THE VALUE OF TECHNETIUM-99-METASTABLE-ETHYLENEDICYSTEINE-DEOXYGLUCOSE (^{99M}Tc-EC-DG) IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Thesis submitted in fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

in Nuclear Medicine

in the Department of Nuclear Medicine

School of Clinical Medicine, Faculty of Health Sciences

University of the Free State

Bloemfontein

October 2023

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DECLARATION

I, Osayande Evbuomwan, declare that the thesis that I herewith submit for the degree Philosophiae Doctor (PhD) in Nuclear Medicine in the Department of Nuclear Medicine at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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Osayande Evbuomwan

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DEDICATION

I dedicate this work to:

My wife, Onyinye, and daughters, Eduwa and Ifueko. Thank you for giving me the opportunity and atmosphere to further my academic career once again. Thank you for your support and prayers. I love you all.

My parents, Dr. and Mrs. Evbuoma. Thank you for your support with my studies through all these years. Without you both, I would not have been here today. I am forever grateful.

ACKNOWLEDGEMENTS

- A big thank you to the Department of Nuclear Medicine staff at Universitas Academic Hospital in Bloemfontein, South Africa, for assisting with the practical implementation of this study.
- A special thanks to all the radiographers for their support and understanding.
- I acknowledge and extend my gratitude to my Head of Department, Dr. Gerrit Engelbrecht, promotor, Dr. Je'nine Horn-Lodewyk, and co-promotors, Dr. Cathryn Driver and Professor Mathys Labuschagne, for their continued support.
- Special acknowledgments to Goorhoo Pryaska and Dr. Daleen Struwig for their invaluable roles played in the success of this work.
- I would also like to acknowledge my mentor, Professor Willy Vangu, HOD, Department of Nuclear Medicine, University of the Witwatersrand, for all he has taught me and his continued support towards my academic career.

EXECUTIVE SUMMARY

Rheumatoid arthritis (RA) is a systemic inflammatory disease that is usually associated with synovitis that can lead to progressive joint damage if not managed appropriately. Prompt diagnosis and early treatment offer a good prognosis in patients with RA. However, treatment monitoring remains challenging for the rheumatologist as it is sometimes difficult to differentiate true remission from subclinical disease. Several modalities are available for assessing disease activity in patients with RA. This usually involves a combination of the clinical assessment of involved joints, laboratory and imaging investigations. Imaging with ultrasound (US) and magnetic resonance imaging (MRI) are considered to be among the most sensitive methods of assessing disease activity. However, these modalities are not without shortcomings. The aim of this study was to investigate the use of technetium-99-metastable ethylenedicysteine-deoxyglucose (^{99m}Tc-EC-DG) in the management of patients with RA. Using ^{99m}Tc-EC-DG to identify synovitis and offer prognostic information was investigated. Its usefulness in assessing treatment response compared to US and the diagnostic accuracy of identifying the disease compared to US was also investigated.

A prospective study was conducted at the Department of Nuclear Medicine of the University of the Free State/Universitas Academic Hospital in Bloemfontein, South Africa. Twenty-two participants seen at the Rheumatology Unit of the Department on Internal Medicine, diagnosed with RA, were enrolled according to the American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) classification criteria. Participants were injected with 20–25 millicurie (mCi) of ^{99m}Tc-EC-DG. Flow, blood pool, whole body, delayed static, and SPECT/CT images were acquired. Known disease sites were qualitatively assessed for the intensity of uptake, and disease severity was graded (Grade 0–3). On the same day, US imaging of the most affected joints was performed in addition to blood samples being obtained from each participant for baseline C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody titre. All imaging and laboratory investigations were performed at baseline, six weeks and six months follow-up after baseline findings in 20 of the participants.

The median (interquartile range) age was 59 (49–68) years, and the majority (n=21; 95.5%) of patients were female. An abnormally increased uptake of ^{99m}Tc-EC-DG was noted in the majority of the sites of known disease, including unknown sites. SPECT/CT imaging localised tracer uptake specifically to the synovial space. Fourteen (63.6%) of the 22 participants had elevated RF and anti-CCP antibody titres. A significant correlation between higher grade

uptake and increased levels of RF and anti-CCP antibodies (p=0.031) was observed. A total of 404 joints were evaluated by ^{99m}Tc-EC-DG and US imaging. The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of ^{99m}Tc-EC-DG SPECT/CT imaging were 86%, 60%, 61%, 85% and 73%, respectively, using US as the gold standard. A sensitivity of 100% was noted in the identification of synovitis in the carpal and knee joints. Disease activity in the distal interphalangeal (DIP) joints was not observed with either ^{99m}Tc-EC-DG or US imaging. The level of agreement between US and ^{99m}Tc-EC-DG imaging in assessing therapy response was 33.3 %, 11.6 % and 6.67 % for the knees, hands and wrist joints, respectively.

^{99m}Tc-EC-DG is a safe radiopharmaceutical that can effectively assess disease activity in the joints of patients with RA, with a strong correlation between high-grade disease on imaging and the presence of RF and anti-CCP antibodies. It has a high sensitivity in detecting synovitis when compared to US imaging. However, it has a poor correlation in the assessment of treatment response in comparison to US findings, which might be attributed to its ability to better detect subclinical disease. Using ^{99m}Tc-EC-DG offers facilities with only SPECT or SPECT/CT cameras an opportunity to investigate patients with RA.

KEY TERMS

^{99m}Tc-glucosamine; ^{99m}Tc-EC-DG; anti-cyclic citrullinated peptide; computed tomography; CT; diagnostic accuracy; Doppler ultrasound; EC-DG; ethylenedicysteine-deoxyglucose; magnetic resonance imaging; MRI; radiolabelled; radiopharmaceutical; rheumatoid arthritis; rheumatoid factor; sensitivity; specificity; single-photon emission computed tomography/computed tomography; SPECT/CT; ultrasound; US

LIST OF ABBREVIATIONS

ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
AI	Artificial intelligence
Anti-CCP	anti-cyclic citrullinated peptide
CDAI	Clinical Disease Activity Index
CRP	C-reactive protein
CT	computed tomography
CUT	Central University of Technology
DAS-28	28-Joint Disease Activity Score
DIP	distal interphalangeal joint
DLBCL	diffuse large B cell lymphoma
DMAD	disease modifying antirheumatic drug
EANM	European Association of Nuclear Medicine
EC-DG (ECDG)	ethylenedicysteine-deoxyglucose
eq	equivalent
ESR	erythrocyte sedimentation rate
FDG	fluorodeoxyglucose
^{18}F-FDG	fluorine-18-fluorodeoxyglucose
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
^{1}H-NMR	proton nuclear magnetic resonance
HPCSA	Health Professional Council of South Africa
HSREC	Health Sciences Research Ethics Committee
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography mass spectrometry
IQR	interquartile range
ITLC-SG	instant thin layer chromatography with silica gel
LEHH	low energy high resolution
mCi	millicurie
MCP	metacarpophalangeal
MHz	megahertz
MIT	multi inter trans
MRC	Medical Research Council
MRI	magnetic resonance imaging
NECSA	South African Nuclear Energy Corporation
NPV	negative predictive value
NRF	National Research Fund

PET	positron emission tomography
PIP	proximal interphalangeal joint
PPV	positive predictive value
RA	rheumatoid arthritis
RCP	radiochemical purity
RF	rheumatoid factor
SASNM	South African Society of Nuclear Medicine
SD	standard deviation
SDAI	Simplified Disease Activity Index
SNMMI	Society of Nuclear Medicine and Molecular Imaging
SPECT	single-photon emission computed tomography
SPECT/CT	single-photon emission computed tomography/computed tomography
$^{99m}TcO_4^-$	technetium-99m-metastable-pertechnetate
^{99m}Tc-ECDG	technetium-99-metastable ethylenedicysteine-deoxyglucose
TLC	thin layer chromatography
UFS	University of the Free State
US	ultrasound

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CHAPTER 1
INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION

In this research, an in-depth study was carried out by the researcher to investigate the value of technetium-99-metastable ethylenedicysteine-deoxyglucose (^{99m}Tc-EC-DG) imaging as a highly sensitive, cost-effective imaging modality for assessing disease activity in patients with rheumatoid arthritis (RA). Rheumatoid arthritis is a chronic inflammatory disease which, if not appropriately treated, leads to irreversible joint damage, deformities, disability, and premature mortality (Hodkinson et al., 2013). Further, RA is an autoimmune disorder of unknown aetiology, characterised by symmetric, erosive synovitis and, in some cases, extra-articular involvement (American College of Rheumatology, 2002; Harris, 1990). Worldwide, RA affects approximately 1% of the entire population (Alamanos et al., 2006; Solomon et al., 1975). Previous reviews of data from developing countries suggest a similar prevalence to that seen in developed countries (Ally & Visser, 2010; Benitha & Tikly, 2007; Mody & Cardiel, 2008). A meta-analysis showed an overall prevalence of RA of 2.5% and 0.07%, respectively, in urban and rural settings in South Africa (Usenbo et al., 2015).

Prompt diagnosis and early treatment offer a good prognosis in patients with RA. However, a major problem in developing countries, is a significant delay in diagnosis and treatment. This usually leads to poor outcomes, morbidity and even mortality. For example, bone erosion is a central feature of this pathology in patients who do not start treatment early and is associated with severe disease and poor functional outcomes (Schett & Gravallese, 2012). Bone erosion has also been found to occur in some patients with low disease activity or in clinical remission (Schett & Gravallese, 2012). It is, therefore, safe to say that whenever RA is diagnosed in patients residing in developing countries, a cost-effective, highly sensitive investigation is needed to accurately monitor disease activity and response to drug therapy. To prevent inappropriate treatment, further morbidity and even mortality, the accurate supervision of patients with RA can determine how these patients are managed. The value of ^{99m}Tc-EC-DG imaging for assessment of disease activity was thus investigated.

1.2 BACKGROUND TO THE RESEARCH PROBLEM

Several advances in the methods of scoring disease activity in RA have been made in recent years. This involves a combination of the clinical assessment of involved joints and laboratory

investigations. The three validated scores currently used in South Africa are the 28-Joint Disease Activity Score (DAS-28), the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI) (Hodkinson et al., 2013). These scores allow for the classification of the patient as being in remission or presenting with low, moderate, or high disease activity, thereby providing a simple tool for the assessment of each patient and guidance towards further therapeutic decisions (Hodkinson et al., 2013; Smolen & Aletaha, 2010). However, non-specific constitutional symptoms such as fatigue and malaise, rather than arthritis, predominate in some patients (Ally & Visser, 2010), and the subjective nature of a clinical assessment reduces its sensitivity. For this reason, diagnostic methods such as ultrasound (US) and magnetic resonance imaging (MRI) are being used increasingly in addition to clinical examination because of their improved sensitivity and specificity (Ally & Visser, 2010).

Laboratory markers are nonspecific tests that are occasionally helpful to distinguish between inflammatory and non-inflammatory conditions. However, these tests are not diagnostic and may be abnormal in a vast array of infectious, malignant, rheumatic, and other diseases (Shojania, 2000; Sox & Liang, 1986). The most used imaging modality in patients with RA is X-ray imaging, despite knowing that structural changes such as erosion may not be evident in early disease (Kgoebane et al., 2018; Manolios et al., 2016; Scheel et al., 2006). X-ray has the best-established role in identifying progressive joint damage but is insensitive in identifying synovitis and early erosive lesions (Backhaus et al., 1999; Backhaus et al., 2002; Wakefield et al., 2000). This lack of sensitivity, therefore, renders X-ray a less favourable imaging modality in identifying patients in complete remission, patients with early treatment response and patients with low disease activity, with the latter being a common dilemma among rheumatologists.

The classification criteria often used to diagnose RA, proposed by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR), incorporated the role of US and MRI in the detection of synovitis, thus enabling earlier diagnosis and correct classification of patients (Aletaha et al., 2010; Kgoebane et al., 2018). In recent years the use of US imaging in the assessment of inflammatory joints especially due to RA has been on the increase. Universally, US is one of the most available, safe, and cost-effective imaging modalities used in the evaluation of patients with RA. In addition to this, it offers the prospect of a more accurate assessment of soft tissue inflammation than conventional clinical examination (D'Agostino et al., 2017). US allows for real-time joint evaluation and provides a useful extension of clinical examination (Ally & Visser, 2010). Synovitis and effusions are readily seen with US, and it has been shown to detect synovitis more accurately than clinical

examination (D'Agostino et al., 2017). When Doppler US is performed, measurement of vascular flow can be used to assess the severity of inflammation and therefore, to monitor the patient's response to treatment (Tehranzadeh et al., 2004). These characteristics make US an imaging modality that detects erosions much earlier than X-rays. However, the drawback of US is that it is operator-dependent (Ally & Visser, 2010; Manolios et al., 2016) and cumbersome, especially when almost every single joint in both hands needs to be evaluated. This could even become more cumbersome if additional joints need evaluation beside those in the hands. Despite US being considered a valid, sensitive, and reliable imaging modality for the detection of synovitis in RA, some studies had earlier indicated that it failed to demonstrate superior sensitivity to change in disease activity, compared to clinical examination (Mandl et al., 2014; Neogi & Felson, 2008). More recent studies have shown this is not the case (D'Agostino et al., 2017; Defaveri do Pradoet al., 2018; Sudot-Szopinska et al., 2014).

These studies confirm that high-resolution musculoskeletal US using a high-frequency linear probe in investigating patients with RA is more sensitive to clinical examination and thus used routinely to complement clinical examination. Its usefulness includes disease monitoring, aiding prognosis, and assessment of treatment response. However, the use of high-frequency linear probes with the correct handling technique is mandatory (McNally., 2008). These high-frequency probes (frequency range of 10–14 MHz) were used by the EULAR task force in a study to standardise the scoring system for disease activity in joints affected with RA (D'Agostino et al., 2017). This scoring system was also applied in this study and elaborated on in the method section of this chapter. The currently accepted practice, however, is to use morphological changes (synovial effusion and hypertrophy) seen on US gray scale imaging and hypervascularity shown on power Doppler to grade disease activity (D'Agostino et al., 2017; Defaveri do Pradoet al., 2018; Sudot-Szopinska et al., 2014). Both the gray scale and power Doppler findings have been shown to be very sensitive to changes in disease activity (D'Agostino et al., 2017).

Conventional radiographs of the hands and feet have traditionally been used in the diagnosis, management, and monitoring of patients with RA. However, they are not sufficiently sensitive to detect changes early in the disease process (Kgoebane et al., 2018). Laboratory markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), may be normal in 20–25% of cases (Kgoebane et al., 2018). The known classification criteria (ACR/EULAR RA classification criteria 2010) incorporate the role of US and MRI in the detection of synovitis, enabling earlier diagnosis and correct classification of patients (Aletaha et al., 2010; Kgoebane et al., 2018). Despite being a valid, sensitive, and reliable imaging modality for detecting

synovitis in RA, US has failed to demonstrate superior sensitivity to change, compared to clinical examination in some studies (Mandl et al., 2014; Neogi & Felson, 2008).

Magnetic resonance imaging has shown strength in identifying early inflammatory changes (Bird et al., 2003; Lassere et al., 2003; Peterfy, 2001). This imaging modality provides greater resolution of joint structure and pathology but has the disadvantage of being able to screen only single joints/regions at a time. MRI is also very expensive, which increases the cost of patient imaging. These are some of the reasons why MRI has still not become a routinely used imaging modality, but rather a problem-solving tool in the diagnosis of RA. Although all these different imaging modalities and clinical or laboratory evaluation techniques have their advantages, there are still disadvantages that warrant the need for a cost-effective, highly reliable, easy and highly sensitive investigation for the early detection and follow-up of patients with RA.

Fluorine-18-fluorodeoxyglucose (^{18}F-FDG) is a well-known positron emission tomography (PET) radiotracer that has been used in oncology, inflammation, and infection imaging for over four decades (Kumar et al., 2007; Hotta M et al., 2020). PET is a nuclear medicine imaging technique that uses a PET camera to image the distribution of positrons in the body. Fluorodeoxyglucose is a glucose analogue with a very high sensitivity for imaging pathological conditions that use glucose as a source of metabolism. Therefore, PET scanning with ^{18}F-FDG offers the best imaging modality for assessing metabolically active tissues that rely heavily on glucose (Manolios et al., 2016). It is useful to detect early inflammation and thus, early synovitis based on the increased metabolic activity before any structural change occurs. The disadvantage, however, is its high cost (Angelides et al., 2014). This high cost makes this modality unavailable in many nuclear medicine centres in low- and middle-income countries, and therefore a more cost-effective and equally sensitive alternative is being pursued.

Technetium-99-metastable ethylenedicysteine-deoxyglucose, also referred to as ^{99m}Tc-glucosamine (both names will be used interchangeably), is a single-photon emission computed tomography (SPECT) radiotracer for functional imaging that in essence is radiolabelled glucosamine (Angelides et al., 2014). SPECT imaging is a nuclear medicine imaging technique that involves imaging the distribution of a SPECT radiotracer in the human body by a gamma camera. Glucosamine is available as an over-the-counter supplement and is safely being used to treat osteoarthritis (Angelides et al., 2014; Kumar et al., 2007; Manolios et al., 2016; Sobal et al., 2009). Furthermore, glucosamine is a natural sugar and a key component of the extra-cellular matrix of cartilage (Manolios et al., 2016; Sobal et al., 2009). Evidence has been reported that glucosamine may serve as the building block to form glycosaminoglycans that

are involved with the formation and repair of cartilage (Kumar et al., 2007). The chemical structure of EC-DG is similar to that of fluorodeoxyglucose (FDG) and glucose, so therefore has similar abilities when radiolabeled, to image inflammation that is dependent on increased glucose trapping (Angelides et al., 2014; Kumar et al., 2007; Manolios et al., 2016). These two properties (being a building block of cartilage and a glucose analogue) are the reasons why EC-DG is believed to be useful in imaging patients with RA (Angelides et al., 2014; Manolios et al., 2016; Sobal et al., 2009).

The inflammation/infection and tumour imaging properties of ^{99m}Tc-EC-DG have been evaluated in lung cancer xenograft mice and New Zealand rabbits during a study performed at the University of the Free State (UFS). The normal biodistribution was also evaluated in baboons. This local research proved ^{99m}Tc-EC-DG to be safe and without any adverse effects (Horn-Lodewyk, 2015). Similar findings have been reported in the literature (Angelides et al., 2014; Ginat et al., 2017; Kumar et al., 2007; Manolios et al., 2016; Sobal et al., 2009). In a study approved by the UFS Health Sciences Research Ethics Committee (HSREC) (Appendix A) and the Free State Province Department of Health (Appendix B), ^{99m}Tc-EC-DG was investigated as a metabolic radiotracer for imaging of patients with breast cancer and lymphoma. In this study, based on personal communication, an intense accumulation of ^{99m}Tc-EC-DG in the joints of patients with known inflammatory joint diseases was observed.

A literature search was conducted to investigate the role of ^{99m}Tc-EC-DG in imaging inflammatory joint disease. Few studies were found, most of them with sample sizes ranging between two and 25 research participants (Angelides et al., 2014; Kumar et al., 2007; Manolios et al., 2016). However, none of these studies evaluated the value of ^{99m}Tc-EC-DG in predicting very early treatment response or compared it head-to-head with US regarding the possible early prediction of treatment response in patients with RA. This is one of the research areas that was explored in the current study. Should ^{99m}Tc-EC-DG be found to be superior or similar to US in terms of sensitivity, it could become the ideal tool for investigating patients with RA, as it can also detect multiple sites of joint involvement and extra-articular disease.

Early diagnosis of RA, including early identification of active disease, provides a window of opportunity for cost-effective therapeutic intervention (Kgoebane et al., 2018). Adherence to the principles of early diagnosis and goal-steered treatment strategies can lead to remission in 30–50% of cases in one year (Bugatti et al., 2018; Gaujoux-Viala et al., 2017; Gremese et al., 2013). The monitoring of RA disease activity remains challenging in both clinical practice and clinical studies (Mandl et al., 2014). Therefore, a valid, reliable, and cost-effective monitoring tool that is sensitive to change in disease activity is needed. Few studies have

shown promising results with ^{99m}Tc-EC-DG imaging in the early detection and follow-up of patients with RA (Angelides et al., 2014, Kumar et al., 2007, Manolios et al., 2016). Kumar et al. (2007) demonstrated increased uptake of ^{99m}Tc-EC-DG in the joints of rats with inflammatory arthritis. Upon removal of the deoxyglucose component from ^{99m}Tc-EC-DG, injection of only ^{99m}Tc-EC resulted in no uptake in the joints affected by inflammatory arthritis. A preliminary observation with a human volunteer in their study also showed increased tracer uptake in the joints involved with inflammatory arthritis, compared to normal joints. They concluded that ^{99m}Tc-EC-DG might be a potential imaging marker for inflammatory arthritis (Kumar et al., 2007).

Using two post-mortem bodies, Sobal et al. (2009) confirmed increased uptake of ^{99m}Tc-EC-DG in degenerated cartilages. They concluded that its use might not be limited to targeting osteoarthritis, but also play a role in the assessing treatment response. Angelides et al. (2014) recruited 11 patients with RA in their study, and ^{99m}Tc-EC-DG accumulation was noted in all clinically known sites of disease. Uptake was more pronounced in patients with active untreated disease and correlated well with disease activity. The conclusion was made that ^{99m}Tc-EC-DG could distinguish between synovial and bone uptake and might play a role in assessing and monitoring rheumatic conditions (Angelides et al., 2014). Finally, Manolios et al. (2016) investigated the use of ^{99m}Tc-EC-DG imaging in 25 patients with RA and 12 patients with ankylosing spondylitis. They concluded that ^{99m}Tc-EC-DG localisation had a significant correlation with disease activity (Manolios et al., 2016).

The literature review section in this study has reported and discussed the few studies available that could assess the role of ^{99m}Tc-EC-DG imaging in RA. Similar to this current research, all these studies demonstrated the safe use of ^{99m}Tc-EC-DG in patients with RA since none of the patients had adverse reactions.

1.3 Reliability

The reliability of the research was established by means of a well-constructed study plan adopted from previous related studies that have been carefully reviewed by the promotor, co-promotors, and experts in this area of research.

1.4 Validity

The validity of this research was maintained through the way the research instruments were designed, as well as the expertise of the supervisors and the supportive expertise provided by

the researcher. The US results were validated by both an experienced sonographer and an experienced radiologist in musculoskeletal ultrasonography, increasing the validity of this modality. The principal researcher and the rheumatologist attended a structured workshop designed on US imaging specifically for RA, which included image interpretation. This will increase the validity of the interpretation of results to determine the more sensitive imaging modality, to identify early responders to conventional therapy in patients with RA.

1.5 PROBLEM STATEMENT

The problem that was addressed is the absence of a highly sensitive, cost-effective, and easy to perform investigation for assessing disease activity in patients with RA, especially for those patients with low disease activity. A valid and reliable tool is necessary for monitoring disease activity in these patients, as this remains a challenge in both clinical studies and practice. The current tools available include clinical assessment, laboratory marker investigations and imaging (X-ray, US, and MRI). Nevertheless, despite these current tools having certain advantages, there are still disadvantages, as highlighted in the literature review that warrant the need for the development of a new and highly sensitive investigative modality.

1.6 RESEARCH QUESTIONS

To address the problem stated, the following main research question were addressed:

> Can ^{99m}Tc-EC-DG imaging serve as a valuable tool in identifying early responders to conventional therapy in patients with RA and thus risk stratifying these patients?

From this research, the following sub-questions have also been addressed:

- Will ^{99m}Tc-EC-DG imaging detect sub-clinical disease activity in patients identified as being in clinical remission based on laboratory and clinical assessment?
- How does ^{99m}Tc-EC-DG imaging compare to US in monitoring disease activity in patients being treated for RA?
- Will ^{99m}Tc-EC-DG imaging be able to detect extra-articular disease in patients with RA and, thus offering further information on prognosis?

These research questions created the point of departure from which this research has been conducted and completed.

1.7 THE OVERALL GOAL, AIM AND OBJECTIVES OF THE STUDY

1.7.1 Overall study goal

The overall goal of the study was to determine the value of ^{99m}Tc-EC-DG as a cost-effective and highly sensitive diagnostic tool in monitoring the disease activity of patients with RA.

1.7.2 Study aim

The aim of this study was to assess the value of ^{99m}Tc-EC-DG imaging in assessing the disease activity of patients with rheumatoid arthritis.

1.7.3 Study objectives

The objectives of this study were:

- to perform an interim ^{99m}Tc-EC-DG scan early on after initiation of RA treatment, to determine if early responders to conventional therapy could be identified;
- to determine if ^{99m}Tc-EC-DG imaging could detect sub-clinical disease;
- to use ^{99m}Tc-EC-DG imaging to assess the changes in disease activity in the affected joints of patients with RA and compare these to changes detected by US to determine which is the more sensitive imaging modality;
- to use ^{99m}Tc-EC-DG imaging to assess the changes in disease activity in the affected joints of patients with RA and compare these to clinical and laboratory changes; and
- to determine if ^{99m}Tc-EC-DG imaging could detect extra-articular disease in patients who would most likely fall into the high-risk category.

1.8 METHODOLOGY

1.8.1 Study location

The study was performed at Universitas Academic Hospital and the UFS Faculty of Health Sciences in Bloemfontein. Clinical assessment took place at the Rheumatology Unit (Department of Internal Medicine), while US imaging was done at the neighbouring Universitas private hospital. ^{99m}Tc-EC-DG imaging was conducted at the Department of Nuclear Medicine.

1.8.2 Study population, sample size and study design

The study population consisted of all patients with diagnosed RA according to the ACR/EULAR classification criteria, who are in early treatment (not more than second week) or yet to start conventional therapy at the Department of Rheumatology, Universitas Academic Hospital, Bloemfontein. A total number of 22 patients was enrolled in this cross-sectional study. Both qualitative data using visual analysis and quantitative data using a computer software program was used to assess disease activity on the ^{99m}Tc-EC-DG images. Qualitative data using visual analysis of disease activity was used to assess disease activity on the ultrasound images.

1.8.3 The research team

The research team for this study included two rheumatology specialists, two nuclear medicine physicians, a radiochemist, a sonographer, a radiologist and a nuclear medicine radiographer. Individual members are listed as follows:

- Dr. Evbuomwan Osayande, a nuclear medicine specialist at Universitas Academic Hospital/UFS and the principal investigator, holds a Master's degree (MMed) in Nuclear Medicine from the University of the Witwatersrand and has vast experience in research. He is also registered with the Health Professions Council of South Africa (HPCSA) and completed a Good Clinical Practice (GCP) course.
- Dr. Je'nine Horn-Lodewyk was the sub-investigator. She is an experienced nuclear medicine radiographer with sufficient knowledge and experience in the safe handling and administration of radioactive substances and has completed a PhD in Clinical Nuclear Medicine. She and other HPCSA qualified nuclear medicine radiographers were responsible for the preparation of ^{99m}Tc-EC-DG and the imaging of the study participants. She is also the main promotor of the PhD candidate, is registered with the HPCSA and completed a GCP course.
- Dr. Gerrit Engelbrecht is the Head Clinical Specialist of the Department of Nuclear Medicine at Universitas Academic Hospital. He is also a qualified nuclear medicine physician and has more than 18 years of experience in the field of nuclear medicine. Dr. Engelbrecht performed most of the tasks of the study physician including monitoring of the research participants and intravenous administration of ^{99m}Tc-EC-DG.

- Professor Mathys Labuschagne is a specialist ophthalmologist registered with the HPCSA. He has vast experience as a researcher and is well-recognised in different fields of postgraduate education. He is also co-promotor for the PhD candidate.
- Dr. Barend Jansen van Rensburg is an experienced rheumatologist at Universitas Academic Hospital/UFS and was involved in patient screening and management.
- Dr. Cathryn Driver is a senior scientist at the South African Nuclear Energy Corporation (NECSA). She holds a PhD in Medical Biochemistry and has experience in organic chemistry, radiochemistry, and the synthesis of radiopharmaceuticals for disease diagnosis. She was responsible for manufacturing the EC-DG. She is also the external co-promotor of the PhD candidate.
- Mrs. Amanda Hendricks is a qualified sonographer. She performed the US scans of the RA patients. She has good experience in musculoskeletal ultrasonography.
- Dr. Ambrosius Swartbooi is a radiologist with vast interest and experience in musculoskeletal ultrasonography. He reported the US scans. He has good experience in musculoskeletal ultrasonography.

1.8.4 Inclusion criteria

The participants included in the study adhered to the following criteria:

- participants who were ≥18 years of age;
- participants should have been diagnosed with RA of the joints in the hands according to the ACR/EULAR classification criteria for RA;
- participants with disease onset less than 6 months prior to the study, as the sensitivity of US in these patients might be reduced due to extensive joint damage; and
- both male and female participants were included.

1.8.5 Exclusion criteria

Participants were excluded from the study if they presented with the following excluding factors:

- participants with chronic RA disease (≥ 6 months);
- pregnant participants.
- participants that were breastfeeding; and

1.8.6 Study method (recruitment, laboratory investigation, imaging and image interpretation)

Twenty-two participants confirmed of having RA using the ACR/EULAR criteria were recruited to participate in the study. Recruitment took place at the Rheumatology Unit at Universitas Academic Hospital, including patients referred from satellite hospitals, as long as the patients adhered to the inclusion criteria. Each research participant received an information document in their language of preference for their consideration before signing an informed consent form attached to the document as they agreed to participate in the study (Appendices C, D and E – Engish, Sesotho, Afrikaans, respectively).

