THE VALUE OF THE FLOW PHASE IN A THREE PHASE BONE SCINTIGRAPHY PERFORMED WITH TECHNETIUM-99M METHYL DIPHOSPHONATE (^{99m}Tc-MDP) IN A PATIENT BEING WORKED UP FOR INFECTION INVOLVING THE MUSCULOSKELETAL SYSTEM

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Medicine (MMed), in Nuclear Medicine.

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DECLARATION

I, Abdul Basit Rahmani, declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine (MMed), in Nuclear Medicine, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at this or any other University.

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Abdul Basit Rahmani Date

ETHICS COMMITTEE APPROVAL

Approval for this study was granted by the Human Research Ethics Committee, University of the Witwatersrand (Clearance Certificate Protocol Number – M171105 / Reference Number R14/49)

DEDICATION

This work is dedicated to my family and all my educators.

ABSTRACT

Bone scintigraphy is one of most frequently performed investigation in many Nuclear Medicine departments. A significant portion of these are performed for the diagnosis of infection involving the musculoskeletal system.

Most departments use preset protocols for planar bone scintigraphy, which involves injection of a diphosphonate labelled with Technetium-99m (^{99m}Tc-MDP). This is followed by imaging over three phases; viz. the initial flow phase, followed immediately by the blood pool phase, and the eventual delayed bone phase performed 2-4 hours after injection.

The sensitivity is high if there is increased uptake in all 3 phases. However, it has been noticed that – especially in the paediatric patient – all 3 phases are not possible due to lack of co-operation; thus, the flow phase is often omitted. Furthermore, in our department it is not always possible to obtain good quality images of the flow phase due to technical reasons, and thus the flow phase is sometimes ignored whilst reporting. Finally, we noticed that if there is increased flow to a specific region during the initial flow phase, there is almost always increased uptake during the blood pool phase as well. This is in relation to its mechanism of uptake being hyperaemia, altered capillary permeability, increased vascularity etc.

Thus, we attempted to determine if the flow phase offered any real advantage over and above the blood pool and delayed phases in this group of patients.

To the best of our knowledge, no study has been performed previously in the literature looking at the role of the flow phase as compared to the blood pool and delayed phases, and comparing these to determine congruency. There has also been no study performed previously to assess if the flow phase offered any real added advantage in patients being worked up for infection involving the musculoskeletal system.

Objective: The aim of this study was to determine the value of the flow phase in three-phase bone scintigraphy performed with technetium-99m methyl diphosphonate (^{99m}Tc-MDP) in a patient being worked up for infection involving the musculoskeletal system.

Methods: This was a case-controlled study comparing retrospectively flow versus blood pool phases, to determine the value of the flow phase in the routine bone scan for patients being worked up for infection involving the musculoskeletal system at two University Hospitals over a 36-month period.

Results: The sample population comprised of 120 patients, ranging from a mere 5-month-old baby to elderly patients in their seventies, with the median age being 56 years.

On analysis of the three-phase bone scintigraphy, we discovered that the findings on the flow phase were congruent with findings on the blood pool phase in 94/120 patients (78%). In all these 94 patients the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

The balance of the 26/120 patients (22%) demonstrated INCONGRUENT findings between the flow and blood pool phases. However, even in these cases, the reporting and management decisions were based more on the findings of the blood pool phase. Part of the reason may be the better delineation that is possible on the blood pool phase, which allows the reporting nuclear physician to provide a better description of uptake with a greater deal of confidence. Again, in this group of patients, the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

Conclusion: On analysis of the congruent and incongruent scans combined, it can be concluded that not a single patient of the 120 patients analysed benefitted additionally from the flow phase. Thus, our recommendation is that the flow phase can be safely omitted in patients being worked up for infection involving the musculoskeletal system without fear of losing crucial information.

This may be beneficial in patients who find it difficult to lie still for the entire duration of the scan. Due to the time constraints applicable to the flow phase, it would thus be more prudent to pay more attention in obtaining good quality blood pool images and omit the flow phase. This would reduce the overall time required for a patient to be lying motionless on the camera, which is especially difficult in the paediatric population. Ultimately, this would also make management of the camera time more efficient in a busy department with limited capacity.

A further prospective study with a larger patient base may yield more conclusive results. Such a study should consider analysing three-phase bone scintigraphy performed for other indications as well. It would also be prudent to include scans with negative findings for a more thorough analysis. **If such a study were to concur with our findings, this may change the practice of medicine in many centres in future, specifically in patients being worked up for infection involving the musculoskeletal system.**

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TABLE OF CONTENTS

LIST OF FIGURES

Page

CHAPTER THREE

LIST OF TABLES

Page

CHAPTER ONE

CHAPTER THREE

NOMENCLATURE/ LIST OF ABBREVIATIONS

AML – Acute myeloid leukaemia

CHBAH – Chris Hani Baragwanath Academic Hospital

CMJAH – Charlotte Maxeke Johannesburg Academic Hospital

CRP – C-reactive protein

CT – Computed Tomography

Cu-64 – Copper-64

DM – Diabetes mellitus

ESR – Erythrocyte sedimentation rate

^{18}F-FDG – ^{18}F-2-fluoro-2-deoxy-D-glucose

G1 group – patients between the ages of 30 to 60 years

G2 group – patients older than 60 years of age

Ga-67 – Gallium-67

Ga-68 – Gallium-68

HAMA – Human anti murine antibody

HIV – Human Immunodeficiency Virus

HMPAO – Hexamethylpropyleneamine oxime

HREC-M – Human Research Ethics Committee – Medical

I-124 FIAU – Iodine-124 fialuridine

IL-8 – Interleukin-8

^{111}In – Indium-111

mCi – milliCuries

MDP – Methyl diphosphonate

M:F – Male to Female ratio

MRI – Magnetic resonance imaging

MSK – Musculoskeletal system

NCA-90 – Non-specific Cross-reacting antigen-90

NCA-95 – Non-specific Cross-reacting antigen-95

PET – Positron emission tomography

SD – Standard deviation

SLE – Systemic lupus erythematosis

SPECT – Single-photon emission computed tomography

SSEA-1 – Stage specific embryonic antigen-1

TB – Tuberculosis

^{99m}Tc – Technetium-99m

^{99m}Tc-MDP – Technetium-99m methyl diphosphonate

UBI – ubiquicidin

US – Ultrasound

WBC – White blood cell

WCC – White cell count

1. Literature review

1.1. Introduction

Osteomyelitis refers to infection involving the bone and may be localized to the periosteum, cortex, marrow or cancellous tissue (Love & Palestro, 2016; Palestro, 2015; Schmitt, 2017). It affects almost 2 million people in the United States annually (Simpfendorfer, 2017). Osteomyelitis can be categorised according to various classifications, viz. (Goldsmith & Vallabhajosula, 2009):

- route of infection (haematogenous or non-haematogenous)
- underlying aetiology (diabetic foot, prosthetic joint infection, non-prosthetic joint orthopaedic hardware infection, spinal infection)
- age of onset (infantile or adult)

These classifications are based on the differing nature of the pathophysiology in the different settings, and ultimately influence the choice of radiopharmaceutical selected (Goldsmith & Vallabhajosula, 2009). The route of infection is further divided into three main mechanisms, including haematogenous seeding from a distant site (predominantly in children), contiguous spread of infection from an adjacent site (usually in the elderly) or direct inoculation of microbes post-trauma or surgery (usually the case in young adults) (Brenner *et al.*, 2012; Love & Palestro, 2016; Montgomery & Epps, 2017; Palestro, 2015; Schmitt, 2017; Sia & Berbari, 2006).

Although osteomyelitis has been reported in almost every bone, the most common sites are the long bones, vertebrae and the compromised foot of the diabetic patient (Schmitt, 2017; Sia & Berbari, 2006). In these sites, many organisms have been implicated in leading to osteomyelitis, with *Staphylococcus aureus* being the most common causative Gram-positive bacterium involved (Goldsmith & Vallabhajosula, 2009).

In the case of haematogenous osteomyelitis, this often involves the metaphysis of long bones (Schmitt, 2017). The presence of vascular loops at the metaphysis allows for the slowing of blood flow, which facilitates deposition of microbes at the metaphysis near the epiphyseal plates (Brenner *et al.*, 2012; Schmitt, 2017). Subsequently, this develops into an infection. If this is left untreated, the inflammatory response would cause pressure in the medullary bone to rise (Schmitt, 2017). As a result of this increased pressure, the infection would eventually break through to the cortex, and even through the periosteum. This would cause a decrease in the blood flow to the periosteum, which would ultimately lead to bone necrosis, and may become a surgical emergency (Brenner *et al.*, 2012; Schmitt, 2017).

In infants, the metaphyseal vessels actually penetrate the epiphysis during the early stages of life (Brenner *et al.*, 2012). This facilitates the spread of infection through the epiphysis into the adjacent joint space (Brenner *et al.*, 2012). After the age of 2 years, very few vessels cross the epiphyseal plate (Brenner *et al.*, 2012). This is a protective mechanism, and protects the epiphysis and adjacent joint from infection (Brenner *et al.*, 2012). Osteomyelitis in this age group now becomes localised to the metaphysis (Brenner *et al.*, 2012).

1.2. Pathophysiology of infection versus inflammation

Inflammation and infection are different processes. Inflammation is a protective response to injury, and is characterised by 4 factors, viz. local hyperaemia causing redness (rubor), heat (calor), oedema or swelling (tumor) and pain (dolor) (Goldsmith & Vallabhajosula, 2009; Petruzzi *et al.*, 2009). If this injury involves living micro-organisms, this leads to the development of an infection (Goldsmith & Vallabhajosula, 2009; Petruzzi *et al.*, 2009).

Inflammation may be classified broadly as acute (lasting up to 10 days) or chronic (may last for several weeks or even years) (Goldsmith & Vallabhajosula, 2009; Love & Palestro, 2016; Montgomery & Epps, 2017). The systemic manifestations of acute inflammation are evidenced by leukocytosis, fever, and an increase in plasma anti-inflammatory proteins such as C-reactive protein, fibrinogen, and haptoglobin; whilst chronic inflammation is characterised by a reduction in the number of polymorphonuclear neutrophils, and an increased infiltration of macrophages, lymphocytes, plasma cells, and fibroblasts (Goldsmith & Vallabhajosula, 2009; Love & Palestro, 2016; Montgomery & Epps, 2017). An infective process, on the other hand, is characterised by enhanced vascular permeability, with subsequent leakage of fluid and small molecules at the affected site, as well as associated transudation or diapedesis of leukocytes leading to localised accumulation of these cells (Petruzzi *et al.*, 2009).

1.3. Clinical presentation

A detailed history and physical examination is crucial in the workup of patients with suspected osteomyelitis. Firstly, the presence of any risk factors should be ascertained. These include a history of prior infection, diabetes, smoking, obesity, malnutrition and other medical co-morbidities (Springer, 2015). In addition, any potential events leading to bacteraemia should be explored, such as surgical procedures or dental work (Springer, 2015).

Physical examination should investigate the affected site thoroughly, with special focus on the presence of warmth, swelling, pain, erythema or wound healing issues (Springer, 2015). The signs and symptoms of osteomyelitis depend on the site of infection, and the mechanism of spread. There is usually chronic pain at the area of bony involvement (Schmitt, 2017; Springer, 2015). If there is advanced infection, fever and chills may occur, although this is not common (Schmitt, 2017). There may be swelling and erythema of the soft tissues; and, in advanced cases, a draining sinus tract may be seen (Schmitt, 2017; Springer, 2015). In patients with

compromised blood supply, there is usually impaired wound healing and formation of an ulcer (Schmitt, 2017). Osteomyelitis involving the vertebrae may eventually lead to compression of the spinal cord, giving rise to typical radiating pain, weakness of the extremities and impaired bladder or bowel function in severe cases (Schmitt, 2017).

1.4. Diagnosis

It is crucial to accurately diagnose osteomyelitis as early as possible, so that effective antibiotic therapy can be initiated to prevent further complications and subsequent damage to the affected site. Although certain signs and symptoms such as fever, pain and general malaise may suggest the presence of infection, these are non-specific (Love & Palestro, 2016). Thus, further biochemical markers, microbiology tests and imaging tests are often performed to confirm or exclude the presence of infection. These tests assist not only assist with confirmation of the disease, but also in determining the extent and severity of the disease, possible complications, as well as in the subsequent follow-up of patients (Sia & Berbari, 2006; Simpfendorfer, 2017; Stumpe & Strobel, 2009). They are thus essential tools utilised to assist with management decisions.

1.4.1. Biochemical markers

The commonly performed blood tests include measurement of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, which are invariably raised (Ouyang *et al.*, 2014; Schmitt, 2017). The white cell count may be normal or raised (Ouyang *et al.*, 2014; Schmitt, 2017). There is usually a normochromic, normocytic anaemia in keeping with a chronic disease pattern (Ouyang *et al.*, 2014; Schmitt, 2017). As can be seen, these laboratory tests are indirect measures of infection and thus lack specificity (Love, Marwin & Palestro, 2009; Springer, 2015).

However, they may be used as screening tools as they are usually cost-effective (Love, Marwin & Palestro, 2009; Springer, 2015).

1.4.2. Imaging tests

The imaging tests include morphologic tests which demonstrate structural changes in tissue resulting either from microbial invasion or the inflammatory response of the host; as well as functional tests which utilise a radioactive material that is either taken up by cells directly, or is attached to a substance which subsequently migrates to the region of interest (Becker & Meller, 2001; Love & Palestro, 2013; Stumpe & Strobel, 2009). Choosing the appropriate combination of imaging tests is not always easy. The choice of imaging tests should factor into account the pathophysiological processes involved, the co-existence of other diseases such as fracture, trauma or cancer, as well as the biodistribution of the radiopharmaceutical, the amount of radiation exposure and the diagnostic accuracy of the tests (Becker & Meller, 2001; Goldsmith & Vallabhajosula, 2009).

1.4.2.1. Morphologic imaging tests

1.4.2.1.1. X-rays

The imaging procedures often begin with x-rays. These are extremely useful in identifying pre-existing conditions, which could influence both the choice, as well as the interpretation of subsequent tests (Montgomery & Epps, 2017; Palestro, 2016; Palestro, Love & Miller, 2006; Simpfendorfer, 2017; Stumpe & Strobel, 2009).

In the identification of osteomyelitis itself, radiological x-rays usually demonstrate a more chronic process, as the articular cartilage may be destroyed in advanced cases, resulting in narrowing of the joint space and subchondral erosion

(Montgomery & Epps, 2017). These changes are easily seen on x-rays. Cortical or metaphyseal bone destruction may also be visualised (Montgomery & Epps, 2017).

1.4.2.1.2. Ultrasound

Ultrasound (US) is a ubiquitous tool, which is rapidly performed and has the added advantage of lacking ionising radiation (Montgomery & Epps, 2017). Due to its very limited ability to assess bone; it is generally not used in diagnosing osteomyelitis (Lee *et al*., 2016). It may, however, play a role in detecting soft tissue or subperiosteal collections (Lee *et al*., 2016). Thus, the main role of US in osteomyelitis is to detect the presence of a joint effusion, especially in areas where effusions cannot be reliably detected by palpation, such as the shoulder and hip regions (Montgomery & Epps, 2017). However, in cases where the US fails to demonstrate an effusion, there may still be associated osteomyelitis causing the symptoms, and advanced imaging with magnetic resonance imaging may be warranted (Montgomery & Epps, 2017).

1.4.2.1.3. Magnetic resonance imaging

Magnetic resonance imaging (MRI) has been recommended in the evaluation of musculoskeletal infections because of the excellent soft-tissue contrast, and the high sensitivity in demonstrating tissue oedema and hyperaemia (Funk & Copley, 2017; Palestro, 2016; Palestro, Love & Miller, 2006; Simpfendorfer, 2017; Stumpe & Strobel, 2009). It has been shown to have greater sensitivity and specificity when compared to computed tomography (CT), and has the additional advantage of identifying inflammation of the bone marrow (Funk & Copley, 2017). Similar to the ultrasound, MRI scans are advantageous in that they also lack ionising radiation (Palestro, Love & Miller, 2006).

MRI may be especially useful in challenging cases, such as spinal infections and diabetic foot infections (Stumpe & Strobel, 2009). It has also been shown to be a great initial screening test and has been recommended in patients with septic arthritis to determine adjacent bone infection prior to arthrotomy (Funk & Copley, 2017).

Although MRI can provide essential information, the cost factor may not justify its widespread use (Funk & Copley, 2017). Furthermore, despite being a great tool in the workup of osteomyelitis when the site of infection is known, choosing a site for MRI imaging may be difficult when the site of infection is not clinically evident (Brenner *et al.*, 2012). Furthermore, this modality is not available ubiquitously, and – even when available – may be contraindicated due to claustrophobia or implanted metallic devices (Simpfendorfer, 2017).

1.4.2.1.4. Computed tomography

Computed tomography (CT) scans have been shown to be excellent in defining bony pathology, as they can provide exquisite detail about sequestration and abscess formation (Funk & Copley, 2017). Thus, they may also have a role to play in monitoring disease progression after initial MRI acquisition (Funk & Copley, 2017). CT scans are not advocated in the diagnosis and management of osteomyelitis in the paediatric population, due to the radiation exposure and the limited visualisation of anatomical and spatial extent of tissue inflammation (Funk & Copley, 2017).

