Use of 99mTc-anti-CD3 scintigraphy in the differential diagnosis of rheumatic diseases

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Abstract

Objectives. The aim of this study was to assess the use of anti-CD3, labelled with technetium-99m scintigraphy, for evaluating the joints of patients with RA, juvenile idiopathic arthritis (JIA), OA and gouty arthritis, and to establish the diagnosis parameters for each disease.

Methods. We evaluated 2044 joints from 77 patients with rheumatic diseases. The clinical evaluation consisted of laboratory assays; examination for joint inflammation (pain and/or oedema); and for patients with RA, the disease activity score of 28 joints. To evaluate the sensitivity and specificity of 99mTc-anti-CD3 in detecting disease activity, patients received an injection of the radiopharmaceutical compound 99mTc-anti-CD3, and underwent a scintigraphy scan 1 h later. Scanning was repeated 3 h later. As a control, after 2 days, the patient was injected with 99mTc-non-specific human immunoglobulins, and scintigraphy scanning performed at 1 and 3 h after the injection. The intensity of uptake and the pattern of activity were defined, and Spearman’s correlation and analysis of variance used for statistical evaluation.

Results. Diagnosis criteria were established for 99mTc-anti-CD3 uptake in different diseases. RA and JIA showed joint uptake with progressive increase in late images. Gouty arthritis showed joint uptake with decrease during the late images. Joint uptake was low or absent in patients with OA, although when present the joint uptake decreased during the examination.

Conclusion. 99mTc-anti-CD3 scintigraphy is a useful method in the differential diagnosis of rheumatic diseases.

Key words: Scintigraphy, Anti-CD3, Rheumatoid arthritis, Juvenile idiopathic arthritis, Osteoarthritis, Gouty arthritis, Differential diagnosis, Rheumatic diseases.

Introduction

Adequate management of patients with RA and juvenile idiopathic arthritis (JIA) sometimes requires differentiation of RA and JIA from other conditions such as OA and gouty arthritis (GA), and regular follow-up care. In clinical practice, a disease activity score (DAS) is commonly used for these purposes in patients with RA. In some cases, even though imaging modalities are desirable, they are not always available, or have low specificity and sensitivity. Until now, imaging methods have been unable to differentiate similar clinical presentations of RA, JIA, OA and GA, or to evaluate disease activity efficiently, with high specificity and sensitivity [1–3].

In European countries as well as in Latin America, DAS of 28 joints (DAS-28) is commonly used as a tool for diagnosis of RA and monitoring disease activity. DAS-28 is endorsed by the European League Against Rheumatism, but it is a subjective method, based mainly on the clinical evaluation of pain and assessment of global health.
Moreover, it may reflect changes that are not related to the inflammatory process [4–8]. Thus, sensitive methods are needed to assess the activity and progression of RA, for improving the patient’s quality of life.

For the past 20 years, several studies have aimed to develop scintigraphic techniques for assessing the degree of arthritis intensity in chronic inflammatory autoimmune diseases such as RA and JIA, and monitor the success of their therapies. The studies have focused on the use of polyclonal antibodies and mAbs labelled with technetium-99m (99mTc) [9–13]. Marcus et al. [10] developed an indirect method for labelling the mAb anti-CD3 with 99mTc for the assessment of disease activity in RA patients. The images showed a strong correlation with clinical outcome and disease activity, which was proposed to be a consequence of specific binding to CD3, in this case to a CD3-receptor–T-cell complex [10]. Subsequently, Martins and co-workers [1, 14] developed a simple, reproducible, high-efficiency technique for labelling anti-CD3 using a direct method. The technique allowed the use of 99mTc-anti-CD3 as a radiopharmaceutical for the evaluation of rheumatological diseases like RA and JIA, in which mature T lymphocytes are associated with the pathological immunological processes. The Martins et al. [1] method gave high-quality images and strong correlation with disease activity, as measured by the DAS-28 [6], and clinical and laboratory parameters. It also had the advantage of not showing the side effects of the Marcus et al. [10] method, in which studies were discontinued when patients experienced tremors and sudoreis. In addition, 99mTc-anti-CD3 scintigraphy has the ability to scan the entire patient at once, and use the uptake pattern as a proxy for measuring disease progression and assessing clinical outcome. The methodology is essential, as there is no cure for RA or JIA, or means to prevent it [1–3, 9, 11, 13].