Baseline routine laboratory investigations (CRP, ESR, rheumatoid factor [RF]) and X-rays of the most symptomatic joints were carried out on each patient seen at the Rheumatology Unit. Baseline disease activity was assessed and scored clinically by the rheumatologists using the DAS-28 assessment instrument, as this is a validated scoring system used in South Africa (Hodkinson et al., 2013). The joints assessed using the DAS28 assessment tool included the shoulders, elbows, wrists, metacarpophalangeal (MCP), proximal interphalangeal (PIP) and knee joints.

Each participant received a baseline US of the joints of the hands, including the wrists, which was carried out by an experienced musculoskeletal sonographer. The Samsung HS70A US machine (Samsung Medison Co. Ltd.; Seoul, South Korea) with a high freqeuncy probe of 15 MHz was used. Each US study was reported afterwards by a radiologist with vast experience in musculoskeletal US imaging. The EULAR scoring system for grading synovitis in RA was applied as follows:

Grade 0: Normal joint (no greyscale detected synovial hypertrophy and no power Doppler signal [within the synovium]);

Grade 1: Minimal synovitis (Grade 1 synovial hypertrophy and ≤ Grade 1 power Doppler signal);

Grade 2: Moderate synovitis (Grade 2 synovial hypertrophy and ≤ Grade 2 power Doppler signal or Grade 1 synovial hypertrophy and a Grade 2 power Doppler signal); and

Grade 3: Severe synovitis (Grade 3 synovial hypertrophy and ≤ Grade 3 power Doppler signal or Grade 1 or 2 synovial hypertrophy and a Grade 3 power Doppler signal).

Once the US study was completed, the participants were sent to the Department of Nuclear Medicine, where ^{99m}Tc-EC-DG was administered and subsequent nuclear medicine imaging performed. Fasting was not a requirement prior to ^{99m}Tc-EC-DG administration, and information on fasting status was gotten from the participants. The tracer EC-DG was produced by NECSA laboratories and was labelled in-house at the nuclear medicine department, Universitas Academic Hospital, with 20 mCi of ^{99m}Tc for each participant by an experienced nuclear medicine radiographer. The SI unit for activity is the megabecquerel, however we will be using the non SI unit (mCi) in this thesis. All scans at the nuclear medicine department were performed using a dual-head gamma camera (Siemens Symbia T16 Truepoint SPECT-CT; Siemens Medical Solutions, Malvern, PA, USA). Dynamic images of the joints of the hands and wrists were acquired at the time of administering the tracer, with a frame rate of 1 frame/second for 60 seconds. This was then followed immediately by blood pool imaging of the same region.

A delayed whole body image was acquired three hours after tracer injection, with dedicated three minutes static images and SPECT/CT images of the joints evaluated with ultrasound. Flash 3D iterative algorithm was used for reconstruction, with four subsets and eight iterations. Gaussian 6.00 filter was used alongside attenuation correction. A scoring system adopted from the study by Angelides et al. (2014) was used to evaluate disease activity in affected joints on the planar images. Radiopharmaceutical activity was correlated with physiologic activity in the neighbouring muscle qualitatively using a four-point (0–3) grading system as follows:

Grade 0: Nil uptake. Defined as no increased/minimally increased radio-pharmaceutical activity in joints (activity less than that of the neighboring muscle tissue).

Grade 1: Mild uptake. Defined as radiopharmaceutical uptake approximates that of the neighboring muscle tissue.

Grade 2: Moderate uptake. Defined as radiopharmaceutical uptake mildly greater than that of the neighboring muscle tissue.

Grade 3: Severe uptake. Defined as radiopharmaceutical uptake markedly greater than that of the neighbouring muscle tissue.

Following all initial assessments, participants were then reassessed at six weeks and six months after the initial assesment, using the same initial assessment criteria.

The US findings were interpreted by a qualified radiologist blinded to the results of the other modalities. The ^{99m}Tc-EC-DG images were also interpreted by a nuclear medicine physician blinded to the findings on the US and the other modalities for assessing disease activity. Results from all investigative modalities were then compared with each other and analysed to assess the degree of correlation. A flow chart showing each participant's movement is illustrated in Figure 1.1.

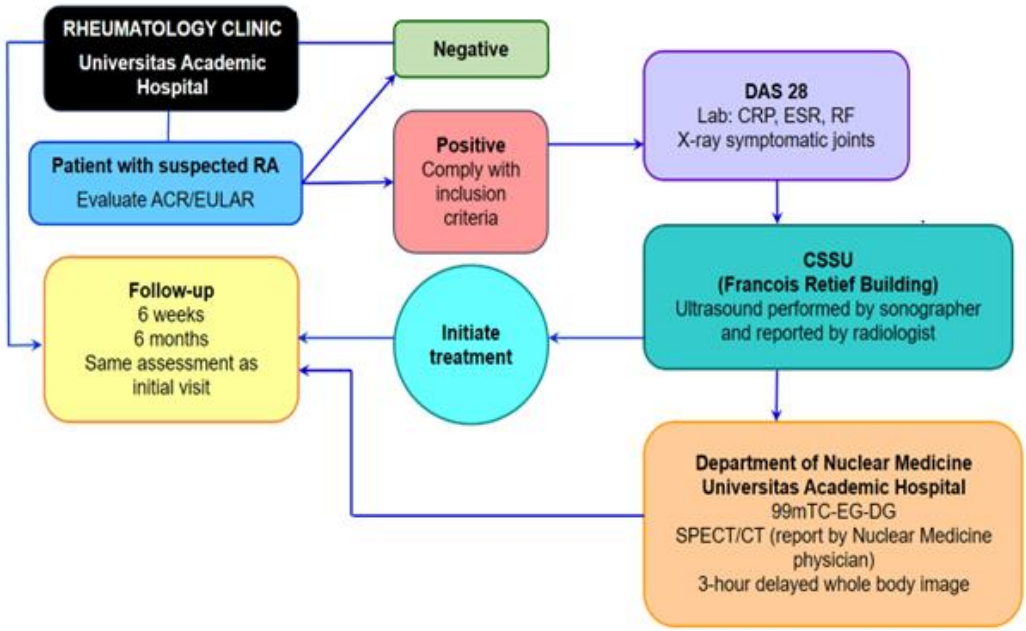

Figure 1.1. Flow chart demonstrating each participant's movement.

1.8.7 ^{99m}Tc-EC-DG general preparation summary

The EC-DG was synthesised at the Nuclear Energy Corporation of South Africa (NECSA) according to standard organic chemistry methods. All commercial reagents and solvents were

purchased from Sigma Aldrich (MilliporeSigma; Burlington, MA, USA) or Merck Millipore (Burlington, MA, USA). All samples for proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy were prepared using deuterated chloroform ($CDCl_3$) or deuterium oxide (D2O), and analysed on a Bruker 300 MHz spectrometer (Bruker Scientific LLC; Billerica, MA, USA). High performance liquid chromatography mass spectrometry (HPLC-MS) analysis was performed using an Agilent Infinity 1200 Series system coupled to Agilent 6100 Series quadrupole MS system (Agilent Technologies, Santa Clara, CA, USA) with radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany). A one-vial-kit containing EC-DG, tin (II) chloride and buffer components was then formulated according to Good Manufacturing Practices (GMP) for radiolabelling with $^{99m}TcO_4^-$. Final preparation of ^{99m}Tc-EC-DG under aseptic conditions for administration to patients was conducted by a qualified nuclear medicine radiographer at Universitas Academic Hospital.

1.8.8 Synthesis of EC-DG

The EC-DG ligand was synthesised according to the procedure described by Yang et al. (2003). Briefly, L-thiazolidine-4-carboxylic acid (30.0 g) was dissolved in liquid ammonia (150 mL), followed by the slow addition of sodium metal (8.0 g, 1.5 equivalent [eq]) resulting in a deep-blue coloured solution. The solution was stirred for 20 minutes at room temperature. The reaction was quenched with ammonium chloride (5.0 g), the ammonia solvent was evaporated, and the resulting residue dissolved in water (200 mL). The pH was adjusted to 3.0 and the resultant precipitate purified by recrystallisation in ethanol to yield ethylenedicysteine (2HCl [EC]) (38% yield).

Ethylenedicysteine (EC) (2.0 g) was dissolved in 2 M sodium hydroxide (NaOH) (30 mL) with ethanol (40 mL) and stirred vigorously for 20 minutes. Benzyl chloride (1.48 g, 2.0 eq) in dioxane (20 mL) was added dropwise to the solution and further stirred for 30 minutes, after which the organic solvents were removed in vacuo. The pH of the resulting aqueous mixture was acidified to pH 3.0 resulting in the precipitation of the hydrochloride salt of S,S'-dibenzyl ethylene dicysteine (Bn-EC) (85% yield), which was used without further purification.

Benzyl chloroformate (4.90 g, 2.5 eq) in dioxane (150 mL) was added to a cooled (0°C) solution of S,S'-dibenzyl ethylene dicysteine.2HCl (6.0 g) dissolved in 10% potassium carbonate (K_2CO_3) solution (150 mL) and the reaction was stirred for 2 hours at 0°C, followed by stirring for 16 hours at 25°C. The solution was extracted with diethyl ether and the crude product precipitated by acidification of the aqueous phase. The product was redissolved and extracted

using ethyl acetate to yield an amorphous solid product N, N'-dibenzyl oxycarbonyl-S,S'-dibenzyl ethylene dicysteine (CBz-Bn-EC) (70% yield) upon drying.

The CBz-Bn-EC (1.34 g) was activated by reaction with ethyl chloroformate (0.41 g, 2.0 eq) in chloroform (30 mL) with triethylamine (0.38 g, 2.0 eq) at –15°C for 15 minutes. A solution of tetra-acetylglucosamine (1.58 g, 2.2 eq) and triethylamine (0.42 g, 2.0 eq) in chloroform (30 mL) was added to this reaction mixture, and the combined reaction mixture was stirred for 1 hour at 0°C and then for 12 hours at 25°C. Following an acid-base workup, the residue was purified by column chromatography (using methanol, ethyl acetate and hexane [MeOH/EtOAc/hexane] at a 1:35:64 volume per volume [v/v] ratio) to afford the fully protected EC-DG (FP-EC-DG) (70% yield).

The FP-ECDG (1.0 g) underwent Birch reduction in liquid ammonia (80 mL) with sodium metal (0.5 g). The deep-blue coloured solution was stirred for 20 minutes at room temperature before quenching with ammonium phenylacetate (1.32 g, 12.0 eq). The resultant milky white solution was dried under a stream of argon gas to afford a strong-smelling, cream-coloured solid. The crude product was handled under an inert atmosphere with the exclusion of light. The ammonium phenylacetate was extracted by stirring twice with isopropanol (50 mL and 25 mL) and separating by centrifugation for 5 minutes at 4 000 revolutions per minute (rpm). Residual isopropanol was removed by stirring and centrifuging with diethyl ether (2 x 50 mL) and the solid product was then dried under argon gas to afford EC-DG (53% yield).

1.8.9 EC-DG Kit preparation

The EC-DG kit preparation was done in a one-vial procedure as described by Zeevaart et al. (2015). All solutions (HCl [0.1 M]), disodium phosphate (Na_2HPO_4) phosphate/citrate buffer solution and $SnCl_2$ solution (1 mg/mL; ensuring the solution was clear and not milky) were freshly prepared with ultrapure, Milli-Q grade (> 18 mega-ohms-centimeter [MΩ-cm]) degassed water before production of the kits. Once prepared, all water and solutions were filtered through a sterile Millex-GP (polyethersulfone, 0.22 µm, 33 mm) syringe filter (Merck; Burlington, MA, USA) into sterilised vials.

Citric acid (0.20 g) was added to a sterile vial containing Na_2HPO_4 (0.284 g) dissolved in water (pH 5.5) with addition of $SnCl_2$ solution (100 µL) and freeze-drying (Christ Alpha I-5 freeze-drier, Type 1050 (MABAG Medizinische Apparate Bau AG; Ahrensburg, Germany) overnight. The EC-DG (5 mg) was weighed into a vial under Ar(g) and dissolved in MeOH (0.75 mL). This solution was immediately transferred to the vial containing the Sn/buffer and flash-frozen in

liquid nitrogen, followed by lyophilisation overnight. The kits were sealed, capped and placed in a –80°C freezer for storage.

1.8.10 Synthesis of ^{99m}Tc-EC-DG

Radiosynthesis of ^{99m}Tc-EC-DG was completed by adding Tc-99m pertechnetate ($^{99m}TcO_4^-$) (50–60 mCi) to the prepared, lyophilised ECDG EC-DG kit vial (5 mg EC-DG, citric acid/Na_2HPO_4, $SnCl_2$). The solution was heated at 75°C for 15 minutes (Zeevaart et al., 2015).

Quality control was performed on the radiolabelled product by testing pH (pH 5.5) and radiochemical purity (RCP) (> 95%). RCP was determined using high performance liquid chromatography (HPLC) (Varian Prostar 325 UV/Vis [Varian Medical Systems Inc.; Palo Alto, CA, USA]) fitted with a radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany) and thin layer chromatography (TLC) (Raytest GmbH; Straubenhardt, Germany). HPLC analysis was done using a C-18 reverse phase column (Agilent Luna-C18 column, 5 µm, 4.6 x 250 mm) with isocratic elution (4.5% acetonitrile/methyl cyanide [MeCN] in 2 mmol ammonium formate [pH 3]) over 35 minutes. TLC analysis was completed using instant thin layer chromatography with silica gel (ITLC-SG) (Agilent Technologies, Santa Clara, CA, USA) and Whatman (MilliporeSigma; Burlington, MA, USA) paper strips (10 cm) developed with saline and acetone as the mobile phase, respectively. Each strip was cut in half and the activity measured for the strip origin and front to determine the percentage colloids (ITLC-SG) and percentage labelled product (Whatman). The stability of the ^{99m}Tc-EC-DG was determined up to 5 hours after preparation using HPLC. The structure of ^{99m}Tc-EC-DG is shown in Figure 1.2.

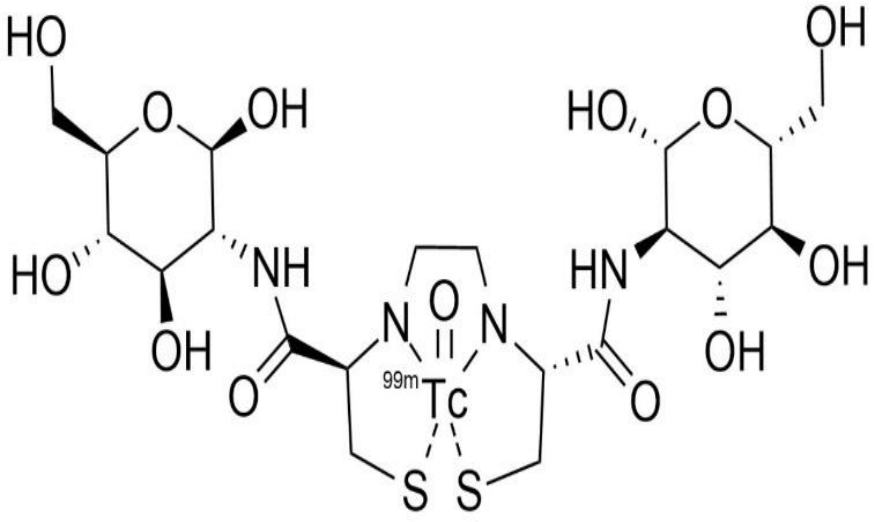

Figure 1.2. Structure of ^{99m}Tc-ethylenedicysteine-deoxyglucose (^{99m}Tc-glucosamine).

1.8.11 Radiopharmaceutical administration

An aseptic dispensing process was followed for the preparation of ^{99m}Tc-EC-DG patient dose. The sterility of the process was checked using air settle plates and finger dab plates that were cultured. The ^{99m}Tc-EC-DG dose was prepared for injection by diluting the prepared radiolabelled solution with saline (approximately 3 mL) and filtering through a sterile filter (Millex-GP, polyethersulfone, 0.22 µm, 33 mm) (Merck; Burlington, MA, USA) into a sealed, sterile vial. Additional saline (sterile, 1 mL) was added to the vial to ensure a final volume of around 5 mL. A patient dose of 20–25 mCi was withdrawn for intravenous administration. Fasting was not a requirement prior to the administration of the radiopharmaceutical. No adverse events were recorded after radiopharmaceutical administration.

1.8.12 Data collection

Information pertaining to the evaluation of participants included in the study was recorded by the principal investigator in a Microsoft Excel spreadsheet immediately after enrollment (Appendix F). These data included participant demographics, clinical and laboratory findings, US and ^{99m}Tc-EC-DG imaging findings. Each participant's name was substituted with a code. The laptop and the Excel document were both password-protected to ensure further confidentiality and data security. The laptop, data sheets and patient information were stored in a secure cupboard in the locked office of the principal investigator.

1.8.13 Data analysis

All data were analysed by the Department of Biostatistics at the UFS. Descriptive statistics were used to describe the calculated mean values ± standard deviation (SD), and percentages from the study variables. The results for variables such as sex were represented in frequency. Age was represented as the median and interquartile range. Fisher's exact test was used to determine associations between groups. Statistical significance was set as a p-value of less than 0.05. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for ^{99m}Tc-glucosamine imaging were also calculated. The level of agreement between the findings on US follow-up imaging and ^{99m}Tc-glucosamine follow-up imaging was expressed as percentages.

1.9 ETHICAL CONSIDERATIONS

This investigation was a prospective study that did not interfere with the normal clinical management of patients. The research was conducted according to the accepted protocol, with reference to the Declaration of Helsinki, GCP, GMP, Health Professions Council of South Africa (HPCSA) and Medical Research Council (MRC) principles. Primary responsibilities included taking care of the wellbeing of the research participants and ensuring that all individuals involved in the study adhered to the general rules of working.

1.9.1 Approval

Ethical approval (Appendix A) for the study was obtained from the University of the Free State's Health Sciences Research Ethics Committee (HSREC) (reference number UFS-HSD2020/1292/2411).

1.9.2 Informed consent

Participants who had indicated their willingness to participate in the study and complied with the inclusion criteria read an information document, which was made available in English, Sesotho and Afrikaans (Appendices C, D and E, respectively). Those who agreed to participate in the study after reading the information document, signed the attached informed consent document also available in English, Sesotho, and Afrikaans (Appendices C, D and E, respectively) before being enrolled in the study. It was also explained to each patient that they had the right to refuse to participate or withdraw their consent at any time. Participants were notified that they would not be penalised should they refuse to participate and/or withdraw from the study, nor would it affect the quality of their care and management at the hospital.

1.9.3 Confidentiality

No names or personal identifiers appeared on any datasheet that was sent for statistical analysis. Study participant codes were used instead. All information was managed in a strictly professional and confidential manner. Patient confidentiality was also maintained strictly when results from the study were submitted for peer review, published, or presented at national or international conferences.

1.9.4 Radiation Control Committee approval

The Radiation Control Committee of the University of the Free State was approached for permission to embark on the study. Written permission (Appendix G) was received from the Radiation Control Committee.

1.10 LAYOUT OF THESIS

This thesis commenced with Chapter 1 providing a description of RA as a clinical entity and the various investigations employed in its management. The thesis comprises a total of six chapters.

Chapter 2 represents an article by the author titled "The Biodistribution and Utility of ^{99m}Tc-Ethylenedicysteine-Deoxyglucose (^{99m}Tc-Glucosamine) in the Identification of Active Disease in Patients with Rheumatoid Arthritis – A Single Center Prospective Study", that was published in ***Nuclear Medicine and Molecular Imaging*** (Appendix H). The scope of the article was to determine if ^{99m}Tc-ethylenedicysteine-deoxyglucose could identify active synovitis, subclinical joint disease, and extra-articular disease in patients with RA. Its comparison with clinical assessment was also evaluated. The biodistribution of this radiopharmaceutical was studied to determine if it was similar to that of the PET glucose analogue ^{18}F-FDG, and if it differed in fasted and non-fasted patients.

Chapter 3 was published in ***Nuclear Medicine Communications*** in an article titled "The prognostic value of ^{99m}Tc-glucosamine imaging in patients with rheumatoid arthritis: a single center prospective study" (Appendix I). This component of the research determined if ^{99m}Tc-EC-DG could also offer prognostic information apart from diagnostic information.

Chapter 4 was submitted to the ***Egyptian Journal of Radiology and Nuclear Medicine*** in a manuscript titled "Head-to-head comparison of ultrasound and ^{99m}Tc-glucosamine SPECT/CT imaging of patients with rheumatoid arthritis – a single center prospective study" (Appendix J). This chapter evaluated the diagnostic accuracy of ^{99m}Tc-glucosamine imaging in the identification of synovitis in patients with RA, comparing it to US, which was used as the gold standard.

Chapter 5 includes the final article submitted to ***Nuclear Medicine Communications***, titled "Prospective six-month follow-up study comparing ^{99m}Tc-glucosamine SPECT/CT imaging and Doppler ultrasound imaging in patients with active rheumatoid arthritis" (Appendix K). This

article discusses the utility of ^{99m}Tc-glucosamine SPECT/CT imaging in the follow-up of patients with synovitis. The aim was to determine if it could be a useful tool in this regard. The results were also compared to that of US.

Chapter 6 provides an overview of the results and main findings presented in Chapters 2 to 5, as well as a conclusion to this work. The limitations, recommendations and overall contribution of this research was also discussed in this final chapter.

1.11 CONFERENCE PRESENTATIONS

The following three conference/congress presentations originated from this research:

1. Society of Nuclear Medicine and Molecular Imaging (SNMMI) Annual Meeting, Chicago, Illinois, USA, 24–27 June 2023. Poster presentation. Title of the abstract (#570): *Imaging of rheumatoid arthritis with the SPECT glucose analogue 99mTc-labelled glucosamine and its correlation with laboratory markers* (Appendix L). This presentation received two awards at the conference (Appendix M): (i) the SNMMI 2023 Best Abstract Award for South Africa; and (ii) third place in the General Clinical Specialties track.

2. 20th Biennial South African Society of Nuclear Medicine (SASNM) Congress, Gqeberha, South Africa, 24–27 August 2023. Poster presentation. Title of the abstract (#3): *^{99m}Tc ethylenedicysteine-deoxyglucose (glucosamine) in the identification of active disease in patients with rheumatoid arthritis – a single center prospective study* (Appendix N).

3. EANM'23 – 36th Annual Congress of the European Association of Nuclear Medicine (EANM), Vienna, Austria, 9–13 September 2023. Oral presentation. Title of the abstract (#992): *Head to head comparison of ultrasound and Tc-99m glucosamine SPECT/CT imaging of patients with rheumatoid arthritis of the knee*. (Appendix O).

***Note to readers**: The referencing styles of the four articles (Chapters 2–5) are according to the specific journal's guidelines and requirements where the articles were submitted and are therefore not similar to the referencing style of Chapter 1 and the complete bibliography.*

CHAPTER 2

THE BIODISTRIBUTION AND UTILITY OF ^{99M}Tc-GLUCOSAMINE (ETHYLENEDICYSTEINE-DEOXYGLUCOSE) IN THE IDENTIFICATION OF ACTIVE DISEASE IN PATIENTS WITH RHEUMATOID ARTHRITIS – A SINGLE CENTER PROSPECTIVE STUDY[1]

Osayande Evbuomwan[1], Gerrit Engelbrecht[1], Cathryn Driver[2], Barend Jansen van Rensburg[3], Mathys Labuschagne[4], Je'nine Horn-Lodewyk[5*]

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[3]Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

[4]Clinical Simulation and Skills Unit, School of Biomedical Sciences, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

[5]Department of Clinical Sciences, Faculty of Health and Environmental Sciences, Central University of Technology Free State, Bloemfontein, South Africa

2.1 ABSTRACT

Purpose. Our objectives were to investigate the utility of ^{99m}Tc-ethylenedicysteine-deoxyglucose (ECDG) in identifying active disease in the joints of patients with rheumatoid arthritis (RA), as well as to evaluate the biodistribution of this radiopharmaceutical.

Methods. A prospective study was conducted at the Department of Nuclear Medicine of University of the Free State/Universitas Academic Hospital in Bloemfontein, South Africa. Twenty-two participants from the rheumatology department diagnosed with RA according to the ACR/EULAR classification criteria were enrolled. Participants were injected with 20–25 mCi of ^{99m}Tc-ECDG. Flow, blood pool, whole body, delayed static and SPECT/CT images were acquired. Known sites of disease were qualitatively assessed for intensity of uptake, and disease severity was graded (Grade 0–3).

Results. Twenty-two participants were studied. The median (interquartile range) age was 59 (49–68) years, and the majority (n = 21; 95.5%) were females. There was abnormal increased uptake of ^{99m}Tc-ECDG noted in the majority of the sites of known disease, including unknown sites. SPECT/CT imaging

[1]Accepted for publication by *Nuclear Medicine and Molecular Imaging* (cf. Appendix H).

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localized radiotracer uptake specifically to the synovial space. Similar biodistribution of tracer was noted in all patients, irrespective of disease severity or fasting status.
Conclusion. ^{99m}Tc-ECDG can efficiently assess disease activity in the joints of patients with RA. It accumulates in sites of both clinical and subclinical disease and might be a very useful tool for the rheumatologist in the management of patients with RA.

Key words: ^{99m}Tc-ECDG; rheumatoid arthritis; SPECT/CT

2.2 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that can lead to irreversible joint damage, deformities, disability and premature mortality, if not treated promptly and properly [1]. Therefore, prompt diagnosis and treatment will offer a very good prognosis in these patients. Bone erosion is a central feature of this pathology in patients who do not start treatment early and is associated with severe disease and poor functional outcomes [2]. Bone erosion has also been found to occur in some patients with low disease activity or those in clinical remission [2]. In order to offer early and proper treatment, investigations with a high sensitivity and specificity are needed to identify active disease.

Several advances in the methods for scoring disease activity in RA have been made in recent years. This involves a combination of the clinical assessment of involved joints and laboratory investigations. The three validated scoring systems currently used in South Africa are the 28-Joint Disease Activity Score (DAS-28), the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI) [1]. These scores allow for the classification of the patient as being in remission or presenting with low, moderate, or high disease activity. However, non- specific constitutional symptoms such as fatigue and malaise may predominate in some patients [3], and the subjective nature of these assessments might reduce their sensitivity. This shortcoming has given rise to the increased use of ultrasound (US) and magnetic resonance imaging (MRI) in the evaluation of these patients, due to their higher diagnostic accuracy [3].

In short, the latest classification criteria often used to diagnose RA, proposed by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR), incorporated the role of US and MRI in the detection of synovitis, thus enabling earlier diagnosis and correct classification of patients [4;5]. However, these imaging modalities are not without their drawbacks. The major drawback of US is that it is operator-dependent and cumbersome, especially when almost every joint in both hands must be evaluated [4;6]. The

inconvenience of this method is further increased if additional joints beside those in the hands need evaluation.

Magnetic resonance imaging has a high diagnostic accuracy in evaluating disease activity in the joints of patients with RA. It has shown strength in identifying early inflammatory changes [7;8]. This imaging modality provides greater resolution of joint structure and pathology but has the disadvantage of being able to screen only single joints/regions at a time. Additionally, MRI is very expensive, which increases the cost of patient imaging.

In the last decade, the use of nuclear medicine imaging to diagnose diseases and provide functional information regarding physiological processes has become increasingly valuable. With respect to RA, conventional planar scintigraphy and single photon computed tomography (SPECT), as well as positron emission tomography (PET), have been applied using a number of radiopharmaceuticals for the evaluation of disease activity [9]. The use of hybrid imaging, combining PET or SPECT imaging with computed tomography (CT), has further increased the diagnostic accuracy of these imaging techniques.

Technetium-99m (^{99m}Tc) diphosphonates, specifically ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP) which accumulates in bones, is most commonly used for evaluation of RA through planar bone scintigraphy and SPECT. While this method can assist in identifying arthritic joints, the disadvantage is low specificity and limited spatial resolution as compared to MRI [9].

The most commonly used PET/CT radiopharmaceutical for the evaluation of patients with RA, is fluorine-18-fluorodeoxyglucose ([^{18}F-]FDG) [10–13]. Some studies have shown [^{18}F-]FDG PET/CT to have a comparable diagnostic accuracy to US and MRI [9;10]. However, not every nuclear medicine center has a PET/CT scanner, and along with the cost and shorter half-life of [^{18}F-]FDG, makes the application of this method slightly more prohibitive.

These drawbacks of the above-mentioned imaging modalities show that there is still a need for a cost-effective, highly reliable, easy to perform and highly sensitive technique for the detection of disease activity in the joints of patients with RA.

^{99m}Tc-ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG), also known in essence as ^{99m}Tc-ECDG (both names to be used interchangeably) is a SPECT radiopharmaceutical for functional imaging of highly metabolic cells and has been investigated for imaging of cancer, similar to [^{18}F-]FDG [14]. Glucosamine is an important basic natural component of cartilage

and synovial fluid [15], and radiolabeled glucosamine may therefore find useful application for joint imaging and evaluation of patients with RA. Very few studies have shown the potential utility of ^{99m}Tc-ECDG imaging in patients with RA [6;14]. The aim of this study was therefore to, in a prospective manner, investigate ^{99m}Tc-ECDG, within a local context, for evaluation of active joint disease in patients with RA, including evaluation of the general whole body biodistribution of the radiopharmaceutical.

2.3 MATERIALS AND METHODS

2.3.1 Ethical considerations

Ethical approval for the study was obtained from our institution's Health Sciences Research Ethics Committee.

2.3.2 Study population and design

This was a prospective cross-sectional study which was conducted at the Department of Nuclear Medicine, University of the Free State/Universitas Academic Hospital in Bloemfontein, South Africa. Twenty-two participants from the rheumatology clinic were recruited into this study. These participants were diagnosed with RA by an experienced rheumatologist (25 years' experience), according to the ACR/EULAR classification criteria. They had disease involving either the wrist, small bones of the hands, and the knees. Recruitment period was between February and August 2022. Signed consent was obtained from all study participants.