The advantages and disadvantages of the various morphologic imaging modalities are summarised in table 1.1 below.

Table 1.1: Summary of the advantages and disadvantages of the various morphologic imaging modalities

IMAGING MODALITY	ADVANTAGES	DISADVANTAGES
X-rays	Relatively inexpensive	Changes visualised late in the disease process
	Readily available	May not demonstrate full extent of disease
	No need for sedation	
	Uncomplicated procedure	
Ultrasound	Relatively inexpensive	Operator dependent
	Lack of ionising radiation	Unable to penetrate bone
	No need for sedation	Depth visualisation may be an issue
	Can detect effusions	
MRI	Excellent for soft tissue visualisation	Not used if metal artefacts
	Lack of ionising radiation	
	Can detect effusions	
CT	Fast	Radiation exposure
	Excellent bony detail	Not great for soft tissue visualisation
	Can detect effusions	Artefacts if prosthesis

Adapted from (Funk & Copley, 2017).

Since conventional radiological imaging modalities have limited value in the early stages of the disease, the more physiological nuclear medicine procedures have gained more prominent roles in the diagnosis of bone and joint infections (Glaudemans, Israel & Slart, 2015; Stumpe & Strobel, 2009). In addition, nuclear medicine techniques routinely allow whole-body imaging, whereas CT and MRI are able to focus only on just a specific part of the body (Becker & Meller, 2001). Furthermore, prosthetic joints or metallic implants in the spine or extremities can cause significant artefacts. These artefacts can degrade image quality sufficiently to make diagnosis impossible on both CT and MRI (Ouyang *et al.*, 2014; Stumpe & Strobel, 2009).

1.4.2.2. Functional imaging tests

Molecular imaging with single-photon emission computed tomography (SPECT) and positron emission tomography (PET) tracers is increasingly being used to diagnose, characterize and monitor disease activity in osteomyelitis (Diaz-Ledezma *et al.*, 2015; El-Maghraby, Moustafa & Pauwels, 2006; Goldsmith & Vallabhajosula, 2009). Commonly performed radionuclide tests include 3-phase bone scintigraphy with technetium-99m (^{99m}Tc)-diphosphonate bone scintigraphy, Gallium-67 (Ga-67) citrate imaging, in vitro labelled leukocyte/ white blood cell imaging, ^{99m}Tc-radiolabeled murine monoclonal antigranulocyte antibodies, ^{99m}Tc-radiolabelled colloids and human immunoglobulins (Love & Palestro, 2016; Stumpe & Strobel, 2009). Although they are useful, each of these tests has limitations. Factors affecting labelling and uptake of these radiotracers include previous intervention, previous insult and other co-morbidities. Thus, the nuclear physician needs to carefully select the most appropriate test based on the grade of inflammation, duration of infection, availability, cost, radiation exposure and confounding factors listed above (Stumpe & Strobel, 2009).

Functional imaging with radiotracers was initiated in the 1970s (Macdonald, 2016), and is able to detect inflammatory processes, as well as monitor response to therapy, with high sensitivity (Basu *et al.*, 2009). The non-specific mechanisms of tracer accumulation include enhanced blood flow, increased vascular permeability, and possible other factors (Basu *et al.*, 2009). The ideal characteristics of an effective radiolabelled imaging agent in this setting include high sensitivity and specificity, ease of preparation, minimal toxicity and radiation dose, and low cost (Basu *et al.*, 2009).

More recently, hybrid imaging systems have led to significant advancements in the field of nuclear medicine. These systems combined SPECT with CT (SPECT-CT) and PET with CT (PET-CT), such that functional and morphological studies are acquired on the same device without having to move the patient (Love & Palestro, 2016). This greatly improved both the diagnostic confidence and test accuracy (Love & Palestro, 2016). By being able to precisely localise uptake of the radiopharmaceutical, these hybrid imaging systems are able to accurately differentiate soft-tissue infections from infections involving bones, and thus guide patient management accurately (Love & Palestro, 2016). Patients in whom infection is excluded, these hybrid imaging systems may also be able to uniquely identify other causes of the patient's symptoms (Becker & Meller, 2001; Love & Palestro, 2016).

1.4.2.2.1. SPECT agents

1.4.2.2.1.1. Three-phase bone scintigraphy

^{99m}Tc bone imaging agents were introduced by Subramanian *et al.* in the early 1970s and have since then stood the test of time (Brenner *et al.*, 2012). Approximately 3.5 million bone scans were performed in the United States alone in the year 2005 (Brenner *et al.*, 2012).

The major advantage of the three-phase bone scintigraphy is that it is widely available and, furthermore, has been shown by many authors to have a sensitivity of more than 90% in non-violated bone (Love & Palestro, 2013; Palestro, 2016; Palestro, Love & Miller, 2006; Simpfendorfer, 2017; Stumpe & Strobel, 2009). It can be positive as early as two days after the onset of symptoms, and has an accuracy rate of more than 90% (Love & Palestro, 2013). However, it must be noted that since the bone scan reflects bone remodelling in general, and is not specific to infection, it may be positive in cases other than infection (Love & Palestro, 2013, 2016; Palestro, 2015, 2016; Simpfendorfer, 2017; Stumpe & Strobel, 2009). These include patients with fractures, orthopaedic hardware, recent surgery or the diabetic patient with neuropathic joint (Love & Palestro, 2013; Palestro, 2015; Palestro, Love & Miller, 2006; Simpfendorfer, 2017). This leads to decreased specificity of the three-phase bone scintigraphy in such patients (Palestro, Love & Miller, 2006; Simpfendorfer, 2017). In these circumstances, the three-phase bone scintigraphy may still be useful as a screening test where, if negative, it would confidently exclude an infection (Love & Palestro, 2013, 2016; Simpfendorfer, 2017).

Various mechanisms have been proposed for the uptake of the radiopharmaceutical. These include local tissue necrosis, damage causing increased tissue calcium deposition, hyperaemia, altered capillary permeability, adsorption onto soft tissue calcium and binding to enzyme receptors or denatured proteins (Brenner *et al.*, 2012). Bony destruction caused by osteomyelitis is followed by an osteoblastic healing response; thus, bone imaging can detect osteomyelitis well before radiographic findings are evident (Becker & Meller, 2001; Brenner *et al.*, 2012). As small as a 5% change in bone turnover can be detected on three-phase bone scintigraphy, whereas at least 40-50% of mineral content must be lost to detect lucency on x-rays or CT scans (Becker & Meller, 2001).

Most departments use preset protocols for the three-phase bone scintigraphy (Naddaf *et al.*, 2004). This involves injection of a diphosphonate (methyl diphosphonate in our centre) labelled with ^{99m}Tc (^{99m}Tc-MDP) (Naddaf *et al.*, 2004).

The three phases refer to (Simpfendorfer, 2017):

- the initial dynamic imaging sequence (blood flow or perfusion phase)
- followed immediately by static imaging (blood pool or soft-tissue phase), and
- the eventual delayed bone phase performed 2-4 hours after injection and includes images of the region of interest

The study is positive for osteomyelitis when there is focal hyperperfusion, focal hyperaemia, and focally increased bone activity in the region of interest on delayed images (Brenner *et al.*, 2012; Love & Palestro, 2013, 2016). The uptake of the radiopharmaceutical depends on the incorporation of the amorphous bone mineral matrix into the calcium moiety, which later matures into hydroxyapatite crystals (Love & Palestro, 2016). The degree of uptake is related to blood flow and rate of new bone formation (Love & Palestro, 2016). In osteomyelitis, there is increased blood flow as well as transcapillary migration of inflammatory cells into the bone, which results in increased delivery to – and uptake of – the radiopharmaceutical in the affected region (Love & Palestro, 2016). It must be noted that focally increased uptake on the delayed phase is the most definitive finding, because there can be no osteomyelitis unless the delayed images are abnormal, even in the setting of increased blood flow and/or blood pool activity (Brenner *et al.*, 2012). A confounding factor in the event of a negative scan (no uptake on the delayed phase) may be the initiation of antibiotic therapy prior to the scan being performed. However, our study excluded negative scans as part of the exclusion criteria.

The patient needs to be prepared adequately to obtain high quality images. The patient should be well hydrated, and the technician will ask the patient to void just before the acquisition begins (Naddaf *et al.*, 2004). The technician will also take the necessary care to ensure optimum patient comfort and proper positioning by using additional supports if necessary (Naddaf *et al.*, 2004). The following equipment settings usually result in superb quality images (Naddaf *et al.*, 2004):

- dual-head camera
- low-energy high resolution collimator
- 20% energy window centred at 140keV
- matrix size of at least 256 x 1024
- perfusion phase acquired at 3-5 seconds per frame
- obtain approximately 1.5 million counts for whole-body images

Although the bone scintigraphy demonstrates similar sensitivity to MRI for diagnosing acute osteomyelitis, the major advantage of the bone scintigraphy is the ability to image the whole body (Glaudemans *et al.*, 2015). This is especially advantageous in the detection of multifocal osteomyelitis (Glaudemans *et al.*, 2015). On the flip side, bone scan results can be positive for at least 2 years after a hip replacement – and up to 5 years after knee prosthesis implantation – due to physiological bone remodelling (Glaudemans *et al.*, 2015). These patients would require additional infection detecting techniques to conclusively diagnose or exclude osteomyelitis, such as those reviewed below.

1.4.2.2.1.2. Labelled leukocyte imaging agents

Complementary inflammation detecting techniques are often utilised to increase the low specificity of MDP, such as the labelled leukocytes (Love & Palestro, 2016). Their uptake is dependent on various mechanisms, including intact chemotaxis, number and types of cells labelled and the cellular component of a particular inflammatory response (Love & Palestro, 2016). The majority of labelled white blood cells (WBC) are usually neutrophils; thus, this technique holds the most promise in identifying neutrophil-mediated inflammatory processes, such as bacterial infections (Love & Palestro, 2016). In disease processes where the predominant cellular response is not neutrophilic in nature, such as tuberculosis, labelled leukocyte imaging is less useful (Love & Palestro, 2016). It is important to take cognisance that a total circulating WBC count of at least 2000/ml is necessary to obtain satisfactory images (Love & Palestro, 2016).

The leukocytes can be labelled either with Indium-111 (^{111}In) or Technetium-99m (^{99m}Tc). The labelling technique was first described by Thakur *et al.* years ago, and was later modified by other authors to increase the labelling efficiency (Becker & Meller, 2001). ^{111}In-WBC imaging can be performed up to 30 hours after reinfusion of labelled cells due to the long half-life of 67 hours (Becker & Meller, 2001). Disadvantages of ^{111}In-WBC include the low-resolution of the images, and the prolonged interval between injection and imaging (Becker & Meller, 2001). The long physical half-life of ^{111}In-oxine results in the patient being subjected to a high radiation burden (Becker & Meller, 2001). Concerns have been raised about the hazards associated with withdrawing of blood, labelling and then re-injecting the cells under non-physiological conditions (Basu *et al.*, 2009; Goldsmith & Vallabhajosula, 2009). The in-vitro leukocyte labelling process requires skilled personnel, is laborious and not always available (Love & Palestro, 2016). It may also not be a feasible procedure in leukopaenic patients and children (Love & Palestro, 2016).

The major advantage of ^{111}In-WBC imaging is that simultaneous dual-isotope may be performed. While the patient is waiting for the white blood cells to be labelled, they can undergo ^{99m}Tc-colloid marrow scintigraphy as part of a simultaneous dual-isotope acquisition protocol (Love & Palestro, 2016). It has also been shown that labelled leukocytes did not accumulate in non-infectious conditions such as heterotopic ossification, metastatic disease and degenerative arthritis (Love, Marwin & Palestro, 2009).

^{99m}Tc-labelled leukocytes, on the other hand, have the advantage of being able to detect abnormalities within a few hours after injection, as well as the high resolution of the images (Love & Palestro, 2016). This is especially useful when adding SPECT-CT to the protocol. Disadvantages include urinary tract activity and bowel activity, which may confound the results (Love & Palestro, 2016). Simultaneous dual-isotope imaging is also not possible, since an interval of 48-72 hours is required between ^{99m}Tc -WBC and ^{99m}Tc-colloid marrow imaging (Love & Palestro, 2016).

Irrespective of the radiotracer being utilised, WBC imaging is the nuclear medicine test of choice for diagnosing complicating osteomyelitis except in the spine (Glaudemans *et al.*, 2015; Love & Palestro, 2016; Palestro, 2015). It is able to accurately differentiate normal post-operative changes from infection (Love & Palestro, 2013). However, due to the physiological biodistribution of labelled WBCs to the bone marrow, up to 50% of the patients with spondylodiscitis may demonstrate photopaenic "cold" areas due to the encapsulation of the infection and the hampered migration of WBCs (Glaudemans *et al.*, 2015; Stumpe & Strobel, 2009). This leads to the low sensitivity and specificity of this test in spinal infections (Glaudemans *et al.*, 2015; Stumpe & Strobel, 2009). Adding SPECT-CT to the protocol can improve localisation of the infectious focus, and better delineate the extent of the disease process (Glaudemans *et al.*, 2015). Complementary ^{99m}Tc-colloid marrow imaging is sometimes required to increase diagnostic accuracy (Love & Palestro, 2016). Other techniques, such as the FDG PET-CT are also promoted as the first line imaging modality for patients with suspected spondylodiscitis (Glaudemans *et al.*, 2015).

1.4.2.2.1.3. Colloid marrow imaging

Labelled leukocyte imaging is the procedure of choice for diagnosing most cases of complicating osteomyelitis (Glaudemans *et al.*, 2015; Love & Palestro, 2016; Palestro, 2015). However, complementary marrow imaging is sometimes required to increase diagnostic accuracy (Love & Palestro, 2016). The rationale behind this is the fact that leukocytes accumulate both in infection as well as in the marrow; thus, it is not always possible to differentiate between the two on WBC images alone (Love & Palestro, 2016; Palestro, 2015). Colloid, on the other hand, accumulates only in the marrow, and thereby facilitates the differentiation between infection and normal marrow (Love & Palestro, 2016; Palestro, 2015). Thus, combined WBC/marrow imaging is considered positive for infection when activity is present on the WBC images without corresponding activity on the marrow images, termed spatially incongruent (Love, Marwin & Palestro, 2009; Love & Palestro, 2016). The

overall diagnostic accuracy of combined WBC/marrow imaging has been shown to be in excess of 90% (Love & Palestro, 2013, 2016; Palestro, 2015).

It is imperative that the correct technique be used to achieve successful leukocyte/ marrow imaging results. If ^{111}In-labelled-leukocytes are being used, marrow imaging may be performed before, after or even during the leukocyte study using the simultaneous dual-isotope acquisition protocol (Love, Marwin & Palestro, 2009). This protocol allows a more direct comparison of the leukocyte and marrow images, as it permits the direct superimposition of one image on top of the other, thereby greatly facilitating interpretation of the study (Love, Marwin & Palestro, 2009). There are, however, some advantages to performing the marrow imaging after the labelled leukocyte imaging, viz. if the labelled leukocyte study fails to demonstrate increased activity around the prosthesis, then the follow-up marrow imaging may not be required at all (Love, Marwin & Palestro, 2009).

On the other hand, if ^{99m}Tc-labeled leukocytes are used instead, simultaneous dual-isotope acquisition is not possible due to the overlap in energy windows (Love, Marwin & Palestro, 2009). When one uses the ^{99m}Tc-labeled leukocytes, there may be persistent activity on the leukocyte images which could confound the results. This activity may last for up to 48 hours after injection (Love, Marwin & Palestro, 2009). Thus, it is best to wait at least 72 hours before performing the marrow imaging.

1.4.2.2.1.4. Labelled antigranulocyte antibody scintigraphy

Constant research is underway to develop alternative radiopharmaceuticals that may overcome some of the limitations of the agents mentioned above. The in-vitro labelling of leukocytes is labour intensive, time-consuming, not always available, requires direct contact with blood, and may not be feasible in leukopaenic patients and children (Love & Palestro, 2016). The additional colloid marrow imaging is inconvenient for many patients, and adds complexity and expense to the procedure

(Love & Palestro, 2016). In-vivo techniques for WBC labelling have been developed as an alternative to in-vitro labelling. This comprises of labelling of antigranulocyte antibodies and antibody fragments (Love & Palestro, 2016; Palestro, 2015). They have been shown to perform well in diagnosing osteomyelitis, specifically in the peripheral skeleton (Stumpe & Strobel, 2009).

^{99m}Tc-Besilesomab (trade name Scintimun) is a murine IgG1 class monoclonal antibody which binds to non-specific cross-reacting antigen-95 (NCA-95) (Love & Palestro, 2016). This antigen is expressed on cell membranes of granulocytes and granulocyte precursors (Love & Palestro, 2016). A significant disadvantage of this agent is the incidence of human anti-murine antibody (HAMA) response, which has been shown to occur in a significant percentage of patients (Love & Palestro, 2016).

This led to the development of antibody fragments, such as the ^{99m}Tc-Sulesomab (trade name Leukoscan) (Love & Palestro, 2016). Leukoscan is a fragment antigen binding (Fab') portion of an IgG1 class murine monoclonal antibody that binds to NCA-90 on leukocytes (Love & Palestro, 2016). Being an antibody fragment, it does not induce a HAMA response (Love & Palestro, 2016).