In rheumatic diseases, especially RA and JIA, methods of diagnostic imaging are used so that physiopathological processes and morphological changes are recognized without the need for invasive procedures such as biopsy. Evaluations must be conducted periodically to determine the disease stage, which is essential for establishing the best therapeutic approach for each individual [3, 15]. In addition to evaluating therapeutic success, imaging is used to differentiate RA and JIA from other rheumatological diseases such as OA and GA, which can eventually present with similar signs, symptoms and radiological findings. Since this is a new scintigraphic method, diagnostic criteria must be established so that it can be used by any nuclear medicine department as a reproducible and routine method for patient evaluation. The aim of this study was to evaluate the use of an anti-CD3 molecule labelled with 99mTc in patients with RA, JIA, OA and GA, and to establish criteria for differential diagnosis of each disease.

Materials and methods

The Committee of Ethics in Research of Hospital Universitário Clementino Fraga Filho (Universidade Federal do Rio de Janeiro) (CIC: 129/03 and CEP: 080/03) approved the study protocol, and written consent was obtained from all patients. We evaluated 77 patients with rheumatic diseases (44 with a clinical diagnosis of RA, 5 with JIA, 15 with OA and 13 with acute GA).

At the initial assessment, medical history, including laboratory assays was considered. A complete physical examination covering symptoms, functional status, objective evidence of joint inflammation (pain and oedema, together or separately), joint mechanical problems and the presence of extra-articular involvement was given. All patients had one or more joints with a clinical pattern of oedema and pain, together or separately, and these were assessed by the same physician in order to standardize articular examinations.

The patients were diagnosed with RA according to the revised criteria of the ACR [15] and DAS-28 score was calculated to determine the pattern of disease activity for this study. Patients with RA were classified into two groups: patients with disease in remission (DAS-28 < 2.6) and patients with disease in activity (DAS-28 > 2.6).

The criteria for JIA was: age < 16 years, mono or poly-articular arthritis for at least 6 weeks, the presence of systemic manifestations, exclusion of other causes of arthritis and changes in ESR [16].

We considered patients with OA as those who presented at the time of scintigraphy with a positive clinical examination for the disease, ESR within reference values of the Westergren method (average rates 0–25 mm/h for men and 0–30 mm/h for women) and negative Waaler Rose and/or latex test.

Patients with GA were characterized clinically and by the presence of hyperuricaemia using conventional values of 1.5–5.5 mg/dl, and moderately increased ESR during acute crisis.

Scintigraphies using 99mTc-anti-CD3 were performed after intravenous injection of 10 mCi (370 MBq) of the radiopharmaceutical. Anterior planar images of the joints were obtained 1 and 3 h after injection, using a dual gamma camera (Millennium MG; GE Medical Systems, Milwaukee, WI, USA) with low-energy, high-resolution, parallel whole collimators. Images were acquired at a matrix size of 256 × 256. Counts were acquired for 10 min in a 15% window centred at 140 KeV. Images taken at 1 and 3 h were evaluated for intensity of uptake and pattern of activity. Increase or decrease of uptake over time was measured as counts per minute, with a correction for decay.

To evaluate the sensitivity and specificity of 99mTc-anti-CD3 scintigraphy in detecting disease activity, a negative control scan was performed on all patients, after an interval of 2 days, using a pool of non-specific human immunoglobulins labelled with Tc-99m. Images were acquired using the same protocol as for 99mTc-anti-CD3.

Spearman’s correlation and analysis of variance were used as statistical tools for comparing scintigraphic findings and clinical and laboratory parameters. Results were considered significant at P < 0.05. All IP and MCP joints were assessed by the same physician in order to standardize articular examinations.
were grouped as one articulation because of vagueness in patient reports about which joint was in pain.

Results

No side effects were observed after administration of the radiopharmaceuticals, even after multiple scintigraphic examinations. In total, 1232 joints were evaluated from patients with RA, 140 from patients with JIA, 360 from patients with OA and 312 from patients with GA (Table 1). We observed that in RA and JIA, the initial scan showed a high uptake per affected joint at the first scan, which increased at the 3-h scan. Joint uptake was absent or minimal in cases of OA, with a subsequent decrease observed in the second scan at 3 h. Moderate joint uptake was observed in the initial scan in patients diagnosed with GA, with the mean uptake decreasing at the second scan. Thus it was possible to differentiate RA (Fig. 1) and JIA (Fig. 2) from OA (Fig. 3) and GA (Fig. 4) according to the uptake pattern. The method was unable to differentiate patients with RA in disease remission, who showed a normal scan, from those with OA.