2.3.3 Radiopharmaceutical preparation

All commercial reagents and solvents were purchased from Sigma Aldrich (Millipore Sigma, USA) or Merck Millipore (USA). All samples for ^{1}H-NMR spectroscopy were prepared using $CDCl_3$ or D_2O and analyzed on a Bruker 300 MHz spectrometer (Massachusetts, USA). High-performance liquid chromatography (HPLC)-MS analysis was done using an Agilent Infinity 1200 Series system coupled to Agilent 6100 Series quadrupole MS system (Agilent, USA) with radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany).

2.3.4 Synthesis of ECDG

The ECDG ligand was synthesized according to the procedure described by Yang et al [16]. Briefly, L-thiazolidine- 4-carboxylic acid (30.0 g) was dissolved in liquid ammonia (150 mL) followed by the slow addition of sodium metal (8.0 g, 1.5 eq) resulting in a deep blue-colored solution. The solution was stirred for 20 minutes at room temperature. The reaction was quenched with ammonium chloride (5.0 g), leading to evaporation of ammonia solvent, and the resulting residue dissolved in water (200 mL). The pH was adjusted to 3.0, and the resultant precipitate was purified by recrystallization in ethanol to yield ethylenedicysteine.2HCl (EC) (38% yield).

EC (2.0 g) was dissolved in 2 M NaOH (30 mL) with ethanol (40 mL) and stirred vigorously for 20 minutes. Benzyl chloride (1.48 g, 2.0 eq) in dioxane (20 mL) was added dropwise to the solution and further stirred for 30 minutes, after which the organic solvents were removed. The pH of the resulting aqueous mixture was acidified to pH 3.0 resulting in the precipitation of the hydrochloride salt of S,S'-dibenzyl ethylene dicysteine (Bn-EC) (85% yield), which was used without further purification.

Benzyl chloroformate (4.90 g, 2.5 eq) in dioxane (150 mL) was added to a cooled (0 °C) solution of S,S'-dibenzyl ethylene dicysteine.2HCl (6.0 g) dissolved in 10% K_2CO_3 solution (150 mL) and the reaction was stirred for 2 hours at 0 °C followed by stirring for 16 hours at 25°C. The solution was extracted with diethyl ether and the crude product precipitated by acidification of the aqueous phase. The product was redissolved and extracted using ethyl acetate to yield an amorphous solid product N, N'-dibenzyloxycarbonyl-S,S'-dibenzyl ethylene dicysteine (CBz- Bn-EC) (70% yield) upon drying.

CBz-Bn-EC (1.34 g) was activated by reaction with ethyl chloroformate (0.41 g, 2.0 eq) in chloroform (30 mL) with triethylamine (0.38 g, 2.0 eq) at -15 °C for 15 minutes. To this reaction mixture, a solution of tetra- acetylglucosamine (1.58 g, 2.2 eq) and triethlyamine (0.42 g, 2.0 eq) in chloroform (30 mL) was added, and the combined reaction mixture was stirred for 1 hour at 0°C and then 12 hours at 25°C. Following an acid-base workup, the residue was purified by column chromatography (MeOH/EtOAc/Hexane = 1:35:64 v/v ratio) to afford the fully-protected ethylenedicysteine-deoxyglucosamine (FP-ECDG) (70% yield).

FP-ECDG (1.0 g) underwent Birch reduction in liquid ammonia (80 mL) with sodium metal (0.5 g). The deep-blue colored solution was stirred for 20 min at room temperature before quenching with ammonium phenylacetate (1.32 g, 12.0 eq). The formed solution was then

dried under argon gas. The ammonium phenylacetate was extracted by stirring twice with isopropanol (50 mL and 25 mL) and separating using centrifugation (5 min, 4000 rpm). Residual isopropanol was removed by stirring and centrifuging with diethyl ether (2 x 50 mL) and the solid product was then dried under argon gas to afford ethylenedicysteine deoxyglucosamine (ECDG) (53% yield). The resultant product was confirmed MS and NMR analysis in accordance with the literature data (16). Calculated m/z 590.665 for $C_{20}H_{38}O_{12}N_4S_2$ [M + H] = 591.1.

2.3.5 ECDG Kit preparation

The ECDG kit preparation was done in a one vial procedure according to Zeevaart et al [17]. All solutions (HCl (0.1 M), Na_2HPO_4 Phosphate/citrate buffer solution and $SnCl_2$ solution (1mg/mL - ensuring the solution is clear and not milky) were freshly prepared with ultrapure, Milli-Q grade (>18MΩ/ cm), degassed water before production of the kits. Once prepared, all water and solutions were filtered through a sterile Millex-GP (polyethersulfone, 0.22 µm, 33 mm) syringe filter (Merck, Massachusetts, USA) into sterilized vials.

Citric acid (0.20 g) was added to a sterile vial containing Na_2HPO_4 (0.284 g) dissolved in water (pH 5.5) with the addition of $SnCl_2$ solution (100 µL) and freeze-drying (Christ Alpha I-5 freeze-drier, Type 1050 (Medizinische Apparatebau; Harz, Germany) overnight. The ECDG (5 mg) was weighed into a vial under Ar(g) and dissolved in MeOH (0.75 mL). This solution was immediately transferred to the vial containing the Sn/buffer and flash frozen in liquid nitrogen followed by lyophilization overnight. The kits were sealed, capped and placed in the –80°C freezer for storage.

2.3.6 Synthesis of ^{99m}Tc-ECDG

Radiosynthesis of ^{99m}Tc-ECDG was completed according to Zeevaart et al. [17] by adding Tc-99m pertechnetate (^{99m}TcO4-) (50 – 60 mCi) to the prepared, lyophilized ECDG kit vial (5 mg ECDG, Citric acid/Na_2HPO_4, $SnCl_2$). The solution was heated at 75°C for 15 min.

Quality control was performed on the radiolabeled product by testing pH (pH 5.5) and radiochemical purity (RCP) (>95%). RCP was determined using HPLC (Varian Prostar 325 UV/Vis (Varian Inc.) fitted with a radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany) and thin-layer chromatography (TLC) (Raytest GmbH; Straubenhardt, Germany). HPLC analysis was performed using a C-18 reverse phase column (Agilent Luna-C18 column, 5um, 4.6 x 250 mm) with isocratic elution (4.5% MeCN in

2 mmol ammonium formate (pH 3)) over 35 min. TLC analysis was completed using ITLC-SG (Agilent, USA) and Whatman (Millipore Sigma, USA) paper strips (10 cm) developed with saline and acetone as the mobile phase, respectively. Each strip was cut in half, and the activity was measured at the strip origin and front to determine the % colloids (ITLC-SG) and % labelled product (Whatman). The stability of the ^{99m}Tc-ECDG was determined up to 5 h after preparation using HPLC. The structure of ^{99m}Tc-ECDG is shown in Fig. 2.1.

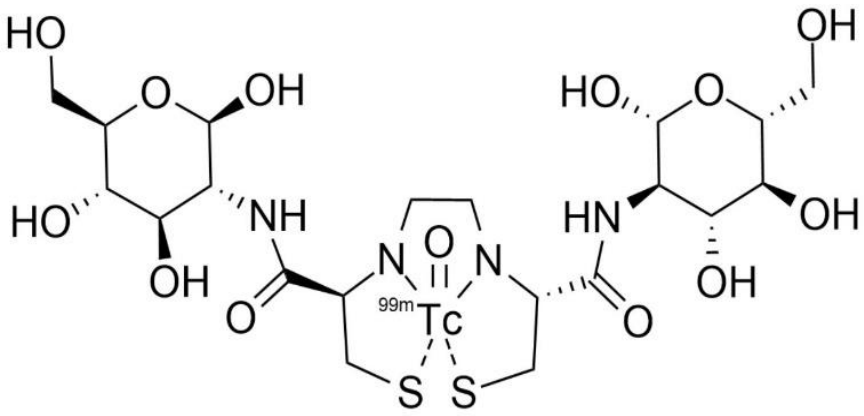

Fig. 2.1. Structure of ^{99m}Tc-ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG).

2.3.7 Radiopharmaceutical administration

An aseptic dispensing process was followed for the preparation of ^{99m}Tc-ECDG patient dose. The sterility of the process was checked using air settle plates and finger dab plates which were cultured. The ^{99m}Tc-ECDG dose was prepared for injection by diluting the prepared radiolabeled solution with saline (approx. 3 mL) and filtering through Millex-GP, a sterile filter (polyethersulfone, 0.22 µm, 33 mm) (Merck, Massachusetts, USA)) into a sealed, sterile vial. Additional saline (sterile, 1 mL) was added to the vial to ensure a final volume of around 5mL. A patient dose of 20–25 mCi was withdrawn for intravenous administration. Fasting was not a requirement prior to the administration of the radiopharmaceutical. No adverse events were recorded after radiopharmaceutical administration.

2.3.8 Imaging protocol

All 22 participants were scanned using a dual-head gamma camera (Siemens Symbia T16 True point SPECT-CT; Siemens Medical Solutions, USA). The SPECT/CT camera was equipped with a low energy, high-resolution collimator (LEHR). Dynamic images of the clinically most symptomatic joints were acquired at the time of administering the

radiopharmaceutical, with a frame rate of 1 frame/second for 60 seconds. This was followed by blood pool imaging of the hands, wrists, and knees. A delayed whole body image was acquired 2 hours after radiotracer injection, followed by dedicated 5 minutes static images of the hands, wrists, and knees. SPECT images of the most clinically symptomatic joint (either the hands/wrist or knees) were also performed at 25 s/stop, with 3° steps, in a 128 X 128 matrix. This was followed by a low dose, non-contrast CT, with the patient in the same bed position.

2.3.9 Image processing and data analysis

Images were processed using the Syngo workstation on the gamma camera. SPECT images were reconstructed using an iterative algorithm and SPECT/CT fusion images were obtained using the multimodality Syngo imaging software on the workstation.

The data of each patient were collected using an Excel 2019 spreadsheet (Microsoft, USA). Statistical analysis was performed using R, version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

2.3.10 Image interpretation

Images were interpreted by a single nuclear medicine physician with 9 years' experience. The dynamic flow images were assessed qualitatively for an increase, a decrease or normal blood flow to the region imaged. The blood pool images were assessed for increased, decreased, or normal blood pool activity. Delayed images were interpreted for disease activity in the joints, using a slight modification of the scoring system used by Angelides et al [14].

Grade 0 – Normal physiological joint uptake, defined as no/minimally increased radiotracer activity in joints (activity same as that of the neighboring muscle tissue).
Grade 1 – Mild radiotracer uptake slightly more than that of the neighboring muscle tissue.
Grade 2 – Moderate radiotracer uptake greater than that of grade 1.
Grade 3 – Severe radiotracer uptake markedly greater than that of grade 1.

The whole body images were qualitatively assessed for the biodistribution of the radiotracer.

2.4 RESULTS

The ECDG ligand was prepared in 5 steps (overall yield of 8 %) and then successfully formulated into buffered kits which were stably stored in a -80°C freezer. Radiolabeling of the ECDG kit with ^{99m}TcO4- saline yielded ^{99m}Tc-ECDG (10-12 mCi/mg) in 97% radiochemical purity as determined by radio-HPLC analysis. ^{99m}Tc-ECDG presented as 4 diastereomeric peaks with retention times of 6.5, 8.5, 10.4 and 11.4 min. TLC analysis indicated almost no colloid formation (< 0.2 %) and > 98 % labelled product. The radiolabeled product formulation was stable over 5 hours at room temperature with no visible degradation and only a 1.5 % loss of bound radioactivity.

Twenty-two participants with diagnosed RA according to the ACR/EULAR classification criteria were recruited into the study. The median (IQR) age was 59 (49–68) years, and the majority (95.5%) were females. Twelve participants had the joints of their hands and wrists clinically examined, as this was their most symptomatic joints, while the remaining ten had their knees clinically examined. So, a total of three hundred and thirty-six joints in both hands, which included the metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints and distal interphalangeal (DIP) joints were evaluated for disease activity. Twenty-four carpal joints and twenty knee joints were also assessed for disease activity. Therefore, a total of 380 joints were evaluated for disease activity.

Overall, the study was well tolerated, with none of the patients presenting with any adverse events. All 22 participants had abnormal increased uptake of the radiopharmaceutical in their affected joints, with SPECT/CT imaging localizing uptake specifically to the synovial space as seen in Fig. 2.2. Of the 22 flow studies, 10 (45%) participants had normal flow to their most symptomatic joints, with 12 (55%) having increased flow to their most symptomatic joints. Increased flow studies were associated with either grade 3 or 2 disease on the delayed static images as shown in Fig. 2.3. Good quality images were obtained 2 hours post ^{99m}Tc-ECDG administration, with an optimal target to background ratio. The majority of the participants had the greatest uptake of radiotracer in their most clinically active joints (n=15, 68%). Majority of the participants had high DAS-28 scores indicating severe disease as seen in Table 2.1 showing the characteristics of the study population. All participants with clinically severe disease had an overall grade 3 or 2 uptake of ^{99m}Tc-ECDG on their scans, with 55% being grade 3 uptake. Twelve participants had evidence of disease in other joints outside the hands, wrists, and knees. These joints included the shoulders (n=9), elbows (n=4) and ankles (n=2). Two participants presented with unilateral knee disease, as seen in Fig. 2.4. No incidental finding of extra-articular disease was noted in any of the 22 participants.

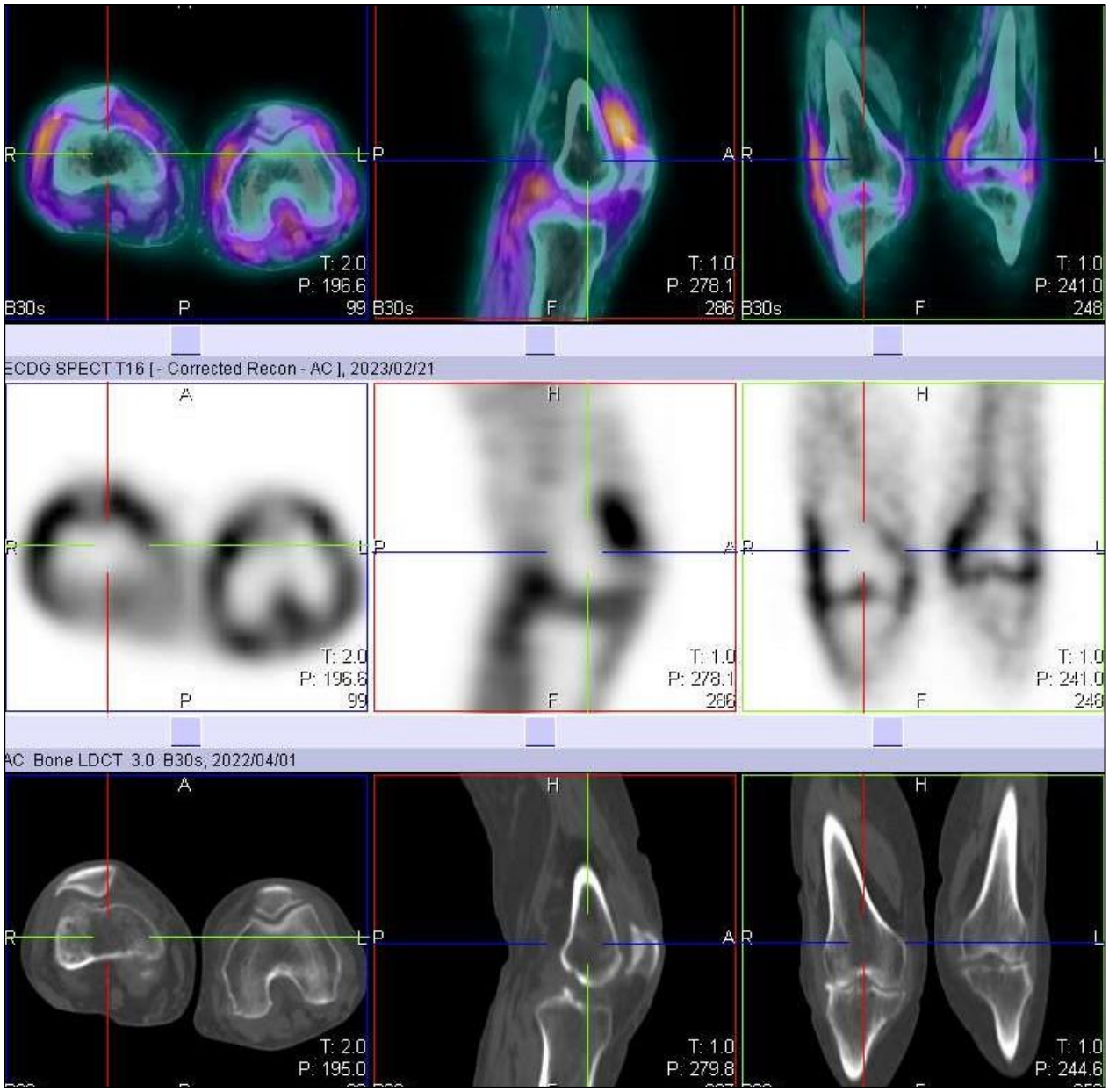

Fig. 2.2. SPECT/CT, SPECT, and CT only images of an 82-year-old female with rheumatoid arthritis of both knees. Note the localization of radiotracer to the synovial space, without bone involvement.

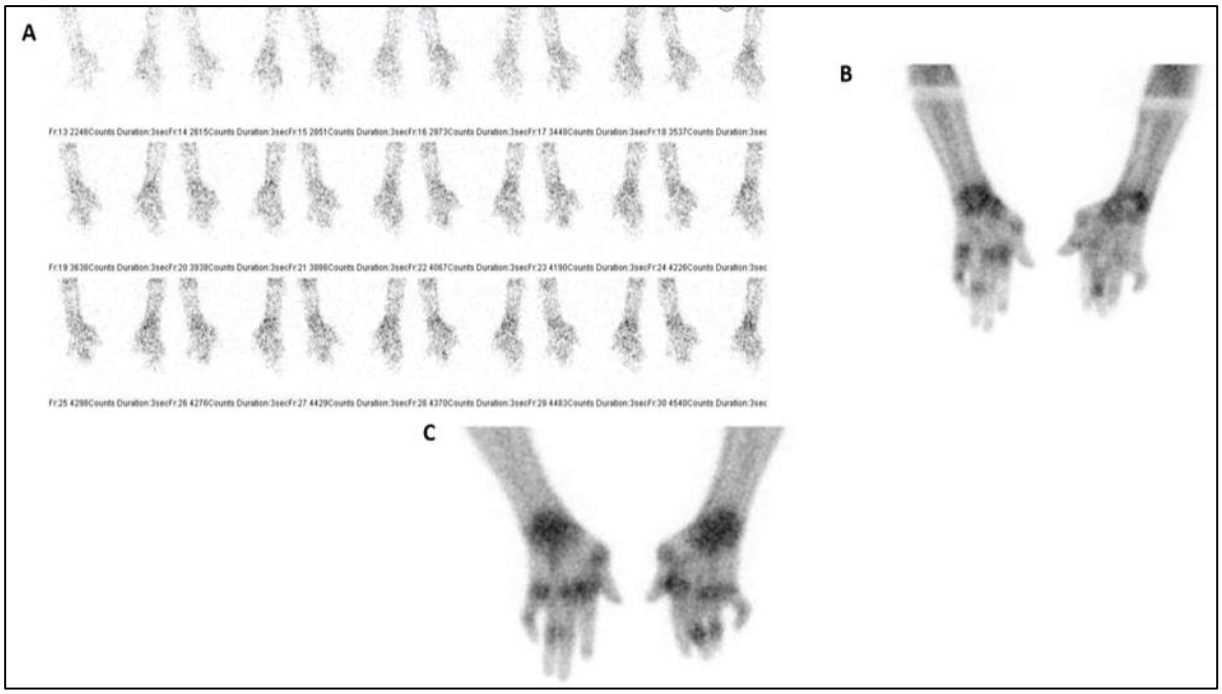

Fig. 2.3. Three phase ^{99m}Tc-ECDG scan of a 58-year-old female with rheumatoid arthritis that developed a flair after discontinuing her medications. The flow images (A) show increased blood flow to the region of the wrists and small bones of the hands. The blood pool and delayed static images (B and C, respectively) show increased blood pool and delayed activity to the wrists and small joints of the hands, respectively.

Table 2.1. Characteristics of the study participants (N=22).

Variable	**n (%)**
Age in years, median (IQR)	59 (49-68)
Sex, n (%)	
Female	21 (95.5)
Male	1 (4.5)
DAS-28 severity, n (%)	
Mild	0 (0)
Moderate	2 (9.1)
Severe	20 (90.9)
^{99m}Tc-ECDG overall uptake grade, n (%)	
Grade 1	2 (9.1)
Grade 2	8 (36.4)
Grade 3	12 (54.5)
Serological markers, median (IQR)	
Anti-CCP (normal/reference value < 3 U/mL)	63 (2.6-201)
Rheumatoid factor (RF) (normal/reference value < 20 IU/mL)	38 (11-81)
Positive (elevated) RF and anti-CCP antibody titers, n (%)	
Yes	14 (63.6)
No	8 (36.4)

IQR, interquartile range; anti-CCP, anticyclic citrullinated peptide; U/mL, units per milliliter; IU/mL, international units per milliliter; mg/mL.

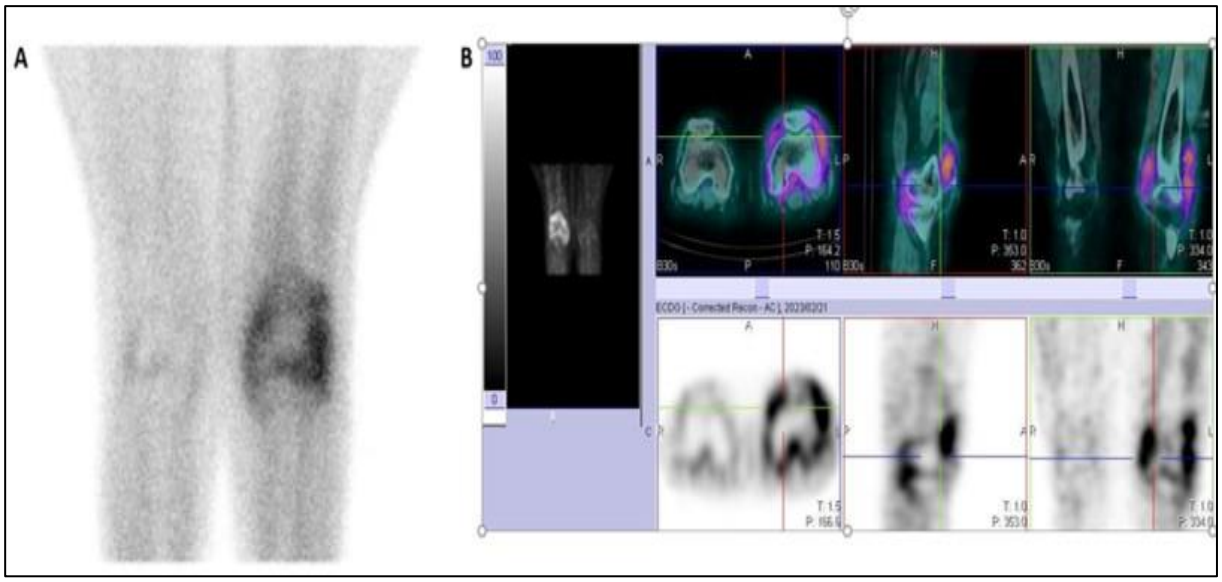

Fig. 2.4. Delayed static (A) and SPECT/CT images of a 51-year-old with unilateral knee disease. Note the normal physiologic uptake of tracer in the right knee.

Comparing the detection of disease activity by clinical evaluation and ^{99m}Tc-ECDG SPECT/CT imaging, interestingly all 12 participants who had the joints of the hands evaluated with both modalities had no detection of disease activity by both modalities in the 96 DIP joints. However, there were some discrepancies in the other 240 joints evaluated, as seen in Table 2.2. The level of agreement, expressed as percentages in the detection of synovitis in the hands, wrists and knees was 56.2, 75 and 85% respectively as seen in Tables 2.2, 2.3 and 2.4.

Table 2.2. Percentage of active disease detected in the joints of the hands by both modalities and their agreement.

Modality	Number of joints assessed	Positive for disease, n (%)
Clinical examination	240	152 (63.3)
^{99m}Tc-ECDG SPECT/CT	240	121 (50.4)

Percentage agreement (tolerance=0) = 56.2

Table 2.3. Percentage of active disease detected in the joints of the wrists by both modalities and their agreement.

Modality	Number of joints assessed	Positive for disease, n (%)
Clinical examination	24	20 (83.3)
^{99m}Tc-glucosamine SPECT/CT	24	22 (91.7)

Percentage agreement (tolerance=0) = 75

Table 2.4. Percentage of active disease detected in the joints of the knees by both modalities and their agreement.

Modality	Number of joints assessed	Positive for disease, n (%)
Clinical examination	20	18 (90.0)
^{99m}Tc-glucosamine SPECT/CT	20	19 (95.0)

Percentage agreement (tolerance=0) = 85

Irrespective of disease activity or fasting state, the general biodistribution of ^{99m}Tc-ECDG was similar on the delayed 2 hour whole body images in all 22 participants. Very mild uptake was noted in the unaffected joints, spine, and skeletal muscle, with occasional mild uptake visible in the lacrimal gland and nasal mucosa. Mild to moderate uptake was noted in the blood pool and liver. The radiopharmaceutical exhibited renal excretion, with moderate activity noted in the kidneys and very intense uptake noted in the urinary bladder. None of the patients had uptake of ^{99m}Tc-ECDG noted in the brain, lungs, and myocardium. Fig. 2.5 shows the distribution of ^{99m}Tc-ECDG in a participant with high disease activity, mild disease activity and in a known non-fasted participant.

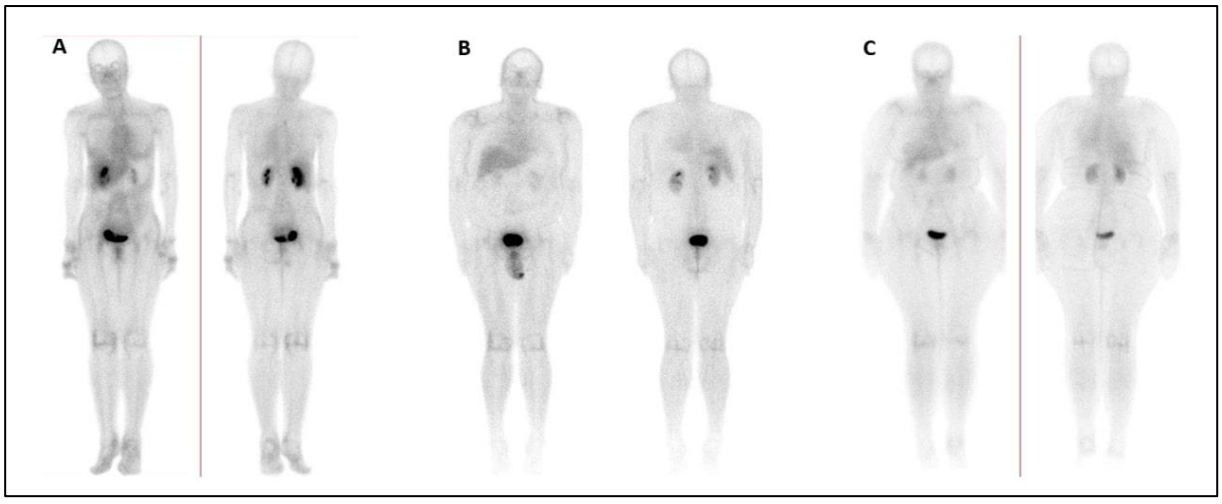

Fig. 2.5. Whole body images of a patient with (A) severe disease, (B) mild disease, and (C) a non-fasted patient. Note the similarities in the biodistribution.

2.5 DISCUSSION

The findings indicated that most of our participants had increased uptake of ^{99m}Tc-ECDG in their affected joints, with varying degrees of uptake. This is not different from the few studies that have used ^{99m}Tc-ECDG to evaluate disease activity in patients with RA [6;14]. However, the level of agreement with the two modalities (clinical evaluation and ^{99m}Tc-ECDG), expressed as percentages in the detection of synovitis in the knees, wrists and hands was 85, 75 and 56.2% respectively. This finding was largely due to the fact that ^{99m}Tc-ECDG imaging detected disease activity in some sites that were negative for disease activity by clinical evaluation, especially in the joints of the wrists and knees. This is likely in keeping with its ability to detect subclinical disease as noted in an earlier study and the fact that clinical evaluation is very subjective [14]. There was however a good correlation between the degree of severity clinically using the DAS-28 scores and the grade of ^{99m}Tc-ECDG uptake.

Our findings also show that all the participants showed similar biodistribution of the radiopharmaceutical. This is also similar to the first study assessing biodistribution, in which the biodistribution of ^{99m}Tc-ECDG was evaluated in patients with non-small lung cell cancer [18]. Just like the study published by Angelides et al. [14], ^{99m}Tc-ECDG was well tolerated by all our participants, with no allergic reactions or side effects. In our study majority of the participants had the greatest uptake of radiotracer in their most clinically active joint. This is also almost similar to the findings of the previously published study by Angelides et al. [14], where the highest radiotracer uptake was noted in the most clinically affected joint. However, even though majority of our participants had these findings, 32% of them did not. This might be attributable to one of the draw backs of clinical estimation of disease activity, whereby its subjective nature might reduce its diagnostic accuracy [19].