An additional advantage of immunoscintigraphy over autologous labelled leukocytes is the simplicity of its use compared to complicated techniques which warrant the isolation of autologous white blood cells (Becker & Meller, 2001). These newer agents, however, have their own limitations, and are not easily available in most centres (Palestro, 2015).

1.4.2.2.1.5. Labelled antimicrobial peptides

Multicellular organisms contain antimicrobial peptides as an essential component of their natural biological defence system (Basu *et al.*, 2009; Love & Palestro, 2016; Palestro, 2015). It was hypothesised that targeting of bacteria using radiolabelled synthetic fragments of ubiquicidin (UBI), a human antimicrobial peptide, would allow

differentiation of infection from sterile inflammation (Basu *et al.*, 2009; Love & Palestro, 2016). A specific role for this tracer is in the setting of spinal infections (Love & Palestro, 2016). It is also used to assess the efficacy of antibacterial agents, and thus may be used to monitor response to treatment (Love & Palestro, 2016; Palestro, 2015).

1.4.2.2.1.6. Labelled human non-specific immunoglobulins

Nonspecific polyclonal human immunoglobulins have a tendency to localize to sites of active infection/inflammation (Petruzzi *et al.*, 2009). This occurs by extravasation of the tracer from the bloodstream due to the induced local hyperaemia (Petruzzi *et al.*, 2009). As such, this agent is non-specific for infection (Becker & Meller, 2001; Petruzzi *et al.*, 2009). A major advantage of this agent is that – being derived from human antibodies – it does not induce a HAMA response (Petruzzi *et al.*, 2009). This agent can be labelled with either ^{111}In or ^{99m}Tc; and, thus, may experience certain limitations related to the biodistribution of these agents (Petruzzi *et al.*, 2009).

1.4.2.2.1.7. Gallium-67 citrate

Gallium-67 (Ga-67) citrate was initially introduced as a tumour imaging agent in the early 1970s (Goldsmith & Vallabhajosula, 2009). Thereafter, its abilities to detect both acute and chronic inflammation were highlighted (Goldsmith & Vallabhajosula, 2009). It was initially used as an adjunct to bone scintigraphy in the workup of osteomyelitis; however, its primary role now is in the setting of spinal infections (Love & Palestro, 2016; Palestro, 2015).

The uptake of Ga-67citrate in infection is related to several factors. The vast majority (approximately 90%) of circulating Ga-67 citrate is bound to transferrin in the plasma (Becker & Meller, 2001; Love & Palestro, 2016). Increased blood flow and vascular membrane permeability result in increased delivery and accumulation of Ga-67

citrate at foci of infection (Becker & Meller, 2001; Love & Palestro, 2016). Another plasma protein, viz. Lactoferrin, is also present in high concentrations in inflammatory exudates (Becker & Meller, 2001; Love & Palestro, 2016). It is hypothesised that Ga-67 citrate is transported via transferrin to inflammatory foci (Becker & Meller, 2001; Love & Palestro, 2016). It then dissociates from transferrin and complexes with the Lactoferrin (Becker & Meller, 2001; Love & Palestro, 2016). Bacteria may also play a role in the uptake of Ga-67 citrate, probably through non-specific binding and facilitated diffusion (Becker & Meller, 2001; Love & Palestro, 2016). The bacteria produce small molecule metal chelates, viz. siderophores, which have been shown to avidly attach to the Ga-67 citrate (Becker & Meller, 2001; Love & Palestro, 2016). This siderophore-Ga-67 complex is then transported into the bacterium, and eventually phagocytosed by macrophages (Becker & Meller, 2001; Love & Palestro, 2016). Some Ga-67 citrate may be bound to, and transported by the leukocytes themselves (Becker & Meller, 2001; Love & Palestro, 2016).

Despite the various mechanisms of uptake, Ga-67 citrate is not an ideal imaging agent. This is related to its unfavourable physical characteristics, viz. the multiple photon energies, the long half-life, as well as the binding to the plasma protein transferrin which results in relatively high background activity and therefore reduces the lesion-to-background contrast (Becker & Meller, 2001; Goldsmith & Vallabhajosula, 2009). There is also a long necessary delay between injection of the radiopharmaceutical and imaging – of up to three days – to allow for the background activity to clear so that acceptable images may be obtained (Love & Palestro, 2016).

Ga-67 citrate accumulates in both septic and aseptic inflammation, in the bone marrow, and in areas of increased bone mineral turnover in the absence of infection (Love, Marwin & Palestro, 2009; Love & Palestro, 2016). Thus, in order to improve the accuracy of both the bone scintigraphy and gallium imaging, the 2 studies are often interpreted together. As explained by Love, Marwin & Palestro (2009), the test is considered to be:

- **positive for osteomyelitis** when “the distribution of the 2 tracers is spatially incongruent, or when the distribution is spatially congruent but the relative intensity of gallium uptake exceeds that of the diphosphonate mineral turnover in the absence of infection”
- **equivocal for osteomyelitis** when “the distribution of the 2 radiotracers is congruent, both spatially and in terms of intensity”
- **negative for osteomyelitis** when “the gallium images are normal, regardless of the bone scan findings or when the distribution of the 2 tracers is spatially congruent and the relative intensity of gallium uptake is less than that of the diphosphonate”

1.4.2.2.1.8. Indium-111 (^{111}In) Biotin

Another radiopharmaceutical that shows promise in the imaging of spinal infections is ^{111}In-Biotin. This is used as a growth factor by certain bacteria, and is a necessary component for cell growth, fatty acid production and metabolism of fats and amino acids (Palestro, 2015).

1.4.2.2.2. PET agents

PET offers several advantages over SPECT radiopharmaceuticals, by being “a high-resolution tomographic technique that enables precise localisation of radiopharmaceutical accumulation” (Becker & Meller, 2001; Love & Palestro, 2013, 2016).

1.4.2.2.2.1. ^{18}F-FDG PET-CT imaging

Over the last several years, 2-[18F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron-emission tomography (PET) with or without computed tomography (CT) imaging has

been shown be of great value in the detection of inflammation, probably based on the focal increase in anaerobic glucose metabolism which is associated with the cellular response (Basu *et al.*, 2009; Goldsmith & Vallabhajosula, 2009).

FDG is transported into cells via glucose transporters and phosphorylated by hexokinase to ^{18}F-FDG-6 phosphate, but is not metabolised further (Becker & Meller, 2001; Love & Palestro, 2016). It accumulates in virtually all leukocytes and its uptake is related to their metabolic rate and the number of glucose transporters (Becker & Meller, 2001; Love & Palestro, 2016). Increased FDG accumulation in infection is presumably due to several factors, as explained by (Becker & Meller, 2001; Love & Palestro, 2016):

- "an increased number of glucose transporters
- an increased expression of these glucose transporters by activated inflammatory cells, and
- an increased affinity of these transporters for FDG in inflammation"

There are several advantages to using ^{18}F-FDG PET-CT in the setting of infection. These include (Becker & Meller, 2001; Love & Palestro, 2016):

- the ability of the small FDG molecule to enter poorly perfused areas rapidly
- the whole procedure being rapidly completed in 1-2 hours (Love & Palestro, 2013)
- the uptake which usually normalises within 3-4 months after trauma or surgery
- the observation that degenerative bone changes show only mildly increased uptake of FDG

^{18}F-FDG has been shown to be very useful in the workup of patients with suspected osteomyelitis. Its reported sensitivity is over 95%, with the specificity being in the range of 75-99% (Love & Palestro, 2016; Palestro, 2015). ^{18}F-FDG has been shown to be especially useful in the setting of spinal infections, where it is able to accurately differentiate degenerative changes from infection (Love & Palestro, 2016; Palestro,

2015). In this setting, ^{18}F-FDG has been shown to be highly sensitive, with a high negative predictive value (Love & Palestro, 2016; Palestro, 2015; Stumpe & Strobel, 2009).

Low-grade and chronic infections are more difficult to diagnose with the current imaging modalities (Stumpe & Strobel, 2009). ^{18}F-FDG PET-CT has also performed well in this clinical scenario, since ^{18}F-FDG is avidly taken up by activated macrophages in the chronic phases of infection (Stumpe & Strobel, 2009). Thus, ^{18}F-FDG-PET has great diagnostic accuracy for confirming or excluding chronic osteomyelitis (Stumpe & Strobel, 2009). A negative ^{18}F-FDG PET-CT scan can virtually rule out osteomyelitis with a great deal of confidence (Love & Palestro, 2016; Stumpe & Strobel, 2009).

It is interesting to note that – unlike in the setting of malignancy – elevated levels of glucose in the blood do not appear to have an adverse effect on the uptake of ^{18}F-FDG in the inflammatory cells (Basu *et al.*, 2009). These factors suggest that ^{18}F-FDG PET-CT has the potential to become a suitable alternative to currently available diagnostic techniques in the workup of osteomyelitis.

1.4..2.2.2.2. Gallium-68 PET-CT imaging

Another PET isotope, Gallium-68 (Ga-68), has been shown to have certain advantages over Ga-67 in the diagnosis of osteomyelitis (Glaudemans *et al.*, 2015). These include its physical half-life of 68 minutes, which is much shorter than that of Ga-67 (half-life of 78 hours) (Love & Palestro, 2016). Consequently, this allows larger doses of radioactivity to be injected, leading to better quality of the images, with less radiation burden to the patient (Love & Palestro, 2016). Imaging is completed within a few hours after injection, in contrast to Ga-67 imaging which may take up to several days (Love & Palestro, 2016). Because Ga-68 is a PET radiopharmaceutical, image quality is superior to that which can be obtained with Ga-67 SPECT images (Glaudemans *et al.*, 2015; Love & Palestro, 2016). Ga-68

citrate has the additional advantages of rapid blood clearance and quick diffusion (Glaudemans *et al.*, 2015).

1.4.2.2.2.3. Iodine-124 fialuridine PET-CT imaging

Bacteria possess a thymidine kinase which is specific to bacteria and different to the human variant (Love & Palestro, 2016). Imaging of this specific thymidine kinase is useful in detecting viable bacteria (Love & Palestro, 2016). Preliminary studies show excellent results when using Iodine-124 fialuridine (I-124 FIAU) PET-CT imaging in the diagnosis of musculoskeletal infection (Love & Palestro, 2016).

As can be seen, there is no single agent that is equally efficacious in all regions of the skeleton. The selection of the appropriate study should, thus, be based on the clinical question posed by the referring physician. Table 1.2 below provides a guide for the specific utility of some of the agents discussed above.

Table 1.2: Different radionuclide tests and their specific clinical indications

Radionuclide test	**Specific indication**
Labelled leukocyte imaging	• complicating osteomyelitis, such as prosthetic joint infection • diabetic pedal osteomyelitis • neuropathic joint
Gallium-67 citrate imaging	• useful adjunct to MRI in spinal infection
18F-2-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET)	• evaluation of spinal infection

Adapted from (Palestro, 2016; Palestro, Love & Miller, 2006)

1.4.2.2.3. Future prospective agents

Tremendous amount of research has focused on finding the ideal radiopharmaceutical for use in the diagnosis of infection. Various criteria were proposed in determining the suitability of the ideal agent. It was suggested that the ideal new agent should “provide high and early uptake in infectious foci and inflammatory tissue, show no accumulation in non-inflamed tissues, have low toxicity and display rapid background clearance” (Becker & Meller, 2001). The current trend is also towards producing smaller peptides (Becker & Meller, 2001).

An IgM antibody was discovered which is directed against the stage-specific embryonic antigen (SSEA-1, anti-CD15) (Becker & Meller, 2001). The very high affinity of this antibody for the leukocyte antigen provides for a high target to background ratio, and thus excellent quality of images (Becker & Meller, 2001). Studies have shown this antibody to have a sensitivity of 95% and a specificity of 96% for the imaging of infection (Becker & Meller, 2001).

Interleukin-8 (IL-8) is a well-known chemotactic cytokine with a high affinity for binding to receptors expressed on activated neutrophils (Petruzzi, Shanthly & Thakur, 2009). This was labelled with ^{99m}Tc, and was shown to have some promising characteristics (Petruzzi, Shanthly & Thakur, 2009). There were no significant side effects noted in initial animal model experiments (Petruzzi, Shanthly & Thakur, 2009).

WBCs have also been labelled in-vitro with PET agents, viz. ^{18}F-FDG, with conflicting results (Glaudemans *et al.*, 2015). The labelling was, however, affected by blood glucose levels and the degree of metabolism of the cells (Glaudemans *et al.*, 2015). Thus, the labelling efficiency was found to be significantly lower than that for ^{99m}Tc-labelled or ^{111}In-labelled WBCs (Glaudemans *et al.*, 2015). It is also not possible to image beyond 4-6 hours after injection (Glaudemans *et al.*, 2015). This is

a major drawback, because late imaging is mandatory for many clinical indications due to the slow accumulation of the WBCs at the sites of infection (Glaudemans *et al.*, 2015). Furthermore, a high activity is required for the labelling process because of the short physical half-life of F-18 of 110 minutes (Glaudemans *et al.*, 2015).

In this regard, Copper-64 (Cu-64) seemed to be a better option for the labelling of WBCs, due to its longer half-life of 12.7 hours (Glaudemans *et al.*, 2015).

Thus, many newer agents appear to be promising in being utilised for imaging of infection in the future. However, it waits to be proven if these agents are specific to infection. Large scale multi-centre trials must be performed to define their role in the future of infection imaging.

It must be noted that these additional investigations are not performed for each and every patient; instead, they are performed only in selected cases, such as in the setting of previously violated bone. Although these additional investigations were not the main focus of our study, the findings of these investigations have been included in the results and discussion for the sake of completeness and only for those selected patients in whom such additional investigations were necessary.

The aim of this study was primarily to determine the value of the flow phase in three-phase bone scintigraphy performed with technetium-99m methyl diphosphonate (^{99m}Tc-MDP) in a patient being worked up for infection involving the musculoskeletal system.

CHAPTER 2

2 MATERIALS AND METHODS

2.1 Study population

The study was a case-controlled study comparing retrospectively the flow phase versus blood pool phase, to determine the value of the flow phase in the routine bone scan performed on patients of all age groups, who were referred for a three-phase bone scintigraphy in the workup of infection involving the musculoskeletal system at two University Hospitals (Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital). The time frame spanned a period of 36 months (from July 2014 to June 2017).

This study was approved by the Human Research Ethics Committee – Medical (HREC - M) of the University of the Witwatersrand (Clearance Certificate Protocol Number – M171105 / Reference Number R14/49).

The inclusion criteria were as follows:-

- All studies performed on patients who were referred for a three-phase bone scintigraphy in the workup of infection involving the musculoskeletal system.
- All ages and gender.

The exclusion criteria were as follows:-

- Study of poor or questionable quality for interpretation.
- Scans with negative findings.
- Scans performed for indications other than osteomyelitis.
- Scans with the flow phase having been performed beyond the stipulated timelines.

2.2. Aims and Objectives

2.2.1. Primary aim

2.2.1.1. To determine the value of the flow phase in three-phase bone scintigraphy performed with technetium-99m methyl diphosphonate (^{99m}Tc-MDP) in a patient being worked up for infection involving the musculoskeletal system.

2.2.2. Secondary objectives

2.2.2.1. To analyse the findings of the flow and blood pool phases.

2.2.2.2. To compare the flow phase versus blood pool phase.

2.2.2.3. To determine if the findings are congruent and if the flow phase offers any real added advantage.

2.2.2.4. To ascertain common presenting signs and symptoms in patients with suspected osteomyelitis.

2.2.2.5. To identify common risk factors for osteomyelitis in our setting.

2.3 Materials and methods

All studies performed on patients referred for a three-phase bone scintigraphy to the Department of Nuclear Medicine at either of the University Hospitals (Charlotte Maxeke Johannesburg Academic Hospital or Chris Hani Baragwanath Academic Hospital) over a period of 36 months were identified (between July 2014 and June 2017).

Clinical notes were obtained from the bone scan request forms and/or hospital records. The bone scan request form is a standard form which provides uniformity in the information requested *(see attached Appendix A).* Information obtained included

demographics, risk factors, presenting signs and symptoms, indications for the scan, results of other investigations and other data (see *Appendices B1 and B2*).

The results of the three-phase bone scintigraphy were obtained from the database of the Nuclear Medicine Department at both above-mentioned academic hospitals. The results of the flow and blood pool phases were analysed. These results were then compared to determine if the findings were congruent and if the flow phase offered any real added advantage *(as per Appendix B1)*. Any follow-up studies performed were also analysed to finally determine the presence or absence of infection in these patients. Follow-up studies included either Gallium-67 citrate imaging, labelled leukocyte imaging with ^{99m}Tc-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) combined with ^{99m}Tc-Nanocolloid scintigraphy, or a variant the Leukoscan which consists of ^{99m}Tc-antigranulocyte antibodies.

All patients were allocated a patient code. This list was kept separately from the data in order to maintain confidentiality (*see Appendix C*).

2.4 Statistical analysis

Our final analysis included a total of 120 patients. The data was captured using Microsoft Excel 2010 and analysed using STATA 14.0 (StataCorp LP, College Station, TX).

The data obtained was also analysed with the assistance of a statistician. Phase 1 involved a descriptive analysis of data. This included drawing up of medians, percentages and frequencies of the study variables. Thereafter, cross tabulation of certain variables was performed e.g. age, gender against risk factors, presenting symptom against final outcome, age against results of scintigraphy etc.