Anterior images of affected joints showed that the 99mTc-anti-CD3 built up as a layer, not only in the joint directly, but also in the periarticular region, suggesting selective uptake by inflamed synovia. The results observed in 99mTc-anti-CD3 scintigraphies strongly correlated with the DAS-28 parameters (Table 2). Table 3 shows a comparison of the uptake of 99mTc-anti-CD3 scintigraphy at 1 and 3 h. No statistically significant differences were observed with regard to individual assessment of joints or their evaluation as a group.

Discussion

Advances in the current knowledge of the physiopathology of RA and JIA 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 have been studied in clinical trials and evaluated by radiological examination to test their effectiveness. The sensitivity and specificity of these imaging methods are not always adequate, reflecting the need for further studies on non-invasive diagnostic methods [17, 18]. Therefore, the uptake of 99mTc-anti-CD3 would be expected to be increasingly positive in joints affected by RA or JIA.

Table 1 Clinical and 99mTc-anti-CD3 scintigraphy evaluation by joint

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Total joints evaluated</th>
<th>Pain</th>
<th>Abnormal uptake of 99mTc-anti-CD3 in patients with pain</th>
<th>Pain + oedema</th>
<th>Abnormal uptake of 99mTc-anti-CD3 in patients with oedema</th>
<th>Abnormal uptake of 99mTc-anti-CD3 in patients with pain + oedema</th>
<th>No clinical complaints</th>
<th>Abnormal uptake of 99mTc-anti-CD3 in patients with no clinical complaints</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>1232</td>
<td>119 (9.7)</td>
<td>83^a (69.7)</td>
<td>80 (6.5)</td>
<td>58^a (72.5)</td>
<td>68 (5.5)</td>
<td>60^a (88.2)</td>
<td>965 (78.3)</td>
</tr>
<tr>
<td>JIA</td>
<td>140</td>
<td>33 (23.6)</td>
<td>25^a (75.7)</td>
<td>3 (2.1)</td>
<td>3^a (100.0)</td>
<td>73 (52.1)</td>
<td>73^a (100.0)</td>
<td>31 (22.1)</td>
</tr>
<tr>
<td>OA</td>
<td>360</td>
<td>280 (77.8)</td>
<td>0</td>
<td>4 (1.1)</td>
<td>1^b (25)</td>
<td>6 (1.7)</td>
<td>1^b (16.6)</td>
<td>70 (19.4)</td>
</tr>
<tr>
<td>GA</td>
<td>312</td>
<td>0</td>
<td>0</td>
<td>10 (3.2)</td>
<td>10^a (100.0)</td>
<td>3 (1.0)</td>
<td>3^a (100.0)</td>
<td>299 (95.8)</td>
</tr>
<tr>
<td>Total joints</td>
<td>2044</td>
<td>432 (21.1)</td>
<td>108 (25.0)</td>
<td>97 (4.7)</td>
<td>72 (74.2)</td>
<td>150 (7.3)</td>
<td>137 (91.3)</td>
<td>1365 (66.8)</td>
</tr>
</tbody>
</table>

Values are represented as n (%). ^aJoints with high uptake that increased at 3-h image; ^bJoints with mild uptake, decreasing at later images; ^cJoints with moderate uptake that decreased at later images.
**Fig. 1** Patient classified with high-activity RA, according to DAS-28.

Scintigraphy with 99mTc-anti-CD3 of the knees shows an increase in the uptake of these areas in late images. Images taken (A) 1 h and (B) 3 h after endovenous injection of the radiopharmaceutical.

**Fig. 2** Patient with active JIA. Scintigraphy with 99mTc-anti-CD3 of the knees shows increase in the uptake of these areas in late images.

Images taken 1 h (A) and 3 h (B) after endovenous injection of the radiopharmaceutical.