Further observations from our study included that apart from symptomatic joints, disease activity was also noted in non-symptomatic joints, including joints outside the hands, wrists, and knees. A finding that is also similar to that published by Angelides et al [14]. We agree that this finding might account for subclinical disease, an entity that is known to be associated with disease progression in otherwise healthy looking patients. This is likely to be an advantage of ^{99m}Tc-ECDG imaging, as it can detect patients with subclinical disease. Our findings are also similar to the published study by Manolois et al. [6], who imaged 25 patients with RA, and concluded that the radiopharmaceutical was well tolerated, and its degree of uptake demonstrated significant correlation with clinically assessed disease activity. They also concluded that it also accumulates in sites of subclinical disease, which is similar to our findings. Both of these previously published studies reported that ^{99m}Tc-ECDG is similar to

[^{18}F-]FDG, and being analogues of glucose, they are metabolized by inflamed tissue. This was based on the information from Yang et al. [20;21], that ^{99m}Tc-ECDG acts as a glucose analogue and can also be used in the imaging of malignancies.

Various authors have also tried to assess the imaging properties of ^{99m}Tc-ECDG in malignancies. Ginat et al. [22], in their study of 9 patients with head and neck squamous cell carcinoma, confirmed increased uptake of the radiotracer in all 9 cases. In a pre-clinical study conducted by Pham et al., they concluded that this radiopharmaceutical was easily taken up both in vivo and in vitro in murine cells by diffuse large B cell lymphoma (DLBCL) cells [23]. However, we had imaged one patient with DLBCL in our facility, known with multiple FDG avid lymph nodes and nodal masses above and below the abdomen, but these lesions had no uptake of ^{99m}Tc-ECDG. Other studies have also investigated the potential utility of this radiopharmaceutical for tumor imaging. Zhang et al. in their study investigated mesothelioma bearing rats and confirmed increased uptake of ^{99m}Tc-ECDG in the tumor [24].

A published study of 17 patients with non-small cell lung cancer compared ^{99m}Tc-ECDG and [^{18}F-]FDG, in the imaging of these patients [25]. They concluded that there was a 100% concordance in the imaging of the primary tumor and 70% concordance in the imaging of metastatic disease with the two radiotracers. All these studies seem to support the idea that ^{99m}Tc-glucosamine, being an analogue of glucose, is metabolized and taken up by tumor cells, with some alluding to the fact that it shares similar properties with [^{18}F-]FDG. However, most of these studies are hampered with the fact that they are either pre-clinical studies or studies involving a very small sample size. Therefore, it cannot still be concluded that this radiopharmaceutical behaves like [^{18}F-]FDG.

Findings from this RA study may suggest that ^{99m}Tc-ECDG and [^{18}F-]FDG do not show similar properties. In our study we noticed that irrespective of the fasting state of the patient, the biodistribution of the radiopharmaceutical remained the same, which is not the case with [^{18}F-]FDG. The biodistribution of [^{18}F-]FDG is associated with intense uptake in the brain, as well as uptake in the myocardium and bone. None of our participants exhibited ^{99m}Tc-ECDG uptake in these areas, although, we noticed very mild uptake in the spine of most of the participants. These findings are relatively similar to those published by Schechter et al [18], however, they postulated that the hydrophilic nature of the radiopharmaceutical might prevent it from crossing the blood brain barrier. This, however, does not explain the absence of uptake in the bone and myocardium.

Although not yet proven, the information obtained from our study has led us to the hypothesis that the uptake of ^{99m}Tc-ECDG in the affected joints of our participant population might not be due to its similar properties to [^{18}F-]FDG, but to the fact that glucosamine is a normal constituent of synovial fluid and that there may be upregulation of glucosamine receptors in the synovium of patients with RA. Further investigation might be required to conclude on the possibility of this hypothesis. But what is clear is that ^{99m}Tc-ECDG has different imaging properties as compared to [^{18}F-]FDG but could be considered as a good alternative for imaging RA in centers that have only SPECT/CT scanners and no PET/CT scanners.

Overall, the findings of this study are similar to the few previously published studies in the imaging of patients with RA, however, a significant limitation of this study is the small sample size. Larger patient numbers would be required to increase the weight of any claims. The varying degree of uptake in our participant population could signify that this radiotracer might be valuable in assessing treatment response and offering prognostic information. We recommend that further studies should be conducted in this regard.

2.6 CONCLUSION

This study has shown that ^{99m}Tc- ECDG is a safe radiotracer that can efficiently assess active disease activity in the joints of patients with RA. It accumulates in sites of both clinical and subclinical disease and might be a very useful tool for the rheumatologist in the management of patients with RA. The study also showed a good correlation between disease severity clinically and degree of ^{99m}Tc-ECDG uptake. ^{99m}Tc-ECDG offers centers with only SPECT or SPECT/CT scanners an opportunity to investigate patients with RA.

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CHAPTER 3
THE PROGNOSTIC VALUE OF ^{99M}Tc GLUCOSAMINE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS – A SINGLE CENTER PROSPECTIVE STUDY[2]

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3.1 ABSTRACT

Objectives: Poor prognostic factors in rheumatoid arthritis (RA) are associated with a more severe form of the disease. Nuclear medicine functional imaging has shown remarkable merit at identifying active disease in patients with RA and is increasingly being used in this regard. However, its prognostic value has not been evaluated thoroughly. We aimed to assess the prognostic value of technetium-99m (^{99m}Tc-) glucosamine imaging in patients with RA.

Methods: Twenty-two participants diagnosed by an experienced rheumatologist with RA were recruited for inclusion in the study. Blood samples were obtained from each participant for baseline C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody titer. On the same day, each participant was injected with 20–25 millicurie (mCi) of ^{99m}Tc-glucosamine. Planar and single-photon emission computed tomography (SPECT/CT) images of known disease sites were acquired up to two hours after radiopharmaceutical administration. Affected joints were qualitatively assessed and graded for ^{99m}Tc-glucosamine uptake and compared with blood results.

Results: All participants affected joints had an increased uptake of the radiopharmaceutical, with 14 (63.6%) having elevated RF and anti-CCP antibody titers. Eight of the 14 patients with increased RF and anti-CCP antibodies had grade 3 uptake of ^{99m}Tc-glucosamine. The remaining 6 had grade 2

[2] Accepted for publication by *Nuclear Medicine Communications* (cf. Appendix I).

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uptake. A significant correlation between higher grade uptake and increased levels of RF and anti-CCP antibodies (p=0.031) was observed.
Conclusion: We found a strong correlation between high-grade disease on imaging and the presence of RF and anti-CCP antibodies in patients with RA.

Keywords: rheumatoid arthritis; ^{99m}Tc-glucosamine; rheumatoid factor; anti-cyclic citrullinated peptide; anti-CCP; prognostic factors

3.2 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that has to be treated appropriately to prevent permanent damage to the affected joints, deformities, disability and premature mortality [1]. It is an autoimmune disorder of which the causes are unknown and presents with symmetric, erosive synovitis and occasionally extra-articular involvement [2]. Bone erosion is common in RA in patients who do not receive treatment at an early stage and is associated with severe disease and poor functional outcomes, even in the event of subclinical disease [3]. Prognostic factors play a significant role in the initiation, type, intensity of treatment and the prediction of joint damage in patients with RA [4]. Several clinical variables and biological markers, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF), constitute prognostic factors in RA [5]. In 2019, the European League Against Rheumatism (EULAR) established poor prognostic factors for RA, including persistently moderate or high disease activity despite therapy, high acute phase reactant levels, more swollen joint counts, high RF and/or anticyclic citrullinated peptide (anti-CCP) antibody titers, and the presence of early bone erosion [6]. It has been reported before that high levels of CRP, RF and anti-CCP positivity are strongly associated with joint damage [4;6].

Technetium 99 metastable (^{99m}Tc) labelled glucosamine is a radiolabelled glucose analogue that has been used for imaging active joint disease in patients with RA [7;8]. It offers an alternative to fluorine-18-fluorodeoxyglucose positron emission tomography computed tomography (^{18}F-FDG PET/CT) imaging of patients with RA in facilities that do not have PET/CT machines. The valuable role of ^{18}F-FDG PET/CT in the management of RA, including the identification of disease activity and the prognosis, is well known [9–16]. With the recognised variable factors that can affect the prognosis of patients with RA, the prognostic value of ^{99m}Tc-glucosamine in patients with RA has not been investigated before. Rheumatoid factor and/or anti-CCP positivity have consistently remained long-term predictors for joint damage [4;17–21]. In this prospective study, we intended to investigate the prognostic value

of ^{99m}Tc-glucosamine in patients with RA by comparing the degree of disease activity on baseline ^{99m}Tc-glucosamine to elevated RF and anti-CCP antibodies, which are known prognostic factors for the development of bone erosion in patients with RA. Our hypothesis was that a good correlation between scan findings and these biological markers might be an indication that baseline ^{99m}Tc-glucosamine imaging might be able to identify patients very early who would need a more aggressive treatment approach.

3.3 MATERIALS AND METHODS

3.3.1 Study design

A prospective cross-sectional study was conducted.

3.3.2 Study population

Twenty-two participants diagnosed with either acute or chronic RA by an experienced rheumatologist (25 years' experience) according to the American College of Rheumatology (ACR)/EULAR classification criteria were recruited to participate in the study between 17 February 2022 and 18 August 2022. Each participant had active synovitis and was recruited from the Rheumatology Clinic at Universitas Academic Hospital UAH) in Bloemfontein, South Africa. Any participant older than 18 years of age was eligible for participation in the study. Pregnant or lactating participants were excluded. Written consent was obtained from each study participant.

3.3.3 Synthesis of ^{99m}Tc-ECDG

Radiosynthesis of ^{99m}Tc-labelled **ethylenedicysteine-deoxyglucose** (ECDG) was completed according to the method described by Zeevaart et al. [17] by adding 50–60 millicurie (mCi) of Tc-99m pertechnetate (^{99m}TcO4-) to a prepared lyophilised ECDG kit vial. The solution was then heated at 75°C for 15 minutes.

3.3.4 Radiopharmaceutical administration

The ^{99m}Tc-ECDG dose was prepared for injection by diluting the prepared radiolabelled solution with 3 mL of saline, which was then filtered into a sealed sterile vial. Additional saline was added to the vial to ensure a final volume of approximately 5 mL. A patient dose of 20–25 mCi was withdrawn for intravenous administration.

3.3.5 Imaging protocol

Prior to imaging on the same day, blood samples were obtained to determine the baseline CRP, ESR, RF and anti-CCP antibody titer for each patient. All 22 participants were scanned using a dual-head gamma camera (Siemens Symbia T16 True Point SPECT-CT; Siemens Medical Solutions USA Inc., Malvern, PA). The camera was equipped with a low-energy high-resolution (LEHR) collimator. Dynamic images of the clinically most symptomatic joints were acquired at the time of administering the radiopharmaceutical, with a frame rate of 1 frame/second for 60 seconds. This was followed by blood pool imaging of the hands, wrists and knees. A delayed whole body image was acquired 2 hours after injection of the radiopharmaceutical, followed by dedicated 5 minutes static images of the hands, wrists and knees. SPECT images of the clinically most symptomatic joint were also performed at 25 seconds per stop, with 3° steps, in a 128 × 128 matrix. Afterwards, a low dose non-contrast CT was performed with the patient in the same bed position.

3.3.6 Image processing

Images were processed using the Syngo Workstation (Siemens Medical Solutions USA Inc., Malvern, PA) on the gamma camera. SPECT images were reconstructed using an iterative algorithm. SPECT/CT fusion images were obtained using the multimodality Syngo imaging software on the workstation.

3.3.7 Data collection and analysis

The data of each patient were recorded in a custom-designed Microsoft Excel (version 2019) spreadsheet and analysed by means of the statistical package Stata, version 16 (StataCorp LLC; College Station, TX, USA). The results for variables such as sex were represented in frequency. Age was represented as the median and interquartile range. Fisher's exact test was used determine associations between groups. Statistical significance was set as a p value of less than 0.05.

3.3.8 Image interpretation

The dynamic flow images were assessed qualitatively for normal, increased or decreased blood flow to the region imaged. The blood pool images were assessed for increased, decreased, or normal blood pool activity. Delayed images were interpreted for disease activity in the joints, using a slight modification of the scoring system reported by Angelides et al [8]. The scoring was interpreted as follows:

- Grade 0 – normal physiological uptake in the joint, which was defined as no or minimally increased radiopharmaceutical activity in the joints (activity same as that of the neighboring muscle tissue).
- Grade 1 – mild radiopharmaceutical uptake; slightly more than that of the neighboring muscle tissue.
- Grade 2 – moderate radiopharmaceutical uptake greater than that of grade 1.
- Grade 3 – severe radiopharmaceutical uptake markedly greater than that of grade 1.

3.3.9 Ethical considerations

In addition to obtaining written informed consent from the patient included in the study, approval for the research was granted by the Health Sciences Research Ethics Committee (HSREC) of the University of the Free State in Bloemfontein, South Africa, under ethics clearance number UFS-HSD2020/1292/2411.

3.4 RESULTS

Twenty-two participants diagnosed with RA according to the ACR/EULAR classification criteria were recruited to participate in the study. No adverse events were recorded after radiopharmaceutical administration. Participants' demographic and some clinical characteristics are summarised in Table 3.1. All 22 participants had abnormally increased uptake of the radiopharmaceutical in their affected joints, with SPECT/CT imaging localising radio-pharmaceutical uptake specifically to the synovial space. Examples are illustrated in Figures 3.1–3.3. The majority of the participants had elevated anti-CCP and RF antibody titers. A significant correlation (p=0.031; Fisher's exact test) was observed between the grade of ^{99m}Tc-labelled glucosamine uptake and the presence of laboratory prognostic markers (anti-CCP and RF). Fourteen (63.6%) of the 22 participants had elevated RF and anti-CCP antibodies, of whom 57.1% (n=8) had grade 3 uptake. The remaining 42.9% (n=6) had grade

2 uptake. There was no participant with elevated RF and anti-CCP antibody levels that had grade 1 uptake. In comparison, 87.5% (n=7) the participants with normal prognostic markers had a combination of grade 1 and 2 uptake of the radiopharmaceutical in their affected joints, as illustrated in Figure 3.1, with only one participant having grade 3 uptake. No significant correlation (p=0.3; Fisher's exact test) was noted between the grade of ^{99m}Tc-labelled glucosamine uptake and the levels of the other inflammatory markers, i.e., ESR and CRP.

Table 3.1. Demographic and clinical variables of patients with rheumatoid arthritis (RA) (N=22).

Variable	
Age in years, median (IQR)	59 (49–68)
Sex, n (%)	
Female	21 (95.5)
Male	1 (4.5)
Prognostic laboratory markers, median (IQR)	
Anti-CCP (normal/reference value < 3 U/mL)	63 (2.6–201)
Rheumatoid factor (RF) (normal/reference value < 20 IU/mL)	38 (11–81)
C-reactive protein (CRP) (normal/reference value < 10 mg/L)	9 (6–19)
Erythrocyte sedimentation rate (ESD) (normal/reference value 0–10 mm/hr)	35 (23–62)
Positive (elevated) RF and anti-CCP antibody titers, n (%)	
Yes	14 (63.6)
No	8 (36.4)

IQR, interquartile range; anti-CCP, anticyclic citrullinated peptide; U/mL, units per milliliter; IU/mL, international units per milliliter; mg/mL, milligram per milliliter; mm/hr, millimeter per hour.

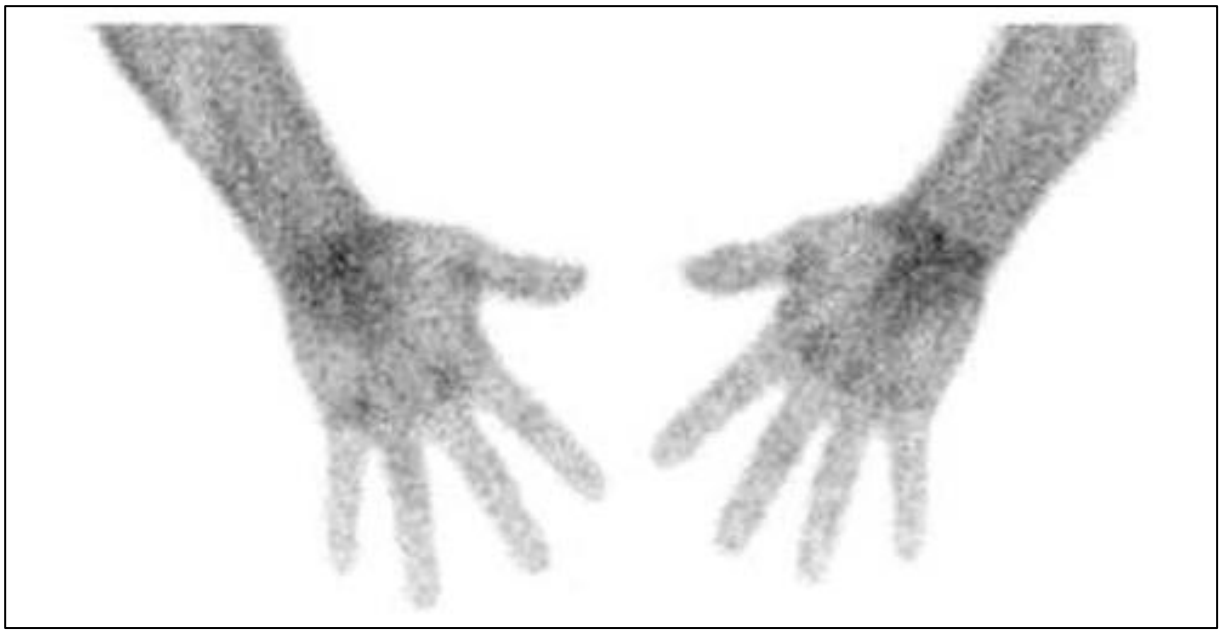

Figure 3.1. A 68-year-old male with low-grade disease in the wrists and fingers. His rheumatoid factor titer was < 10 IU/mL (negative), with an anti-CCP antibody titer of 1.1 U/mL (negative).

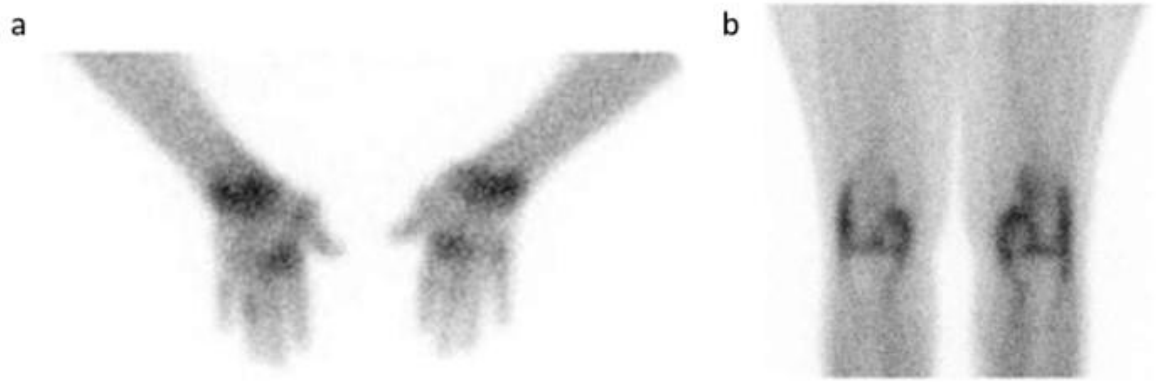

Figure 3.2. A 61-year-old female with high grade (grade 3) disease in **(a)** wrists and hands and **(b)** knees. Her rheumatoid factor titer was 333 IU/mL, with an anti-CCP antibody titer of 201 U/mL.

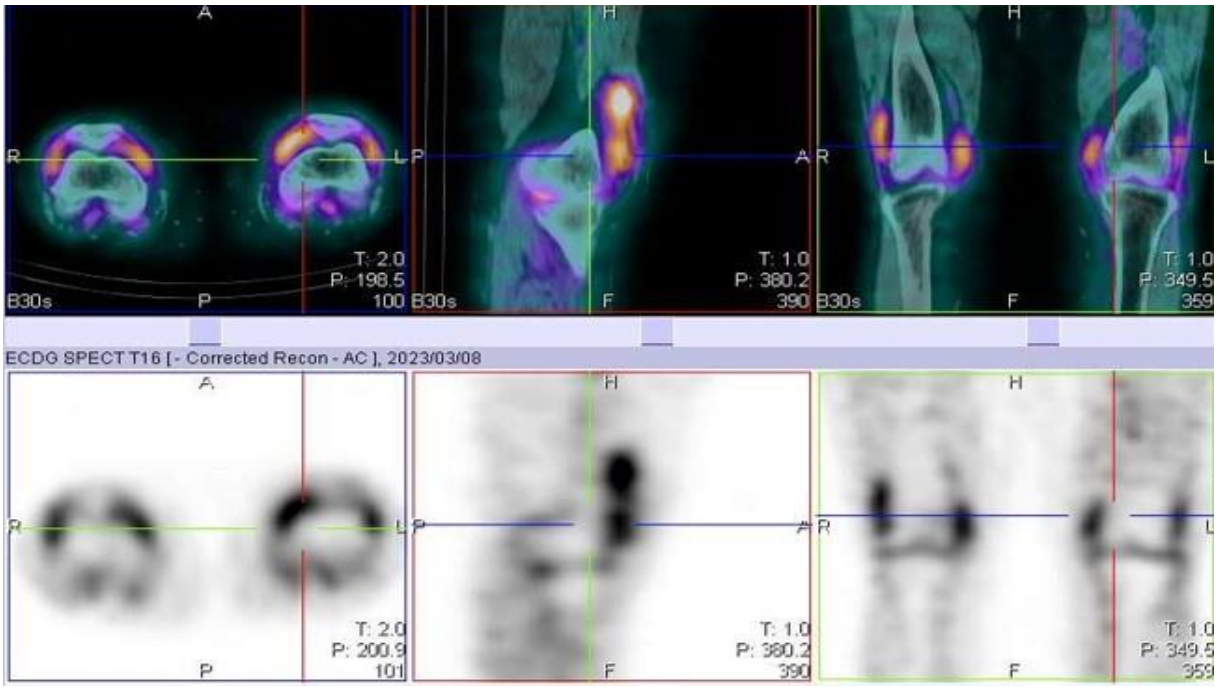

Figure 3.3. SPECT/CT images of the same patient in Figure 3.2, showing localisation of the intense uptake of ^{99m}Tc-labelled glucosamine in the knee to the synovial space, without bone uptake.

3.5 DISCUSSION

Prognostic factors are used to make decisions pertaining to the treatment of patients with RA. Our findings indicated a strong association between participants with elevated RF and anti-CCP values and high-grade ^{99m}Tc-labelled glucosamine uptake, as shown in Figure 3.2 and 3.3. The autoantibody anti-CCP has earlier been predicted to offer a prognostic role in patients with RA by identifying those patients who might eventually have radiological damage, hence needing more aggressive treatment [20;21]. Vencovsky et al. confirmed that when increased RF levels are combined with anti-CCP antibodies, the prediction for early structural damage is more accurate [19]. It has also been concluded that both RF and anti-CCP antibodies are clinically valuable biomarkers for the diagnosis and prognosis of patients with RA [18]. Today, of all the poor prognostic factors in patients with RA, RF and anti-CCP antibodies are among the most consistently relevant indicators of disease severity [4;6]. In our study, all patients with positive RF results also had positive anti-CCP antibody titers, implying that they more likely fell into the category of patients prone to early structural damage.

Limited information about the prognostic utility of nuclear medicine metabolic imaging in patients with RA is available in the literature [22;23]. The ability of ^{18}F-FDG PET/CT imaging to identify complications of RA is believed to contribute to the prognostication of patients [22]. Methotrexate, a disease-modifying drug for the treatment of RA, has been known to be

associated with lymphoproliferative disorders [24;25]. Some evidence is available in the literature suggesting that ^{18}F-FDG PET/CT imaging could be useful for predicting the prognosis of and spontaneous regression in these patients [24;25]. Its ability to also detect unexpected disease activity very early, thus ensuring rapid initiation of treatment and a more favourable prognosis, has also been reported in the literature [10]. However, no studies have been conducted to directly correlate the uptake of ^{18}F-FDG in the joints of patients with RA and the presence of well-known and recognised poor prognostic markers such as RF and anti-CCP titers.

To the authors' knowledge, our study is the first to compare the intensity of ^{99m}Tc-labelled glucosamine uptake with the RF and anti-CCP status of patients with RA. All the participants with elevated RF and anti-CCP antibodies had either grade 3 or 2 uptake score, with 57.1% of them having a grade 3 uptake score. Only one participant with a grade 3 uptake score had no indication of elevated RF and anti-CCP antibody levels. This result may imply that grade 3 ^{99m}Tc-labelled glucosamine uptake might be associated with a worse prognosis in patients with RA.

Other imaging modalities such as ultrasound (US) and magnetic resonance imaging (MRI) have also offered prognostic information in patients with RA by early identification of joints at risk of damage [5;6;26–29]. However, in their retrospective study of 138 patients, Sun et al. concluded that a high gray scale synovitis score at baseline does not always indicate a worse prognosis of RA [6]. However, Ziegelasch et al. established a correlation between prognostic US findings and the presence of anti-CCP antibodies in patients with RA, concluding that bone erosion detected by US was an independent predictor for the development of clinical arthritis [29]. Similar findings have been reported with MRI. Hetland et al. observed that MRI-detected bone oedema, when associated with the elevated anti–CCP antibodies, was able to predict radiographic progression at five years [30].

It could be argued that imaging might not be needed for prognostication if RF and anti–CCP antibodies are reliable. However, not all centers might offer these investigations and they are not able to demonstrate the true extent of the disease in all the affected joints.

Limitations

A limitation in our study was using only visual assessment to grade disease activity in the small sample size, which might not be robust enough to confirm the conclusion that grade 3 uptake of ^{99m}TC-labelled glucosamine was a poor prognostic marker radiological detection of joint damage. A recommendation would be to devise quantitative methods to assess disease

activity in these patients, or better still look at the possibility of evaluating them with a glucosamine labeled PET radioisotope. The lack of long-term follow-up in these patients to assess for radiologically confirmed progression of joint damage also contributed to the limitations of the study. We recommend that long-term follow-up for the radiological detection of possible joint damage in patients with RA should be investigated further.

3.6 CONCLUSION

Our findings have shown a statistically significant correlation between high-grade disease on a baseline ^{99m}Tc-glucosamine scan and the presence of high levels of RF and anti-CCP antibodies in patients with RA. This radiopharmaceutical might be of value in identifying patients requiring more aggressive treatment in the management of their RA to prevent bone erosion and irreversible joint damage.

Data availability statement

Data are available from the corresponding author upon reasonable request.

Funding

Funds were received from the Multi-, Inter- and Trans- (MIT) Disciplinary Collaborative Research Grant under the University of the Free State (UFS) and Central University of Technology Free State (CUT) research collaboration project, and the South African National Research Foundation (NRF) (grant number 129902).

Disclosure of conflicting interest statement

The authors do not have any conflicting interests to disclose.

Ethics approval

The research was approved by the Health Sciences Research Ethics Committee (HSREC) of the University of the Free State, Bloemfontein, South Africa.

Acknowledgements

The authors acknowledge all the radiographers, nurses and staff of the Department of Nuclear Medicine and staff of the Division of Rheumatology, Department of Internal Medicine, Universitas Academic Hospital. The authors also acknowledge Goorhoo Pryaska for preparation of the radiopharmaceutical, and Dr. Daleen Struwig, medical writer, for technical and editorial preparation of the article.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative artificial intelligence (AI) and AI-assisted technologies have been used in the writing of this article.

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CHAPTER 4
HEAD-TO-HEAD COMPARISON OF ULTRASOUND AND ^{99M}Tc-GLUCOSAMINE SPECT/CT IMAGING OF PATIENTS WITH RHEUMATOID ARTHRITIS – A SINGLE CENTER PROSPECTIVE STUDY[3]

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4.1 ABSTRACT

Background

Rheumatoid arthritis (RA) is an inflammatory disease that can lead to progressive joint damage. Early identification of synovitis is key in the management of patients with RA. The aim of this study was to assess synovitis in patients with RA using ^{99m}Tc-glucosamine single photon emission computed tomography/computed tomography (SPECT/CT) imaging and compare this radiopharmaceutical's diagnostic performance with ultrasound (US) imaging.

Methods

This prospective study included 22 participants with active RA and 380 joints were assessed with SPECT/CT and US imaging. SPECT/CT imaging of the joints of interest was performed in each participant three hours after injection of ^{99m}Tc-glucosamine, with US imaging of the same joints performed on the same day. The affected joints were qualitatively assessed for ^{99m}Tc-glucosamine uptake and compared with the findings on US imaging.

[3] Submitted to *Egyptian Journal of Radiology and Nuclear Medicine* (cf. Appendix J).

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Results

Abnormal increased uptake of ^{99m}Tc-glucosamine, localised specifically to the synovial space, was noted in the affected joints. The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of ^{99m}Tc-glucosamine SPECT/CT imaging were 86%, 60%, 61%, 85% and 73%, respectively. A sensitivity of 100% was noted in the identification of synovitis in the carpal and knee joints. Disease activity in the distal interphalangeal (DIP) joints was not observed with either SPECT/CT or US.

Conclusions

SPECT/CT imaging with ^{99m}Tc-glucosamine is a valuable tool for assessing disease activity in the joints of patients with RA. It has a very high sensitivity in detecting synovitis and it correlates very well with US imaging in this regard.

