Original article

99mTc-anti-TNF-α scintigraphy in RA: a comparison pilot study with MRI and clinical examination

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Abstract

Objective. To compare the use of radiolabelled human monoclonal anti-TNF-α scintigraphy with clinical examination and MRI of hands and wrists joints in patients with active RA.

Methods. Eight patients with active RA, 28-joint DAS (DAS-28) > 3.2 and a healthy volunteer underwent whole body and hand/wrist scintigraphy after the administration of anti-human TNF-α labelled with technetium-99m (99mTc). One hundred and ninety-eight joints were examined. Patients were also given clinical examinations in addition to MRI of the hands and wrists.

Results. Of the 198 joints examined, signs of inflammation were detected by MRI in 49 (24.7%) and by scintigraphy in 48 (24.2%) joints, with agreement between the two methods in 44 joints. In five joints, MRI was positive and scintigraphy negative. In another four joints, scintigraphy was positive and MRI negative for signs of inflammation. MRI and scintigraphy were in agreement for negative results for 145 joints. The sensitivity and specificity of scintigraphy was 89.8 and 97.3%, respectively. When clinical parameters (presence of swelling and tenderness of joints) were compared with the MRI findings, lower correlation coefficients were observed (sensitivity of 59.2% and 65.3%, respectively).

Conclusions. Scintigraphy using 99mTc-anti-TNF-α showed high correlation with the presence of inflammatory signs detected by MRI in the hands and wrists of patients with active RA, and demonstrated a greater sensitivity than clinical examination. These results can assist in better understanding of anti-cytokine therapy and support the achievement of evidence-based biologic therapy.

Key words: Anti-TNF-α, Labelling, Technetium-99m, Diagnosis, MRI, Rheumatoid arthritis, Scintigraphy, Joints, Activity.

Introduction

RA is a systemic disease characterized by chronic inflammatory synovial involvement that causes pain and, if not treated early and intensively, progressive joint destruction and incapacity. The early diagnosis of synovitis, even in its early stages, is essential in order to prevent or delay complications and to avoid irreversible incapacity [1].

TNF-α has a central role in the chronic inflammatory and destructive process of RA. A number of studies have detected this cytokine in the rheumatoid joint and shown the ability of TNF-α to amplify and perpetuate synovitis [2-4]. Following enormous efforts by the pharmaceutical community to generate new drugs targeted to such molecules, the development of specific anti-TNF-α therapeutics has revolutionized the clinical management of RA. Currently, TNF-α inhibitor drugs come in the form of either soluble receptors (etanercept) or mAbs (infliximab, adalimumab and, more recently, golimumab) [5].

The clinical development of immunobiological therapy has proved to be complex and challenging. In some
cases, clinical assessment may reflect changes that are not related to the inflammatory process; thus unnecessary treatment may be advised for patients whose disease is in remission. The technology of radiolabelling immunobiological molecules may allow us to identify the accumulation of labelled mAbs against TNF-α inside the affected joint, indicating the presence of this cytokine. The use of radiolabelled antibodies thus provides a reliable method to objectively identify the presence of cytokines in the rheumatoid joint, and allows for the possibility of image-based biologic therapy [6, 7].

Non-specific immunoglobulin G (IgG) labelled with 99mTc has long been used to image inflammation [8]. The localization of IgG to inflammatory foci is mainly due to non-specific mechanisms such as increased vascular permeability. The selectivity of IgG and other radiopharmaceutical agents can therefore be increased by targeting them with specific molecules. Adalimumab (Abbott Laboratories) is a fully human monoclonal anti-TNF-α antibody with high affinity and specificity [9, 10]. The therapeutic use of adalimumab provides a rapid clinical improvement in the majority of patients and is capable of preventing or retarding joint destruction [11].