**Fig. 3** Patient with OA.

Images of 99mTc-anti-CD3 scintigraphy showing a normal pattern (no joint uptake) in a patient with OA, 1 h after injection of the radiopharmaceutical (A). The same pattern, observed in the late images (3 h), considered a normal examination according to the parameters established in this study (B).
Joints affected by GA contain monourate monosodium crystals, which lead to an inflammatory process. Recent studies provide new knowledge about the physiopathology of the inflammatory response elicited by GA. The urate crystals bind to innate immune receptors, leading to cell activation, typically of phagocytic cells, as well as the release of cytokines and chemokines that orchestrate the initial inflammatory response [19]. Based on these data, a possible explanation for the 99mTc-anti-CD3 uptake, as observed in the early scan taken 1 h after injection, is increased vascularity and cell infiltration in patients for whom the main complaint was oedema, and for whom pain and oedema occurred together. Since T lymphocytes are not activated by the TCR–CD3 complex, a decrease in uptake is observed in later images. In painful joints with GA, the inflammatory process may not be present, which explains the absence of radiopharmaceutical uptake in the affected joints.

OA is a chronic degenerative disease in which the inflammation process is not acute, and is therefore independent of the activation of T lymphocytes by the TCR–CD3 complex. Consequently, significant uptake of anti-CD3 labelled with technetium was not expected.

During the remittance phases of RA, the inflammatory process caused by activation of T lymphocytes by the TCR–CD3 complex diminishes or disappears [20]. Consequently, 99mTc-anti-CD3 uptake is expected to be minimal or absent. For this reason, this method cannot differentiate patients with RA in remission from those with OA. Synovitis in patients with OA indicates activation of T lymphocytes by the TCR–CD3 complex, which is difficult to differentiate from RA. In these cases, however, the finding of an abnormal scan may be an indication for early treatment of these patients, in order to avoid bone and cartilage destruction.

Current methods of assessing the degree of RA and JIA disease activity do not allow a precise diagnosis, because they lack specificity, and indirectly assess disease activity based on painful and oedema joints. The assessment of pain is directly related to trauma or inflammation, and oedema may or may not be present in affected joints. Moreover, in some studies, pain was found to be occasionally associated with bone destruction, while oedema was related only to events of synovitis or inflammation [3]. These findings are consistent with the negative results of 99mTc-anti-CD3 scintigraphies observed in 36 joints of patients with RA, 8 joints of patients with JIA and all joints of patients with OA and GA. Therefore, the use of less subjective methods for diagnosis and monitoring is essential.

In patients with RA, 99mTc-antiCD3 scintigraphy showed an increased joint uptake in 83 (69.7%) of 119 painful joints. For the presence of oedema, increased uptake of the radiopharmaceutical was observed in 58 (72.5%) of 80 joints. When oedema and pain are considered together, scintigraphy and clinical findings were concordant in 60 (88.2%) of 68 joints. The 99mTc-anti-CD3
scintigraphy showed a significant correlation \((P < 0.05)\) between radiopharmaceutical uptake and joints with oedema or pain, allowing the differentiation of patients with active synovitis from those in disease remission scored by DAS-28. No correlation was seen between age or sex and 99mTc-anti-CD3 scintigraphy results. Table 2 shows the comparison between DAS-28 parameters and 99mTc-anti-CD3 scintigraphy. In patients with JIA, 99mTc-anti-CD3 scintigraphic findings were consistent in most cases. From 109 joints of patients with JIA with clinical complaints of pain and/or oedema, 101 were detected by 99mTc-anti-CD3. The eight joints with discordant results were those in which the clinical complaint was pain.

For joints of OA patients in which clinical examination showed the presence of pain, all scintigraphies were considered normal, with no radiopharmaceutical uptake. In joints \((n = 4)\) where the presence of oedema was the main complaint, in only one joint did the scan show mild uptake of the radiopharmaceutical. For six joints with pain and oedema, only one scintigraphy showed mild uptake of radiopharmaceutical, and 21.4% of joints without changes by clinical examination showed mild radiopharmaceutical uptake. One patient clinically diagnosed with OA presented an ESR above the reference value, and corresponding 99mTc-anti-CD3 scintigraphic images showed uptake areas in the knees and hands, with increased uptake in delayed images, which was considered an indication of RA. In these joints \((n = 4)\), the patient had pain and oedema on physical examination. This case was referred to the Department of Rheumatology for further clinical evaluation, where a diagnosis of RA was confirmed. For all patients with GA, clinical and physical examinations were compliant with changes in the 99mTc-anti-CD3 scintigraphy for joints with oedema \((n = 10)\) and pain and oedema \((n = 3)\).