Keywords: rheumatoid arthritis; disease activity; synovitis; ultrasound; ^{99m}Tc-glucosamine; SPECT/CT; single photon emission computed tomography

4.2 BACKGROUND

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by progressive damage to the joints. Joint damage begins early in the course of the disease as a consequence of the active inflammation and can lead to progressive and irreversible disability [1]. Therefore, prompt diagnosis and early treatment are imperative in patients with RA. For early diagnosis to be feasible, an investigation that can offer an accurate assessment of disease activity, with very high sensitivity even in the setting of subclinical disease, is required. Several methods to evaluate disease activity in RA are available. These methods involve clinical assessment of involved joints, laboratory and imaging investigations, or a combination of two or more methods.

A number of validated clinical scoring systems, including the 28-Joint Disease Activity Score (DAS-28), the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI), allow for the classification of the patient as being in remission or presenting with low, moderate, or high disease activity [2;3]. However, non-specific constitutional symptoms such as fatigue and malaise, rather than arthritis, predominate in some patients [4] and the subjective nature of a clinical assessment reduces the diagnostic accuracy of these validated clinical scoring methods. Laboratory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are nonspecific tests that are occasionally helpful in distinguishing between inflammatory and non-inflammatory conditions. However, these tests are not diagnostic for RA and may be abnormal in many infectious, malignant, and other disease conditions [5]. Several imaging modalities can also be used and have been shown to be useful

in the management of patients with RA. The most commonly used modality is X-ray imaging, despite knowing that structural changes such as erosion may not be evident in early disease [6–8]. X-ray has the best-established role in identifying progressive joint damage. However, its sensitivity is inadequate for identifying synovitis and early erosive lesions [8;9].

The new classification criteria often used to diagnose RA, proposed by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR), incorporated the role of ultrasound (US) and magnetic resonance imaging (MRI) in the detection of synovitis, thus enabling earlier diagnosis and correct classification of patients [3;6]. In recent years, the use of US imaging in the assessment of inflammatory joints, especially due to RA, has increased. Universally, US is one of the most widely available, safe and cost-effective imaging modalities used for evaluating patients with RA. In addition, it offers the prospect of a more accurate assessment of soft tissue inflammation than conventional clinical examination [10]. Synovitis and effusions are readily observed with US, and it has been shown to detect synovitis more accurately than clinical examination [10]. However, the drawback of US is that it is operator-dependent [4;11] and cumbersome, especially when almost every single joint in both hands needs to be evaluated. Performing the procedure could become even more labour-intensive and time-consuming when additional joints besides those in the hands also need to be examined. The currently accepted practice, however, is to use morphological changes (synovial effusion and hypertrophy) occurring on US gray scale imaging and hypervascularity observed by power Doppler to grade disease activity [12]. Both US gray scale and power Doppler findings have been shown to be very sensitive to changes in disease activity [10].

Positron emission tomography/computed tomography (PET/CT) imaging of RA using fluorine-18-fluorodeoxyglucose (^{18}F-FDG) is becoming a popular nuclear medicine imaging modality in patients with RA. Evidence has been reported extensively in the literature that PET-CT is very sensitive in evaluating disease activity [13–19]. Technetium-99-metastable ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG), also referred to as ^{99m}Tc-glucosamine, is a single-photon emission computed tomography (SPECT) radiotracer for functional imaging [20]. This radiopharma-ceutical has not been as widely investigated as ^{18}F-FDG in evaluating disease activity in patients with RA. However, few investigators have reported good sensitivity of ^{99m}Tc-glucosamine in evaluating the disease activity of patients with RA [11;20]. Not every nuclear medicine center has access to a PET/CT camera, making ^{99m}Tc-glucosamine imaging an alternative in centers with only SPECT/CT cameras. However, ^{99m}Tc-glucosamine must be compared extensively to one of the valid, sensitive, and reliable imaging modalities, such as US, before it should be considered a good alternative to ^{18}F-FDG imaging in patients with RA.

No studies that extensively evaluated the diagnostic performance of this radiopharmaceutical in RA have been reported in the literature. Therefore, the aim of this prospective study was to compare the diagnostic outcome of ^{99m}Tc-glucosamine with US in the evaluation of disease activity in patients with RA.

4.3 METHODS

4.3.1 Study design and population

For this prospective cross-sectional study, 22 patients diagnosed with RA by an experienced rheumatologist (25 years' experience) according to the ACR/EULAR classification criteria, were recruited to participate. The participants were recruited from the rheumatology clinic at our institution between February and October 2022. Twelve participants had confirmed RA affecting the hands, while 10 had confirmed RA affecting the knees. With the exception of pregnant or lactating female participants, any participant older than 18 years of age was eligible for inclusion in the study.

4.3.2 Synthesis of the radiopharmaceutical ^{99m}Tc-glucosamine

The ECDG ligand was synthesised according to the procedure described by Yang et al. [21]. The ECDG ligand was successfully formulated into buffered kits that were stably stored in a -80°C freezer. Fifty to sixty millicurie (mCi) of Tc-99m pertechnetate (^{99m}TcO4-) was added to a prepared, lyophilised ECDG kit vial. The solution was then heated at 75°C for 15 minutes. Quality control was performed on the radiolabeled product by testing pH (pH 5.5) and radiochemical purity (> 95%). Radiochemical purity was determined by using high-performance liquid chromatography on a Varian Prostar 325 UV/Vis HPLC machine (Varian Medical Systems Inc.; Palo Alto, CA, USA) fitted with a radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany) and thin-layer chromatography (Raytest GmbH; Straubenhardt, Germany) [22].

4.3.3 ^{99m}Tc-glucosamine administration

The prepared radiolabeled solution was added to 3 mL of sterile saline and then filtered into a sealed sterile vial. Additional saline was added to the vial to ensure a final volume of approximately 5 mL. A participant activity of 20–25 mCi was withdrawn for intravenous administration [22].

4.3.4 Imaging protocol and processing

All 22 participants were scanned using a dual-head gamma camera (Siemens Symbia T16 True Point SPECT-CT; Siemens Medical Solutions; Malvern, PA, USA). The SPECT/CT camera was equipped with a low-energy, high-resolution collimator (LEHR). Three hours after radiopharma-ceutical administration, SPECT images of the relevant joints (either the hands/wrist or knees) were performed at 25 s/stop, with 3° steps, in a 128 × 128 matrix. This was followed by a low dose, non-contrast CT with the patient in the same bed position. Images were processed using the Syngo workstation (Siemens Medical Solutions; Malvern, PA, USA) on the gamma camera. SPECT images were reconstructed using an iterative algorithm and SPECT/CT fusion images were obtained using the multimodality Syngo imaging software on the workstation.

4.3.5 Ultrasound imaging

Ultrasound was performed by an experienced musculoskeletal ultrasonographer on the same day as the ^{99m}Tc-glucosamine using a Samsung HS70A (Innovative Ultrasound Imaging, Inc.; San Diego, CA, USA) high frequency 15 MHz transducer with power Doppler functions.

4.3.6 Data collection and analysis

The data of each patient were collected using an Excel 2019 spreadsheet (Microsoft Corporation; Redmond, WA, USA) and analysed by means of the statistical package Stata version 16 (StataCorp LP; College Station, TX, USA). Results for variables such as sex were presented as frequencies and percentages. Age was presented as the median and interquartile range. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for ^{99m}Tc-glucosamine imaging were calculated.

4.3.7 Image interpretation

The attenuated corrected and non-corrected SPECT images were qualitatively assessed for an abnormally increased uptake of the radiopharmaceutical localised in the joint space on CT by an experienced nuclear medicine physician (7 years' experience). Abnormally increased uptake of ^{99m}Tc-glucosamine suggestive of disease activity was identified as activity exceeding that of the surrounding muscle. On US imaging, synovitis was identified by an experienced musculoskeletal radiologist (10 years' experience) by assessing synovial hypertrophy detected

on US grey scale and a positive power Doppler signal. Both the nuclear medicine physician and radiologist were blinded to each other's findings.

4.3.8 Ethical considerations

Ethics approval was obtained from the institutional Health Sciences Research Ethics Committee (HSREC) (reference number UFS-HSD2020/1292/2411). Written informed consent was obtained from each participating patient.

4.4 RESULTS

The median age was 59 years (interquartile range [IQR] 49–68 years) and the vast majority of patients were female (n=21; 95.5%). Optimal images with a good target-to-background ratio were obtained for all the participants. All the participants with positive disease on ^{99m}Tc-glucosamine imaging had an abnormally increased uptake of the radiopharmaceutical localised to the synovial space, with no bone uptake. An example is illustrated in Figure 4.1, showing ^{99m}Tc-glucosamine SPECT/CT and SPECT images of the knee of a 72-year-old female with abnormal increase uptake of the radiopharmaceutical in both knees, localised to the synovial space, with no abnormal bone uptake. Twelve (54.5%) participants had the joints of their hands and wrists evaluated, while in the remaining 10 (45.5%) patients, the knee joints were evaluated. A total of 380 joints were evaluated for disease activity, representing 360 joints in both hands, which included the metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints and distal interphalangeal (DIP) joints. Twenty-four carpal joints and 20 knee joints were also assessed for disease activity. Each knee joint was further subdivided into four regions (suprapatellar, lateral, medial and popliteal regions). Both US and ^{99m}Tc-glucosamine imaging did not detect disease activity in any of the DIP joints.

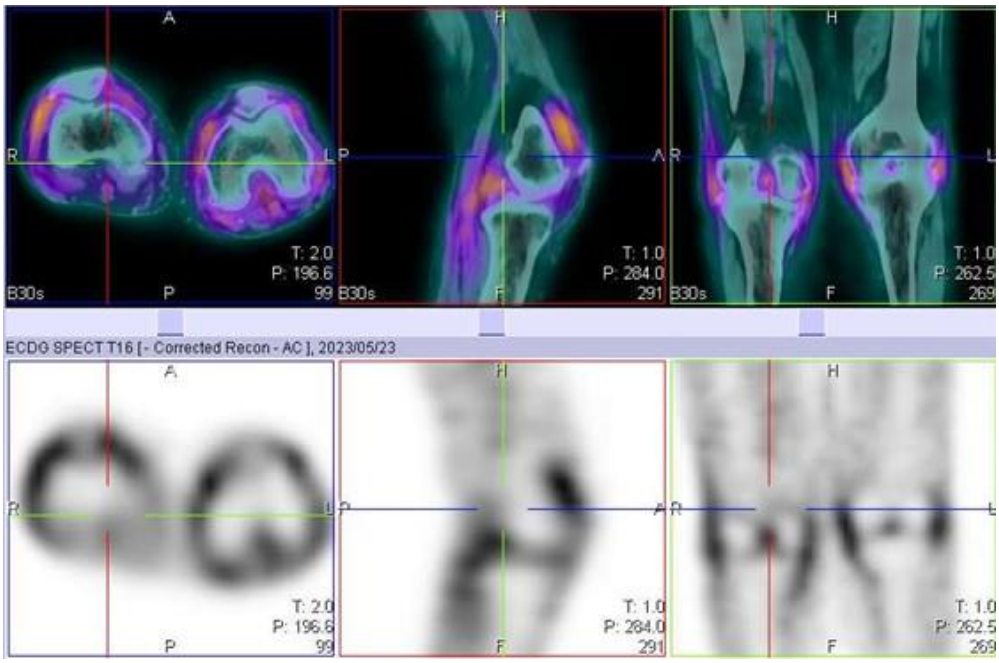

Fig. 4.1. ^{99m}Tc-glucosamine SPECT/CT and SPECT images of the knee of a 72-year-old female showing abnormal increased uptake of the radiopharmaceutical in both knees, localised to the synovial space, with no abnormal bone uptake

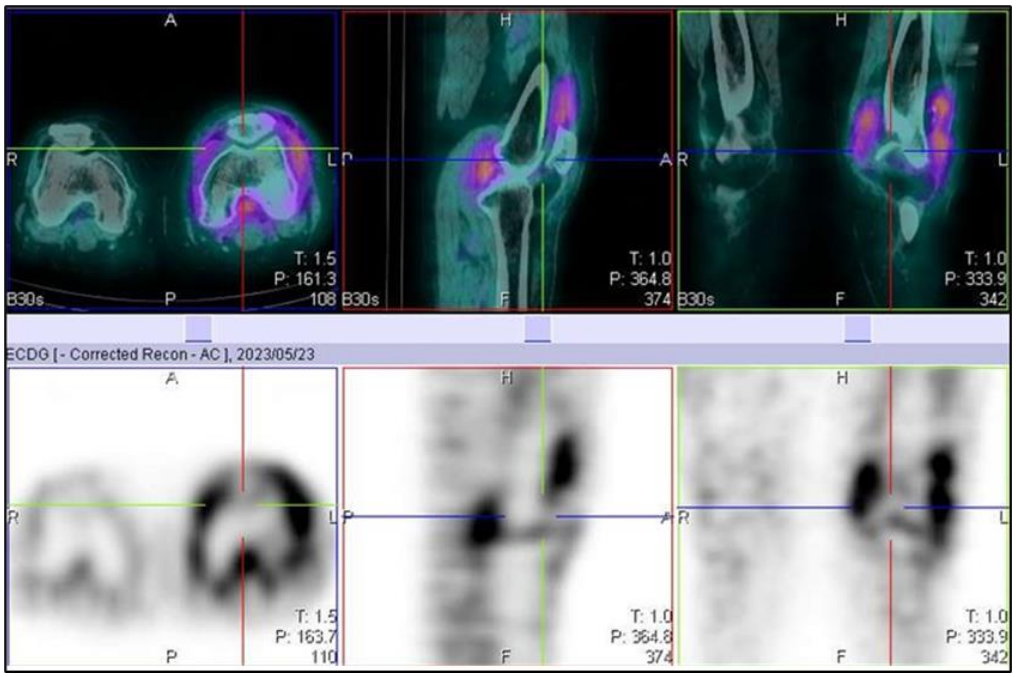

Fig. 4.2. ^{99m}Tc-glucosamine SPECT/CT and SPECT images of the knee of a 51-year-old female showing abnormal increase uptake of the radiopharmaceutical in all four regions of the left knee joint. Baseline ultrasound imaging was initially negative for disease in the popliteal, medial and lateral regions of the joint, but positive on subsequent 6 weeks follow-up imaging

Assessing the total number of joints and regions, ^{99m}Tc-glucosamine imaging had an overall sensitivity of 86%, specificity of 60%, PPV of 61%, NPV of 85% and accuracy of 73%. The diagnostic performance of ^{99m}Tc-glucosamine imaging in the various group of joints is summarised in Table 4.1. The diagnostic accuracy of ^{99m}Tc-glucosamine imaging was 70% when considering all four regions in each of the knee joints (a total of 80 locations), as shown in Table 4.2.

Table 4.1. Diagnostic performance of ^{99m}Tc-glucosamine imaging in the different joint groups

Joint group	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Accuracy (%)
Hand (n=336)	83	65	70	80	74
Carpal (n=24)	100	73	25	100	86
Knee (n=20)	100	90	33	100	95

[a]PPV, positive predictive value; [b]NPV, negative predictive value

Table 4.2. Diagnostic performance of ^{99m}Tc-glucosamine imaging in the different regions of the knee joint

Region	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Accuracy (%)
All regions (n=80)	96	44	44	96	70
Suprapatellar (n=20)	75	100	100	86	86
Lateral (n=20)	100	17	44	100	58
Medial (n=20)	100	15	33	100	57
Popliteal (n=20)	100	25	25	100	63

[a]PPV, positive predictive value; [b]NPV, negative predictive value

4.5 DISCUSSION

To the authors knowledge, this has been the first prospective study to directly compare SPECT/CT ^{99m}Tc-glucosamine imaging with US imaging in the evaluation of disease activity in joints affected by RA. Using arthroscopy or MRI as the gold standard, a number of studies found that grey scale and Doppler US imaging had a very high diagnostic accuracy in the evaluation of synovitis in patients with RA (sensitivity of over 85% and specificity of over 95%) [8;23]. We observed an overall sensitivity of 86% for ^{99m}Tc-glucosamine SPECT/CT imaging in identifying active disease in patients with RA compared to US imaging. This finding was consistent with an earlier hypothesis by Manolios et al. [11], who confirmed that ^{99m}Tc-glucosamine imaging of patients with RA had a good correlation with CRP and clinical

assessment (Spearman's correlation coefficient, p=0.048 and p=0.003 respectively) and could identify synovitis in all affected joints [11]. In our study, the sensitivity of ^{99m}Tc-glucosamine imaging reached the 100% in the knee and carpal joints, compared to 83% in the joints of the hands. This finding might be suggestive of US being slightly more sensitive in detecting disease activity in the small joints of the hand than ^{99m}Tc-glucosamine imaging.

The overall specificity of ^{99m}Tc-glucosamine imaging in our study was 60%. Angelides et al. [20] reported that ^{99m}Tc-glucosamine uptake was present at sites of osteoarthritis (OA) and subclinical OA. We suspect that the specificity of 60% might be associated with radiopharmaceutical uptake in unknown sites of osteoarthritis, subclinical disease, or a combination of both. The site with the highest level of specificity (90%) were the knees when considered as a single site, and not split into the four separate regions. However, when broken down into the different regions, the specificity dropped to 44%, which was most probably due to abnormal ^{99m}Tc-glucosamine uptake in the medial and lateral regions, with specificities of 15% and 17%, respectively, as shown in Table 4.2. Again, this might be attributed to the identification of subclinical disease not observed on the US imaging, or non-specific uptake in co-existing OA. What might have favored the identification of subclinical disease was the fact that in six of the ten participants who had a follow-up US six weeks after their baseline US, sonographic evidence of synovitis was discovered in a total of 10 knee regions previously negative for synovitis on the baseline US examination, although it had been positive on the baseline ^{99m}Tc-glucosamine imaging. An example of this observation is illustrated in Figure 4.2, which shows ^{99m}Tc-glucosamine SPECT/CT and SPECT images of the knee of a 51-year-old female with abnormal increase uptake of the radiopharmaceutical in all four regions of the left knee joint. Baseline ultrasound imaging was initially negative for disease in the popliteal, medial and lateral regions of the joint, but positive on subsequent 6 weeks follow-up imaging.

It has been reported in the literature that various nuclear medicine imaging modalities compare well with conventional imaging in identifying active disease in patients with RA [13;14;24–30]. The most popular modality is ^{18}F-FDG PET/CT imaging, which seems to be similar to ^{99m}Tc-glucosamine imaging, as both radiopharmaceuticals are radiolabeled analogues of glucose. Beckers et al. determined that positive ^{18}F-FDG PET findings correlated well with US imaging in detecting active disease in the joints of patients with RA [17], similar to the finding observed in our study. We, however, do recommend that a more robust study similar to ours be performed with ^{18}F-FDG PET/CT.

A limitation of this study was not identifying and/or excluding participants with possible co-existing OA. It has previously been shown that patients with OA might have mildly increased

^{99m}Tc-glucosamine uptake [20], a finding that might be difficult to differentiate from subclinical RA. Another very important limitation is the absensce of a recognised gold standard, like MRI or tissue biopsy in this study that would have been used to compare ^{99m}Tc-glucosamine and US imaging findings. This would have given a more accurate picture on the diagnostic accuracy of ^{99m}Tc-glucosamine imaging. It would have also addressed some of the shortcomings of US, like its operator dependent nature.

4.6 CONCLUSIONS

This study demonstrated that SPECT/CT imaging with ^{99m}Tc-glucosamine had a high sensitivity in detecting synovitis in patients with RA, which compares well with US imaging. However, the specificity was low, which could be attributed to nonspecific uptake of the radiopharmaceutical in patients with co-existing OA or the identification of subclinical disease that had been missed on US imaging. We are of the opinion that this is an area that might warrant more research for clarity. Overall, ^{99m}Tc-glucosamine might have a role to play in the evaluation of disease activity in patients with RA, especially in centers that do not have PET/CT cameras for ^{18}F-FDG PET/CT imaging.

Acknowledgements: The authors acknowledge all the radiographers, nurses and staff of the Department of Nuclear Medicine and staff of the Division of Rheumatology, Department of Internal Medicine, Universitas Academic Hospital. The authors also acknowledge Goorhoo Pryaska for preparation of the radiopharmaceutical.

Funding: Funds were received from the Multi-, Inter- and Trans- (MIT) Disciplinary Collaborative Research Grant under the University of the Free State (UFS) and Central University of Technology Free State (CUT) research collaboration project, and the South African National Research Foundation (NRF) (grant number 129902).

Data availability: Data are available from the corresponding author upon reasonable request.

Ethics approval: Ethics approval was obtained from the institutional Health Sciences Research Ethics Committee (HSREC) (reference number UFS-HSD2020/1292/2411).

Consent to participate: Written informed consent was obtained from each participating patient.

Consent for publication: Patients were informed that research findings will be published.

Competing interests: The authors do not have any competing interests to declare

Declaration of generative AI and AI-assisted technologies in the writing process: The authors declare that no generative artificial intelligence (AI) and AI-assisted technologies have been used in the writing of this article.

List of abbreviations

^{18}F-FDG	Fluorine-18-fluorodeoxyglucose
^{99m}Tc-ECDG	Technetium-99-metastable ethylenedicysteine-deoxyglucose
$^{99m}TcO_4^-$	Tc-99m pertechnetate
ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
CDAI	Clinical Disease Activity Index
CRP	C-reactive protein
DAS-28	28-Joint Disease Activity Score
DIP	distal interphalangeal
ESR	erythrocyte sedimentation rate
HSREC	Health Sciences Research Ethics Committee
IQR	interquartile range
LEHR	low-energy, high-resolution collimator
mCi	millicurie
MCP	metacarpophalangeal
MRI	magnetic resonance imaging
NPV	negative predictive value
OA	osteoarthritis
PET/CT	positron emission tomography/computed tomography
PIP	proximal interphalangeal
PPV	positive predictive value
RA	rheumatoid arthritis
SDAI	Simplified Disease Activity Index
SPECT	single-photon emission computed tomography
US	ultrasound

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CHAPTER 5
PROSPECTIVE SIX-MONTH FOLLOW-UP STUDY COMPARING ^{99M}Tc-GLUCOSAMINE SPECT/CT IMAGING AND DOPPLER ULTRASOUND IMAGING IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS[4]

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5.1 ABSTRACT

Introduction. Rheumatoid arthritis (RA) is a systemic inflammatory disorder characterised by the development of joint synovitis, usually associated with pain and swelling. Treatment monitoring remains challenging for rheumatologists as it may be difficult to differentiate true remission from subclinical disease. The aim was to carry out a prospective six-month follow-up study comparing ^{99m}Tc-glucosamine SPECT/CT imaging and Doppler ultrasound (US) imaging in the treatment monitoring of patients with RA on disease-modifying antirheumatic drugs (DMAD).

Methods. This prospective study included 20 participants diagnosed with active RA. Each participant had their affected joints assessed with ^{99m}Tc-glucosamine SPECT/CT and US imaging on the same day at baseline, six weeks and six months after commencing therapy. The affected joints were qualitatively assessed for ^{99m}Tc-glucosamine uptake and compared with the findings on US imaging at all three different time points to evaluate treatment response.

[4] Submitted to *Journal of Nuclear Medicine* (cf. Appendix K).

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Results. In total, 348 joints were evaluated by both imaging modalities. Eleven participants had the joints of their hands and wrists evaluated, while the remaining nine had the knee joints evaluated. The level of agreement between US and ^{99m}Tc-glucosamine imaging in assessing therapy response was 33.3%, 11.6% and 6.7% for the knees, hands, and wrists, respectively.
Conclusion. The results of this study suggest that ^{99m}Tc-glucosamine SPECT/CT imaging could be a valuable tool in the assessment of disease activity in patients with RA undergoing treatment. However, it had a poor correlation in this assessment when compared to US findings.

Keywords: rheumatoid arthritis; ultrasound; ^{99m}Tc-glucosamine; SPECT/CT; follow-up

5.2 INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease, which, if not treated properly, can lead to irreversible joint damage, deformities and disability (*1*). Early detection of synovitis is crucial for initiating treatment and preventing progression of the disease (*2*). However, monitoring disease activity in clinical practice is always challenging (*3*). Bone erosion, a common characteristic of RA, has been shown to occur even in patients considered to be in clinical remission or those presenting with normal radiological findings (*4*). For this reason, it is vital to have a diagnostic modality that is very sensitive in identifying active disease in patients in clinical remission.

In individuals with subclinical disease and normal radiological findings, magnetic resonance imaging (MRI) and Doppler ultrasound (US) imaging are able to detect inflammatory soft tissue changes and even bone changes (*5*). These imaging modalities offer the rheumatologist the opportunity of not discontinuing medication in these patients, thus preventing continuous bone erosion and other complications of RA. However, these imaging modalities are not without shortcomings. Ultrasound is operator-dependent (*6,7*) and cumbersome, especially when the operator must evaluate almost every joint in both hands. Despite US being considered a valid, sensitive and reliable imaging modality, some studies had earlier indicated that it failed to demonstrate superior sensitivity to change in disease activity, compared to clinical examination (*3,8*).

Magnetic resonance imaging, on the other hand, has the limitation of being able to screen only a single joint/region at a time. MRI is also very expensive, which increases the overall cost of patient management.

A limited number of studies have shown that technetium 99m-glucosamine (^{99m}Tc-glucosmaine) may be a useful radiopharmaceutical in detecting early synovitis and following

up patients with RA (*6,9*). ^{99m}Tc-glucosmaine is a single-photon emission computed tomography (SPECT) radiopharmaceutical and is also known as ^{99m}Tc-ethylenedicysteine-deoxyglucose (ECDG). Both these terms are used interchangeably in this article. Furthermore, ^{99m}Tc-glucosmaine is a radiolabeled analogue of glucose, and is thought to have properties that are similar to the positron emission tomography (PET) radiopharmaceutical fluorine 18-fluorodeoxyglucose (^{18}F-FDG) (*9*).

Numerous studies in the literature have shown the potential value of ^{18}F-FDG PET computed tomography (CT) in the management of patients with RA (*10–18*). However, it is important to emphasise that not all nuclear medicine facilities have PET/CT cameras in addition to their SPECT or SPECT/CT cameras. We have therefore decided to conduct a prospective six-month follow-up study comparing ^{99m}Tc-glucosamine SPECT/CT imaging and Doppler US imaging on the treatment monitoring of patients with RA on disease-modifying antirheumatic drugs (DMAD). The hypothesis was that ^{99m}Tc-glucosmaine SPECT/CT imaging might be a sensitive tool in identifying changes in disease activity of patients with RA on treatment, recognising subclinical disease and therefore offering an opportunity to centers with only SPECT/CT cameras for the imaging of these patients.

5.3 MATERIALS AND METHODS

5.3.1 Study design and population

This prospective cross-sectional study was conducted at a single center in South Africa. Twenty-two participants with confirmed RA, diagnosed by an experienced board-certified rheumatologist (25 years' experience) according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria, were selected. The participants were recruited from the rheumatology clinic at our institution between February and October 2022. Each participant was intended to have two follow-up visits at six weeks and six months, respectively, after their baseline visit. Of the original 22 patients, 12 participants had confirmed RA affecting the hands/wrists, while 10 had confirmed RA involving the knees. However, only 20 participants attended both follow-up visits and were included in the final analysis. The two participants excluded suffered from RA of the hand/wrist and knee joints, respectively, consequently resulting in 11 patients with affected hands/wrists and nine with affected knees.

Both US and ^{99m}Tc-glucosamine SPECT/CT imaging were performed on the same day in each participant on day one, and on their six weeks and six months follow-up visits. The specific joints evaluated included the metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints, distal interphalangeal (DIP) joints, carpal joints and knee joints. The knee joints were, however, further categorized into four regions, namely suprapatellar, lateral, medial and popliteal regions. Disease activity was evaluated in each of these four regions.

5.3.2 Inclusion criteria

Participants were included in the study if they were ≥18 years of age, had proven active RA and had either not started DMAD or were non-compliant with their medications, and those who had baseline, six weeks and six months follow-up studies performed.

5.3.3 Exclusion criteria

Pregnant and breastfeeding participants were excluded from the study. For the current component of the study, two patients who did not present for both six weeks and six months follow-up visits were also excluded.

5.3.4 Synthesis of the radiopharmaceutical ^{99m}Tc-glucosamine

The ECDG ligand was synthesised according to the procedure described by Yang et al. (*19*). The ECDG ligand was successfully formulated into buffered kits that were stably stored in a –80°C freezer. Fifty to 60 millicurie (mCi) of ^{99m}Tc pertechnetate ($^{99m}TcO4^{-}$) was added to a prepared, lyophilised ECDG kit vial. The solution was then heated at 75°C for 15 minutes. Radiochemical purity (>95%) was determined by using high-performance liquid chromatography (HPLC) on a Varian Prostar 325 UV/Vis HPLC machine (Varian Medical Systems Inc.; Palo Alto, CA, USA) fitted with a radiometric GABI Star gamma detector (Raytest GmbH; Straubenhardt, Germany) and thin-layer chromatography (Raytest GmbH; Straubenhardt, Germany) [20].

5.3.5 ^{99m}Tc-glucosamine administration

The prepared radiolabeled product was added to 3 mL of sterile saline and then filtered into a sealed sterile vial. A final volume of approximately 5 ml was ensured by the addition of more saline. An activity of 20–25 mCi for each participant was withdrawn for intravenous administration [20].

5.3.6 Imaging protocol and processing

All 20 participants were scanned using a dual-head gamma camera (Siemens Symbia T16 True Point SPECT-CT; Siemens Medical Solutions; Malvern, PA, USA). The SPECT/CT camera was equipped with a low-energy, high-resolution (LEHR) collimator. Three hours after administration of the radiopharmaceutical, SPECT/CT images of the relevant joints (either the hands/wrist or knees) were performed at 25 s/stop, with 3° steps, in a 128 × 128 matrix. This was followed by a low-dose, non-contrast CT with the patient in the same bed position. Images were processed using the Syngo workstation (Siemens Medical Solutions; Malvern, PA, USA) on the gamma camera. SPECT/CT images were reconstructed using an iterative algorithm, and fusion images were obtained using the multimodality Syngo imaging software on the workstation.

