the SUV_{max} of ^{18}F-FDG in the large joints before and after treatment ($r=0.60$ and 0.36, respectively; both $p<0.001$). Additionally, the SUV_{max} of ^{68}Ga-PRGD2 was significantly correlated with TJC and SJC before and after treatment ($p<0.001$) (table 1).

Three months after treatment, the patients with RA who repeated PET/CT were assigned to a responder group (n=9) or a poor-responder group (n=3) according to the cDAI good

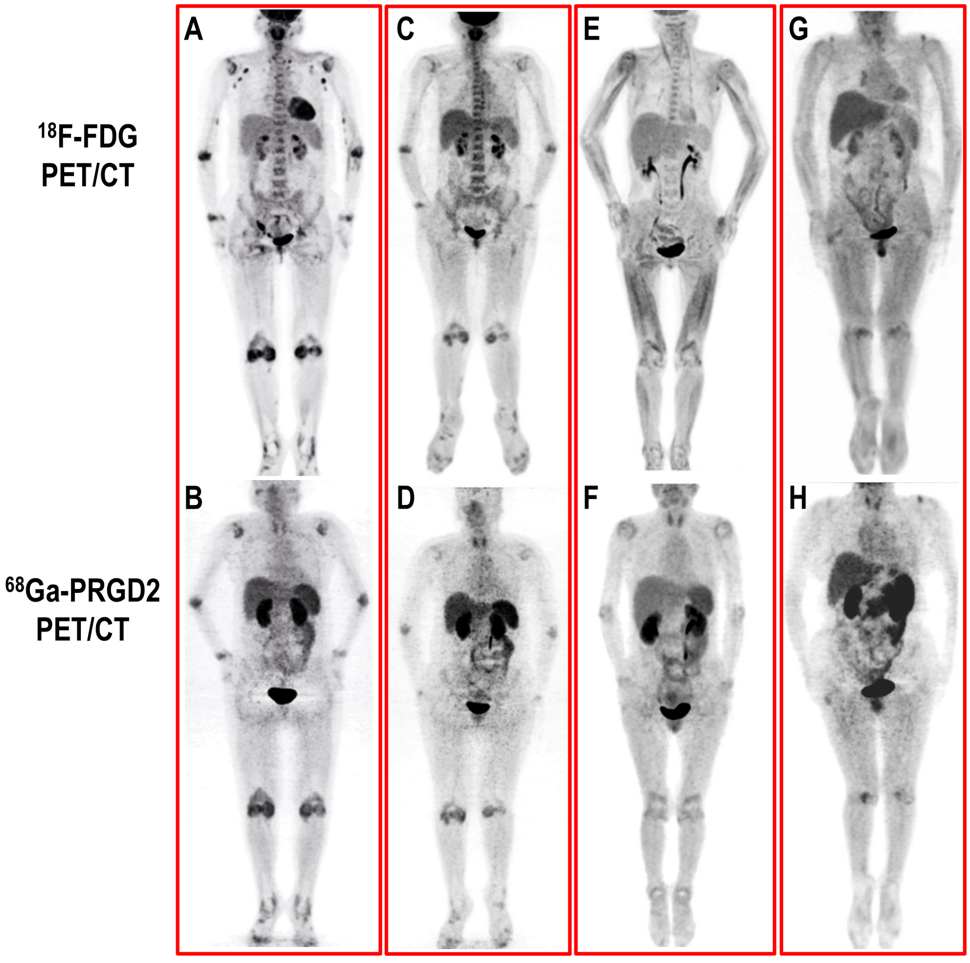

Figure 1 Comparison of the distribution of ^{18}F-FDG and ^{68}Ga-PRGD2 in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). (A, B) In a patient with RA (F, 48 years) with a clinical disease activity index (cDAI) of 28.0, intense ^{18}F-FDG uptake was observed in the inflammatory synovia and axillary lymph nodes, whereas ^{68}Ga-PRGD2 accumulated specifically in the synovia. (C, D) After successful treatment (cDAI=6.0), ^{18}F-FDG uptake and ^{68}Ga-PRGD2 accumulation significantly decreased in the joints. (E, F) In another patient with RA, the joint pain caused intense ^{18}F-FDG uptake in the muscles; this accumulation significantly influenced the evaluation of disease using ^{18}F-FDG positron emission tomography/computed tomography (PET/CT) but had no effect on the distribution of ^{68}Ga-PRGD2. (G, H) The regional uptake of ^{18}F-FDG and the accumulation of ^{68}Ga-PRGD2 in the shoulders and knees of an OA patient (M, 60 years) were significantly different from the diffuse synovial involvement in the patients with RA.

Table 1 Correlation between the uptake of ^{18}F-FDG and the accumulation of ^{68}Ga-PRGD2 and the tender joint count and swollen joint count in patients with rheumatoid arthritis

		Pretreatment (n=20)		Post-treatment (n=12)	
		TJC	SJC	TJC	SJC
SUV_{max} of ^{68}Ga-PRGD2	r	0.44	0.22	0.43	0.33
	p	<0.001	<0.001	<0.001	<0.001
SUV_{max} of ^{18}F-FDG	r	0.37	0.12	0.44	0.39
	p	<0.001	<0.001	<0.001	<0.001

SJC, swollen joint count; SUV_{max}, maximal standardised uptake value; TJC, tender joint count.

response criteria (defined as achieving ≥50% improvement of cDAI or cDAI ≤2.8 after treatment). The SUV_{max} of ^{18}F-FDG and ^{68}Ga-PRGD2 decreased significantly after treatment in the responder group ($p<0.001$). In contrast, the SUV_{max} of ^{18}F-FDG and ^{68}Ga-PRGD2 increased significantly in the poor-responder group ($p=0.001$ and $p=0.002$, respectively) (see online supplementary table S2).