Categorical variables were analysed using the transformation t test (non-normal distribution of a single sample). Non-categorical variables were analysed using chi squared test (2 groups, independent) *(see attached statistics as Appendix D*).

The study was a simple comparison of two variables (flow phase versus blood pool phase), thus a t-test was used as a method of comparison. Other statistical methods were simple measurements for tabulation of data (thus the use of percentages and frequencies), as well as the measurement of centricity on the distribution of data (thus the use of median).

To the best of our knowledge, no study has been performed previously in the literature looking at the role of the flow phase as compared to the blood pool and delayed phases, and comparing these to determine congruency. There has also been no study performed previously to assess if the flow phase offered any real added advantage in patients being worked up for infection involving the musculoskeletal system.

CHAPTER 3

3 RESULTS

We identified 325 patients in total who had been referred for a three phase bone scintigraphy to the Department of Nuclear Medicine at either of the two University Hospitals over a period of 36 months. However, some of these patients had been referred for indications other than assessment of infection involving the musculoskeletal system. These indications included assessment of avascular necrosis, workup of non-specific pain, assessment of bony lesions, etc. Thus, these patients were excluded from the study. Furthermore, scans with negative findings and scans of poor or questionable quality for interpretation were also excluded as per the exclusion criteria. This left a total of 120 patients for final analysis (see figure 3.1).

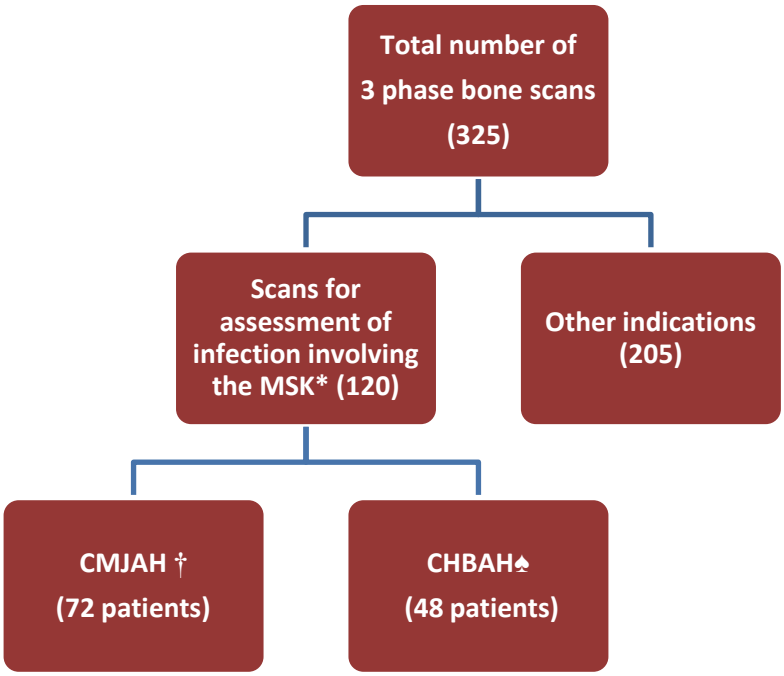


Figure 3.1: Graphical representation of the number of patients included in final analysis

*MSK = Musculoskeletal system

†CMJAH = Charlotte Maxeke Johannesburg Academic Hospital

♠CHBAH = Chris Hani Baragwanath Academic Hospital

The three-phase bone scintigraphy had been performed using various imaging systems, viz. SPECT cameras, Forte and Brightview (Philips Medical), and SPECT-CT cameras, Infinia Hawkeye and Discovery Infinia 640 (GE Medical) and Brightview XCT (Philips Medical).

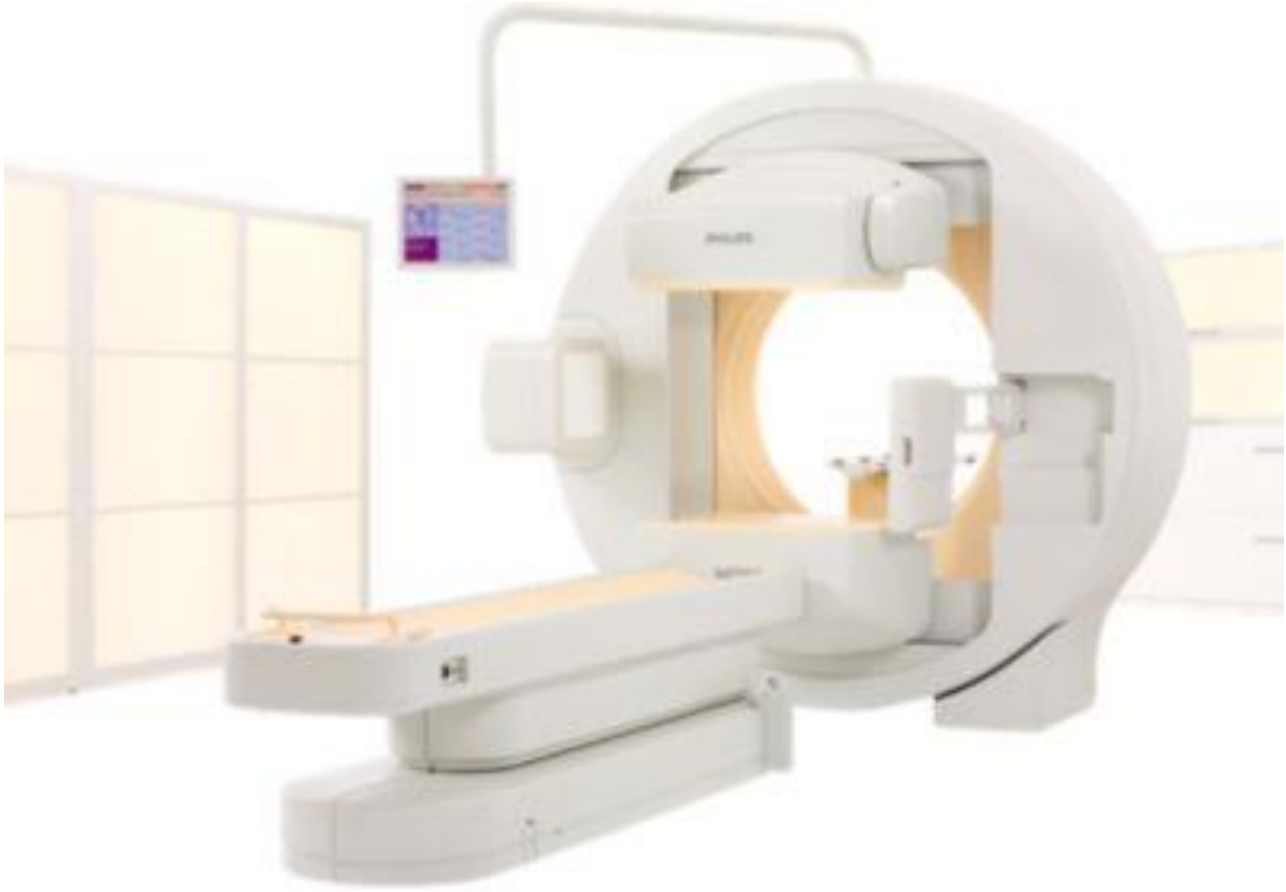


Figure 3.2: Image of the Philips Brightview XCT SPECT/CT Dual Head Gamma Camera *(Source: Philips, 2019)*

Figure 3.3: Image of GE Discovery Infinia 640 SPECT/CT Gamma Camera
(Source Blockimaging, 2019)

The three-phase bone scintigraphy was performed within 1 – 2 weeks of presentation to the hospital. However, the symptoms had been ongoing for varying lengths of time, from as little as one month to over twenty years in some cases prior to the patient seeking medical care. This is elaborated upon later in the discussion.

The study consisted of the early phase (which was sub-divided into the flow phase and the blood pool phases) performed within the first ten minutes of injection of methyl diphosphonate labelled with Technetium-99m (^{99m}Tc-MDP) and the delayed phase performed 2-4 hours after injection. This timing protocol was standardised at both centres.

The technicians had followed a standardized protocol at both centres. The patients had been well hydrated. They had been asked to void prior to the acquisition of the delayed images. The settings on the imaging cameras had been optimised as

described by Naddaf *et al.*, (2004) to ensure superb quality images. A low-energy high-resolution collimator had been used, with a 20% energy window centred at 140keV. Both the departments had administered a standardised dose of 20mCi of ^{99m}Tc-MDP for adult patients. The dose had been adjusted according to the body weight for the paediatric patients. The technician had ensured optimum patient comfort during the scan acquisition.

The results of the three-phase bone scintigraphy were subsequently analysed. We determined asymmetrically increased vascularity on the flow phase images in 64/120 patients (53%) on the side of the pathology. This was regarded as positive. There was no increased vascularity on the side of the pathology in 56/120 patients (47%) and was regarded as negative. The blood pool phase images demonstrated asymmetrically increased vascularity on the side of the pathology in 90/120 patients (75%). This was regarded as positive. There was no increased vascularity on the side of the pathology in 30/120 patients (25%) and was regarded as negative. These findings are tabulated in table 3.1 below.

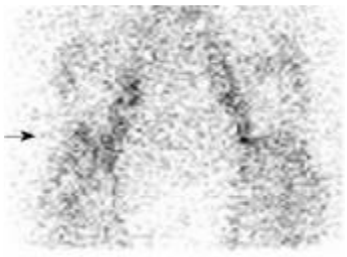
Table 3.1: Comparison of findings during the flow and blood pool phases

	Positive findings	**Negative findings**
Flow phase	53% (6 patients only mildly increased)	47%
Blood pool phase	75% (12 patients only mildly increased)	25%

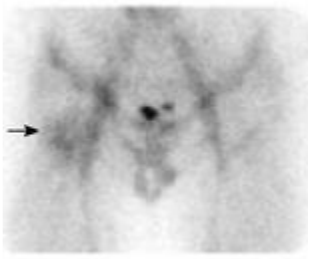
We then compared directly the results of the flow and blood pool phases to determine congruency, as outlined in table 3.2 below. If the findings of the flow phase and the blood pool phase were similar (either both positive or both negative), this was termed congruent. If there was a discrepancy (negative flow phase but positive blood pool phase), this was termed incongruent or flow-blood pool discordance. None of the patients had the reverse discrepancy (i.e. positive flow phase but negative blood pool phase).

Table 3.2: Direct comparison of flow and blood pool phases to determine congruency

Number of patients	**Flow phase**	**Blood pool phase**	**Conclusion**
64 patients	Positive	Positive	Congruent
30 patients	Negative	Negative	Congruent
26 patients	Negative	Positive	Incongruent
0 patients	Positive	Negative	Incongruent



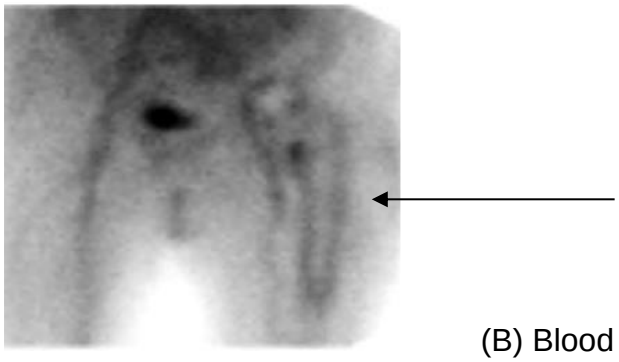
(A) Summed Flow phase image



(B) Blood pool phase image

Figure 3.4: Example of a congruent scan where both the flow and blood pool phases are positive (asymmetrically increased vascularity in the right proximal femur on both the flow phase (A) as well as the blood pool phase (B))

(A) Flow phase



(B) Blood pool phase

Figure 3.5: Example of an incongruent scan where the flow phase is negative but the blood pool phase is positive (no asymmetrically increased vascularity in the left proximal femur on the flow phase (A); however, there is asymmetrically increased vascularity in the left proximal femur on the blood pool phase (B))

The study was a simple comparison of two variables (flow phase versus blood pool phase), thus a t-test was used as a method of comparison, as expounded later under the discussion. Other statistical methods were simple measurements for tabulation of data (thus the use of percentages and frequencies), as well as the measurement of centricity on the distribution of data (thus the use of median).

All of the 120 patients demonstrated asymmetrically increased uptake of tracer on the side of the pathology during the delayed phase. This was to be expected, as patients with negative scans had been excluded from our study. This decision was based on the suggestions of Brenner *et al.*, (2012), who documented that focally increased uptake on the delayed phase is the most definitive finding, because there can be no osteomyelitis unless the delayed images are abnormal, even in the setting of increased blood flow and/or blood pool activity.

We analysed the final diagnosis in patients that demonstrated positive findings on all 3 phases of the three-phase bone scintigraphy (64 patients in total). Only 17/64 patients (26.5%) were diagnosed as positive for infection, while 30/64 patients (47%) were diagnosed as having NO infection despite all 3 phases demonstrating increased uptake of tracer. The balance of the 17/64 (26.5%) patients had inconclusive results. These findings are tabulated in table 3.3 below.

Table 3.3: Final diagnosis in patients demonstrating positive findings on all 3 phases of the three-phase bone scintigraphy *(n = 64)*

Final diagnosis	**Number of patients**
Positive for infection	17/64 patients (26.5%)
Negative for infection	30/64 patients (47%)
Inconclusive	17/64 patients (26.5%)

We found that patients had as many as 5 sites of abnormal uptake on the three-phase bone scintigraphy (the average being 2 sites of uptake usually involving the femur and acetabulum). Frequent sites of abnormal uptake included the femori, acetabulae, knees, tibiae and tarsal bones (see table 3.4).

Table 3.4: Frequency of sites of abnormal uptake

SITES OF ABNORMAL UPTAKE	**NO. OF PATIENTS**	
LOWER LIMBS	**LEFT SIDE**	**RIGHT SIDE**
Femur	33	22
Acetabulum	24	13
Knees	18	20
Tibia	13	11

Foot	10	12
Hips	6	5
Fibula	3	4
UPPER LIMBS		
Shoulder	1	
Humerus	3	
Sternoclavicular joint	1	
Clavicle	2	
Glenoid	1	
Thumb		1
OTHERS		
Facial bones and skull	1	
Manubrium and sternum	1	
Spine	5	
Pelvis	3	

Results were further analysed according to the age groups, i.e. paediatric patients versus adult population. The paediatric group was defined as patients below the age of 16 as per hospital and departmental policies (personal communication on 14 November 2018 at 09h00 – Prof M Mulaudzi, HOD Paediatrics, CMJ Academic Hospital). In our sample population, 8/120 patients (6.7%) fell into the paediatric sub-category. The characteristics of these patients are tabulated in table 3.5 below.

Table 3.5: Characteristics of the paediatric population

	Parameter	**No of patients**
n = 8		
Hospital	CMJAH	2
	CHBAH	6
Gender	Females	5
	Males	3
Age Median age (range)	8 years (5 months to <16 years)	
Signs/ Symptoms	Pain	4
	Abscess	2
	Sepsis	2
	Swelling	1
Risk factors	Previous surgery	1
	Previous trauma or fracture	1
	Pre-existing medical condition	1

We also analysed the results of the three-phase bone scintigraphy in this sub-group of paediatrics. The results are tabled below in table 3.6.

Table 3.6: Comparison of findings during the flow and blood pool phases in the paediatric population

n = 8	**Positive findings**	**Negative findings**
Flow phase	7 patients	1 patient
Blood pool phase	7 patients	1 patient

We then compared directly the results of the flow and blood pool phases to determine congruency, as outlined in table 3.7 below.

Table 3.7: Direct comparison of flow and blood pool phases to determine congruency in the paediatric population (*n = 8*)

Number of patients	**Flow phase**	**Blood pool phase**	**Conclusion**
7 patients	Positive	Positive	Congruent
1 patient	Negative	Negative	Congruent

We also looked at the sites of abnormal uptake in this sub-category of paediatric patients (see table 3.8).

Table 3.8: Frequency of sites of abnormal uptake in the paediatric population

SITES OF ABNORMAL UPTAKE	NO. OF PATIENTS	
LOWER LIMBS	**LEFT SIDE**	**RIGHT SIDE**
Femur	3	
Acetabulum	1	
Knees	3	
Tibia	3	3
Foot	1	2
Hips	2	
Pelvis	1	

Thereafter, we moved to other end of the spectrum and analysed the much older age category. This was defined as patients older than 60 years of age. Since it is well-known that older patients may have a compromised blood supply, we envisaged that there may be some differences in adults older than 60 years of age versus adults below the age of 60 years. When we re-assessed our sample population, we realised that the vast majority of our population (108/120 patients, 90%) was above the age of 30 years (see figure 3.10). We thus divided the adults into patients between the ages of 30 to 60 years (G1 group) and patients older than 60 years of age (G2 group).

We analysed the findings on the three-phase bone scintigraphy in both these sub-groups and compared them to determine levels of congruency and any significant differences between the two groups (see tables 3.9 and 3.10 below).