5.3.7 Ultrasound imaging

Ultrasound was performed by an experienced musculoskeletal ultrasonographer (15 years' experience) on the same day as the ^{99m}Tc-glucosamine using a Samsung HS70A (Innovative Ultrasound Imaging, Inc.; San Diego, CA, USA) high-frequency 15 MHz transducer with power Doppler functions.

5.3.8 Data collection and analysis

The data of each patient were collected using an Excel 2019 spreadsheet (Microsoft Corporation; Redmond, WA, USA). Statistical analysis was performed by a statistician using R, version 4.3.0 (R Foundation for Statistical Computing; Vienna, Austria). For variables such as sex, the results were presented as frequencies and percentages. Age was presented as the median and interquartile range. The level of agreement between the findings on US follow-up and ^{99m}Tc-glucosamine follow-up was expressed as percentages.

5.3.9 Image interpretation

The attenuated corrected and non-corrected SPECT images were qualitatively assessed for an abnormally increased uptake of the radiopharmaceutical localised in the joint space on CT by an experienced nuclear medicine physician (7 years' experience). Abnormally increased uptake of ^{99m}Tc-glucosamine suggestive of disease activity was identified as activity exceeding that of the surrounding muscle. This was also graded from 0 to 4, with grade 0 being activity similar to that of the surrounding muscle and grade 4 representing activity markedly greater

than that of the surrounding muscle. On US imaging, synovitis was identified by an experienced musculoskeletal radiologist (10 years' experience) by assessing synovial hypertrophy detected on US grey scale and a positive power Doppler signal. The nuclear medicine physician and radiologist were blinded to each other's findings.

5.3.10 Evaluation of treatment response

Participants were classified into six groups according to their responses at six weeks and six months. These groups included patients showing either no response, transient response (response only at six weeks but not sustained), sustained response (response at six weeks and six months), worsened disease, fully resolved disease and new disease. Any reduction in the initial grade of uptake on ^{99m}Tc-glucosamine on the initial follow-up imaging was recognised as at least transient response, and sustained response if it remained the same or was further reduced to a lower grade at the six-month imaging.

5.3.11 Ethical considerations

Ethical approval for the research was obtained from the institutional Health Sciences Research Ethics Committee (HSREC) (reference number UFS-HSD2020/1292/2411). Written informed consent was obtained from each participating patient.

5.4 RESULTS

The vast majority of participants were female (n=19; 95.5%), and the median age of the study population was 59 years (interquartile range [IQR] 49–68 years). Eleven (55%) participants had the joints of their hands and wrists evaluated, while in the remaining nine (45%) participants, the knee joints were evaluated. In total, 308 joints in both hands were evaluated, which included the MCP joints, PIP joints, DIP joints and 22 carpal joints. Eighteen knee joints (72 knee regions) were also evaluated for disease activity. This came to a total of 348 joints that were evaluated with US and ^{99m}Tc-glucosamine SPECT/CT imaging at baseline and follow-up at six weeks and six months.

Both imaging modalities did not detect active disease in all the DIP joints. However, this is expected as it is well known that the DIP joints are rarely affected. Overall, ^{99m}Tc-glucosamine SPECT/CT imaging detected more joints with active disease on baseline evaluation in all three joint groups compared to US imaging, as shown in Table 5.1. An important finding was that six of the participants had new disease observed in three joints in the hand and seven in knee

regions on their six weeks follow-up US (Table 5.2 and 5.3, respectively). These observations were evident previously on their baseline ^{99m}Tc-glucosamine scan but not their baseline US examination. Tables 5.2, 5.3 and 5.4 show the percentages of the various response outcomes using both imaging modalities. The level of agreement between US and ^{99m}Tc-glucosamine imaging in assessing therapy response was 33.3%, 11.6% and 6.7% for the knees, hands, and wrists, respectively.

Table 5.1. Percentage of joints/regions of active disease detected at baseline with both imaging modalities.

Joint group	Imaging modality	
	Doppler ultrasound	^{99m}Tc-glucosamine
	n (%)	n (%)
Hand (n=308)	125 (40.5)	151 (49.2)
Wrist (n=22)	15 (68.2)	20 (90.9)
Knee regions (n=72)	24 (33.3)	54 (75.0)

Table 5.2. Percentage of the various response outcomes involving the joints of the hands detected on baseline by both modalities.

Response outcome	Imaging modality	
	Doppler ultrasound (n=92)	^{99m}Tc-glucosamine (n=115)
	n (%)	n (%)
No response	37 (40.2)	22 (19.1)
Transient response	3 (3.3)	29 (25.2)
Sustained response	16 (17.4)	42 (36.5)
Worsened disease	1 (1.1)	11 (9.6)
Fully resolved disease	32 (34.8)	4 (3.5)
New disease	3 (3.3)	7 (6.1)

Table 5.3. Percentage of the various response outcomes involving the joints of the wrists detected on baseline by both modalities.

Response outcome	Imaging modality	
	Doppler ultrasound (n=15)	^{99m}Tc-glucosamine (n=21)
	n (%)	n (%)
No response	4 (26.7)	8 (38.1)
Transient response	0 (0)	2 (9.5)
Sustained response	1 (6.7)	10 (47.6)
Worsened disease	0 (0)	1 (4.8)
Fully resolved disease	10 (66.7)	0 (0)
New disease	0 (0)	0 (0)

Table 5.4. Percentage of the various respo
nse outcomes involving the joints and regions of the knees detected on baseline by both modalities.

Response outcome	Imaging modality	
	Doppler ultrasound (n=31)	^{99m}Tc-glucosamine (n=58)
	n (%)	n (%)
No response	11 (35.5)	6 (10.3)
Transient response	3 (9.7)	5 (8.6)
Sustained response	3 (9.7)	23 (39.7)
Worsened disease	0 (0)	18 (31.0)
Fully resolved disease	7 (22.6)	6 (10.3)
New disease	7 (22.6)	0 (0)

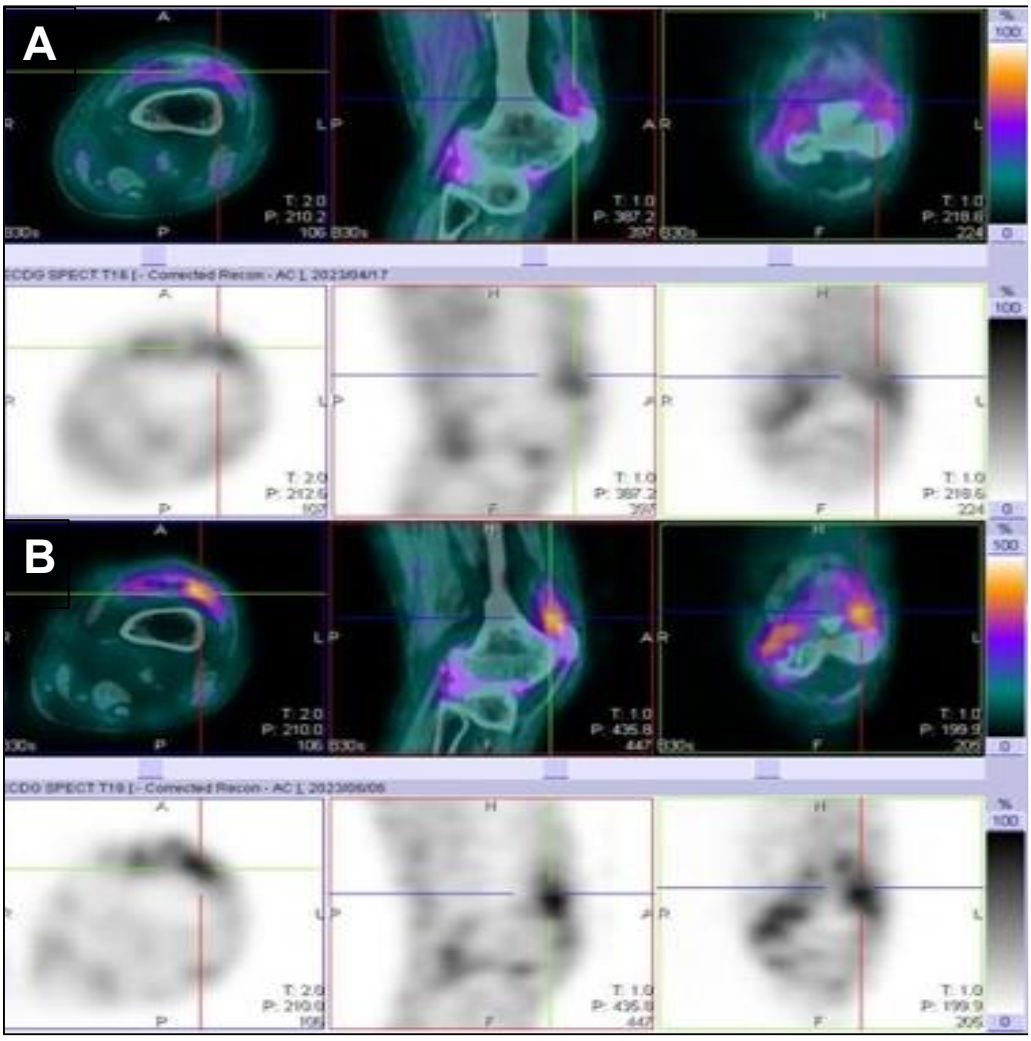

Figure 5.1. 40-year-old female with worsened disease. SPECT/CT and SPECT images in the top row (A) show increased grade 2 uptake in the medial and lateral compartment of the left knee joint, in keeping with medial and lateral synovitis. SPECT/CT and SPECT images performed after 6 months (B) show worsening of disease with grade 3 uptake. Also note the persistent grade 1 uptake in the popliteal region in keeping with persistent low grade popliteal synovitis.

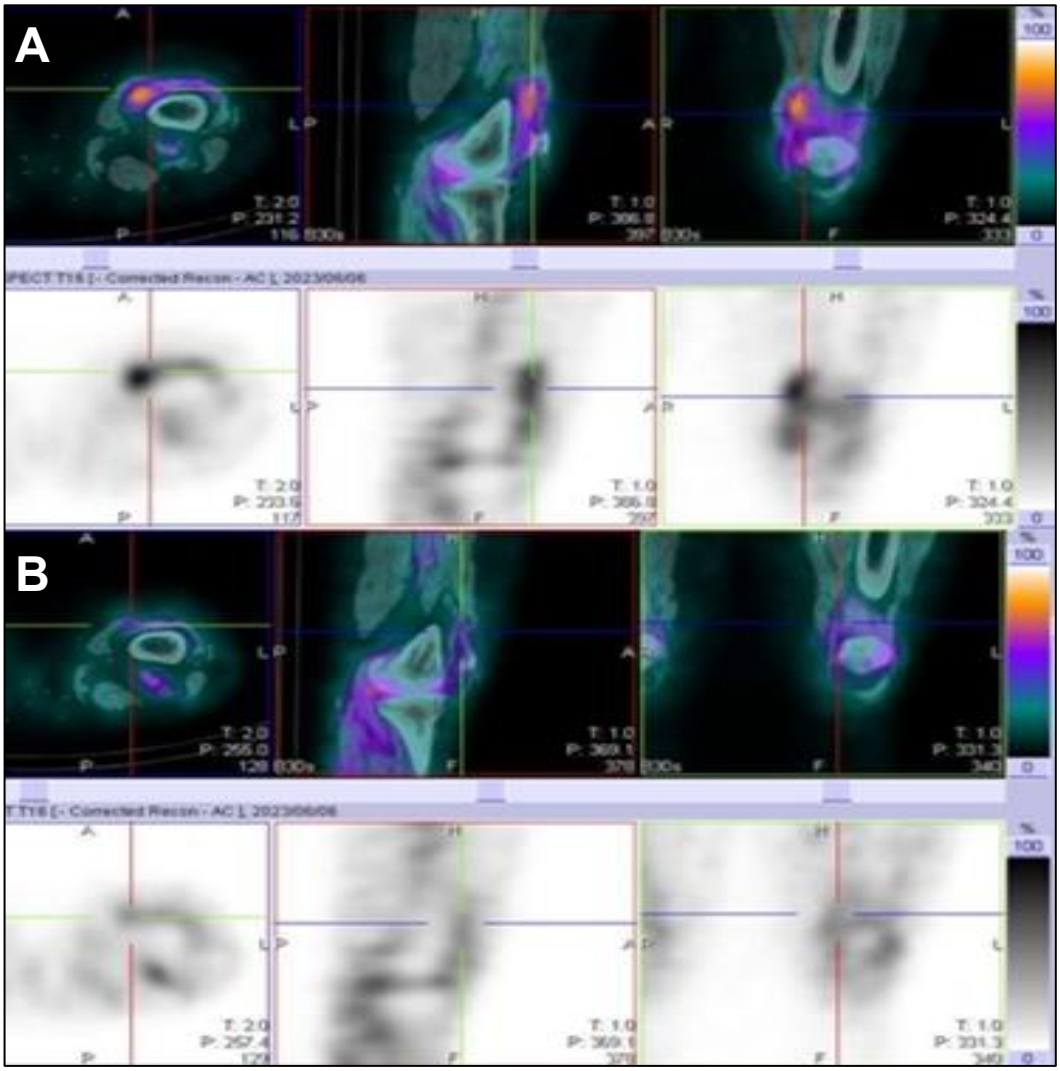

Figure 5.2. 61-year-old female with fully resolved disease. SPECT/CT and SPECT images in the top row (A) show increased grade 3 uptake in the medial compartment of the left knee joint, in keeping with medial synovitis. SPECT/CT and SPECT images performed after 6 months (B) show fully resolved disease

5.5 DISCUSSION

This study reports the first prospective follow-up study comparing ^{99m}Tc-glucosamine SPECT/CT imaging and Doppler US imaging in the treatment monitoring of patients with RA. The study showed that in all three different joint regions (hands, wrists, and knees) ^{99m}Tc-glucosamine SPECT/CT imaging detected more sites of active synovitis compared to Doppler US imaging, indicating that it might be a more sensitive modality in this regard. The available data also showed that ^{99m}Tc-glucosamine imaging could detect more patients with worsened disease in all three joint regions, as seen in Figure 5.1, which might further buttress the point that it might be more sensitive to changes in the degree of synovitis. The finding of fewer fully resolved disease (Figure 5.2) in all three joint regions with ^{99m}Tc-glucosamine might be in keeping with the ability to detect subclinical disease that would otherwise be normal on Doppler US. However, we cannot conclude with certainty on its accuracy of detecting subclinical disease, as mild osteoarthritis, a possible false positive result (*9*), was not ruled out in these participants.

A significant finding in this study was the poor agreement on the treatment response in the follow-up studies with both modalities evaluating individual joints/regions. To the best of our knowledge, no previous prospective studies have compared SPECT functional metabolic imaging and US in the assessment of treatment response in patients with RA. However, as far back as 2006, Beckers et al. reported a noteworthy agreement between changes in the metabolic activity on serial ^{18}F-FDG PET/CT studies and various MRI parameters in the assessment of treatment response in patients with RA (*18*). A more recent study confirmed that ^{18}F-FDG PET/CT is a reproducible and accurate tool for evaluating disease activity in RA, with a good correlation when compared with US findings (*13*). Although ^{99m}Tc-glucosamine and ^{18}F-FDG are both radiolabeled glucose analogs, they might not share exactly the same imaging characteristics for active synovitis. This could be why we might be getting a different result with US findings in this comparative follow-up study. ^{99m}Tc-glucosamine might be more sensitive and specific for joint synovitis, and more sensitive to disease change and detection of subclinical disease. These are all hypothetical arguments and a comparative study between both metabolic agents might be necessary in this regard.

Nevertheless, a few limitations were associated with this study. We had not been able to follow these patients up for a longer period of time to correlate metabolic response to the final outcome. Furthermore, we also did not rule out osteoarthritis in this study population, as it might be associated with potential false positive results. A very important limitation is that we were unable to identify the true diagnostic accuracy of ^{99m}Tc-glucosamine imaging as there is

still no consensus on a widely accepted reference standard. Although the literature seems to favor MRI as the modality with the highest diagnostic accuracy, both ^{99m}Tc-glucosamine and US imaging were not correlated with MRI findings. We intend to address most of these limitations with future studies.

5.6 CONCLUSION

^{99m}Tc-glucosamine SPECT/CT imaging is a valuable tool in identifying disease activity and assessing treatment response in patients with RA. The findings in this study seem to suggest that it outperforms US imaging in this regard. However, more prospective studies using a recognised valid gold standard reference are necessary. This modality definitely offers centers without PET/CT cameras the opportunity to image patients with RA.

Ethical approval and consent to participate: The study was approved by the institutional review board from the University of The Free State Health Sciences Research Ethics Committee (**UFS-HSD2020/1292/2411**), and each participant read and signed a consent form before participation. All procedures performed in studies involving human participants were in accordance with the Helsinki declaration as revised in 2013 and its later amendments.
Consent for publication: Consent was obtained for publication.
Availability of data and material: Contact the corresponding author for data requests.
Disclosure: The authors do not have any conflict of interest to declare.
Funding: Funds were received from the National Research Foundation (NRF) and the Multi-, Inter- and Trans- (MIT) Disciplinary Collaborative Research grant under the University of the Free State (UFS) and Central University of Technology Free State (CUT) Research Collaboration Project.
Authors' contributions: The study was designed by Evbuomwan Osayande. Material preparation and data collection were performed by Evbuomwan Osayande, Barend Jansen Van Rensburg and Cathryn Driver. The first draft of the manuscript was written by Evbuomwan Osayande, with Cathryn Driver contributing to the materials and methods. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
Acknowledgements: The authors, with their permission would like to acknowledge all the radiographers, nurses, and staff of the Department of Nuclear Medicine, all the staff of the Department of Rheumatology, Universitas academic hospital. The authors, with her permission would also like to acknowledge Goorhoo Pryaska.

Key points

QUESTION: Is ^{99m}Tc-glucosamine SPECT/CT imaging a useful modality in assessment of treatment response and follow up of patients with rheumatoid arthritis?

PERTINENT FINDINGS: This was a prospective cross-sectional study comparing ^{99m}Tc-glucosamine SPECT/CT imaging and Doppler ultrasound imaging in the follow up of patients with active rheumatoid arthritis on treatment. The study showed that ^{99m}Tc-glucosamine SPECT/CT imaging is a sensitive tool in the assessment of treatment response of patients with rheumatoid arthritis. However, in this study there was a poor correlation with Doppler ultrasound in the assessment of treatment response of the study participants.
IMPLICATIONS FOR PATIENT CARE: The high sensitivity of ^{99m}Tc-glucosamine SPECT/CT imaging gives the rheumatologist an opportunity of detecting synovitis in patients with low disease activity, thus preventing premature cessation of treatment, which might be detrimental to these patients.

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CHAPTER 6
CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

Rheumatoid arthritis (RA) is a systemic disease that usually presents with synovitis and can be debilitating if not appropriately managed. Identification of synovitis, even in the setting of clinical remission, is crucial in the management of these patients, as prompt diagnosis and treatment are usually associated with a better prognosis. Of all the investigative modalities used that could be employed in the management of RA, we decided to explore the use of ^{99m}Tc-EC-DG, otherwise known as ^{99m}Tc-glucosamine, in patients with RA.

6.2 OVERVIEW OF THE STUDY

The problem that was addressed is the lack of a highly sensitive, cost-effective, and easy-to-perform investigation for assessing disease activity in patients with RA, especially in those patients with low disease activity. Notably, a valid and reliable tool is necessary to monitor disease activity in these patients. The aim of this study was to assess the value of ^{99m}Tc-EC-DG imaging in the assessment of disease activity of patients with RA. To achieve this aim, the work was divided into four chapters with publishable articles, which addresses the objective of the thesis.

6.2.1 Research questions

The primary research question was stated as:

Can ^{99m}Tc-EC-DG imaging serve as a valuable tool in identifying early responders to conventional therapy in patients with RA and thus risk stratifying these patients?

The following sub-questions were also addressed:

i. Will ^{99m}Tc-EC-DG imaging detect sub-clinical disease activity in patients identified as being in clinical remission based on laboratory and clinical assessment?
ii. How does ^{99m}Tc-EC-DG imaging compare to US in monitoring disease activity in patients being treated for RA?

iii. Will ^{99m}Tc-EC-DG imaging be able to detect extra-articular disease in patients with RA and thus offering further information on prognosis?

6.2.2 Research objectives

The following objectives were perused to address these sub-questions:

- to perform an interim ^{99m}Tc-EC-DG scan early on after initiation of RA treatment to determine if early responders to conventional therapy could be identified;
- to determine if ^{99m}Tc-EC-DG imaging could detect sub-clinical disease;
- to use ^{99m}Tc-EC-DG imaging to assess the changes in disease activity in the affected joints of patients with RA and compare these to changes detected by US to determine which is the more sensitive imaging modality;
- to use ^{99m}Tc-EC-DG imaging to assess the changes in disease activity in the affected joints of patients with RA and compare these to clinical and laboratory changes; and
- to determine if ^{99m}Tc-EC-DG imaging could detect extra-articular disease in patients who would most likely fall into the high-risk category.

Chapter 2 (article 1) reported on the ability of ^{99m}Tc-EC-DG imaging to **detect active synovitis**. Furthermore, it also focused on the normal biodistribution of the radiopharmaceutical. This chapter revealed that ^{99m}Tc-EC-DG is a safe radiopharmaceutical and could efficiently detect joints with active synovitis. However, participants had varying degrees of uptake in affected joints, which was likely based on the disease severity. The chapter also went further to show that the uptake of ^{99m}Tc-EC-DG was specific to the synovial space, with no abnormal accumulation in the bone. The presence of abnormal ^{99m}Tc-EC-DG uptake in asymptomatic joints was also reported in this chapter. A finding that might be in keeping with this radiopharmaceutical's ability to **detect subclinical disease** was one of the objectives of this study. There was no **abnormal extra-articular uptake** noted in all the participants, which was also an objective of the research. However, there might be reasons for this finding, which include the small number of study participants. Another important reason is that the SPECT/CT camera has a lower sensitivity and resolution compared to a PET/CT camera. Therefore, a higher possibility exists that subtle extra-articular disease, such as aortitis, could be missed. The biodistribution of ^{99m}Tc-EC-DG was closely similar to that of ^{18}F-FDG, according to the existing literature on ^{18}F-FDG biodistribution. However, of importance was the fact that the fasting state of the patient did not seem to influence the biodistribution of ^{99m}Tc-EC-DG. In conclusion, ^{99m}Tc-EC-DG is a safe radiopharmaceutical that can efficiently assess disease activity in the joints of patients with RA.

Chapter 3 (article 2) focused on the possible prognostic value of ^{99m}Tc-EC-DG imaging in patients with RA. This chapter compared the degree of ^{99m}Tc-EC-DG uptake in joints with active disease and the presence of known poor prognostic markers, such as RF and anti-CCP antibodies. A significant correlation was found between higher grade uptake of ^{99m}Tc-EC-DG and increased levels of RF and anti-CCP antibodies, with a p-value of 0.031. There was, however, a weak correlation between the grade of uptake and increased CRP and ESR values, which might be expected due to the very poor specificity of these inflammatory markers. In conclusion, ^{99m}Tc-EC-DG imaging might be of value in the prognostication of patients with RA. This was one of the most significant findings described in the thesis and an abstract from this particular work received two awards at the 2023 Society of Nuclear Medicine and Molecular Imaging (SNMMI) international scientific meeting in Chicago, USA (cf. Appendix M).

Chapter 4 (article 3) described the findings on how ^{99m}Tc-EC-DG imaging compared to US imaging in the identification of synovitis. It has already been well known that Doppler US imaging is a very sensitive tool in identifying synovitis. In this chapter, an abnormally increased uptake of ^{99m}Tc-EC-DG was localised specifically to the synovial space in the affected joints, with an overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of 86%, 60%, 61%, 85% and 73%, respectively, when compared to US. We suspect that the specificity of 60% might have resulted from subclinical disease not detected by US imaging. The next chapter also highlighted this finding, which is explained further in this summary. A sensitivity of 100% was noted in the identification of synovitis in the carpal and knee joints. It was noteworthy that both ^{99m}Tc-EC-DG and US imaging did not detect disease activity in the DIP joints of any of the participants. This chapter further addressed the point that ^{99m}Tc-EC-DG has a very high sensitivity in detecting synovitis. It also correlates very well with US imaging in this regard.

Chapter 5 (article 4) focused on a very important aspect in the management of patients with RA, which is the **assessment of treatment response**. This is very important, as you want to follow these patients up with a modality that can detect subclinical disease. In this chapter, 348 joints were evaluated by both ^{99m}Tc-EC-DG and US imaging at baseline, and again at six weeks and six months from baseline, including joints of the hands, wrists and knees. The level of agreement between US and ^{99m}Tc-EC-DG imaging in assessing therapy response was 33.3%, 11.6% and 6.7% for the knees, hands, and wrists, respectively. We suspected that this poor agreement was because ^{99m}Tc-EC-DG imaging remained positive in joints that were negative on US. We are of the opinion that this observation might be attributed to the identification of subclinical disease by ^{99m}Tc-EC-DG imaging. It was in favour of this finding

that six participants had new disease seen on their six week follow-up US, which was previously seen on their baseline ^{99m}Tc-glucosamine scan but not their baseline US. The conclusion was that ^{99m}Tc-EC-DG imaging is a valuable tool in identifying disease activity and assessing treatment response in patients with RA. The findings in this study appear to suggest that it outperforms US imaging in assessing therapeutic response in patients with RA. However, both modalities would need to be compared with a gold standard such as MRI, clinical outcome, or tissue biopsy, to come to a concrete conclusion.

6.3 LIMITATIONS

Although this research has shown the value of ^{99m}Tc-EC-DG imaging in the management of patients with RA, it is not without limitations. A larger participant population would increase the weight of any of the claims. A longer follow-up period was not performed to better correlate treatment response with the outcome of these patients and should be considered in future research. The outcome of the prognostic value of ^{99m}Tc-EC-DG imaging was also not investigated, as it is well known that patients with a poorer prognosis end up with complications such as joint destruction and deformities. A significant limitation was that concomitant osteoarthritis in the joints of these participants had not been ruled out, thus making it difficult to differentiate between subclinical synovitis and mild osteoarthritis. In terms of the assessment of synovitis on ^{99m}Tc-EC-DG imaging, a very significant limitation is the absence of quantitative assessment of radiopharmaceutical uptake in affected joints. It is known that quantitative assessment is more reproducible and would enhance the assessment of clinical images. However, the shortcoming of most of the SPECT cameras and software is the absence of quantitative software tools for assessing uptake of radiopharmaceuticals in various regions of interest. However, this shortcoming should be addressed in future works, as newer SPECT/CT cameras with better detectors and semiquantitative abilities are now available.

6.4 RECOMMENDATIONS

Findings from this thesis open the door for future research that may include studies without some of the limitations we experienced. PET/CT imaging has been found to be more sensitive than SPECT/CT imaging, due to its ability to perform quantitative assessment, better spatial resolution, and sensitivity. If EC-DG can be labelled with a PET radioisotope such as ^{18}F, a similar study can be performed using ^{18}F-EC-DG. This will present a more sensitive metabolic imaging approach, with validated quantitative parameters, which can most likely offer more accurate prognostic information and assessment of patients' treatment response. Another study would be to compare the diagnostic accuracy between the well-known ^{18}F-FDG PET/CT

and ^{99m}Tc-EC-DG imaging, with the hypothesis that ^{99m}Tc-EC-DG, even though it might be less sensitive, it might be more specific in the identification of synovitis.

A similar study to the one conducted can also be performed with a larger patient cohort where osteoarthritis has been ruled out deliberately. This would most likely confidently confirm the presence of subclinical disease in asymptomatic patients with RA, and/or patients with negative findings on Doppler US. Researchers can also perform studies using ^{99m}Tc-EC-DG imaging to assess the efficacy of newer drugs for the treatment of RA, including biologics. We are in the era of theranostics – using one radioactive substance to identify or diagnose a disease in combination with a second radioactive substance to deliver therapy – an area that could also be explored in the future, where labelling of EC-DG with a therapeutic radiopharmaceutical for the potential treatment of patients with active synovitis due to RA may be investigated. As MRI is often considered the gold standard imaging modality for detecting synovitis, we also recommend that future work could be done in comparing ^{99m}Tc-EC-DG imaging with MRI in patients with synovitis.

6.5 CONTRIBUTION AND SIGNIFICANCE OF THE RESEARCH

This research has proven highly significant and of interest to the nuclear medicine community. One of the indicators as to how significant or important this research has been, is the evidence of the two awards received from one of the conference abstracts submitted. Being selected as the best international abstract from South Africa at the 2023 SNMMI international meeting, and the third best abstract in the category General Clinical Specialties at the same international meeting, is testimony to the significance of this research.

Feedback from the Rheumatology Unit in the Department of Internal Medicine, University of the Free State, has also been encouraging as they support the implementation of this technology as a standard of care in the management of patients with RA in our institution. The project also provided some of the researchers with an opportunity to present abstracts at scientific meetings. A total of three conference abstracts have been accepted and presented, and four articles have been written from this project for publication thus far (of which two have recently been published; cf. Appendices H and I), with at least another two of each pending. We ultimately feel satisfied with the opportunities this project has generated. However, most importantly, we trust that the local healthcare facility would provide the necessary funds required for ^{99m}Tc-EC-DG imaging to become a routine standard of care in managing patients with RA.

