

# Use of $^{99m}\text{Tc}$ -labelled Anti-TNF Monoclonal Antibodies to Assess Patients Affected by Inflammatory Arthropathies

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## Abstract

Nuclear medicine techniques have been applied for many years to assess the activity in patients with different inflammatory arthropathies. The recent development of specific radiopharmaceuticals suggested the possibility to use nuclear medicine in order to clarify the disease mechanisms and predict response to treatment. In this review, we aimed at presenting data concerning the use of  $^{99m}\text{Tc}$ -labelled anti-TNF monoclonal antibodies. Few studies have been conducted in patients affected by Rheumatoid Arthritis and Spondyloarthropathies, treated for systemic or mono-articular involvement, showing the possible use of this specific scintigraphy.

## Introduction

Nuclear medicine techniques have been applied for many years to assess the activity status in patients affected by inflammatory arthropathies. Recently, the development of specific radiopharmaceuticals suggested the possibility to use nuclear medicine techniques not only in the assessment and monitoring of patients, but also to clarify the disease mechanisms. Moreover, these new imaging modalities could help the physician for therapy decision-making and follow-up [1]. In particular, recent studies evaluated the possible application of scintigraphy with a  $^{99m}\text{Tc}$ -anti-tumor necrosis factor (TNF) monoclonal antibody (mAb) in the assessment of patients affected by inflammatory bowel diseases and inflammatory arthropathies, such as rheumatoid arthritis (RA) [2-3]. Biological agents directed against TNF revolutionized the therapeutic approach of these patients, due to the innovative mechanism of action, based on the ability to recognize specific molecular or cellular targets involved in the disease pathogenesis. The administration of anti-TNF drugs determined a dramatic improvement in the prognosis of patients refractory or intolerant to conventional disease modifying anti-rheumatic drugs (DMARDs) treatment. Randomized controlled trials demonstrated the efficacy of these drugs to prevent the development of bone erosive damage, with consequent better profile in terms of remission status and prevention of long-term disability [4]. The identification of pivotal role of pro-inflammatory cytokines in the pathogenesis of inflammatory diseases determined the attempt to develop drugs specifically targeting the molecules involved in the disease processes. Indeed, TNF is a pro-inflammatory cytokine able to promote synovial inflammation and bone and cartilage erosions [5]. Produced by several different immune and non-immune cell types, it was recognized as a key factor in the inflammatory process of arthropathies, such as RA, psoriatic arthritis (PsA) and ankylosing spondylitis (AS) [6]. To date, five different anti-TNF drugs have been developed: infliximab, adalimumab, golimumab, certolizumab pegol, which are mAbs or fragments thereof, and etanercept, a genetically engineered fusion protein composed of a dimer of the extracellular portions of human TNFR2 fused to the Fc portion of a human IgG1 [6]. Despite the recognized efficacy of anti-TNF agents, approximately one third of patients have to discontinue the treatment due to inefficacy or intolerance. In these cases, the switching to another anti-TNF agent could represent a valid option due to significant differences in terms of molecular structure, pharmacokinetics, interactions with TNF,

generation of antibodies, induction of apoptosis, and dosing regimen among the TNF antagonists [4]. More recently, other molecular or cellular targets involved in the pathogenesis of the autoimmune diseases have been identified, such as the pro-inflammatory cytokines interleukin-1 and interleukin-6, CTLA-4, and molecules involved in the activation, differentiation and maturation of B cells. These drugs have been used in patients that do not benefit from anti-TNF drugs, obtaining good results in terms of remission and long-term prognosis [4]. In the light of the possibility to choose among different biological drugs, with specific targets, the establishment of a personalized treatment is a critical issue. Several efforts have been performed in order to identify biomarkers able to predict the response to treatment and guide the physician in the therapy decision-making. The most promising candidate is TNF, targeted by the mAb infliximab.

## $^{99m}\text{Tc}$ Radiolabelled Infliximab Scintigraphy

The chimeric mouse/human antibody infliximab, demonstrated a greater efficacy in the treatment of patients with inflammatory arthropathies due to its ability to bind TNF with high affinity and specificity both in soluble and membrane form. As demonstrated by recent studies,  $^{99m}\text{Tc}$ -infliximab scintigraphy could be used to evaluate the presence of TNF at the articular level, in order to evaluate the possibility to treat patients with drugs specifically directed against this molecule [7]. The mAb was radiolabeled by a direct method after reduction of disulphide bond with 2-mercaptoethanol. This allows the binding of  $^{99m}\text{Tc}$  using methylene-diphosphonate as a weak competitor. The use of such radiopharmaceutical was reported in the study of Conti et al. in a patient affected by SpA with a mono-arthritis resistant to conventional intra-articular and systemic treatment [7]. After i.v. injection of 370 – 550 MBq (100 – 150  $\mu\text{g}$ ) of  $^{99m}\text{Tc}$ -infliximab, planar images of the involved joints were acquired at 6 h and 24 h.