Table 3.9: Comparison of findings during the flow and blood pool phases in the 2 sub-groups of adult patients

		30 – 60 years G1 *(n = 57)*	**61 – 80 years G2 *(n = 51)***
Flow phase			
	Positive findings	29 patients	25 patients
	Negative findings	28 patients	26 patients
Blood pool phase			
	Positive findings	42 patients	38 patients
	Negative findings	15 patients	13 patients

Table 3.10: Direct comparison of flow and blood pool phases to determine congruency in the 2 sub-groups of adult patients

		Flow phase	**Blood pool phase**	**Conclusion**
30 – 60 years G1 *(n = 57)*				
	29 patients	Positive	Positive	Congruent
	15 patients	Negative	Negative	Congruent
	13 patients	Negative	Positive	Incongruent
61 – 80 years G2 *(n = 51)*				
	25 patients	Positive	Positive	Congruent
	13 patients	Negative	Negative	Congruent
	13 patients	Negative	Positive	Incongruent

Overall, of the 120 patients analysed, most patients had follow-up studies (see figure 3.6). Follow-up studies included either Gallium-67 citrate imaging, labelled leukocyte imaging with ^{99m}Tc-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) combined with ^{99m}Tc-Nanocolloid scintigraphy, or a variant the Leukoscan which consists of ^{99m}Tc-antigranulocyte antibodies.

Only 32/120 patients (27%) had no additional investigations performed. Of these, 11 were diagnosed with infection based on the three-phase bone scintigraphy alone and in 1 patient the diagnosis of infection was excluded. The balance of the 20 patients had inconclusive findings as they defaulted follow-up appointments for additional imaging studies specific for infection.

The other 88/120 patients (73%) had specific infection imaging, either the labelled leukocyte scan, Leukoscan or Gallium-67 citrate scan. Of these, no additional imaging was required in 39 patients. In the vast majority of these patients (38/39 patients), infection was excluded without any additional studies, while only 1 patient was diagnosed as being positive for infection.

The balance of the 49 patients required additional colloid imaging for definitive confirmation or exclusion of infection. In 35 patients, infection was excluded based on the colloid imaging findings. Ten patients were diagnosed as having infection based on the colloid imaging findings. Four patients defaulted follow-up appointments and a definitive conclusion could not be provided.

This information is depicted graphically in figure 3.6 below.

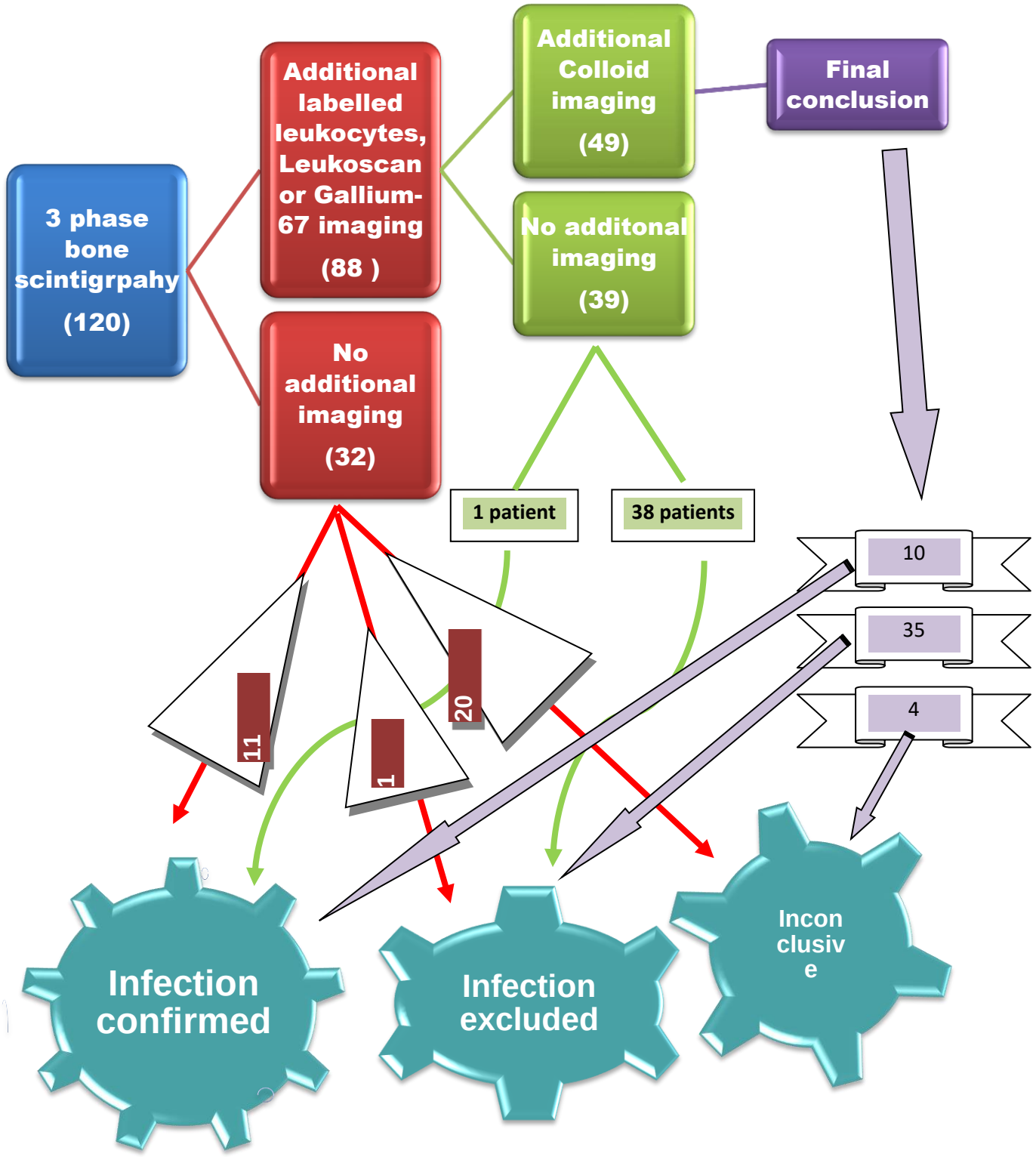


Figure 3.6: Graphical representation of the overall results of the three-phase bone scintigraphy and additional imaging studies

Table 3.11 below summarises the final overall diagnoses in our sample population of 120 patients.

Table 3.11: Summary of final diagnoses in our sample population

Final conclusion	**No. of patients**
Positive for infection	22 patients
Negative for infection	74 patients
Inconclusive	24 patients
TOTAL	*120 patients*

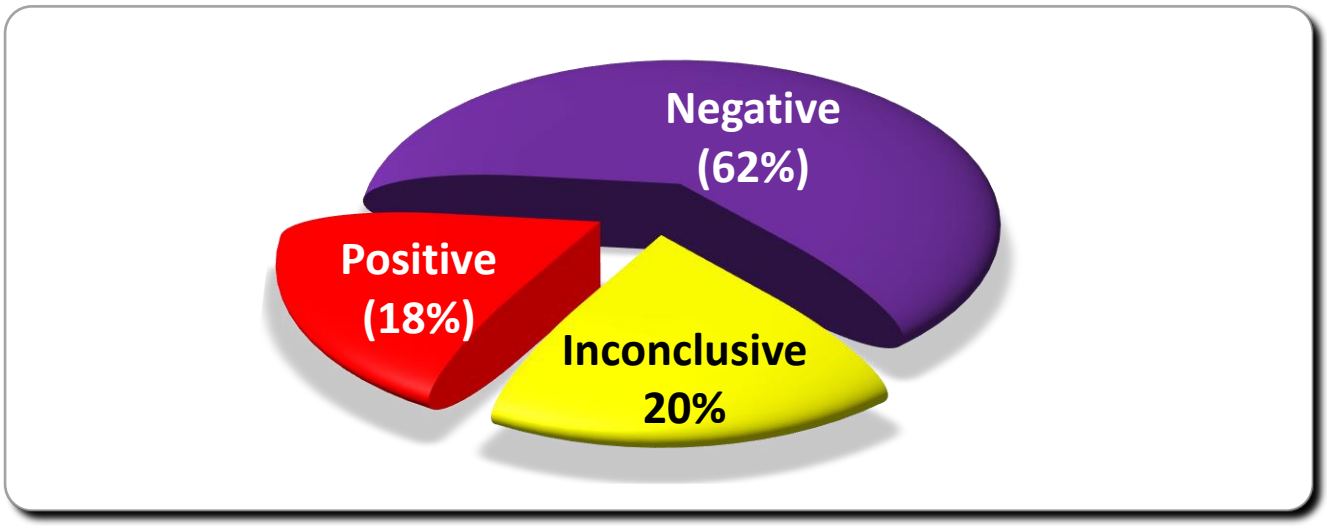


Figure 3.7: Relative distribution of final diagnoses in our sample population

We divided our sample population into the paediatric group and the adult group. We then compared directly the final diagnoses to the findings of the flow phase and the blood pool phases in these two groups. In the paediatric population, 6/7 patients (86%) with confirmed infection had positive findings on the flow and blood pool phases. On the other hand, in the adult population, only 12/57 patients (21%) with

confirmed infection had positive findings on the flow phase. When comparing the blood pool phase, again, only 14/83 adult patients (17%) with confirmed infection had positive findings on the blood pool phase.

The results appeared to fare better when analysing the negative findings. In the adult population, 44/55 patients (80%) with negative findings on the flow phase had no evidence of infection on subsequent follow-up imaging. Similarly, 22/29 patients (76%) with negative findings on the blood pool phase had no evidence of infection on subsequent follow-up imaging. These results are depicted in table 3.12 below

Table 3.12: Direct comparison of final diagnoses to the findings of the flow phase and the blood pool phases in the paediatric and adult population

Age		**Flow phase**	**Blood pool phase**		**Final**	**Conclusion**	
					Positive for infection	**Negative for infection**	**Inconclusive**
Paediatrics		Positive = 7	Positive = 7		6	1	
(n = 8)		Negative= 1	Negative= 1				1
Adults		Positive= 57			12	29	16
(n = 112)		Negative=55			4	44	7
			Positive= 83		14	51	18
			Negative=29		2	22	5

We then attempted to calculate the number of true positives and true negatives in the two groups, as displayed in tables 3.13 and 3.14 below.

Table 3.13: Number of true positives in the paediatric population

Paediatrics		
Flow phase	Infection confirmed	Infection excluded
Positive	**6**	1
Negative	0	0

Table 3.14: Number of true negatives in the adult population

Adults				Adults		
Flow phase	Infection confirmed	Infection excluded		Blood pool phase	Infection confirmed	Infection excluded
Positive	12	29		Positive	14	51
Negative	4	**44**		Negative	2	**22**

We further scrutinised the final diagnosis in the 30 patients with negative findings on both the flow and the blood pool phases as per table 3.2. Again, it was demonstrated that the majority of these patients (22/30 patients, 73.3%) had no evidence of infection on the subsequent follow-up imaging. This is portrayed in table 3.15 below.

Table 3.15: Final diagnosis in patients with negative findings on both the flow and the blood pool phases *(n = 30)*

Final diagnosis	**Number of patients**
Positive for infection	2/30 patients (6.7%)
Negative for infection	22/30 patients (73.3%)
Inconclusive	6/30 patients (20%)

Finally, we analysed the final diagnosis in the 26 patients with incongruent findings on the flow and blood pool phases (flow-blood pool discordance) as per table 3.2. We realised that the majority of these patients (22/26 patients, 84.6%) also had no evidence of infection on the subsequent follow-up imaging. This is specified in table 3.16 below.

Table 3.16: Final diagnosis in patients with flow-blood pool discordance (negative flow, positive blood pool) *n = 26*

Final diagnosis	**Number of patients**
Positive for infection	2/26 patients (7.7%)
Negative for infection	22/26 patients (84.6%)
Inconclusive	2/26 patients (7.7%)

In terms of the demographics, of the 120 patients, 72 patients were from Charlotte Maxeke Johannesburg Academic Hospital (60%) and 48 from Chris Hani Baragwanath Academic Hospital (40%) – see figure 3.8. These hospitals were the two sites of investigation.

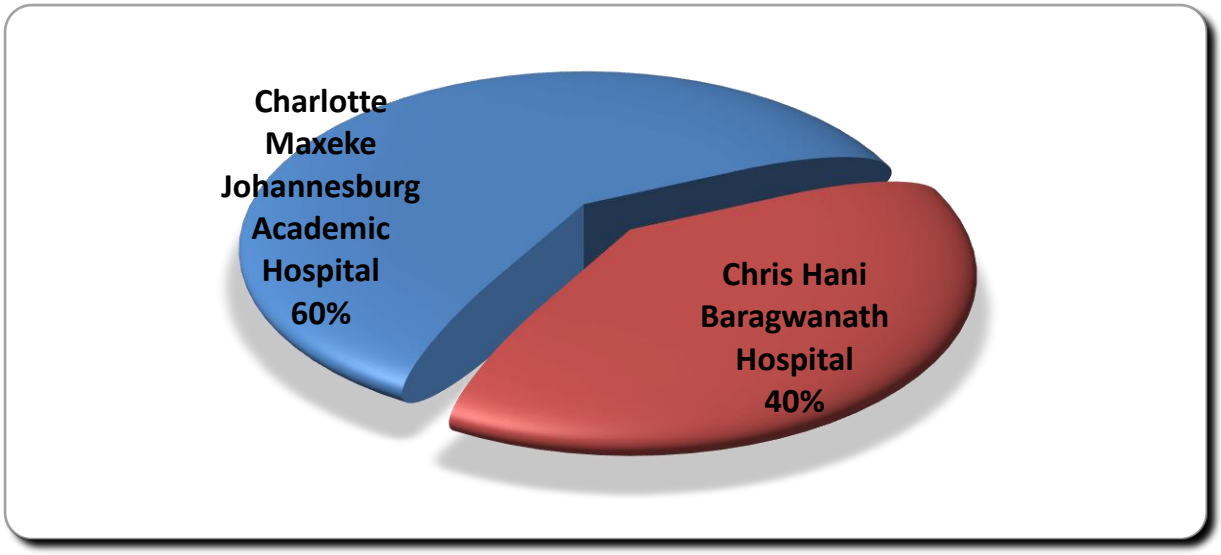


Figure 3.8: Distribution of patients from the two hospitals

There were 70 females and 50 males, with a female to male ratio of 1.4:1.

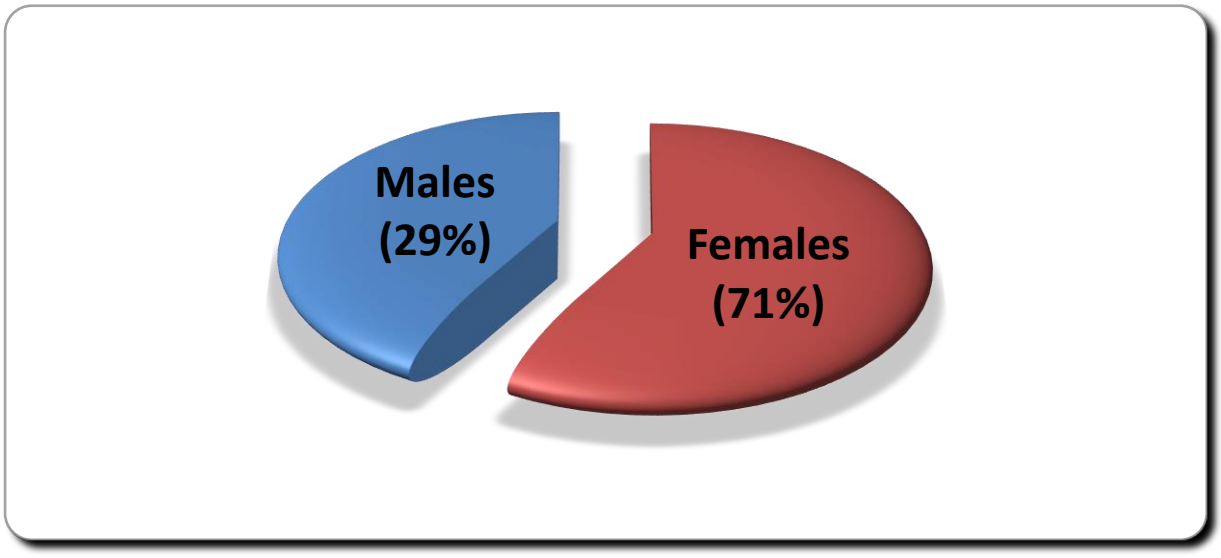


Figure 3.9: Female to male ratio

This female preponderance was noted mainly to be from Charlotte Maxeke Johannesburg Academic Hospital, as indicated in table 3.17 below.

Table 3.17: Gender ratio at the different hospitals

HOSPITAL	FEMALES	MALES	TOTAL
Chris Hani Baragwanath Academic Hospital	27	21	48
Charlotte Maxeke Johannesburg Academic Hospital	43	29	72

The median age was 56 years, with a wide range from as young as 5 months to as old as 78 years. There was a significant peak in the seventh decade (see figure 3.10). Patients in the 4th to 8th decades of life made up the vast majority (90%) of the sample.