6.6 CONCLUDING REMARKS

^{99m}Tc-EC-DG, also known as ^{99m}Tc-glucosamine, proved to be a highly sensitive and valuable tool in the assessment of synovitis in patients with RA. The intensity of this radiopharmaceutical's uptake in affected joints has been shown to be related to the presence or absence of poor prognostic laboratory markers. It has also been shown to be valuable in the assessment of treatment response in patients with RA.

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APPENDIX A

ETHICAL APPROVAL BY THE HEALTH SCIENCES RESEARCH ETHICS COMMITTEE (HSREC)

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

13-Nov-2020

Dear Dr Osayande Evbuomwan

Ethics Clearance: **THE VALUE OF TECHNETIUM-99-METASTABLE-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS**

Principal Investigator: **Dr Osayande Evbuomwan**

Department: **Nuclear Medicine Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2020/1292/2411**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 0001992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa

APPENDIX B

PERMISSION FROM THE FREE STATE PROVINCE DEPRTMENT OF HEALTH

health
Department of Health
FREE STATE PROVINCE

06 November 2020

Dr O Evbuomwan
Dept. of Nuclear Medicine
UFS

Dear Dr O Evbuomwan

Subject: The value of technetium-99-metastable-ethylenedicysteine-deoxyglucose imaging in patients with rheumatoid arthritis.

- Please ensure that you read the whole document. Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of **Universitas Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeelats@fshealth.gov.za / makenamr@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- **Please discuss your study with Institution Manager on commencement for logistical arrangements see 2nd page for contact details.**
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- **As part of feedback you will be required to present your study findings/results at the Free State Provincial health research day**

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 6/11/2020

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

APPENDIX C

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT (ENGLISH)

PATIENT INFORMATION AND CONSENT FORM

Study title

THE PROGNOSTIC VALUE OF ^{99m}Tc-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS

Principal Investigator: Dr. Evbuomwan Osayande
Telephone number work: 051 405 3488
Cell phone number: 074 286 9731
Email address: moreli14@yahoo.com

Supervisor: Dr. J. Horn-Lodewyk
Telephone number work: 051 507 4073
Cell phone number: 082 859 4533
Email address: jeninehorn@gmail.com

Head of Department: Dr. G.H.J. Engelbrecht
Telephone number work: 051 405 3488
Cell phone number: 082 928 1206
Email address: engelbrechtgh@ufs.ac.za

Site Address: Department of Nuclear Medicine
Universitas Academic Hospital
1 Logeman Street
Bloemfontein

Background

You are being asked to participate in a research study on the comparison of technetium-99-metastable-ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG) imaging with ultrasound in participants (patients) with rheumatoid arthritis. ^{99m}Tc-ECDG imaging will be used in the assessment of disease activity and its role in prognostication will be evaluated. You will also be asked to undergo a standard routine clinical and laboratory assessment and ultrasound procedure. Thereafter you will undergo ^{99m}Tc-ECDG imaging. All these assessments will be done at baseline before you commence treatment, and will be repeated at 6 weeks and again at 6 months after initiation of treatment. ^{99m}Tc-ECDG is a radiotracer that has been used for almost two decades in the imaging of inflammation and tumors and it has been proven to be safe, similar to all radioisotopes used for imaging by departments of nuclear medicine.

Participant's initials _____

Who can answer my questions?

You may contact the principal investigator or supervisor of the study at any time and ask any question you have about this study. The telephone number is 051 405 3488, with full contact details at the beginning of this information document. You may also wish to discuss the details of this study and your participation in this study with your attending physician.

How many patients will take part in the study?

This study is expected to enrol approximately 20 participants who meet certain criteria.

How long will I be in the study?

Each study participant would be evaluated at baseline and again at 6 weeks and 6 months on treatment. This means you will be through with the study 6 months after starting treatment.

Why is this study being done?

This study is being done to see whether ^{99m}Tc-ECDG imaging can give us a more specific answer on the level of disease activity present 6 months after treatment, and early predict those patients with rheumatoid arthritis who would respond better or poorly to initial therapy. These findings would help prevent low disease activity from being untreated, which eventually can lead to bone destruction. It can also alert the attending physician early on the need to change or alter therapy early for a better outcome.

What will happen during the study?

The referring physician will determine if you are eligible to participate in this study and then conduct routine assessment and investigation prior to ^{99m}Tc-ECDG imaging. If eligible, you will receive a copy of this informed consent form (ICF) on your first appointment with your attending doctor. The ICF must then be read, understood and signed before you will be enrolled into the study.

Participant's initials _____

What preparation is required of me for the ^{99m}Tc-ECDG SPECT/CT scan?

You must have fasted for at least 6 hours before the time of your scheduled ^{99m}Tc-ECDG scan appointment. If you are a diabetic, you may take your medication as necessary with a light meal at least 4 hours before the ^{99m}Tc-ECDG scan. If you are a female within the reproductive age group, a urine pregnancy test will be performed first to exclude pregnancy, as pregnancy is an exclusion criterion for this study.

You will be encouraged to drink a lot of water before you are injected with a small amount of ^{99m}Tc-ECDG. Just before imaging you will be advised to empty your bladder.

How will images be taken?

About 2 hours after being injected with ^{99m}Tc-ECDG, you will be imaged by means of a gamma camera. The whole imaging time should be approximately 30 minutes.

What are the possible risks to me from the study?

As with any research study, there is a risk of side-effects. You should discuss this with your attending physician. Not all patients will experience side-effects. The principal investigator or a nuclear medicine technician will monitor you closely to see if you experience any side-effects. Side-effects that have been experienced with ^{99m}Tc-ECDG include itching and very mild skin rash. Most side-effects resolve within a few hours. Redness of the skin might also develop at the sight of the injection after the administration of ^{99m}Tc-ECDG, which will resolve by itself. If you experience any unusual signs or symptoms, please report them to the principal investigator.

Participant's initials _____

RADIATION RISK

The ^{99m}Tc-ECDG scan involves exposure to a low diagnostic dose of radiation. In our day-to-day activities, we are exposed to various types of radiation that occurs naturally and artificially in the environment. There is insufficient information to determine the risk to the unborn foetus or child, which is why all women participating in this research will receive a pregnancy test and would be excluded if found to be pregnant.

Are there benefits to taking part in the study?

The literature has shown the ability of ^{99m}Tc-ECDG to detect low disease activity in patients with rheumatoid arthritis that would have been missed by laboratory/clinical assessment. If this happens to be the case with you, continuation of treatment will prevent bone destruction and a poorer treatment outcome. Future patients would definitely benefit from this study if ^{99m}Tc-ECDG imaging has been found to be a strong prognostic tool.

What other choices do I have if I do not participate in the study?

If you choose not to participate, your usual course of medical care will continue. You may also opt to drop out of the study at any time, without any consequences.

What are the costs of tests and procedures?

No cost to you. The study is being supported by a financial grant.

Will I receive any compensation for my participation in the study?

You will be compensated with transport money and a light meal when you come in at 6 months into your treatment for evaluation. There will be no other monetary or financial compensation for participating in this study.

Participant Initials _____

What are my rights if I take part in the study?

Taking part in the study is voluntary and it is your decision to participate. You do not have to take part in this study, but if you do, you may drop out of the study at any time. This will not affect the standard medical treatment you receive now or in the future.

How will my privacy be protected?

Data obtained from this study may be published. However, your name and other identifying information will be kept confidential and will not be made available publicly. Representatives from the Republic of South Africa Department of Health (RSA DoH) and University of the Free State Health Sciences Research Ethics Committee may review your personal and medical records if requested.

All scanning data will be maintained at the Department of Nuclear Medicine, Universitas Academic Hospital in Bloemfontein, for a period of least ten years, and governed by the privacy policies of RSA DoH. Your medical records related to this study will also be provided to your attending physician.

Participant's initials _____

FURTHER INFORMATION

If you experience any unusual signs or symptoms or side-effects, or if you experience a research-related injury and need medical treatment, please immediately contact the principal investigator at 074 286 9731 or go to the nearest hospital emergency department. **The Department of Health cannot be held accountable for injuries and physical or monetary claims caused during or after participation in this study. Participant insurance was taken out at Marsh (Pty) Limited with policy number P1297114.**

The ethical aspects of this protocol has been reviewed by University of the Free State (UFS) Health Sciences Research Ethics Committee and the UFS Radiation Control Committee. You may contact the Secretariat of the Health Sciences Research Ethics Committee, UFS, at telephone number 051 401 7794/5 if you have questions about your rights as a research subject.

My signature below means I have read the patient information and consent form, my questions have been answered to my satisfaction, and that I agree to participate in this study. A copy of this signed patient information and consent form will be given to me. I understand that even thought I have read and signed this consent form, I can withdraw from the study at any time if I so do wish. I will not donate blood while I am in the study and for at least 30 days thereafter.

_____
Research participant: Name (print)

_____ _____
Participant: Signature Date

_____
Witness: Name (print)

_____ _____
Witness: Signature Date

VERBAL PATIENT INFORMED CONSENT

(applicable when a patient cannot read or write)

I, the undersigned, Dr. _____, have read and have explained fully to the participant named _____ the participant information leaflet, which is indicating the nature and purpose of the study in which I have asked the participant to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The participant indicated that he/she understands that he/she will be free to withdraw from the study at any time for any reason.

I hereby certify that the participant has agreed to participate in this study.

_____ _____

Participant: Signature Date

_____

*Witness: Name (print)

_____ _____

Witness: Signature Date

_____

Investigator: Name (print)

_____ _____

Investigator: Signature Date

*Witness – sign that he/she has witnessed the process of informed consent.
Consent procedure should be witnessed whenever possible.

APPENDIX D

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT (SESOTHO)

TLHAHISOLESEDING HO MOKUDI LE FOROMO YA TUMELLO

Sehlooho sa ho ithuta

THE PROGNOSTIC VALUE OF ^{99m}Tc-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS

Mofuputsi ya ka sehlooho: Dr Evbuomwan Osayande
Mohala wa mosebetsing: (051) 405 3488
Mohala wa letheka: 0742869731
E-mail address: moreli14@yahoo.com

Mohlokomedi: Dr. J. Horn-Lodewyk
Mohala wa mosebetsing: (051) 507 4073
Mohala wa letheka: 082 859 4533
E-mail address: jeninehorn@gmail.com

Hlooho ya lefapha: Dr G.H.J. Engelbrecht
Mohala wa mosebetsing: (051) 405-3488
Mohala wa letheka: 082 928 1206
E-mail address: engelbrechtgh@ufs.ac.za

Site Address: Department of Nuclear Medicine
Universitas Academic Hospital
1 Loggemans Street
Bloemfontein

Semelo

O kopuwa ho nka karolo ho dibatlisiso tsena tse batlang ho sheba Technetium-99-meta-stable-pertechnetate ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG) imaging le ultrasound ho banka karolo ba ramatiki (rheumatoid arthritis). ^{99m}Tc-ECDG imaging e tla sebediswa ho sheba bohloko ha bo tswela pele hape le ho leka ho ka shebela pele bohloko. O tla kopuwa ho ka nka diteko tsa laboratory/tjhebisiso ya tsa bongaka le tse kenyeletsang ultrasound le MRI jwaloka ha di etswa kgafetse ho bakudi. Feela o tla etswa ^{99m}Tc-ECDG imaging ka mora ultrasound. Tsena tsohle di etsetswa hore ho be le sebaka sa mathomo pele kalafo e qala, mme tsena tse boletswe ditla phetwa hape ka mora kgwedi tse pedi le tse tseletseng ka mora kalafo. ^{99m}Tc-ECDG e sebedisitswe ho feta lemo tse mashome a mabedi ho hlahlojwa ho ruruha le ho sheba mofetshe ho bakudi, e bonahetse e bolokehile bakeng sa ho sebediswa ho bakudi jwaloka tseding tse sebediswang ho ka bona bohloko kapa o na mafu.

Participant Initials _____

Ke mang ya ka arabelang dipotso tsa ka?

O ka ikopanya le mofuputsi ya ka sehlooho kapa mohlokomedi wa dipatlisiso tsena ka nako engwe le engwe ha o na le dipotso. Nomoro ya mohala ke (051) 405 3488, dintla tse feletseng di ka hodimo ho maqhepe lena. O ka buisana le ngaka e o alafang ha o batla mabapi le dintlha tsa dithuto tsena le ho nka karola ha hao.

Ke bakudi ba bakae batlo nka karolo dithutong tsena?

Ho lebeletswe banka-karolo ba mashome a metso e meraro ho dithuto tsena.

Ke tlo nka nako e kae ke le ho dithuto tsena?

Monka-karolo e mong le e mong o tla hlahlojwa ha kalafo di qala le ka mora kgwedi tse pedi le tse tsheletsen. Hona ho bolela hore o tla nka karolo kweding tse tsheletseng dipatlisisong ka mora ho qala ka kalafo.

Dipatlisiso tsena di etsetswang?

Dipatlisiso tsena di etsetswa ho sheba hore ^{99m}Tc-ECDG e ka fana ka karabo e nepahetse ho lefu la ramatiki ka mora kgwedi tse tsheletseng pekolo e qadile, hape le ho batlisisa hore ho ka etswa tjhebelopele hore ke bakudi bafeng batla dumelwa ke kalafo le batlo sa tlang ho dumelwa ke kalafo nakong ya qalo ya kalafo. Tse tlang ho fumanwa dipatlisisong tsena di tla thusa hore ho fumanwe hore bakudi ba nang le lefu la ramatiki e nyane ba se fete ba sa fumana kalafo, hobane hona ho etsa hore ramatiki ena e baka tshenyeho ya masapo. Hona ho thusa le ngaka e o alafang hore e tsebe ho ka fetola mokgwa oo kalafo e fuwang ka teng e sa le qalong ya kalafo hore ho fumanwe sephetho se betere.

Ho tla etsahalang ha dipatlisiso di tswelapele?

Ngaka ya hao e tla etsa qeto ya hore wa sthwaneleha ho ka nka karolo dipatlisisong tsena le ho o tlhahloba pele o ka fumana ^{99m}Tc-ECDG. Haeba o kgethilwe ho ka nka karolo, o tla fuma foromo ya tumello eo ho yona o tla fana ka tumello ya ho nka karolo, ha o ya ngakeng ya hao eo phekolang kgetlong la pele. Lengolo la tumello ya hao le tlameha ho balwa le hore o le utlwisise pele o ka nka karolo dithutong, ka mora moo a le saene.

Participant initials _____

Ho hlokahala eng honna hore ke itukisetse tlhahlobo ya ^{99m}Tc-ECDG SPECT/CT?

O tshwanela ho qetela hoja hora tse tsheletseng pele ho tlhabobo ya ^{99m}Tc-ECDG. Ha o na le lefu la tswekere, o n+wa meriana ka dijo tse bobebe hora tse nne pele ho tlhabobo ya ^{99m}Tc-ECDG. Ha o l e mosadi wa dilemp tsa ho pepa, teko ya boimana e tlameha ho etswa ka thhanobo ya motshetse. Ka ha boimana bo a kgethollwa ho etsa teko.

O kgotha lletswa ho mwa metsi, a mangata pele o tlhabuwa ka ^{99m}Tc-ECDG. Pele o nkwa ditshwantsho o tla kupuwa ho ntsha metsi.

Ditshwantsho di tla nkuwa jwang?

O tla nkuwa ditshwantsho ka gamma camera ka mora hora tse pedi hore o entuwe ka ^{99m}Tc-ECDG. Ho nka ditshwantso hotla nka metsotso e ka bang mashome a mararo.

Ekaba ke kotsi tse feng tse ka lebellwang ho dithuto tsena?

Jwaloka dipatlisiso kapa dithuto tse ding letse ding, ho na le kgonahalo ya kotsi e ka hlahang e sa lebellwa. O tlameha ho buisana le ngaka ya hao mabapi le sena. Ha se bakudi kaofela ba ka hlahelwang ke kotsi. Mofuputsi ya ka sehlohong kapa monka ditshwantsho (radiographer) o tla beha maemo leihlo hore haho kotsi e bonahalang kapa eo o e utlwang. Dikotsi tse tlalehuweng ka ^{99m}Tc-ECDG dikenyaletsa ho tjhwatjhwasela le ho tswa makgopo letlalong. Boholo ba dikotsi tse tlalehilweng di nyamela ka mora nako ya dihora dise kae. Bokgubedu ba letlalo le bona ke kotsi e ka hlahang moo nale e keneng teng, fela bo tsamae ka mora nako e nyane. Ha o ka utlwa ntho e sa tlwaelehang mmeleng wa hao bolella mofuputsi ya ka sehloohong.

Participant initials _____

Kotsi tsa Radiation

^{99m}Tc-ECDG scan e kenyaletsa ho pepeswa ho diagnostic dose ya radiation etlase. Letsatsing le leng le leng re fumana kapa hona pepeswa mekgwang e fapaneng ya radiation ya tlhaho le e iketseditsweng. Ho na le tlhahisoleseding e sa fellang ho bontsha kotsi ho ngwana ya sokang a hlaha ka ho moemana, ke ka hoo basadi ka nkang karolo dipatlisisong batlang ho fumana teko yah ore ke baimana, ha ba fumanwa e le baimana batla tloswa dipatlisisong.

Ho na le melomo ka ho nka karolo ho dithuto tsena?
Dingolwa tsa mahlale a bongaka di bontsa hore ^{99m}Tc-ECDG e kgona ho fumana ramatiki ya rheumatoid ha e sa qala eo e tlabeng e sa bonwa ke laboratori kapa tlhatlhobo ya bongaka. Haeba hona ho etsahala ho wena, ho tla tswellwa ka kalafo ho thibela tshenyeho ya masapo le ho thibale sepheto sebe sa kalafo. Leha ho le jwalo, ha hosa fumanwe ramati ha e qala, ho na le kgoneho e kgolo hare o keke wa una molemo ka ho nka karolo dithutong tsena. Bakudi ba tlang nakong e tlang baka kgola molemoho ha ho ka gumanwa hore dithuto tsa ^{99m}Tc-ECDG imaging di atleha hoba sesebediswa se bontsha ramati ha e qala.

Ke kgetho tsefe tseo ke nang le tsona ha ke kgetha ho se nke karolo diphuputsong tsena?
Ha o kgetha ho se nke karolo, o ka tswelapele ka tlhokomelo ya bongaka e tlwaelehileng. Hape o ka kgetha ho tswe hara diphuputso tsena nako engwe le engwe, o keke wa supuwa ka monwana kapa hwa ba le ditlamorao.

Ekaba ke bokae ditshenyehelo tsa diteko le tsebetso?
Ha hona letho leo o tla lepatala. Diphuputso tsena ditseheditswe ka tjhelete.

Nka labella moputso ka ho nka karolo diphuputsong tsena?
O tla thuswa ka tjhelete ya dipalangwang le ya dijo ha o tla ka mora kgwedi tse pedi ho hotla hlahloba hore peko/kalafo ya hao. Ha hona tjhelete engwe eo o tla e fumana ka ho nka karolo diteko tsena.

Participant Initials _____

Ditokelo tsa ka ke tsefeng ha ke kgetha ho nka karolo dithutong tsena?

Ho nka karolo ke boithaopo hape ke kgetho ya hao. Ha oa qobelwa ho nka karolo dipatlisisong tsena, empa ha o kgetha ho nka karolo, o ka tlohella nako engwe le engwe eo o batlang. Hona hokeke ha sitisa kapa wa kgetholwa bakeng sa kalafo eo o e fumanang le eo o tlang ho e fumana nakong e tlang.

Ekaba lekunutu la kalafo ya ka le tla tshireletswa jwang?

Dintla/dipalopalo tse nkilweng diphuputsong tsena di ka phahlalatswa ka mokgwa wa dingolwa tsa mahlale. Empa lebitso le dintla tse ding tseo di ka hlahisa ditla bolokwa e le lekunutu, hona hobolela hore setjhaba se ka se ditsebe. Bakgethwa ba Republic ya aforika Borwa ba lefapha la tsa bophelo (RSADoH) le ba Univesiti ya Foreisitata ba lefapha la mahlale a tsa Bophelo Ethics Committee ba ka hlahloba ditokomane tsa hao le tsa bongaka ha ho kopuwa.

Dintla tsa hao tsa ditshwantso le tseding di tla bewa lefaphend la Nuclear Medicine, Universitas Hospital in Bloemfontein nako e ka etsang dilemo tse leshome, lekunutu le tla buswa ke maano a lekunutu a lefapaha la Bophelo Aforika Borwa. Direkoto tsa hao tsa bongaka tse tswang mona di tla fuwa ngaka eo e hlahlobang lefu la hao.

Participant initials _____

Tlhahisoleseding ho ya pele

Ha o ka utlwa o na le dipontsho kapa matswao ao o sa a tlwaelang hape o hloka tsa bongaka, ikopanye kapelepele le mofuputsi moholo mohaleng ona 074 286 9731 kapa o ye sepetleleng se haufinyana le wena. kapa If you experience any unusual signs or symptoms or side effects, or if you experience a research-related injury and need medical treatment, please immediately contact the principal investigator at 0742869731 or go to the nearest hospital emergency department. Lefapaha la Bophelo le keke la nka boikarabelo bakeng sa dikotsi tse ka o hlahelang mmele kapa tseko ya tjhelete tse ka hlahang nakong e oho etswa diphuputso kapa ka mora tsona. Inshorennse ya monka-karolo e hlophisitswe le Marsh (Pty) Limited with policy number: P1297114.

Dintlha tsa boitswaro mabapi le diphuputso tsena di lekotswe ke University of the Free State (UFS) Health Sciences Ethics Committee and the UFS Radiation Control Committee. Ho saena ha ka fatshe leqepeng ho bolela hore ke badile tlhahisolesding ya mokudi le foromo ya ho dumela, hape dipotso tsa ka di arabuwe ka tsela e kgotsofatsang le hore ke dumela ho nka karolo dithutong tsena. O tla fuwa kopiso ya tlhahisolesding ya mokudi le foromo ya tumello e saenilweng. Ke utlwisisa hantle hore le ha ke badile le ho saena foromo ya tumello, nka ikgula nako engwe le engwe dithutong tseng ha ke batla. Ke dumela hore nkeke ka fana ka madi ha ke ntse ke nka karolo dithutong tsena, bakeng sa nako ya matsatsi a mashome a mararo bonyane kamora dithuto tsena. I will not donate blood while I am in the study and for at least 30 days thereafter.

_____

Lebitso la monka-karolo diphuputsong (ngola ka tlhaku tse kgolo)

_____ _____

Monka-karolosaena Letsatsi

_____

Lebitso la paki(ngola ka tlhaku tse kgolo)

_____ _____

Paki saena Letsatsi

TUMELLO YA MOKUDI YA MOLOMO (fela ha mokudi a sa tsebe ho bala kapa ho ngola) Nna, ya qotsitsweng mona _____, ke badile le ho hlalosetsa monka-karolo wa lebitso, dikateng tsa tlhahisoleseding e ka hara sengolwa sa monka-karolo e bontsitseng tlhaho le morero ya dithuto moo ke kopileng monka-karolo hore a nke karolo. Tlhaloso eo ke faneng ka yona e bonstitse ka bopedi dikotsi le melemo ya dithuto tsena. Monka-karolo o bontsiste hore yena o utlwisitse hore o na le matla a ho ka ikgula dithutong tsena nako engwe le engwe ka lebaka le leng le leng.

I hereby certify that the participant has agreed to participate in this study.

_____ _____

Monka-karolo saena Letsatsi

_____

Lebitso la paki (ngola ka tlhaku tse kgolo)

_____ _____

Paki saena Letsatsi

_____

Lebitso la mofuputsi (ngola ka tlhaku tse kgolo)

_____ _____

Mofuputsi saena Letstatsi

*Witness – sign that he/she has witnessed the process of informed consent.
Consent procedure should be witnessed whenever possible.

APPENDIX E

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT (AFRIKAANS)

Bladsy 1 van 8	Protokol No.
Ingeligte Toestemmingsvorm	Januarie, 2020

PASIËNTINLIGTING- EN TOESTEMMINGSVORM

Studietitel*

THE PROGNOSTIC VALUE OF ^{99m}Tc-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS

Hoofnavorser: Dr. Evbuomwan Osayande
Telefoonnommer werk: 051 405 3488
Selfoonnommer: 074 2869731
E-posadres: moreli14@yahoo.com

Promotor: Dr. J. Horn-Lodewyk
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Departementshoof: Dr. G.H.J. Engelbrecht
Telefoonnommer werk: 051 405 3488
Selfoonnommer: 082 928 1206
E-posadres: engelbrechtgh@ufs.ac.za

Navorsingsadres: Departement Kerngeneeskunde
Universitas Akademiese Hospitaal
Logemanstraat 1
Bloemfontein

***Titel in Afrikaans:** DIE PROGNOSTIESE WAARDE VAN ^{99M}TC-ETILEENDISITEÏN-DEOKSIGLUKOSE BEELDING IN PASIËNTE MET RUMATOÏEDE ARTRITIS

Agtergrond

U word genooi om deel te neem aan 'n navorsingstudie oor die vergelyking van technetium-99-metastable ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG) beelding met ultraklank in deelnemers (pasiënte) met rumatoïede artritis. ^{99m}Tc-ECDG beelde sal gebruik word vir die evaluering van siekte-aktiwiteit en die rol daarvan in die prognostiese voorspelling van die siekteverloop. U sal ook gevra word om 'n standaard, roetine kliniese en laboratoriumevaluering en ultraklankprosedure te ondergaan. Ná hierdie ondersoeke sal u die ^{99m}Tc-ECDG beelding ondergaan. Al hierdie ondersoeke word voor die tyd as basislyn-evaluering (intreefase) gedoen voordat met standard behandeling vir rumatoïede artritis begin word. U sal dieselfde ondersoeke weer ondergaan op 6 weke en weer op 6 maande ná aanvang van die behandeling. ^{99m}Tc-ECDG is 'n radioaktiewe spoorder wat al langer as twee dekades vir die beelding van inflammasie en tumore gebruik word en is bevestig as veilig, soortgelyk aan alle radioaktiewe spoorders wat vir beelding deur kerngeneeskunde-departmente gebruik word.

Deelnemer se voorletters _____

Wie kan my vrae beantwoord?

U mag die hoofnavorser of die promotor van die studie enige tyd kontak as u vrae het in verband met die studie. Die plaaslike telefoon nommer is 051 405 3488, met volle kontakbesonderhede aan die begin van hierdie inligtingstuk. U is ook welkom om u deelname aan die studie met u behandelende geneesheer (dokter) te bespreek.

Hoeveel pasiënte sal aan die studie deelneem?

Die studie behoort 20 deelnemers in te sluit wat aan sekere kriteria voldoen.

Hoe lank sal ek deel wees van die studie?

Elke deelnemer sal geëvalueer word op intreefase (basislyn), en weer ná 6 weke n 6 maande op behandeling. Dit beteken dat u ná 6 maande sal klaar wees met deelname aan die studie.

Hoekom word hierdie studie gedoen?

Die studie word gedoen om te evalueer of die ^{99m}Tc-ECDG beelding 'n meer spesifieke antwoord kan gee oor die siekte-aktiwiteit 6 maande ná die begin van behandeling, en of dit vroeg kan voorspel watter pasiënte met rumatoïede artritis beter of swakker reageer op die aanvanklike behandeling. Die bevindinge sal help om te voorkom dat lae-vlak siekte-aktiwiteit onbehandeld bly, wat uiteindelik kan lei tot beenvernietiging. Dit kan ook vroeër onder die aandag van die behandelende geneesheer gebring word as die pasiënt nie op die behandeling reageer nie, om dan die behandeling te verander vir 'n beter uitkoms.

Wat sal tydens die studie gebeur?

Die verwysende dokter sal bepaal of u aan die insluitingskriteria voldoen om aan die studie te kan deelneem en sal vooraf die roetine ondersoeke doen vóór die ^{99m}Tc-ECDG beelding. Indien u ingesluit kan word vir deelname aan die studie, sal u 'n inligtingstuk (IS) ontvang tydens die eerste besoek aan u behandelende geneesheer. Die IS moet gelees word en u moet die inligting verstaan. As u bereid is om aan die studied deel te neem, sal u gevra word om die ingeligte toestemmingsvorm te onderteken.

Deelnemer se voorletters _____

Watter voorbereiding word van my verwag vir die ^{99m}Tc-ECDG SPECT/CT beelding?

U moet ten minste vir 6 ure voor die geskeduleerde ^{99m}Tc-ECDG skandering vastend wees. As u 'n diabeet is, kan u u medikasie soos nodig met 'n ligte ete neem 4 ure vóór die ^{99m}Tc-ECDG beelding. Indien u 'n vrou in die reproduktiewe ouderdomsgroep is, sal u uriene getoets word om swangerskap uit te skakel, want swangerskap is 'n uitsluitingskriterium vir die studie. U sal aangemoedig word om baie water te drink voordat u met 'n klein hoeveelheid ^{99m}Tc-ECDG ingespuit word vir beelding. Net vóór die beelding sal u gevra word om u blaas te ledig.

Hoeveel beelde sal geneem word?

Ongeveer 2 ure ná toeding van die ^{99m}Tc-ECDG, sal u beelding gedoen word met behulp van 'n gamma-kamera. Die totale beeldingstyd sal ongeveer 30 minute neem.

Wat is die moontlike risiko's vir my om aan die studie deel te neem?