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Radiolabelled infliximab scintigraphy was useful to assess a high presence of TNF in the right knee justifying the use of anti-TNF drugs. Indeed, the patient was treated by intra-articular infliximab (100 mg) and underwent a control examination at the end of the treatment showing the disappearance of TNF from the inflamed joint. Moreover, the clinical and ultrasonography assessment confirmed a total remission. After this case report, a larger population was evaluated including 12 patients with active refractory mono-arthritis (11 knees and 1 ankle), affected by RA (5 patients) and SpA (7 patients) (8). At baseline and after 12 weeks of treatment with intra-articular infliximab (100 mg for the knee and 50 mg for the ankle), a  $^{99m}\text{Tc}$ -infiximab scintigraphy was performed. Quantitative analysis of TNF in the inflamed sites was performed using the OSIRIS software. Target to background (T/B) ratios were calculated drawing circular region of interests (ROIs) on the affected joints (target) and rectangular ROIs approximately five cm below the knee or above the ankle [8]. The same ROIs were used to analyse both pre- and post-therapy images. In order to obtain the T/B ratio for each joint, the activity in the ROIs was normalized and divided by the background activity. The T/B ratio was calculated for affected and non-affected joints on images acquired at 6 h and at 20 h. Authors reported an increase in the T/B ratio from 6 to 20 hours. By using this method, the  $^{99m}\text{Tc}$ -infiximab binding to TNF in the joints was expressed in absolute values and as the percentage increase at 20 h with respect to 6 h (%T/B 20h/6h). The results of these studies confirmed the promising role of  $^{99m}\text{Tc}$ -infiximab scintigraphy in monitoring the response to anti-TNF therapies. Indeed, a significant decrease in the T/B ratio of the affected joints was associated with complete remission as a confirmation of the success of the treatment. Overall, responder patients showed a significantly greater increase in uptake from 6h to 20h p.i. compared to non-responders. Therefore, the high level of TNF confirmed by  $^{99m}\text{Tc}$ -infiximab scintigraphy in the inflamed joints is associated with better efficacy of intra-articular administration of infliximab.

### $^{99m}\text{Tc}$ Radiolabelled Adalimumab Scintigraphy

Adalimumab is the first 'fully human' mAb against TNF approved for the management of moderate to severe active RA and psoriatic arthritis, showing less potential side effects and antigenicity compared with infliximab. The administration of this drug seems to be able to down regulate the expression of other pro-inflammatory cytokines, such as IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor [9].

Adalimumab could be radiolabelled with  $^{99m}\text{Tc}$  directly or via an indirect method, using succinimidyl-hydrazinonicotinamide (S-HYNIC) as a bi-functional chelator, tricine as a co-ligand and stannous chloride as a reducing agent. Data from the literature described the comparison between  $^{99m}\text{Tc}$ -adalimumab and radiolabelled infliximab.

The study conducted by Barrera et al. aimed at assessing the sensitivity and bio-distribution after i.v. administration of the radiopharmaceutical for TNF in 10 RA patients [2]. Each patient performed two scintigraphic examinations by the administration of 740 MBq (0.1 mg); whole-body and joint-specific images were acquired after 5 min, 4 h, and 24 h.

Results demonstrated that inflamed joints were clearly visualised at 4 and 24 h p.i. with a median increased uptake of 30% at 24 h. The uptake was not evident in smaller inflamed joints, suggesting the absence of TNF in some joints. As expected, normal joints did not show uptake in any patients. Patients were then divided into two groups;

the first one received a therapeutic dose (10 mg/kg) of unlabelled anti-TNF- $\alpha$  mAb immediately before the  $^{99m}\text{Tc}$ -anti-TNF- $\alpha$  mAb administration for competition studies. The second one received an intramuscular injection of corticosteroid (120 mg) 2 days before the second scintigraphy to check the sensitivity to reflect decreased inflammation. Results from the first group revealed that co-injection of unlabelled anti-TNF mAb was able to reduce the uptake of  $^{99m}\text{Tc}$ -adalimumab by a median of 25% as a percentage of injected doses after 24 h. In this way, it was possible to confirm the specificity of the radiopharmaceutical for its target in inflamed joints. In the second group, systemic corticosteroid administration proved to reduce the disease activity, decreasing uptake of the radiopharmaceutical. This study demonstrated the possibility to use this radiopharmaceutical to detect clinically relevant changes in disease activity [2]. In 2008 Malviya et al. performed a study for therapy decision-making and follow-up with both  $^{99m}\text{Tc}$ -labelled infliximab and adalimumab in RA patients [10]. Two groups of patients underwent scintigraphic examination with  $^{99m}\text{Tc}$ -infiximab (12 patients) and  $^{99m}\text{Tc}$ -adalimumab (9 patients), respectively. Imaging evaluation was performed before and 3 months after intra-articular therapy with infliximab or systemic therapy with adalimumab. After injection of 370 MBq  $^{99m}\text{Tc}$ -infiximab or  $^{99m}\text{Tc}$ -adalimumab, planar anterior and posterior images of inflamed joints were acquired at 6 and 20 h p.i. and T/B ratio was calculated in all affected joints. The authors did not identify differences in the bio-distribution between the two radiopharmaceuticals. A variable degree of joint uptake (T/B ratio ranging from 1 to 4) has been observed, which was not always correlated with joint pain or swelling. After the therapy with unlabelled anti-TNF mAb, a reduction in the joint uptake of  $^{99m}\text{Tc}$ -anti-TNF mAb correlated with the reduction of clinical symptoms. Moreover, patients with higher uptake prior to therapy had more benefit than patients with lower uptake in the inflamed joints. The study conducted by Barrera et al., performing a  $^{99m}\text{Tc}$ -adalimumab scintigraphy in patients with active RA before and after treatment with unlabelled anti-TNF mAb or corticosteroids, obtained similar results [2].

### Conclusion

$^{99m}\text{Tc}$ -labelled anti-TNF mAb scintigraphy is a promising imaging tool that could help to manage and follow-up patients affected by inflammatory arthropathies.  $^{99m}\text{Tc}$ -infiximab and  $^{99m}\text{Tc}$ -adalimumab could be candidate for therapy decision-making purposes, predicting the response to anti-TNF treatment in RA patients.

### Competing Interests

The authors declare that they have no competing interests.

### Author Contributions

All the authors substantially contributed to the literature review, drafting the manuscript and approve the final version of the manuscript.

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