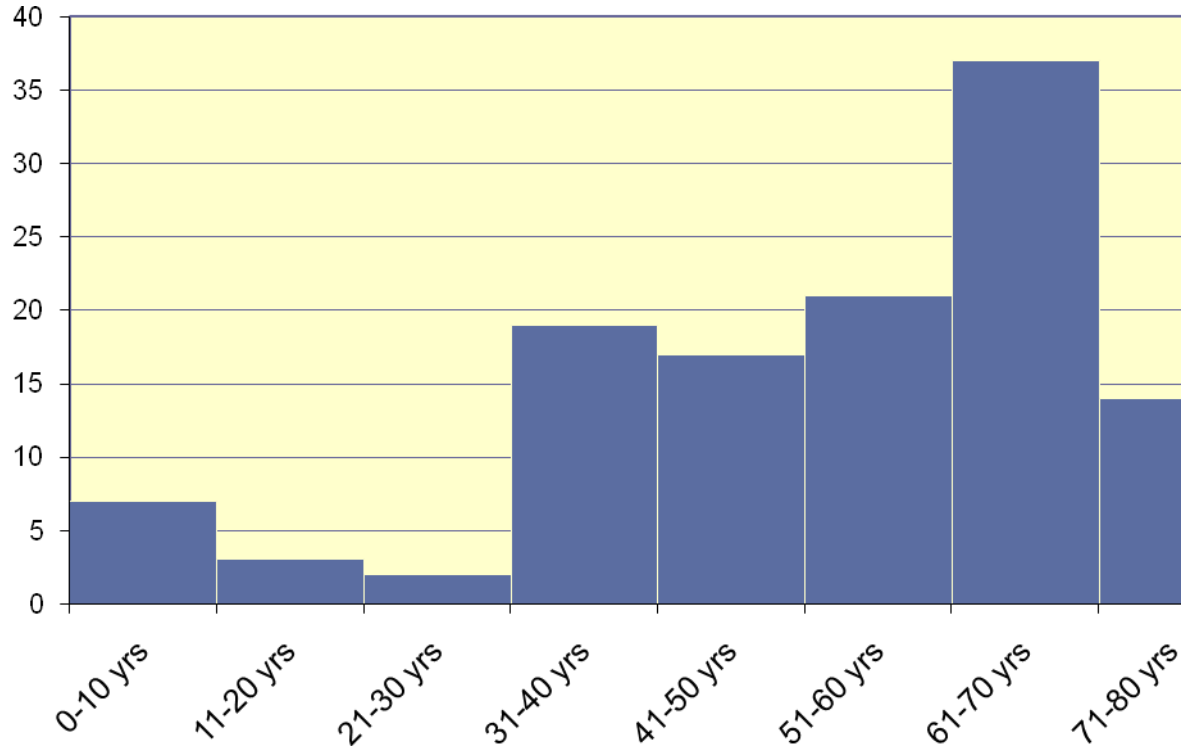


Figure 3.10: Distribution of patient numbers over the various age groups

The age groups were further analysed according to the different hospitals, and we discovered contrasting differences in the age groups, as elicited in table 3.18 below. As can be vividly elicited from table 3.18 below, using the two sample t-test, we found that patients from Chris Hani Baragwanath Academic Hospital tended to be younger (mean age 45.69 ± 19.87) as compared to their counterparts from Charlotte Maxeke Johannesburg Academic Hospital (mean age 56.40 ± 14.79) [p = 0.01]. We realised that this dataset appeared not to be normally distributed; thus, we further submitted the difference in ages between the two institutions to a 2-sample Wilcoxon rank sum test, which still showed significant differences in the ages (p = 0.002). The median age now was calculated as 46 years for patients from Chris Hani Baragwanath Academic Hospital versus 60.5 years for patients from Charlotte Maxeke Johannesburg Academic Hospital.

Table 3.18: Age analysis as per the different hospitals

	Charlotte Maxeke Johannesburg Academic Hospital	**Chris Hani Baragwanath Academic Hospital**
Age range	6 years – 78 years	5 months – 76 years
Median (age)	60.5 years	46 years
Peak	7th decade	4th and 7th decades
Mean (age)	56.40 years	45.69 years
Patients under 35 yrs	3	15
Patients over 70 yrs	11	3

Pain was the most common presenting symptom, occurring in 77/120 patients (64%). The other signs and symptoms were observed in only a small number of patients (see table 3.19 below). None of the patients presented with fever.

Table 3.19: Frequency of presenting signs and symptoms in the sample population

SIGNS & SYMPTOMS	No. of patients	Percentage
Pain	77/120	64%
Abscess or oozing pus*	10/120	8%
Malunion or non-union	9/120	7.5%
Sepsis*	7/120	6%
Swelling	7/120	6%
Ulcer	3/120	2.5%
Instability	2/120	1.7%

**Abscess referring to localised infection while sepsis referring to more generalised widespread infection.*

Of the 77 patients presenting with pain in our sample population, only half of these patients (36/77 patients, 48%) demonstrated increased flow during the early vascular phase. We analysed the final conclusion in patients presenting with pain as their primary symptom, as demonstrated in table 3.20 below. We did not analyse the other signs and symptoms as these occurred in less than 10% of patients.

Table 3.20: Comparison of findings on the flow phase to the final diagnoses in patients presenting with pain

Symptom	Flow phase	Final Conclusion		
		Positive for infection	Negative for infection	Inconclusive
Pain *(n = 77)*	Positive = 36	6	20	10
	Negative = 41	3	30	8

The patients had a history of various risk factors for developing infection, viz. previous surgery in 74/120 patients (62%), history of trauma or fracture in 35/120 patients (29%), and pre-existing medical conditions or malignancies in 17/120 patients (14%). Patients with a history of previous surgery tended to be older as compared to patients with pre-existing medical conditions or a history of trauma or fracture (see table 3.21).

Table 3.21: Comparison of risk factors and median age

RISK FACTOR	**NUMBER OF PATIENTS**	**MEDIAN AGE**
Previous surgery	74/120 patients (62%)	62 years
Trauma or fracture	35/120 patients (29%)	44 years
Pre-existing medical conditions or malignancies	17/120 patients (14%)	40 years

Of the 74 patients with a history of previous surgery – mainly a total hip or knee replacement – only 38% (28/74 patients) demonstrated increased flow during the early vascular phase. In these patients with increased flow during the early vascular phase, the average time delay from surgery to the presentation of symptoms was 5.44 years (range 5 months to 23 years).

On the other hand, of the 35 patients with a history of previous trauma or fracture, more than half of the patients (21/35 patients, 60%) demonstrated increased flow during the early vascular phase. The differences between these 2 sub-categories are listed in table 3.22 below.

Table 3.22: Differences in the number of positive findings on flow phase in patients with different risk factors

Patient sub-group	**Increased flow phase**
Previous surgery	38%
Previous trauma or fracture	60%

Table 3.23 below lists the different types of surgeries that had been performed in these patients.

Table 3.23: Types of surgery performed in the sample population

TYPE OF SURGERY	**NO. OF PATIENTS**
Total hip replacement	36 patients
Total knee replacement	18 patients
Open reduction and internal fixation	7 patients
Fusion surgeries (hip, ankle, thoraco-lumbar spine, etc)	6 patients
External fixator/ K-wire insertion	3 patients
Partial knee replacement	1 patient
Mitral valve replacement	1 patient
Multi-ligament reconstruction in knee	1 patient

As noted from table 3.23 above, total hip or total knee replacements were the most common type of surgeries that had been performed (54/74 patients – 73%). Two-third of these 54 patients had previous total hip replacements (36 patients), while the balance of one-third had previous total knee replacements (18 patients).

Of the patients with total hip replacements (36 patients), 12 patients had surgery only on the right hip, 15 patients only on the left hip, and the balance of the 9 patients had surgery on both hips. This relative distribution is presented graphically in figure 3.11 below. Patients presented with symptoms on an average of 7.1 years after the initial surgery (range from 1 year to 23 years post surgery).

Of the 18 patients with total knee replacements, 9 patients had surgery only on the right knee, 4 patients had surgery on the left knee and the remaining 5 patients had surgery bilaterally. Patients in this sub-group presented with symptoms on an average of 3.6 years post surgery (range from 8 months to 11 years after the surgery).

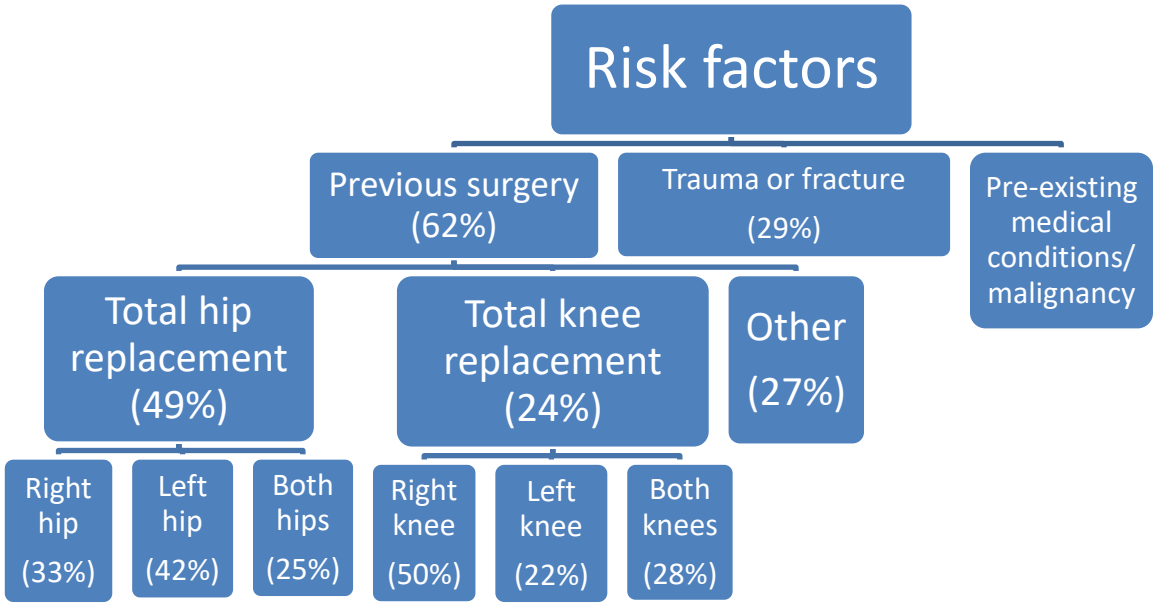


Figure 3.11: Relative distribution of the affected side of surgical prostheses

We thereafter determined the ages of the patients with a history of total hip replacements and compared these to the ages of the patients with a history of total knee replacements (see table 3.24 below).

Table 3.24: Comparison of age range of patients with total knee replacements versus total hip replacements

Procedure	**Median age**	**Age range**
Total knee replacement	67 years	48 – 76 years
Total hip replacement	62 years	38 – 76 years

We also tabulated the amount of time in years that had lapsed from the time of surgery to the presentation of symptoms, with specific focus on the two most common types of surgeries, i.e. total hip replacements and total knee replacements. The results are tabulated below in table 3.25.

Table 3.25: Comparison of time lapse after surgery for presentation of symptoms in patients with total knee replacements versus total hip replacements

Type of surgery	**Average time lapse**	**Range**
Total hip replacement	7.1 years	1 – 23 years
Total knee replacement	3.6 years	8 months – 11 years

Besides surgery, other risk factors comprised of a history of trauma or fracture in 35/120 patients (29%), and pre-existing medical conditions or malignancies in 17/120 patients (14%) – see table 3.21. The pre-existing medical conditions included systemic lupus erythematosis (SLE), polyarthritis, tuberculosis (TB), Charcot's arthropathy, osteoarthritis, malignant otitis externa, diabetes mellitus (DM), cellulitis or rheumatoid arthritis. Some patients had been diagnosed with various malignancies, including Hodgkin's lymphoma, multiple myeloma or acute myeloid leukemia (AML).

We finally analysed the final outcomes in patients with the various risk factors and compared these to the findings on the flow and blood pool phases, as elaborated in table 3.26 below.

Table 3.26: Analysis of final diagnoses in patients with the various risk factors in comparison to the findings on the flow phase

Risk factors	**Flow phase**	**Final Conclusion**		
		Positive for infection	**Negative for infection**	**Inconclusive**
Previous surgery	Positive= 28	2	16	10
(n = 74)	Negative= 46	4	35	7
Trauma/ previous fracture	Positive= 21	3	14	4
(n = 35)	Negative= 14	1	12	1
Medical conditons/ malignancies	Positive= 12	7	1	4
(n = 17)	Negative= 5		4	1

Haematological investigations had been carried out in only 12/120 patients (10%). All 12 patients had documented CRP values, 7 of which were high whilst the other 5 were within the normal range. ESR levels had been recorded in 11 patients, 8 of which were high whilst the other 3 were within the normal range. In the 12th patient, the white cell count (WCC) had been measured instead of ESR levels, and was

found to be normal. We compared the levels of the biomarkers to the results of the three-phase bone scintigraphy, as elucidated in table 3.27 below. These results were both surprising and unexpected.

Table 3.27: Analysis of biochemical markers versus three-phase bone scintigraphy findings

BIOCHEMICAL MARKERS	**PARAMETERS**	**NO. OF PATIENTS**	**FINAL CONCLUSION**
Congruent results	Both CRP and ESR levels raised	6 patients	None of the scans were positive for infection ***(surprising)***
	Both CRP and ESR within normal range	3 patients	None of the scans were positive for infection ***(expected)***
Discrepant results	Normal CRP, high ESR	2 patients	Only 1 scan positive for infection.
Incomplete results	Only CRP measured, together with WCC (high CRP, normal WCC)	1 patient	Positive for infection ***(surprising)***
	Total patients	**12 patients**	

We further defined a sub-group of patients with a localised abscess or generalised sepsis as the presenting sign, and correlated these findings with the levels of biomarkers in these patients, as shown in table 3.28 below.

Table 3.28: Correlation of patients with abscess or sepsis and biomarker levels

Abscess or sepsis	**Both biomarkers normal (CRP & ESR)**	**Both biomarkers abnormally high**	**Only 1 biomarker abnormally high**
Yes	-	1	2
No	3	5	1
Total	3	6	3

Three patients had been tested for the Human Immunodeficiency Virus (HIV) with consent. Two were found to be positive and one negative.

It was disappointing to note that only 22/120 patients (18%) had supplementary radiological investigations, viz. x-rays. In half of these patients (11 patients), the x-rays demonstrated features of loosening of the prosthesis, while in approximately 20% (4/22 patients) features of infection were demonstrated.

Other relevant information provided by the x-rays included mal-union or non-union of fractures (4 patients), osteoarthritic changes (1 patient) or osteo-degenerative changes (1 patient). The x-rays also appeared to be useful in excluding pathology. No overt bone changes were visualised in 3 patients, whilst 1 patient had no evidence of sepsis.

The demographics and other characteristics of the paediatric population has been mentioned previously. Table 3.29 below lists the characteristics and a comparison of the 2 age categories of adult patients.

Table 3.29: Characteristics and a comparison of the 2 age categories of adult patients

		30 – 60 years G1 *(n = 57)*		**61 – 80 years G2 *(n = 51)***
Gender				
	Males	28		18
	Females	29		33
Hospital				
	CHBAH	24		15
	CMJAH	33		36
Signs/ Symptoms				
	Pain	37		35
	Abscess	7		1
	Mal-union/ non-union	5		3
	Swelling	3		3
	Ulcer	1		2
	Sepsis	1		3
	Instability	0		1

Biomarkers (ESR, CRP, WCC)				
	Both biomarkers normal			3
	Both biomarkers high	2		3
	At least 1 biomarker high	2		1
Risk factors				
	Previous surgery	32		40
	Previous trauma or fracture	25		7
	Pre-existing medical conditions or malignancies	11		2
HIV positive		2		0

CHAPTER 4

4. DISCUSSION

The most commonly performed radionuclide procedure is the three-phase bone scintigraphy. Most departments use preset protocols for planar bone scintigraphy (Naddaf *et al.*, 2004). This involves injection of a diphosphonate (methyl diphosphonate in our centre) labelled with Technetium-99m (^{99m}Tc-MDP) (Naddaf *et al.*, 2004).

The three phases refer to (Simpfendorfer, 2017) :-

- the initial dynamic imaging sequence (flow or perfusion phase) performed immediately after tracer injection
- followed by static imaging (blood pool or soft-tissue phase) acquired within ten minutes of tracer injection, and
- the eventual delayed bone phase performed 2-4 hours after injection

The sensitivity is high if there is increased uptake in all three phases (Ouyang *et al.*, 2014; Trevail *et al.*, 2016). However, it has been noticed that – especially in the paediatric patient – all three phases are not possible due to lack of co-operation; and thus it is often converted to a 2-phase bone scintigraphy. Furthermore, in our department it is not always possible to obtain good quality images of the flow phase due to technical reasons, and thus the flow phase is sometimes ignored whilst reporting. Finally, we noticed that if there is increased flow to a specific region during the initial flow phase, there is almost always increased uptake during the blood pool phase as well. This is in relation to its mechanism of uptake being hyperaemia, altered capillary permeability, increased vascularity etc. (Brenner *et al.*, 2012; Petruzzi *et al.*, 2009).

In an attempt to reduce unnecessary technical issues and the length of procedure, we amassed the findings of the flow and blood pool phases in appropriately identified patients. This was followed by analysis of the findings to assess congruency. Thereafter, we determined if the flow phase offered any ADDITIONAL USEFUL information over-and-above the blood pool and delayed phases. The rationale was to establish if the flow phase could be totally obviated in such patients. If this proved to be true, we could then pay more attention in obtaining good quality blood pool images instead, due to the time constraints applicable to the flow phase. This would reduce the overall time required for a patient to be lying motionless on the imaging gamma camera, which is especially difficult in the paediatric population. Ultimately, this would also make management of the gamma camera time more efficient in a busy department with limited capacity.