For the past 20 years, studies have aimed at developing scintigraphic techniques to assess the presence and degree of arthritis in chronic inflammatory diseases such as RA and JIA, and to monitor the success of therapies. These studies have focused on the use of polyclonal and mAbs labelled with 99mTc. Martins and co-workers developed a simple, reproducible and highly efficient technique for labelling anti-CD3 with 99mTc using a direct method [12, 13]. This advancement allows for the use of 99mTc-anti-CD3 as a radiopharmaceutical for the evaluation of diseases like RA and JIA, in which mature T lymphocytes are associated with the pathological immunological processes [12, 13]. This radiopharmaceutical method gave high-quality images that strongly correlated with disease activity. These data clearly revealed the feasibility of targeting antibodies to image the joints of patients with RA, JIA and gout arthritis. Based on this work, we developed a technique to label a human monoclonal anti-TNF-α antibody (adalimumab) with 99mTc. TNF-α represents a specific target that plays an important role in RA pathophysiology, and the functional characterization of TNF-α in affected joints by molecular imaging would fulfil the early diagnosis of disease activity, thereby providing an important clinical tool for planning therapy and precisely monitoring the disease. We also compared scintigraphic results with those from MRI study in patients with active RA.

**Patients and methods**

Eight patients (seven with RA, one with JIA) according to the ACR 1987 criteria for RA, aged >18 years, with active disease as defined by 28-joint DAS (DAS-28 ≥ 3.2) [14] and a healthy volunteer control subject were enrolled in this study. The median age of the patients was 47.6 years (range 35–67 years) and the healthy subject was 33 years old. The median disease duration was 8 years. All patients were positive for RF and two had used anti-TNF-α drugs in the past. They were being treated with DMARDs—MTX, LEF—and anti-malarial drugs and low-dose prednisone.

The inclusion criteria for this study consisted of at least one swelling joint of the hands and/or wrists as well as the regular use of anti-rheumatic drugs. Exclusion criteria were pregnancy, lactation, active infection, concomitant severe disease, or a history of drug allergy. The Institutional Ethics Committee in Research and the National Ethics Committee approved the study (no. 711/2008). Patients were informed about the nature of the procedure and written informed consent was obtained from all patients.

**Study protocol**

Clinical examinations of patients’ hands and wrists were performed on the same day as the 99mTc-anti-TNF-α scintigraphy and by the same rheumatologist throughout the study in order to standardize examinations. MRI was performed at an interval of <48 h following scintigraphy.

**MRI**

All MRI procedures were performed in an OrthOne device (1.0T) from ONI Medical Systems, Inc. (Wilmington, MA, USA). The device was equipped with a coil-phased array surface and the patient sat in an anatomical chair with their arms extended, abducted and placed in the supine position. Axial and coronal cohorts were obtained in T1-weighted spin echo (TR/TE/thickness: 600–700 ms/15 ms/3 mm) before and after intravenous contrast 0.1 mmol/kg body weight gadolinium (gadoteric acid; Dotarem, Guerbert, France). A 224 × 256 matrix was used and the field of view was 140 mm. We also used the short tau inversion recovery (STIR) sequence on axial and coronal plans (TR/TE/TI/thickness: 4000–3250 ms/53 ms/150 ms/3 mm). Synovitis was identified, according to the definitions developed by the Omeract MRI collaborative subgroup [rheumatoid arthritis magnetic resonance imaging score (RAMRIS)] [15], as tenosynovitis, joint swelling and bone oedema in wrists, MCP and PIP joints bilaterally. Two radiologists, in consensus, interpreted the MRI images without prior knowledge of the clinical status of the patients or the scintigraphic findings.

**99mTc-anti-TNF-α scintigraphy**

Based on a previous experience [12, 13, 16] in which we successfully labelled anti-CD3, monoclonal human anti-body anti-TNF-α (150 µl) (Abbott Laboratories, IL, USA) was labelled with 99mTc. Briefly, anti-TNF-α was incubated for 10 min with a reducing agent and then 370 MBq (10 mCi) of 99mTc was added. Filtration with a 0.22 µm Millipore sterile filter was used to sterilize the radiopharmaceutical. The total amount of administered anti-TNF-α was 15% of a treatment dose, without pharmacological effect.

Scintigraphs using 99mTc-anti-TNF-α were obtained 30 min and 3 h after intravenous injection of the radiopharmaceutical. Whole body and anterior planar images of hands and wrists were obtained using a dual gamma
In patients using adalimumab, the saturation of TNF-α counts per minute in the uptake of 99mTc-anti-TNF-α observed an increase in both visual parameters and graphs of the first and seventh day of drug therapy. We sites in affected joints was evaluated by comparing scintigraphs on Days 1 and 7 following s.c. injection of 40 mg of adalimumab to compare the saturation of TNF-α with the unlabelled anti-TNF-α mAb. Images were acquired 30 min, 3 and 24 h after the administration of 259 MBq (7 mCi) of the radiopharmaceutical. Increases or decreases of uptake over time were measured as counts per minute, with a correction for decay. The carpal region was not analysed. Each joint was scored as present or absent depending on the uptake pattern: present (definite uptake maintained in the late images after 3 h) or absent (no uptake or faint uptake with no accumulation of the radiopharmaceutical in the 3 h image).