We analysed the correlation between the change in SUV_{max} (ΔSUV_{max}) and the change in clinical parameters after treatment. We observed that the reduction in ^{68}Ga-PRGD2 uptake in the affected joints was significantly correlated with the ΔPtGA, ΔPyGA and ΔcDAI ($p<0.05$), whereas the reduction in ^{18}F-FDG uptake after treatment was significantly correlated with ΔPtGA and ΔPyGA ($p<0.05$) but not with ΔcDAI ($p=0.083$) (table 2).

Histopathological features of the RA synovium

We examined the histopathology and expression of $\alpha_v\beta_3$-integrin in the synovia of two patients with RA to corroborate relevant findings with the ^{68}Ga-PRGD2 PET/CT findings. In agreement with the intense ^{68}Ga-PRGD2 accumulation in the affected joint synovium (see online supplementary figure S1A,B), high levels of $\alpha_v\beta_3$-integrin were selectively expressed on the endothelial cells of the synovial blood vessels (see online supplementary figure S1C). An extensive vascular network with ongoing angiogenesis and proliferation was observed in the synovium, as demonstrated by the positive staining of VEGF, CD34 and Ki67 (see online supplementary figure S1D–S1F).

DISCUSSION

Synovial angiogenesis and pannus formation are major histopathological findings in patients with RA.[1 2] The development of a new and reliable approach is needed to assess synovial neovasculature and its response to treatment.[12] In the last decade, numerous studies have demonstrated that ^{18}F-FDG PET/CT is a sensitive technique for evaluating disease activity and treatment response in patients with RA.[13–15] However, the mechanism behind ^{18}F-FDG uptake is only associated with elevated glucose metabolism.[16]

^{68}Ga-PRGD2 is specifically designed to target the endothelial cells of neovasculature that express $\alpha_v\beta_3$-integrin at high levels.[17 18] Therefore, ^{68}Ga-PRGD2 PET/CT represents a specific method for evaluating angiogenesis. As demonstrated by the present study, ^{68}Ga-PRGD2 was found to be typically distributed in a diffuse manner along the lining of the synovium of the affected joints and tendon sheaths of patients with RA, whereas the accumulation of ^{68}Ga-PRGD2 was confined to a specific diseased area in patients with OA. Interestingly, we also found that ^{68}Ga-PRGD2 did not accumulate in the ^{18}F-FDG-avid axillary lymph nodes commonly observed in patients with RA.[19] In patients with intense ^{18}F-FDG uptake in muscles caused by arthritic pain, we observed that ^{68}Ga-PRGD2 PET/CT was better able to evaluate disease severity than ^{18}F-FDG PET/CT. Additionally, in response to therapeutic intervention, the changes of ^{68}Ga-PRGD2, not ^{18}F-FDG, significantly correlated with clinical measures of changes in the form of cDAI.

To the best of our knowledge, this is the first study conducted in humans to investigate the use of integrin imaging (specifically ^{68}Ga-PRGD2 PET/CT) for the non-invasive measurement of synovial angiogenesis in patients with active RA. We compared the findings of this technique with the ^{18}F-FDG PET/CT findings of the same patients. We provided histopathological confirmation showing high-level expression of $\alpha_v\beta_3$-integrin on the neovasculature endothelial cells of the ^{68}Ga-PRGD2-avid RA synovium that were consistent with previous immunohistochemical findings in synovial tissue.[9 20]

Some limitations apply to the present study. First, the number of enrolled patients with RA was small. However, each patient underwent ^{68}Ga-PRGD2 PET/CT and ^{18}F-FDG PET/CT scanning, and 12 patients repeated the scans after 3-month treatment; thus, the preliminary results of this study support a proof-of-concept study. Second, the study lacks a sufficient number of control patients with OA or other forms of arthritis. An additional study is required to recruit a wide variety of patients with arthritis to determine the sensitivity, specificity and accuracy of ^{68}Ga-PRGD2 PET/CT in diagnosing RA. Studies with more cases are needed to correlate the image findings related to post-treatment changes with the clinical response and final prognosis of patients with RA.

In conclusion, this prospective cohort study demonstrates that ^{68}Ga-PRGD2 PET/CT is a specific method for identifying and assessing inflammatory synovial angiogenesis in patients with RA. In contrast to ^{18}F-FDG, ^{68}Ga-PRGD2 did not accumulate in areas such as the axillary lymph nodes with reactive hyperplasia and the strenuous skeletal muscles. Therefore, ^{68}Ga-PRGD2

Table 2 Correlation between the changes in SUV_{max} in ^{68}Ga-PRGD2 PET/CT and ^{18}F-FDG PET/CT and the changes in clinical parameters of patients with RA (n=12) before and after 3-month treatment

	ΔSUV_{max} of ^{68}Ga-PRGD2		ΔSUV_{max} of ^{18}F-FDG	
	r	p Value	r	p Value
ΔVAS	0.39	0.215	0.65	0.021
ΔPtGA	0.62	0.033	0.61	0.034
ΔPyGA	0.60	0.040	0.72	0.009
ΔcDAI	0.60	0.039	0.52	0.083

Δ, changes between the baseline and the post-treatment evaluation; cDAI, clinical disease activity index; r, correlation coefficient; PET, positron emission tomography; PtGA, patient's global assessment of overall well-being; PyGA, physician's global assessment; RA, rheumatoid arthritis; SUV_{max}, maximal standardised uptake value of a tracer; VAS, visual analogue scale of joint pain.

PET/CT is a useful tool for assessing synovial angiogenesis and monitoring treatment responses in patients with RA.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study has been approved by the Institutional Review Board of Peking Union Medical College Hospital (S-532).

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