We reviewed the records of all patients being worked up for infection involving the musculoskeletal system that were referred to the Department of Nuclear Medicine in either of the two academic hospitals, viz. Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital. As these are the two largest public sector hospitals in the southern Gauteng region, it was hoped that patients included in the study would be representative of a variety of different backgrounds and with different socio-economic circumstances. The imaging study under discussion was a three-phase bone scintigraphy performed with Technetium-99m methyl diphosphonate (^{99m}Tc-MDP). The time frame spanned a period of roughly 36 months (from July 2014 to June 2017).

We discovered that almost 110 three-phase bone scintigraphy scans were being performed per annum at the two university hospitals combined for various indications. The majority of these were performed as elective procedures, whilst some had been slotted in as urgent cases. Since the two hospitals combined possess five SPECT imaging systems as detailed previously, it is often possible to obtain a booking for a three-phase bone scintigraphy scan within 1-2 weeks, despite the enormous patient load from other scans being performed.

Of the 325 three-phase bone scintigraphy scans identified during this period, we included a total of 120 scans into our final analysis. Various factors led to the exclusion of the balance of the 205 patients. These factors included other indications not relevant to our study, such as assessment of avascular necrosis, workup of non-specific pain, assessment of bony lesions, etc. Scans of poor or questionable quality for interpretation were also excluded. The quality of the scan was assessed by the registrar in consultation with the attending consultant. Consultants have varying levels of experience, ranging from 5 years to over 20 years of experience in the field. However, there appeared to be consistency amongst the consultants in assessing the quality of the scans. Furthermore, scans with negative findings were also excluded as it is well known that there can be no osteomyelitis unless the delayed images are abnormal, even in the setting of increased blood flow and/or blood pool activity (Brenner *et al.*, 2012).

It must be stressed that the results of the three-phase bone scintigraphy were analysed in detail. Firstly, the results of the flow and blood pool phases were recorded and analysed. These results were then compared to determine if the findings were congruent and if the flow phase offered any real added advantage *(see Appendices B1 & B2)*. Any follow-up studies performed were also analysed to finally determine the presence or absence of infection in these patients.

The study was a simple comparison of two variables (flow phase versus blood pool phase), thus a t-test was used as a method of comparison. Other statistical methods were simple measurements for tabulation of data (thus the use of percentages and frequencies), as well as the measurement of centricity on the distribution of data (thus the use of median).

In approximately half of the patients (64/120 patients, 53%), the three-phase bone scintigraphy demonstrated asymmetrically increased vascularity on the side of the pathology during the flow phase, although in 6 patients this uptake was only mildly increased. In contrast, the three-phase bone scintigraphy demonstrated asymmetrically increased vascularity on the side of the pathology during the blood

pool phase in three-quarters of the patients (90/120 patients, 75%). In this second group, the uptake during the blood pool phase was only mildly increased in 12 patients. All of the 120 patients demonstrated asymmetrically increased uptake of tracer on the side of the pathology during the delayed phase. This was to be expected, as patients with negative scans had been excluded from our study as per the exclusion criteria based on the well-known fact that there can be no osteomyelitis unless the delayed images are abnormal, even in the setting of increased blood flow and/or blood pool activity (Brenner *et al.*, 2012).

We further discovered that the findings on the flow phase were congruent with findings on the blood pool phase in 94/120 patients (78%), as demonstrated in table 3.2. In these patients, the flow and blood pool phases were BOTH positive in 64/94 patients (68%). Similarly, the flow and blood pool phases were BOTH negative in 30/94 patients (32%). Since the findings were congruent – either positively congruent or negatively congruent – in all these 94 patients the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

ALL the patients with increased vascularity on the flow phase also demonstrated increased vascularity on the blood pool phase. However, the reverse was not true, i.e. some patients with increased vascularity on the blood pool images demonstrated NO increased vascularity on the flow phase images. These 26/120 patients (22%) demonstrated INCONGRUENT findings between the flow and blood pool phases. It is a crucial point that in each and every one of these cases, the clinician still regarded the combined phases as positive as per the blood pool phase. This gives rise to the notion that three-phase bone scintigraphy reporting and management decisions were based more on the findings of the blood pool phase. Part of the reason may be the better delineation that is possible on the blood pool phase, which allows the reporting nuclear physician to provide a better description of uptake with a greater deal of confidence. Again, in this group of patients, the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

Thus, looking at the congruent and incongruent scans combined, it can be concluded that not a single patient of the 120 patients analysed benefitted additionally from the flow phase.

We thereafter added the findings of the delayed phase to our analysis and realised that 64/120 patients (53%) had positive findings on all 3 phases. We compared these findings to the final conclusion provided in the reports (see table 3.3). Only 17/64 patients (26.5%) had a final diagnosis of being positive for infection despite all 3 phases being regarded as positive on the bone scan. This was extremely surprising since the literature reports that positive findings on all 3 phases have an accuracy rate of more than 90% in diagnosing osteomyelitis (Brenner *et al.*, 2012; Love & Palestro, 2013, 2016). However, it must be noted that this high accuracy rate holds true only under certain conditions.

In our sample population, just under half of the patients were diagnosed as having NO infection despite all 3 phases demonstrating increased uptake of tracer on the bone scan (30/64 patients – 47%). The balance of the 16/64 patients (25%) had inconclusive reports – due to either the follow-up labelled leukocyte scan or the follow-up colloid scan not being performed. This was mainly because patients defaulted on their appointments. One patient with inconclusive report was referred for histological correlation to differentiate between non-benign and advanced infective changes as the findings were not definitive on the three-phase bone scintigraphy, and it was felt that additional follow-up studies would not be helpful.

In the patients with positive findings, we further evaluated the sites of abnormal uptake (see table 3.4). We realised that many patients had more than one site of abnormal accumulation of radiotracer, the average being two sites with some patients demonstrating as many five sites. Sites of abnormal uptake were mostly located around the lower limbs. The most frequent sites included the femori, acetabulae, knees, tibiae and tarsal bones. Some patients demonstrated abnormal uptake in the upper limbs. In very few patients other sites were visualised. These included the spine, pelvis, skull and facial bones. A limited number of patients (7/120

patients, 6%) demonstrated abnormal uptake bilaterally, mainly in both knees or in both femori and acetabulae. These tend to pose a diagnostic challenge for the nuclear physician reporting the scan without adequate clinical information.

We further divided the sample population into two categories, i.e. adults versus paediatric population. The paediatric group was defined as patients below the age of 16 as per hospital and departmental policies. We found a very small number of paediatric patients in our sample population (8/120 patients, 6.7%). Most of these paediatric patients (6/8 patients, 75%) were from Chris Hani Baragwanath Academic Hospital. There were 5 females and 3 males, with a median age of 8 years (range from as young as 5 month old baby to just under 16 years of age).

Pain was the most common sign/symptom, followed by abscess, sepsis and swelling (see table 3.5). This corresponds to findings documented in the literature by various authors (Schmitt, 2017; Springer, 2015). None of the paediatric patients had documented biomarker levels measured. Only 1 patient had a history of previous surgery (left hip fusion), 1 patient had a history of trauma and 1 patient had pre-existing acute myeloid leukaemia.

On analysis of the results of the three-phase bone scintigraphy in the paediatric population (see tables 3.6 and 3.7), we found that of the total number of 8 paediatric cases, 7 patients had positive findings on both the flow and blood pool phases, while 1 patient had negative findings on both the flow and blood pool phases. This gave us a congruency rate of 100% in the paediatric population.

Six patients had the diagnosis of infection confirmed based solely on the three-phase bone scintigraphy as the increased uptake of ^{99m}Tc-MDP occurred in non-violated bone. In the 7th patient with a history of previous trauma, further imaging was required and infection was excluded on these subsequent imaging tests. The 8th and final paediatric patient demonstrated negative findings on both the flow and blood pool phases. The delayed phase, however, demonstrated increased uptake of tracer. These findings required further infection imaging, but this was not performed as the

patient defaulted follow-up. This would have been crucial to her final diagnosis, as this patient had a history of a previous left hip fusion. Thus, her results remain inconclusive.

Since the congruency rate in the paediatric population was 100%, this begs the question of whether the flow phase is required at all in the paediatric population, or can be safely omitted without loss of crucial information. As the number of paediatric patients in our sample population was very small (only 8 patients), a larger study is required to confirm these findings.

In terms of the sites of abnormal uptake, when the sub-category of paediatrics was analysed (see table 3.8), we discovered that the majority of these patients had only 1 site of abnormal uptake (range 1 to 4 sites). This contrasted with the results from the adult population that had an average of 2 sites of abnormal uptake, with some patients demonstrating as many as 5 sites of abnormal uptake. The most frequent sites involved in the paediatric population were similar to their adult counterparts and were mainly concentrated around the lower limbs (see table 3.8). These included mainly the femori, knees and tibiae and were mainly focused on the left side. The reasons for this are not immediately apparent. In contrast to their adult counterparts, however, none of the paediatric patients demonstrated any uptake in the upper limbs, nor in the skull, spine or facial bones. Again, the reasons for these differences are not very clear.

Finally, we moved over to other end of the spectrum and analysed the geriatric age category. Since it is well-known that older patients usually have a compromised blood supply, we envisaged that there may be some differences in adults older than 60 years of age versus adults below the age of 60 years. Since the vast majority of our population (108/120 patients, 90%) was above the age of 30 years (see figure 3.10), we thus divided the adults into patients between the ages of 30 to 60 years (termed group 1 or G1) and patients older than 60 years of age (termed group 2 or G2).

Upon comparison of the results of the three-phase bone scintigraphy in these two groups (see table 3.9), we found that during analysis of the flow phase, half of the patients had positive findings and the other half had negative findings in both groups, whereas approximately three-quarters of the patients had positive findings on the blood pool phase in both groups. We further analysed the results to determine the level of congruency in the two groups (see table 3.10). We found a congruency rate of 77% (44/57 patients) in the younger age group, and a rate of 75% (38/51 patients) in the older age groups. To our surprise, these rates appeared to be quite similar in both the age groups. We had expected the congruency rate to be lower in the older age group due to the pathophysiology being different in the older patients with compromised blood supply.

The majority of the paediatric patients had infection confirmed or excluded based purely on the three-phase bone scintigraphy, since this has been shown to have a sensitivity of more than 90% in non-violated bone (Palestro, 2016; Palestro *et al.*, 2006; Simpfendorfer, 2017). Additionally, the three-phase bone scintigraphy can be positive as early as two days after the onset of symptoms (Love & Palestro, 2013). This was not the case with most of the adult patients, who had a history of previous surgery, previous trauma or fracture etc. Since the bone scintigraphy reflects bone remodelling in general, and is not specific to infection, it may be positive in cases other than infection (Palestro *et al.*, 2006; Simpfendorfer, 2017). These include patients with fractures, orthopaedic hardware, recent surgery or the diabetic patient with neuropathic joint (Palestro *et al.*, 2006; Simpfendorfer, 2017). This leads to decreased specificity of the three-phase bone scintigraphy in such patients (Palestro *et al.*, 2006; Simpfendorfer, 2017). These patients required additional imaging studies to conclusively determine the presence or absence of infection, such as labelled leukocytes, Leukoscan, Gallium-67 imaging or colloid scans (see figure 3.6).

Overall, of the 120 patients analysed, only 27% (32/120 patients) had no additional investigations performed. In 10 of these patients, the three phase bone scintigraphy alone was sufficient to diagnose infection as these patients had presented with non-violated bone. One patient was diagnosed with septic arthritis and thus did not

require additional imaging tests. In one patient, infection was excluded based on the blood pool phase being negative. The balance of the 20 patients had inconclusive studies – 18 patients had defaulted on the follow-up labelled leukocyte imaging studies, one patient had defaulted on the follow-up Gallium-67 citrate scan and one patient was referred for histological correlation to differentiate between non-benign and advanced infective changes.

Eighty-eight patients had additional labelled leukocytes, Leukoscan or Gallium-67 citrate imaging. The time delay between the three-phase bone scintigraphy and these additional scans ranged from as early as three days, to a prolonged 183 days. However, the average delay in obtaining the additional scan was approximately three weeks. The prolonged delay was usually the result of patients defaulting appointments or due to the non-availability of the specific imaging agent. Gallium-67 citrate is not always available within the Nuclear Medicine departments; instead, it needs to be ordered on a per-patient basis.

Thirty-seven patients demonstrated no abnormal accumulation of tracer and were regarded as negative. Infection was safely excluded in these patients. One patient had Gallium-67 citrate imaging which was spatially congruent and infection was also excluded; thus, this patient did not require confirmation with colloid imaging. One patient was diagnosed as positive for infection based purely on the intensity of uptake.

The balance of the 49 patients required additional colloid imaging for positive confirmation. If the findings on the colloid scan were spatially congruent to that seen on labelled leukocyte imaging, this was regarded as positive and infection was excluded. If the colloid scan was negative or was spatially incongruent to the labelled leukocyte imaging, this confirmed infection in the patient. In 35 patients, infection was excluded based on the colloid imaging findings. Ten patients were diagnosed as having infection based on the colloid imaging findings. Four patients defaulted follow-up appointments and a definitive conclusion could not be provided.

The time delay between the labelled leukocyte imaging and the colloid scan ranged from as little as two days to a long delay of 36 days. On average, however, the colloid scan was performed within a week of the labelled leukocyte imaging.

Love, Marwin & Palestro (2009) have mentioned that if ^{111}In-labelled-leukocytes are being used, marrow imaging may be performed before, after or even during the leukocyte study using the simultaneous dual-isotope acquisition protocol. However, when one uses the ^{99m}Tc-labeled leukocytes – as is the case in our department – there may be persistent activity on the leukocyte images which could confound the results. This activity may last for up to 48 hours after injection (Love, Marwin & Palestro, 2009). Thus, it is best to wait at least 72 hours before performing the marrow imaging.

Although the time delay for the colloid scan was not as prolonged as the labelled leukocyte imaging, some patients had to wait over a month. This was surprising as the ^{99m}Tc-nanocolloid tracer is usually available in-house in most Nuclear Medicine departments.

It should be borne in mind that in only 12/120 patients (10%), the three-phase bone scintigraphy on its own was sufficient to confidently confirm or exclude infection (see figure 3.6). The majority of the patients required additional imaging. The reason being that the three-phase bone scintigraphy is very sensitive only in non-violated bone (Palestro, 2016; Palestro *et al.*, 2006; Simpfendorfer, 2017). This is usually the case in the paediatric population, a very small portion of our sample population. Since the bone scan reflects bone remodelling in general, and is not specific to infection; thus, it may be positive in cases other than infection, including fractures, orthopaedic hardware, recent surgery or the diabetic patient with neuropathic joint (Palestro *et al.*, 2006; Simpfendorfer, 2017). This leads to decreased specificity of the three-phase bone scintigraphy in such patients. Since the majority of our patients were adults, and many had a history of either previous surgery, previous trauma or fractures; most of these patients invariably required additional imaging over and above the three-phase bone scintigraphy to confidently confirm or exclude infection.

This begs the question of whether the three-phase bone scintigraphy was required at all in such patients, or could the final diagnosis have been made just as easily by omitting the three-phase bone scintigraphy. If these results are confirmed in a large multi-centre trial, we would propose that three-phase bone scintigraphy still play a crucial role in patients with non-violated bone only. However, in patients with previous surgery, previous trauma or fractures, the three-phase bone scintigraphy may be safely omitted without losing crucial information. Such patients may go on to have labelled leukocyte imaging directly, and subsequent colloid imaging if required. This would go a long way in reducing the very prolonged delays noted in obtaining the required additional imaging – in some cases this delay was over six months.

Out of the total number of 120 patients analysed, infection was eventually excluded in the majority of the patients with additional imaging tests (74/120 patients, 62%). Only 22/120 patients (18%) were finally diagnosed as positive for infection (see table 3.11). The balance of the 24/120 patients (20%) had inconclusive findings (see figure 3.7). The main reasons for the inconclusive findings were patients defaulting follow up appointments for additional imaging.

We divided the patients into the paediatric group and the adult group. We then compared directly the final diagnoses to the findings of the flow phase in these two subgroups. We found that the majority of the paediatric population (6/7 patients, 86%) with confirmed infection had positive findings on the flow phase; whilst only 12/57 adult patients (21%) with confirmed infection had positive findings on the flow phase (see table 3.12). The results appeared to fare better when analysing the negative findings. The majority of the adult patients with negative findings on the flow or the blood pool phases had no evidence of infection on subsequent follow-up imaging (80% and 76% respectively) (see table 3.12).

We further analysed the findings in terms of true positives and true negatives. From this analysis, it may appear that it is imperative to perform the flow phase imaging in the paediatric population (true positive rate of 1.0) (see table 3.13). The sensitivity is

thus 100%. However, it must be noted that the blood pool phase was also positive in all these patients; thus, excluding the flow phase would not result in loss of crucial information. It must be noted that these findings are based on a small group of paediatric patients (only 8 patients). Therefore, these findings need to be confirmed in a larger study prior to definitive recommendations being suggested.

The analysis also demonstrated that, in the adult population, the findings on the flow phase do not predict the presence of infection (false positive rate of 0.40 or 40%) (see table 3.14). Similarly, the findings on the blood pool phase in the adult population predict the presence of infection to an even lesser degree (false positive rate of 0.70 or 70%) (see table 3.14). However, the negative predictive value of the flow phase in the adult population was quite high (0.92 or 92%). Similarly, the negative predictive value of the blood pool phase in the adult population was also very high (0.92 or 92%).