### Statistical analysis

The κ-coefficient of agreement and the P-value of McNemar’s chi-square test were used as statistical tools to compare clinical examination (swelling and tenderness of joints), scintigraphy scans and MRI. Sensitivity and specificity were also calculated. MRI was considered the gold standard against which the other methods were compared.

### Results

The infusion of the radiopharmaceutical was well tolerated, without any side effects. No uptake of 99mTc-anti-TNF-α was observed in the joints of the healthy volunteer. In patients using adalimumab, the saturation of TNF-α sites in affected joints was evaluated by comparing scintigraphs of the first and seventh day of drug therapy. We observed an increase in both visual parameters and counts per minute in the uptake of 99mTc-anti-TNF-α on the seventh day.

**Correlation of MRI with 99mTc-anti-TNF-α scintigraphy**

The presence of synovitis, joint swelling, tenosynovitis and bone oedema in MRI was considered the gold standard for the presence of inflammation. These findings were correlated with 99mTc-anti-TNF-α scintigraphy and clinical examination (swelling and tenderness) (Fig. 1). The carpal region was not analysed.

Of 198 joints examined, MRI detected signs of inflammation in 49 (24.7%) and scintigraphy in 48 (24.2%) with agreement between the methods in 44 joints (sensitivity of 89.8%). There were five false-negative and four false-positive scintigraphs. One hundred and forty-five joints truly correlated as negative for active disease, resulting in a specificity of 97.3% (Table 1). Tenderness and swelling were calculated to have 65.3% and 59.2% sensitivity and 75.2% and 95.3% specificity, respectively, when compared with MRI.

**Correlation of 99mTc-anti-TNF-α scintigraphy with clinical assessment**

The correlation between the absence of swelling and negative scintigraphy was 96% (Table 2). Six joints had swelling but were negative at scintigraphy; among these, two joints had a positive MRI. Within the 18 joints positive for scintigraphy and negative for oedema, MRI was positive in 17. Tenderness had a lower correlation with scintigraphy (Table 2).

### Discussion

Advances in the current knowledge of the pathophysiology of RA are leading to the development of new medicines that offer patients the opportunity to stabilize the progression of their disease and improve their quality of life. Several drugs [17, 18] have been studied in clinical trials and evaluated for effectiveness by clinical and radiological examination. The sensitivity and specificity of the imaging methods used are not always adequate to establish disease activity [19, 20], reflecting the need for further studies on non-invasive diagnostic methods.

Scintigraphy assays are no longer used as a diagnostic tool for RA by rheumatologists. This is likely due to the high cost of the examinations, limited availability of the necessary apparatus, lack of specific tracers and low number of studies performed with scintigraphy in comparison with MRI and US.

Scintigraphic bone scanning agents, such as 99mTc-MDP, have poor specificity for synovial inflammation and are unable to reliably distinguish between active and inactive RA [21]. In the 1980s, various studies demonstrated the comparative advantage of 99mTc-labelled non-specific immunoglobulin (99mTc-HIG) in imaging inflammatory arthritis [8, 22]. This method, however, has low specificity, as the uptake of this radiopharmaceutical is related to increases in vascularization, extracellular fluid volume, endothelial permeability within inflamed synovium and the presence of RF (immunoglobulin that binds to the Fc portion of IgG) [23].

The development of techniques to label mAbs directed against specific molecules such as adhesion molecules, activation markers, cytokines or receptors has proved useful. These labelled antibodies may provide a more sensitive and specific diagnosis of synovitis related to disease activity and indirectly identify the presence of their antigens in the affected tissues [7]. Several radiopharmaceuticals labelled with 99mTc, such as anti-CD3, anti-CD4 and anti-E-selectin mAbs, are already used in the evaluation...
FIG. 1 (A) Right hand with fourth PIP joint swelling and capsular bulging. (B) Hand scintigraphy scan, showing increase in uptake of $^{99m}$Tc-anti-TNF-$\alpha$ in the fourth right (PIP) (arrow), third and fourth left PIP and wrists. (C and D) Right-hand MRI coronal (C) and axial (D) slices showing fourth PIP synovitis.