Soos met enige navorsingstudie is daar 'n risiko op newe-effekte. U moet dit met u behandelende geneesheer bespreek. Nie alle pasiënte wat deelneem aan die studie sal newe-effekte ondervind nie. Die hoofnavorser of 'n kerngeneeskundige tegnikus sal gesondheid monitor vir enige aanduiding van newe-effekte. Newe-effekte wat al aangedui is, is jeukerigheid en 'n baie matige veluitslag. Die meeste newe-effekte klaar binne 'n paar uur op. Rooiheid van die vel kan moontlik in die area van die inspuiting waar die ^{99m}Tc-ECDG toegedien is ontwikkel, maar sal dan vanself opklaar. Indien u enige buitengewone tekens of simptome ervaar, rapporteer dit asseblief aan die hoofnavorser.

Deelnemer se voorletters _____

BESTRALINGSRISIKO

Die ^{99m}Tc-ECDG beelding sluit 'n lae diagnostiese dosis van bestraling in. In ons daaglikse aktiwiteite word ons blootgestel aan natuurlike en onnatuurlike bestraling wat in die omgewing voorkom. Daar is nie voldoende inligting beskikbaar oor die risiko van bestraling aan 'n ongebore baba of kind nie, en daarom moet vroulike deelnemers aan die navorsing 'n swangerskap toets ondergaan en sal uitgesluit word indien swangerskap bevestig word.

Is daar enige voordele vir my om aan die studie deel te neem?

Die literatuur het die vermoë bewys van ^{99m}Tc-ECDG om lae siekte-aktiwiteit in pasiënte met rumatoïede artritis op te spoor, wat gemis kon word met laboratorium/kliniese evaluering. As dit gebeur in u situasie, sal voorsetting met die standaard behandeling kan lei tot beenvernietiging en 'n swakker behandelingsuitkoms. Ongelukkig as dit nie die geval is met u, sal daar moontlik nie 'n behandelingsvoordeel vir u deelname aan die studie wees nie. Toekomstige pasiënte sal definitief voordeel trek uit die studie indien gevind word dat ^{99m}Tc-ECDG beelding 'n sterk prognostiese hulpmiddel is.

Watter ander keuses het ak as ek nie aan die studie deelneem nie?

Indien u kies om nie aan die studie deel te neem nie, sal u die standaard mediese behandeling ontvang en u behandeling sal normaalweg voortgaan. U het ook die keuse om enige tyd tydens die studie te besluit om u deelname te onttrek, sonder enige gevolge.

Wat is die kostes vir die toetse en die prosedures?

Daar sal geen kostes vir u as deelnemer wees nie. Die studiekostes word deur 'n finansiële toekenning gedek.

Sal ek enige vergoeding ontvang vir my deelname aan die studie?

U sal vergoed word vir u vervoerkostes en 'n ligte ete ontvang tydens u evalueringsbesoek ná 8 weke wat u behandeling ontvang. U sal geen finansiële vergoeding of kompensasie ontvang vir u deelname aan hierdie studie nie.

Deelnemer se voorletters _____

Wat is my regte as ek aan die studie deelneem?

Deelname aan die studie is vrywillig en dit is u eie keuse. U hoef nie deel te neem aan die studie nie, maar indien u sou, kan u enige tyd besluit om u deelname aan die studie te onttrek. Indien u sou onttrek, sal dit nie nou of in die toekoms u standaard mediese sorg affekteer nie.

Hoe sal my privaatheid beskerm word?

Die resultate van die studie sal moontlik gepubliseer word. U naam en enige ander identifiseerbare inligting sal egter vertroulik gehou word en nie aan die publiek bekend gemaak word nie. Verteenwoordigers van die Republiek van Suid Afrika Department van Gesondheid (Department of Health) (RSA DoH) en die Universiteit van die Vrystaat Gesondheidswetenskappe Navorsingsetiekkomitee mag op versoek u persoonlike en mediese rekords evalueer.

Al die beeldingsinligting sal by die Department Kerngeneeskunde, Universitas Akademiese Hospitaal in Bloemfontien gehou word vir 'n tydperk van minstens 10 jaar, en word beheer deur die privaatheidsbeleid van RSA DoH. U mediese rekords wat met die studie verband hou, sal ook aan u behandelende geneesheer verskaf word.

Deelnemer se voorletters _____

VERDERE INLIGTING

Indien u enige ongewone tekens of simptome of newe-effekte, of 'n studie-verwante besering ervaar en mediese behandeling benodig, skakel asssеblief dadelik die hoofnavorser by 074 286 9731 of gaan na u naaste hospitaal se noodgevalle-afdeling. **Die Departement van Gesondheid kan nie aanspreeklik gehou word vir beserings en fisiese en geldelike eise, as gevolg van u deelname aan die studie of daarna nie. Deelnemerversekering is uitgeneem by Marsh (Pty) Limited onder polisnommer P1297114.**

Die etiese aspekte van die protokol is deur die Universiteit van die Vrystaat (UVS) Gesondheidswetenskappe Navorsingsetiekkomitee en die UVS Bestralingsbeheerkomitee. U kan die Sekretariaat van die Gesondheidswetenskappe Navorsingsetiekkomitee kontak by die telefoon nommer (051) 401 7794/5 indien u enige vrae het oor u regte as 'n deelnemer aan 'n navorsingstudie.

My handetekening hieronder beteken dat ek het die pasiëntinligting- en toestemmingsvorm gelees het, my vrae bevredigend beantwoord is, en dat ek toestem om aan die studie deel te neem. 'n Kopie van hierdie getekende pasiëntinligtings- en toestemmingsvorm sal aan my verskaf word. Ek verstaan dat alhoewel ek die inligtingsdokument gelees en die toestemmingsvorm geteken het, ek enige tyd van die studie kan onttrek indien ek sou verkies. Ek sal nie tydens die studie en vir ten minste 30 dae ná afloop daarvan bloed skenk nie.

_____
Navorsingsdeelnemer: Naam (drukskrif)

_____ _____
Deelenemer: Handtekening Datum

_____

Getuie: Naam (drukskrif)

_____ _____
Getuie: Handtekening Datum

PASIËNT SE MONDELINGE INGELIGTE TOESTEMMING
(van toepassing as 'n pasiënt nie kan lees of skryf nie)

Ek, die ondergetekende, Dr. _____, het vir die deelnemer genaamd _____ die pasiëntinligtingstuk gelees en volledig verduidelik wat die aard en doel is an die studie, waaraan ek die deelnemer versoek het om deel te neem.. Die verduideliking wat ek aan die deelnemer voorsien het sluit moontlike risikos van deelname aan die studie, sowel as die voordele van deelname aan die studie, in. Die deelnemer het aangedui dat hy/sy verstaan dat hy/sy enige tyd vry is om vir enige rede aan die studie te mag onttrek.

Hiermee sertifiseer ek dat die deelnemer toestemming gegee het om aan die studie deel te neem.

_____ _____

Deelenemer: Handtekening Datum

_____

*Getuie: Naam (drukskrif)

_____ _____

Getuie: Handtekening Datum

_____

Navorser: Naam (drukskrif)

_____ _____

Navorser: Handtekening Datum

*Getuie – teken dat hy/sy die proses van ingeligte toestemming waargeneem het.
Die toestemmingsprosedure moet sover as moontlik deur die geluie waargeneem word.

APPENDIX F

BASELINE DATA COLLECTION INSTRUMENT

Code*	Age	Sex	Date	Rh (< 20 IU/mL)#	CRP (< 10 mg/L)#	ESR (< 10 mm/hr)#	Anti-CCP (< 3 U/mL)#	DAS-28	Most symptomatic joint	ECDG hands flow	ECDG hands blood pool	ECDG hand delayed	ECDG wrist flow	ECDG wrist blood pool	ECDG wrist delayed	ECDG knee flow	ECDG knee blood pool	ECDG knee delayed	ECDG feet flow	ECDG feet blood pool	ECDG feet delayed	ECDG ankle flow	ECDG ankle blood pool	ECDG ankle delayed	Joint with highest activity	Other joint involvement	Incidental findings	US hand	US wrist	US knee	US feet	US ankle
1																																
2																																
3																																
4																																
5																																
....																																
....																																
....																																
....																																
....																																
....																																
22																																

*Participant number; #normal/reference value; Rh, rheumatoid factor ; CPR, C-reactive protein; ESR, erythrocyte sedimentation rate; Anti-CCP, anti-cyclic citrullinated peptide; DAS-28. 28-joint Disease Activity Score; ECDG, ethylenedicysteine-deoxyglucose; US, ultrasound.

Note: The table has been formatted to adhere to the page size. On the original data collection sheet, sufficient space was available to record the respective findings.

APPENDIX G
Radiation Control Committee approval

health
Department of Health
FREE STATE PROVINCE

09 July 2020

Dr. Evbuomwan Osayande
Department of Nuclear Medicine
P.O. Box 339 (Private Bag G50)
University of the Free State
Bloemfontein
9300

Permission to continue with PhD study involving imaging of patients with Rheumatoid arthritis with Technetium-99-metastable ethylenedicysteine-deoxyglucose

Dear Dr. Evbuomwan Osayande (student number: 2002128862), on behave of the Radiation Control Committee (RCC), you are hereby granted permission to perform your study in *THE VALUE OF TECHNETIUM-99-METASTABLE-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS.*

No significant additional radiation risks is foreseen to Nuclear Medicine personnel. The total patient dose is calculated as 12 mSv which poses no risk.

With Kind regards

Dr FCP du Plessis

Chairman of the RCC

APPENDIX H

CHAPTER 2: ARTICLE PUBLISHED *NUCLEAR MEDICINE AND MOLECULAR IMAGING*

Your article in Nuclear Medicine and Molecular Imaging is now online

From: do-not-reply@springernature.com

To: moreli14@yahoo.com

Date: Monday, September 25, 2023 at 10:43 AM GMT+2

SPRINGER NATURE

Congratulations

Dear Osayande Evbuomwan

We are pleased to inform you that your article has just been published:

Title

The Biodistribution and Utility of 99mTc-Ethylenedicysteine-Deoxyglucose (99mTc-Glucosamine) in the Identification of Active Disease in Patients with Rheumatoid Arthritis—a Single Center Prospective Study

Journal

Nuclear Medicine and Molecular Imaging

DOI

10.1007/s13139-023-00823-4

Publication Date

2023-09-25

Your article is available online here https://doi.org/10.1007/s13139-023-00823-4 or as a PDF here https://link.springer.com/content/pdf/10.1007/s13139-023-00823-4.pdf.

Nuclear Medicine and Molecular Imaging
https://doi.org/10.1007/s13139-023-00823-4

ORIGINAL ARTICLE

The Biodistribution and Utility of ^{99m}Tc-Ethylenedicysteine-Deoxyglucose (^{99m}Tc-Glucosamine) in the Identification of Active Disease in Patients with Rheumatoid Arthritis—a Single Center Prospective Study

Osayande Evbuomwan[1] · Barend Jansen Van Rensburg[2] · Gerrit Engelbrecht[1] · Cathryn H. S. Driver[3] · Mathys Labuschagne[2] · Joseph Sempa[2] · Je'nine Horn-Lodewyk[4,5]

Received: 13 July 2023 / Revised: 21 August 2023 / Accepted: 25 August 2023
© The Author(s) 2023

Abstract

Purpose Our objectives were to investigate the utility of ^{99m}Tc-ethylenedicysteine-deoxyglucose (ECDG) in identifying active disease in the joints of patients with rheumatoid arthritis (RA), as well as to evaluate the biodistribution of this radiopharmaceutical.

Methods A prospective study was conducted at the Department of Nuclear Medicine of the University of the Free State/Universitas Academic Hospital in Bloemfontein, South Africa. Twenty-two participants from the rheumatology department diagnosed with RA according to the ACR/EULAR classification criteria were enrolled. Participants were injected with 20–25 mCi of ^{99m}Tc-ECDG. Flow, blood pool, whole body, delayed static, and SPECT/CT images were acquired. Known sites of disease were qualitatively assessed for intensity of uptake, and disease severity was graded (Grade 0–3).

Results Twenty-two participants were studied. The median (interquartile range) age was 59 (49–68) years, and the majority (n = 21; 95.5%) were females. There was abnormal increased uptake of ^{99m}Tc-ECDG noted in majority of the sites of known disease, including unknown sites. SPECT/CT imaging localized radiotracer uptake specifically to the synovial space. Similar biodistribution of radiotracer was noted in all patients, irrespective of disease severity or fasting status.

Conclusion ^{99m}Tc-ECDG can efficiently assess disease activity in the joints of patients with RA. It accumulates in sites of both clinical and subclinical disease and might be a very useful tool for the rheumatologist in the management of patients with RA.

Keywords ^{99m}Tc-ECDG · Glucosamine · Rheumatoid arthritis · SPECT/CT

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that can lead to irreversible joint damage, deformities, disability, and premature mortality, if not treated promptly and properly [1]. Therefore, prompt diagnosis and treatment will offer a very good prognosis in these patients. Bone erosion is a central feature of this pathology in patients who do not start treatment early and is associated with severe disease and poor functional outcomes [2]. Bone erosion has also

APPENDIX I

CHAPTER 3: ARTICLE PUBLISHED IN *NUCLEAR MEDICINE COMMUNICATIONS*

NMC Decision

From: Nuclear Medicine Communications (em@editorialmanager.com)

To: moreli14@yahoo.com

Date: Monday, July 31, 2023 at 12:41 PM GMT+2

31-07-2023

RE: NMC-11-4858R2, entitled "The prognostic value of 99mTc glucosamine imaging in patients with rheumatoid arthritis – a single center prospective study"

Dear Dr Evbuomwan,

I am pleased to inform you that your work has now been accepted for publication in Nuclear Medicine Communications. All manuscript materials will be forwarded immediately to the production staff for placement in an upcoming issue. You will receive an email from the typesetters within 3 weeks to inform you when the proofs will be ready for your approval.

Original article

Nuclear Medicine Communications

The prognostic value of ^{99m}Tc-glucosamine imaging in patients with rheumatoid arthritis: a single center prospective study

Osayande Evbuomwan[a], Gerrit Engelbrecht[a], Cathryn Driver[b], Barend Jansen van Rensburg[c], Mathys Labuschagne[d] and Je'nine Horn-Lodewyk[e,f]

Objectives Poor prognostic factors in rheumatoid arthritis (RA) are associated with a more severe form of the disease. Nuclear medicine functional imaging has shown remarkable merit at identifying active disease in patients with RA and is increasingly being used in this regard. However, its prognostic value has not been evaluated thoroughly. We aimed to assess the prognostic value of technetium-99m (^{99m}Tc-) glucosamine imaging in patients with RA.

Methods Twenty-two participants diagnosed by an experienced rheumatologist with RA were recruited for inclusion in the study. Blood samples were obtained from each participant for baseline C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody titer. On the same day, each participant was injected with 20–25 millicurie (mCi) of ^{99m}Tc-glucosamine. Planar and single-photon emission computed tomography images of known disease sites were acquired up to 2 hours after radiopharmaceutical administration. Affected joints were qualitatively assessed and graded for ^{99m}Tc-glucosamine uptake and compared with blood results.

Results All participants affected joints had an increased uptake of the radiopharmaceutical, with 14 (63.6%) having elevated RF and anti-CCP antibody titers. Eight of the 14 patients with increased RF and anti-CCP antibodies had grade 3 uptake of ^{99m}Tc-glucosamine. The remaining 6 had grade 2 uptake. A significant correlation between higher grade uptake and increased levels of RF and anti-CCP antibodies ($P = 0.031$) was observed.

Conclusion We found a strong correlation between high-grade disease on imaging and the presence of RF and anti-CCP antibodies in patients with RA. *Nucl Med Commun* XXX: XXXX–XXXX Copyright © 2023 Wolters Kluwer Health, Inc. All rights reserved.

Nuclear Medicine Communications XXX, **XXX**:XXXX–XXXX

Keywords: ^{99m}Tc-glucosamine, anti-cyclic citrullinated peptide, prognostic factors, rheumatoid arthritis, rheumatoid factor

[a]Department of Nuclear Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein, [b]Nuclear Energy Corporation of South Africa, Pretoria, [c]Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, [d]Clinical Simulation and Skills Unit, School of Biomedical Sciences, Faculty of Health Sciences, University of the Free State, [e]Department of Clinical Sciences, Faculty of Health and Environmental Sciences, Central University of Technology Free State, Bloemfontein, South Africa and [f]Current affiliation: Hawke's Bay Fallen Soldier Memorial Hospital, Te Whatu Ora Health, Hastings, New Zealand

Correspondence to Dr. Osayande Evbuomwan, Department of Nuclear Medicine Faculty of Health Sciences University of the Free State, 205 Nelson Mandela Drive Bloemfontein 9300, South Africa
Tel: +27 51 405 3487, +27 74 286 9731; e-mail: moreli14@yahoo.com

Received 13 May 2023 **Accepted** 31 July 2023.

APPENDIX J

CHAPTER 4: ARTICLE SUBMITTED FOR PUBLICATION: *EGYPTIAN JOURNAL OF RADIOLOGY AND NUCLEAR MEDICINE*

From: Egyptian Journal of Radiology and Nuclear Medicine Editorial Office (em@editorialmanager.com)

To: moreli14@yahoo.com

Date: Saturday, June 17, 2023 at 10:21 PM GMT+2

EJRN-D-23-00337
Head-to-head comparison of ultrasound and 99mTc-glucosamine SPECT/CT imaging of patients with rheumatoid arthritis – a single center prospective study.
Osayande Evbuomwan; Gerrit Engelbrecht; Cathryn Driver; Joseph Simpa; Barend Jansen van Rensburg; Mathys Labuschagne; Je'nine Horn-Lodewyk
Egyptian Journal of Radiology and Nuclear Medicine

Dear Dr Evbuomwan,

Thank you for submitting your manuscript 'Head-to-head comparison of ultrasound and 99mTc-glucosamine SPECT/CT imaging of patients with rheumatoid arthritis – a single center prospective study.' to Egyptian Journal of Radiology and Nuclear Medicine.

The submission id is: EJRN-D-23-00337
Please refer to this number in any future correspondence.

During the review process, you can keep track of the status of your manuscript by accessing the following website:

https://www.editorialmanager.com/ejrn/

If you have forgotten your username or password please use the "Send Login Details" link to get your login information. For security reasons, your password will be reset.

Best wishes,

Editorial Office
Egyptian Journal of Radiology and Nuclear Medicine
ejrnm.springeropen.com

APPENDIX K

CHAPTER 5: ARTICLE SUBMITTED FOR PUBLICATION: *NUCLEAR MEDICINE COMMUNICATIONS*

NMC Submission Confirmation for Prospective six-month follow-up study comparing 99mTc-glucosamine SPECT/CT imaging and...

From: Nuclear Medicine Communications (em@editorialmanager.com)

To: moreli14@yahoo.com

Date: Saturday, September 16, 2023 at 11:38 AM GMT+2

16-09-2023

Dear Dr Evbuomwan,

Your submission entitled "Prospective six-month follow-up study comparing 99mTc-glucosamine SPECT/CT imaging and Doppler ultrasound imaging in patients with active rheumatoid arthritis" has been received by the journal editorial office.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.

https://www.editorialmanager.com/nmc/

Your username is: moreli
https://www.editorialmanager.com/nmc/l.asp?i=254525&l=3N0AFGZZ

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

Kind Regards,

Nuclear Medicine Communications

APPENDIX L

CONFERENCE PRESENTATION: SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING

2023 SNMMI Annual Meeting Submission Notification

From: SNMMI Abstracts (noreply@xcdsystem.com)

To: moreli14@yahoo.com; moreli14@yahoo.com

Date: Friday, March 24, 2023 at 03:54 PM GMT+2

SNMMI SOCIETY OF NUCLEAR MEDICINE & MOLECULAR IMAGING

March 24, 2023

Dear Osayande Evbuomwan,

Congratulations! On behalf of the SNMMI Scientific Program Committee, I am pleased to inform you that your abstract submission, Imaging of rheumatoid arthritis with the SPECT glucose analogue 99mTc- labelled glucosamine and its correlation with laboratory markers (#570), has been accepted for presentation at the SNMMI 2023 Annual Meeting in Chicago, Illinois on June 24-27, 2023.

Final decisions on session assignments and type of presentation (either poster or oral presentation) will be sent in early April.

EARLY-BIRD REGISTRATION: Register by April 27, 2023, to receive the early-bird registration rate. Please click here to register for the SNMMI 2023 Annual Meeting.

Again, we congratulate you on your abstract acceptance for this year's SNMMI Scientific Program and look forward to seeing your scientific research at the upcoming SNMMI Annual Meeting in Chicago!

Sincerely,

Heather Jacene, MD,
Chair, SNMMI Scientific Program Committee

APPENDIX M

AWARDS RECEIVED: SNMMI 2023, CHICAGO, ILLINOIS, USA

← **SNMMI 2023 International Best Abstract Award**

Riester, Holly
to Me
Today, 12:58

Dear Osayande Evbuomwan,

Congratulations! I am writing to let you know that your submission, *Imaging of rheumatoid arthritis with the SPECT glucose analogue 99mTc-labelled glucosamine and its correlation with laboratory markers*, has been selected to receive the SNMMI 2023 International Best Abstract Award for South Africa.

If you will be attending the annual meeting in person, please stop by the Science Pavilion information booth Monday or Tuesday to pick up your certificate. If you are not able to attend in person, I will send you an electronic version after the meeting is over.

Thank you so much for your participation in SNMMI 2023 and once again, congratulations!

Holly Riester

SNMMI SOCIETY OF NUCLEAR MEDICINE & MOLECULAR IMAGING

Senior Program Manager, Scientific Programs

phone: 703.667.5121

email: hriester@snmmi.org

2023 SNMMI Poster Award Notification

Riester, Holly
to Me
12 Jul, 22:51

Congratulations! I am very pleased to tell you that your poster, *#570 - Imaging of rheumatoid arthritis with the SPECT glucose analogue 99mTc-labelled glucosamine and its correlation with laboratory markers,* has been awarded **Third place in the General Clinical Specialties** track poster awards at the 2023 SNMMI Annual Meeting.

Your award certificate is attached, and the full list of poster award winners will be posted on our website and in the meeting app shortly.

Thank you so much for your participation in our first fully digital poster hall and once again, congratulations!

Holly Riester

SNMMI SOCIETY OF NUCLEAR MEDICINE & MOLECULAR IMAGING

Senior Program Manager, Scientific Programs

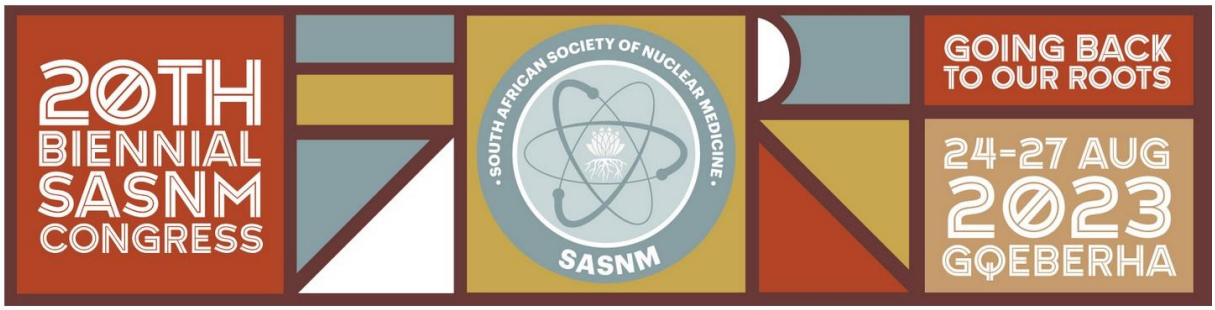
phone: 703.667.5121

email: hriester@snmmi.org

www.snmmi.org

APPENDIX N

CONFERENCE PRESENTATION: SOUTH AFRICAN SOCIETY OF NUCLEAR MEDICINE



SASNM 2023 Abstract Outcomes - Successful

From: SASNM 2023 Congress Programme Secretariat (mail@eventsairmail.com)

To: moreli14@yahoo.com

Date: Thursday, June 1, 2023 at 10:56 AM GMT+2

Dear Osayande,

Thank you for submitting an abstract for the 20th Biennal South African Society of Nuclear Medicine (SASNM) Congress taking place in Gqeberha (formerly Port Elizabeth) from 24 to 27 August 2023.

The scientific programme committee has reviewed your submission listed below and we are pleased to advise that this submission has been **ACCEPTED**.

The following steps are now required:

1. Please confirm that you are indeed still able to present at the Congress.

2. Please register for the Congress by the **15 June 2023** and make use of the discount code SASNMC011 to take advantage of the early bird rates. Follow your direct link to the registration site: SASNM Congress 2023 Registration. Your completed registration will be the final confirmation to the Scientific Committee that you are able to present at the 20th Biennal South African Society of Nuclear Medicine (SASNM) Congress.

PRESENTATION FORMAT DETAILS ARE AS BELOW

- Oral Presentation - Specifications for oral presentations will be sent in a follow-up communication
- Poster Presentation - Posters will not be digital. Specifications for the poster will be sent out next week in a follow-up communication

Abstract Submission Outcome

Title	99mTc ETHYLENEDICYSTEINE-DEOXYGLUCOSE (GLUCOSAMINE) IN THE IDENTIFICATION OF ACTIVE DISEASE IN PATIENTS WITH RHEUMATOID ARTHRITIS – A SINGLE CENTER PROSPECTIVE STUDY

APPENDIX O

CONFERENCE PRESENTATION: EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE

EANM'23 – 36th Annual Congress of the European Association of Nuclear Medicine

Vienna, Austria

from 09/09/2023 until 13/09/2023

From: abstracts <abstracts@eanm.org>
Sent: Tuesday, June 20, 2023 3:38 PM
To: Osayande Evbuomwan <EvbuomwanO@ufs.ac.za>
Subject: EANM'23 - Abstract Notification

Dear Dr. Osayande Evbuomwan,

Thank you very much for submitting your work for the EANM'23 - Annual Congress of the European Association of Nuclear Medicine.
(September 9-13, 2023 in Vienna/Austria).

I am pleased to inform you that your abstract (Control Number: **# 992**):

"Head to head comparison of ultrasound and Tc - 99m glucosamine SPECT/CT imaging of patients with rheumatoid arthritis of the knee."

was accepted as a Top Rated Oral Presentation (TROP) within the Scientific Programme of EANM'23.

Please find below your presentation details:

Presentation Number: OP-249
Session Number: 608
Session Title: Inflammation & Infection Committee - TROP Session: Infection and Inflammation Imaging: New Frontiers
Session Date: Sunday, September 10, 2023
Session Time: 4:45:00 PM - 6:15:00 PM
Session Hall: Hall F2

Within your session you will be presenter number: **2**
Therefore your oral presentation time is: **4:55:00 PM - 5:05:00 PM**

APPENDIX P

SUMMARY OF TURNITIN REPORT

Submission date: 05-Oct-2023 10:16AM (UTC+0200)
Submission ID: 2186294953
File name: Osayande_Evbuomwan_PhD_TEXT_ONLY_for_Turnitin.docx (89.06K)
Word count: 14026
Character count: 80657

Dr O Evbuomwam draft

ORIGINALITY REPORT

12%	7%	13%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

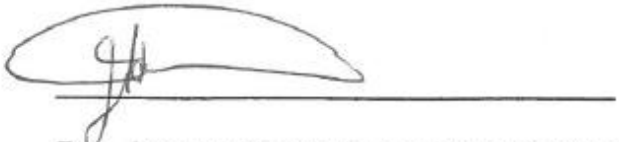
9%

★ "2015 ACR/ARHP Annual Meeting Abstract Supplement", Arthritis & Rheumatology, 2015.

Publication

PROMOTOR'S MOTIVATION FOR EXCEEDING 10% SIMILARITY INDEX (SI)

The similarity index exceeds 10% due to the highly technical nature of the research. Consequently, there is extensive use of terminology related to rheumatoid arthritis (RA), its diagnosis and clinical management, the modalities used in the diagnosis and assessment of patients' degree of disease and treatment response, including Doppler ultrasound and SPECT/CT, and a detailed description of the methodology related to the preparation and administration of the radiopharmaceutical ^{99m}Tc-glucosamine. Fixed terms, names of international institutions and societies, and lists of specific symptomatology and the classification of RA cannot be transcribed or paraphrased. On these grounds, I trust that the 12% SI is acceptable and that the thesis is approved for submission.



DR. JE'NINE HORN-LODEWYK, PROMOTOR

Date: 6 October 2023

APPENDIX Q

DECLARATION OF LANGUAGE EDITING

TO WHOM IT MAY CONCERN

I hereby declare that with regard to the following document:

Author: Dr. Osayande Evbuomwan
Title: THE VALUE OF TECHNETIUM-99-METASTABLE-ETHYLENEDICYSTEINE-DEOXYGLUCOSE IMAGING IN PATIENTS WITH RHEUMATOID ARTHRITIS

- I have performed the language editing (grammar, vocabulary and syntax).
- I assisted the author with the technical preparation of the thesis, including the layout and formatting.
- I verified the accuracy of the citations in the bibliography.
- Where applicable, I obtained and verified the most recent active uniform resource locator (URL) for internet-based references.

Notice: The verification of the accuracy of citations in the list of references (authors and title of the article, journal name, date, volume, issue and page numbers, and DOI; or similar information and the URL for internet references; or textbooks used as references) does not include authentication of the content that was cited from the reference. The editor cannot be held accountable for any changes made to the text, including language, formatting and layout, or any addition or deletion of text performed by the author after the document has been returned by the editor. The application or rejection of queries, comments and recommendations made during the editing process remains the responsibility of the author and is decided based on the author's discretion. It is the author's obligation to adhere to the similarity index requirements and policies of the UFS. The author is responsible to review the final document prior to submission for assessment and cannot hold the editor accountable for any errors occurring in the document if not communicated prior to submission.

DR. DALEEN STRUWIG
BSc, BSc (Hons), MMedSc, PhD

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Email address: daleenstruwig@gmail.com

Date: 9 October 2023