The ESR, despite being widely used for monitoring patients with RA and JIA, is questionable, as it leads to inconclusive results, with many false positives and false negatives reported even in healthy patients [21]. In this study, 99mTc-anti-CD3 scintigraphy had no significant correlation with ESR, consistent with previous studies in which the value of ESR for assessing RA and JIA was found to be debatable [21]. The ESR is not considered a standard test for the diagnosis of RA and JIA, since changes in ESR do not accurately characterize the stage of disease. In addition, some discordant examinations of elderly patients indicated that deviations can be attributed to other diseases or even be inherent in these patients [21].

Currently, polyclonal antibodies and mAbs labelled with radionuclides by different methods, such as 99mTc-anti-CD3, have been used for the diagnosis of disease by immunoscintigraphy [1, 10–12]. In many cases, for example in the Marcus et al. study [10], uptake of 99mTc-anti-CD3 by the joint indicated synovitis before clinical evidence of the condition. However, this study was discontinued because of side effects. Although these reversed spontaneously within a few hours, they are a limiting factor to using 99mTc-anti-CD3 marked by that method. In this study, using a direct method, we obtained results consistent with clinical findings. Uptake of the radiopharmaceutical was observed in joints affected by the disease, without the observation of any side effects after scintigraphy.

Scintigraphy with 99mTc-polyclonal immunoglobulin G has been used for high-specificity detection of synovial inflammation in RA, and for distinguishing disease activity and remission, although the correlation of scintigraphic shoulders with clinical findings is low [11]. In this study, significant correlation was observed for RA and pain (69.7%), oedema (72.5%) and when oedema and pain were considered together (88.2%); and for JIA and pain (75.7%), oedema (100.0%) and oedema and pain (100.0%). These findings are probably attributable to the high specificity of anti-CD3 for the physiopathology of RA and JIA. The use of non-specific immunoglobulins labelled with 99mTc has also been reported in RA and JIA patients. However, because uptake of these radiopharmaceuticals is non-specific, results varied depending on blood flow and, in cases of synovitis, endothelial permeability, limiting their use [12].

Scintigraphy results with 99mTc-anti-CD3 showed a high correlation between clinical findings and laboratory assays in the evaluation of MCP and IP joints, which are considered difficult to diagnose. However, a limitation of the method was the evaluation of the hip joints and spine, because the normal biodistribution of 99mTc-anti-CD3 in the liver and kidneys obscures diagnosis in these regions. Another limitation of the method is the inability

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Number of joints clinically affected</th>
<th>Number of joints with abnormal uptake of 99mTc-anti-CD3 in 1-h image</th>
<th>Number of joints with abnormal uptake of 99mTc-anti-CD3 in 3-h image</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>267</td>
<td>235 (88.0)</td>
<td>201 (75.3)</td>
</tr>
<tr>
<td>JIA</td>
<td>109</td>
<td>101 (92.7)</td>
<td>101 (92.7)</td>
</tr>
<tr>
<td>OA</td>
<td>290</td>
<td>17 (0.34)</td>
<td>0</td>
</tr>
<tr>
<td>GA</td>
<td>13</td>
<td>13 (100)</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are represented as n (%), unless otherwise mentioned. aWith pain and/or oedema.
to differentiate a patient without disease, who shows normal scan, from a patient with OA.

The establishment of the diagnostic parameters developed in this study made it possible to differentiate RA and JIA from the other rheumatic diseases such as OA and GA. Moreover, the method is easily reproducible using these criteria. In addition, the costs of using 99mTc-anti-CD3 for routine examination of patients with RA and JIA are relatively low, since most of the reagents are routinely used.

In conclusion, this study demonstrates that 99mTc-anti-CD3 scintigraphy is a useful tool for diagnosis and follow-up of different rheumatic diseases, using the criteria established here. The technique has the advantages of simplicity, reproducibility and reliability, particularly since no other specific, non-invasive methods are available for adequate evaluation of these patients.

**Rheumatology key messages**

- 99mTc-anti-CD3 can help in the differential diagnosis of rheumatic diseases.
- 99mTc-anti-CD3 strongly correlates with clinical findings for patients with RA.

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

**References**