**TABLE 1** Agreement of scintigraphy and clinical assessment (pain and oedema) with MRI in the 198 joints analysed

<table>
<thead>
<tr>
<th>Assessment</th>
<th>MRI</th>
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<tbody>
<tr>
<td></td>
<td>Positive $n = 45$; 24.7%</td>
<td>Negative $n = 149$; 75.3%</td>
<td>Total $n = 194$</td>
<td>$P$-value</td>
<td>$\chi^2$ of McNemar</td>
<td>Sensitivity, %</td>
<td>Specificity, %</td>
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<tr>
<td>Scintigraphy</td>
<td>44 (89.8)</td>
<td>4 (2.7)</td>
<td>48 (24.2)</td>
<td>0.877</td>
<td>1.000</td>
<td>89.8</td>
<td>97.3</td>
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<tr>
<td>Pain</td>
<td>5 (10.2)</td>
<td>145 (97.3)</td>
<td>150 (75.8)</td>
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<tr>
<td>Positive</td>
<td>32 (65.3)</td>
<td>37 (24.8)</td>
<td>69 (34.8)</td>
<td>0.356</td>
<td>0.009</td>
<td>65.3</td>
<td>75.2</td>
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<tr>
<td>Negative</td>
<td>17 (34.7)</td>
<td>112 (75.2)</td>
<td>129 (65.2)</td>
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<tr>
<td>Oedema</td>
<td>29 (59.2)</td>
<td>7 (4.7)</td>
<td>36 (18.2)</td>
<td>0.598</td>
<td>0.019</td>
<td>59.2</td>
<td>95.3</td>
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<tr>
<td>Negative</td>
<td>20 (40.8)</td>
<td>142 (95.3)</td>
<td>162 (81.8)</td>
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of RA. In comparison with 99mTc-HIG, these mAbs are more specific in detecting joint inflammation. Kinne et al. [24] using 99mTc-anti-CD4 scintigraphy, and Jamar et al. [23] using 99mTc-anti-E-selectin scintigraphy, found advantages in the use of specific labelled antibodies compared with the labelling of non-specific polyclonal human immunoglobulin in RA. Labelled anti-CD4 was also shown to be informative about the presence of Th lymphocytes and macrophages in inflamed synovium. 99mTc-anti-CD3 scintigraphy is also a promising tool for the diagnosis and follow-up of rheumatic diseases [13] and has the advantages of simplicity, reproducibility and reliability. Problematic, however, is the use of an anti-CD3 mAb that has a murine origin. In addition, in our experience, studies using 99mTc-anti-TNF-α have demonstrated a higher image quality with this labelled mAb compared with 99mTc-anti-CD3. As a great number of RA patients treated with traditional DMARDs fail to achieve remission or lower disease activity [17, 18], the next option in treatment should be a choice of the available biological agents.

The ability of 99mTc-anti-TNF-α to identify high levels of TNF-α in the inflamed joint or tenosynovium in RA has major practical implications. In this study, it was possible to evaluate the specific targeting of 99mTc-anti-TNF-α to TNF-α in affected regions, as we observed a decrease in the uptake of the radiopharmaceutical when unlabelled anti-TNF-α (adalimumab) was administered previously to the patient. These patients had lower uptake of 99mTc-anti-TNF-α on Day 1 compared with Day 7 following treatment, in spite of unchanged findings from hand and wrist examinations made by the same physician. These results are in agreement with Barrera et al. [25] and Malviya et al. [7], who observed that joint saturation with anti-TNF-α therapy reduces labelling with 99mTc-anti-TNF-α. These findings support the concept that the uptake of 99mTc-anti-TNF-α in the joint is, at least in part, due to its binding with TNF-α.

In a pilot study, Malviya et al. [7] compared anti-CD20 and anti-TNF-α scintigraphs in the same RA patient and found different intensities of uptake in different joints with the two radiopharmaceuticals. This finding was interpreted by the authors as indicating differences in the mechanisms responsible for inflammation. Additionally, patients with high pre-therapy uptake of 99mTc-anti-TNF-α had more therapeutic benefit with anti-TNF drugs than patients with low pre-therapy uptake of the same tracer [7].

In RA patient management, identification of joint inflammation is an essential goal. Clinical examination has limitations; however, the use of US and MRI increases the accuracy of diagnosis. Our study demonstrates the feasibility of the addition of scintigraphy to current practice, by showing that scintigraphy with 99mTc-anti-TNF-α was well tolerated and that the uptake of the labelled mAb was limited to diseased joints. MRI is well known as an imaging method that detects early synovitis in patients with RA, but its acquisition takes much longer than other modalities, such as scintigraphy [26].

Validation of synovitis on MRI has been extensively addressed using arthroscopy and synovial biopsy and comparing these with MRI synovial volume estimates. This has been performed in knee as well as in the MCP joints using miniarthroscopy, macroscopic evaluation and histology [27]. Ostendorf et al. [28] found that synovial enhancement post-gadolinium contrast [gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA)] on MRI correlated with macroscopic signs of synovitis, and joint space narrowing on MRI was significantly correlated with bone changes on arthroscopy. Like MRI and US, 99mTc-anti-TNF-α scintigraphy demonstrates a greater sensitivity compared with clinical examination. In the current study, a high correlation between 99mTc-anti-TNF-α scintigraphy and MRI was observed in the detection of joint inflammation.

Effective doses in diagnostic nuclear medicine investigations are generally low. As compared with other imaging modalities scintigraphy with Tc-99m emits less radiation to the patient based on its physical properties [140 keV gamma ray with no associated primary particle radiation, physical half-life of 6 h, effective dose equivalent (mSv/MBq) equal to 8.2E-03 and effective dose of 0.022 rem/mCi] [29].

Scintigraphy scans have the advantage of a lower acquisition time and also provide images of the whole body in 15 min in contrast to MRI, where the evaluation of both hands and wrists can take up to 2 h. Moreover, patients

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**Table 2** Agreement of clinical assessments (pain and oedema) with scintigraphy in the 198 joints analysed

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>Scintigraphy</th>
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<tr>
<td></td>
<td>Positive (n = 48; 24.2%) n (%)</td>
</tr>
<tr>
<td>Pain</td>
<td>30 (62.5)</td>
</tr>
<tr>
<td></td>
<td>18 (37.5)</td>
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<tr>
<td>Oedema</td>
<td>30 (62.5)</td>
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<td>18 (37.5)</td>
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reported that the scintigraphy scan was more comfortable than MRI, despite the fact that scintigraphy does not require contrast medium, different from MRI.

The five false negative findings using scintigraphy in this study can likely be explained by the absence of significant concentrations of TNF-α in those inflamed joints. In these cases, inflammation may be due to another major mechanism rather than TNF-α. Three out of four false positive patients had tenderness and two of them had discrete swelling. These findings suggest that different results can be due to different sensitivity between the two methods.

To our knowledge, this is the first study comparing scintigraphy using a human monoclonal ⁹⁹ᵐTc-labelled antibody with MRI of the joints in patients with RA. We show scintigraphy with ⁹⁹ᵐTc-anti-TNF-α to be more sensitive and specific than clinical examination for detection of joint inflammation, and to be comparable to MRI. The main limitation of this study was the small number of patients and the delay between scintigraphy and MRI (up to 48 h).

In conclusion, the labelling of human anti-TNF-α could be useful in various ways. It can allow the direct identification of the mAb in the joints and correlate its presence in synovial structures, allowing for evidence-based therapy. Finally, our study demonstrates that the use of this method enables a more accurate diagnosis of joint inflammation than clinical examination. It was possible to rapidly evaluate the whole body and less time was needed when compared with MRI. We suggest that ⁹⁹ᵐTc-anti-TNF-α has the potential to be a reliable biomarker of joint inflammation in RA. More studies with ⁹⁹ᵐTc-anti-TNF-α in RA are now ongoing.

### Rheumatology key messages

- ⁹⁹ᵐTc-anti-TNF-α can help in more accurate diagnosis of disease activity in RA.
- ⁹⁹ᵐTc-anti-TNF-α can easily evaluate the whole body in less time than MRI, being more comfortable.
- ⁹⁹ᵐTc-anti-TNF-α is a promising method to evaluate active inflammation.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**