Upon studying the final diagnosis in the 30 patients with negative findings on both the flow and blood pool phases, we found that the majority of these patients (22/30 patients, 73.3%) had no evidence of infection on subsequent follow-up imaging (see table 3.15). This is in keeping with the high negative predictive value of both the flow phase and blood pool phase as elaborated upon above. A similar pattern was demonstrated in the 26 patients with incongruent findings or what was termed flow-blood pool discordance (i.e. negative flow phase, positive blood pool phase). We again found that the majority of these patients (22/26 patients, 84.6%) had no evidence of infection on the subsequent follow-up imaging (see table 3.16). This again corroborates our previous findings of a very high negative predictive value of the flow phase images; suggesting that if the flow phase is negative, the possibility of infection is very low. However, the numbers are not enough to come up with a firm recommendation. These findings need to be verified in a study with a larger sample size or a multi-centre study.

In terms of the demographics, our sample population consisted of over half of the patients being from Charlotte Maxeke Johannesburg Academic Hospital (60%), while the balance of 40% of patients were from Chris Hani Baragwanath Academic Hospital (see figure 3.8).

It was impossible to determine the incidence of musculoskeletal infection in the southern Gauteng region from our study as it is well known that many patients are diagnosed clinically with the assistance of biochemical tests, and treatment initiated without diagnostic imaging. As this was a retrospective study analysing the results of diagnostic imaging, these patients would not have been included in our study. Furthermore, our study looked solely at patients undergoing a three-phase bone scintigraphy. Thus, patients referred directly for other diagnostic imaging modalities such as x-rays, CT scans, labelled leukocyte imaging (without having undergone a three-phase bone scintigraphy first), MRI scans, etc would also not be included in our study.

Overall, just over two-third of the patients were females (71% females versus 29% males) – see figure 3.9. Although there appeared to be a more equal split in terms of gender at Chris Hani Baragwanath Academic Hospital, there was a notable female preponderance mainly at Charlotte Maxeke Johannesburg Academic Hospital (see table 3.17).

The race of the patients was not documented and thus could not be analysed.

The sample population comprised of patients ranging from a mere 5 month old baby to elderly patients in their seventies, the oldest being a female of 78 years old (see figure 3.10). The median age was 56 years, with a significant peak in the seventh decade. Almost one-third of the sample population (30%) was in the 7^{th} decade of life (36/120 patients), with the vast majority (90%) of the sample population being in the 4^{th} to 8^{th} decades of life.

The age groups were further analysed according to the different hospitals, and we discovered contrasting differences in the age groups, as elicited in table 3.18. Using the two sample t-test, we found that patients from Chris Hani Baragwanath Academic Hospital tended to be younger (mean age 45.69 ± 19.87) as compared to their counterparts from Charlotte Maxeke Johannesburg Academic Hospital (mean age 56.40 ± 14.79) [p = 0.01]. We realised that this dataset appeared not to be normally distributed; thus, we further submitted the difference in ages between the two institutions to a 2-sample Wilcoxon rank sum test, which still showed significant differences in the ages (p = 0.002). The median age now was calculated as 46 years for patients from Chris Hani Baragwanath Academic Hospital versus 60.5 years for patients from Charlotte Maxeke Johannesburg Academic Hospital.

Only 3 of the patients from Charlotte Maxeke Johannesburg Academic Hospital were under 35 years of age. Comparison of the same age category, i.e. under 35 years revealed a whopping 15 patients from Chris Hani Baragwanath Academic Hospital. In contrast, 11 patients from Charlotte Maxeke Johannesburg Academic Hospital were over the age of 70, versus only 3 from Chris Hani Baragwanath Academic Hospital. The reasons for these differences are not clear, and may allude to referral bias by the referring physicians.

It was found that pain was the most common presenting sign/symptom (see table 3.19), occurring in more than 60% of patients (77/120). This concurs with findings reported in the literature (Schmitt, 2017). In many instances, this pain was quite severe and was the driving force behind the patients seeking medical attention. The pain usually occurred at the site of previous surgery, mainly hip or knee replacement surgery. Despite pain being the commonest presenting sign/symptom, only 9 patients with pain (12%) were conclusively diagnosed with infection (see table 3.20). It must, however, be borne in mind that a significant number of cases (18/77 patients, 23%) had inconclusive results due to the follow-up imaging not having been performed. This was mainly a result of patients defaulting follow-up appointments. Had this not been the case, it is possible that many more such patients would have been diagnosed with infection in the final analysis.

Several authors have documented chronic pain at the area of bony involvement (Schmitt, 2017; Springer, 2015). This is characterised by enhanced vascular permeability (Petruzzi *et al.*, 2009). Of the 77 patients presenting with pain in our sample population, only half of these patients (36/77 patients, 48%) demonstrated increased flow during the early vascular phase. Of these, only 6/36 patients (16.7%) were eventually diagnosed with infection (see table 3.20). This alludes to the fact that even in symptomatic patients experiencing significant amounts of pain, the findings of increased flow on the flow phase images do not reliably predict the presence of infection (false positive rate of 0.40 or 40%).

The other signs and symptoms each occurred in less than 10% of our patients. These included sepsis, swelling, mal-union or non-union of previous fracture and abscesses. These findings are similar to that documented in the literature (Schmitt, 2017; Springer, 2015). We differentiated abscess formation and full-blown sepsis on the basis of the extent of the infection – abscess referring to a localised infection, while sepsis referred to more severe generalised widespread infection. Formation of an ulcer and instability were reported in very few cases (3 and 2 patients respectively).

None of the patients presented with fever. Schmitt (2017) reports that fever and chills are not common signs/symptoms and usually develop only in advanced cases of infection. However, it was surprising that even in patients presenting with symptoms of chills as the main complaint, there was no documentation of fever in these patients. Since sepsis refers to more severe generalised widespread infection, it is likely that these patients in fact did have fever; however, this was not properly documented.

Some patients presented with other signs and symptoms indicative of advanced infection. These included a history of a draining sinus tract or pus oozing from the wound site, as well as impaired wound healing with subsequent formation of an ulcer (Schmitt, 2017). Although diabetes mellitus is an important risk factor in the

development of cellulitis, in impairing wound healing and subsequently contributing to the formation of ulcers as well as in the development of Charcot's arthropathy, this important medical history was documented in only three patients (2.5%). It is commonly known that the incidence of diabetes mellitus in our country is much higher (International Diabetes Federation, 2017) and often contributes to development of infections, thus we presume that this important information was, again, actually missed during the history taking session by the referring physician, rather than diabetes mellitus actually not being very prevalent in the sample population with suspected infection. The vast majority of our patients complained of only one symptom, with pain being the most common as previously mentioned. Only 13 patients (11%) presented with two or more symptoms, the combinations usually included either swelling and pain or abscess together with sepsis. These symptoms would often be expected to occur together and are self-explanatory due to their pathophysiology, i.e. swelling causing the pain as a result of building up of pressure, and untreated abscesses progressing on to generalised widespread infection or sepsis.

It has been reported that patients with osteomyelitis involving the vertebrae may present with typical radiating pain, weakness of the extremities and impaired bladder or bowel function as a result of compression of the spinal cord (Schmitt, 2017). None of the patients in the sample population had these symptoms. However, it is well known from the literature that many such patients would have an MRI scan as the primary investigation of choice rather than the three-phase bone scintigraphy as this would enable the clinicians to more accurately assess the degree of spinal cord compression and instigate appropriate therapeutic measures immediately (Lee *et al*., 2016; Pineda, Espinosa & Pena, 2009). Only three patients in our study had suspected vertebral osteomyelitis, none of which proved to be positive for osteomyelitis in the final analysis. While in two of these patients, imaging tests did not demonstrate any evidence of osteomyelitis, the third patient defaulted follow-up imaging for confirmation. These negative findings may be the reason for the lack of these specific symptoms in our sample population.

Various risk factors were identified in these patients being worked up for infection involving the musculoskeletal system, such as previous surgery (62%), history of trauma or fracture (29%), and pre-existing medical conditions or malignancies (14%) – see table 3.21. Patients with a history of previous surgery tended to be older, with a median age of 62 years. Almost three quarters of these patients (54/74 patients, 73%) were over the age of 50. On the other hand, patients with a history of trauma or fracture tended to be in the younger age groups, with a median age of 44 years. This concurs with findings in the literature which mentions that direct inoculation of microbes post-trauma usually occurs in young adults (Schmitt, 2017; Sia & Berbari, 2006). Patients with pre-existing medical conditions or malignancies also happened to be relatively younger, with a median age of 40 years. These medical conditions consisted of benign disease processes such as SLE, osteoarthritis, TB, cellulitis, malignant otitis externa, rheumatoid arthritis, Charcot's arthropathy and diabetes mellitus; as well as malignant conditions including Hodgkin's lymphoma, multiple myeloma and AML.

Of the 74 patients with a history of previous surgery – mainly a total hip or knee replacement – only 38% (28/74 patients) demonstrated increased flow during the early vascular phase (see table 3.22). In these patients with increased flow during the early vascular phase, the average time delay from surgery to the presentation of symptoms was 5.44 years (range 5 months to 23 years).

On the other hand, of the 35 patients with a history of previous trauma or fracture, more than half of the patients (21/35 patients, 60%) demonstrated increased flow during the early vascular phase. The differences between these 2 sub-categories are listed in table 3.22.

In terms of previous surgery, majority of the patients in this sub-group had either total hip replacement or total knee replacement (two-thirds versus one-third, respectively) – see table 3.23. Of the 36 patients with total hip replacements, a quarter of the patients had the procedure on both hips – see figure 3.11. In the balance of the patients, there seemed to be roughly an equal distribution between pathology

affecting either the right or the left hip (12 patients versus 15 patients, respectively). In contrast to the 18 patients with total knee replacements, a quarter of these patients had the procedure bilaterally, another quarter had surgery only on the left knee, while the remaining half had surgery only on the right knee.

It was interesting to note that patients with a history of total knee replacements happened to be older than patients with total hip replacements (see table 3.24). This seems to suggest that the native knees tend to last longer in patients than native hips by a period of roughly 10 years. Of course, there may be various other factors for this anomaly which have not been explored in our study.

There was a very wide range in the time lapsed from the time of surgery to the presentation of symptoms, as tabulated previously in table 3.25. In the sub-group of patients with total hip replacements, this varied from as early as one year after the surgery to a prolonged period of 23 years after the surgery in one case, with an average time span of the patient presenting with symptoms 7.1 years after the surgery. On the other hand, the time frame from the time of surgery to the presentation of symptoms in the other sub-group of patients with total knee replacements was shorter (almost half as compared to the sub-group of patients with total hip replacements), and varied from as soon as eight months after the surgery up to 11 years post-surgery (average time lapse being 3.6 years). In fact, the majority of these patients presented within the first two years after surgery (one-third presented within one year after surgery with symptoms, and another third presented in the second year post surgery). The interpretation of the three-phase bone scintigraphy results in such patients is further complicated by the fact that bone scan results can be positive for at least two years after a hip replacement – and up to five years after knee prosthesis implantation – due to physiological bone remodelling (Glaudemans *et al.*, 2015). It seems to be appear from our analysis that patients with total knee replacements may be at higher risk of developing infections in the region of the prosthesis quite early after the procedure. This may be attributed to the fact that knee surgery is a more complicated procedure, or that the implants

have a higher risk of failure once infection develops. This aspect needs to be explored further in a larger multi-centre trial.

We once again compared directly the findings on the flow phase to the final diagnoses in patients with the various risk factors (see table 3.26). The number of patients with a history of previous surgery was almost double as compared to the number of patients with a history of previous trauma or fracture (74 and 35 patients respectively). Despite this, when performing the analysis using the chi squared test, we found a significant difference in the flow phase between patients with a history of previous surgery versus patients with a history of previous trauma or fracture ($p = 0.03$). The flow phase did not contribute to the diagnosis of infection in patients with a history of previous surgery or in patients with a history of previous trauma or fracture (positive predictive value being 11% and 18% respectively). However, the negative predictive value in these patients was 90% and 92% respectively. Many more patients with a history of previous surgery had inconclusive findings as compared to patients with a history of previous trauma or fracture (17 versus 5 patients respectively). Of note was the very high positive predictive value of the flow phase in patients with a history of medical conditions or malignancies (88%).

Only 10% of patients (12/120 patients) had documented biochemical tests performed on them. These included measurement of the C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white cell count (WCC). In two-third of these patients, the ESR levels were raised, while just half the patients had raised CRP levels. Of these 12 patients, 9 patients had congruent results, i.e. 6 patients had high levels of both CRP and ESR, while in the other 3 patients both the CRP and ESR values were within the normal range (see table 3.27). We were astonished to discover that – of the 6 patients with high levels of both CRP and ESR – none of these patients were eventually diagnosed with infection. This was surprising since literature suggests that despite CRP and ESR being non-specific, these biomarkers are invariably raised in the setting of infection (Schmitt, 2017). However, our findings may be explained by the fact that in these cases, the elevated biomarkers may have been more indicative of inflammation rather than infection. Thus, we would have expected that in the

majority of these patients, infection would be confirmed on the three-phase bone scintigraphy. On the other hand, the three-phase bone scintigraphy conclusively excluded infection in patients that had demonstrated both CRP and ESR values to be within the normal range. This was to be expected.

Two patients had discrepant results, i.e. normal CRP value but high ESR levels. In these patients, only the one patient had evidence of infection while the second patient did not. In the last patient, only the CRP level had been measured, omitting the ESR level. Instead, the WCC had been recorded. In this patient, even though the CRP level was high, the WCC was found to be within the normal range. This patient was diagnosed positive for infection. It is worth noting that a normal WCC on its own does not exclude infection as it may be related to the patient's immune status.

These findings suggest that in our setting, biochemical markers are not a reliable indicator to suggest the presence or absence of infection, and often give a mixed confusing picture. However, it needs to be stressed that this is based on a very small number of patients – only 12 patients. Thus, larger studies addressing this specific question need to be performed prior to making firm recommendations in this regard.

What was surprising, though, is that only 1 out of the 120 patients had a documented WCC. This is a major concern as abnormal levels of WCC may influence the labelling efficiency of ^{99m}Tc-HMPAO and consequently may also affect the quality of the scans. We recommend that WCC levels be obtained routinely in patients undergoing further imaging with ^{99m}Tc-HMPAO.

Upon further analysis of a sub-group of patients with a localised abscess or generalised sepsis as the presenting sign/symptom, we correlated these findings with the levels of biomarkers in these patients (see table 3.28). Again, it was surprising that of the 17 cases of abscess or sepsis (10 cases of abscess and 7 cases of sepsis), only 3 patients from this sub-group had documented levels of ESR and CRP. In 2 of the 3 patients, at least one biomarker was found to be abnormally high, whilst in the third patient both the biomarkers were abnormally elevated. This

produced a true positive rate of 100%. Conversely, in the 6 patients with high levels of at least one biomarker, there was no associated evidence of abscess or sepsis. Again, these results suggest that in our setting, biochemical markers are not a reliable indicator to advise on the presence or absence of infection, and often give a mixed confusing picture. They may, however, play a role in defining levels of true positives. However, as stressed previously, this is based on a very small number of patients. Poor documentation may be the leading cause for this misnomer. It should also be borne in mind that even if the larger studies confirm our findings, the clinician should not totally abandon the practice of evaluating the biomarker levels, since these may still be used as screening tools as they are usually cost-effective (Love, Marwin & Palestro, 2009; Springer, 2015).

Only three patients (2.5%) had undergone HIV testing, and two were found to be reactive. Again, this is surprising given the prevalence of HIV in our country. We believe that HIV testing should have been promoted more extensively in these patients, as it is often an important cause of non-specific sepsis. It is possible, though, that HIV testing had actually been performed in a larger number of patients, but the results were not documented in the clinical notes for reasons of confidentiality.

Even though only a limited number of patients had been referred for measurement of biochemical markers as stated above, many more patients had been referred for supplementary radiological investigations, the main investigation being x-rays. These x-rays demonstrated features of loosening in approximately half of these patients, while in less than a quarter of the patients the x-rays exhibited features of infection. The x-rays also served to reveal pre-existing conditions, such as mal-union or non-union of fractures, osteoarthritic changes or osteo-degenerative changes.

When comparing the x-ray findings to the results of the three-phase bone scintigraphy, we discovered that two patients with no significant changes on the x-ray were diagnosed as having infection on the three-phase bone scintigraphy. This possibly reflects the increased sensitivity of the three-phase bone scintigraphy as it

demonstrates bone remodelling which occurs well before definitive anatomical changes are visualised (Becker & Meller, 2001; Brenner *et al.*, 2012). As small as a 5% change in bone turnover can be detected on three-phase bone scintigraphy, whereas at least 40-50% of mineral content must be lost to detect lucency on x-rays or CT scans (Becker & Meller, 2001).

The opposite was also true. A patient with features of infection on the x-rays underwent three-phase bone scintigraphy which proved that there was in fact no evidence of infection in this patient. Only in one patient did the three-phase bone scintigraphy concur with the x-ray findings as both demonstrated features of infection. In two patients we were unable to offer a definitive diagnosis on the three-phase bone scintigraphy as the follow-up labelled leukocyte scans were not performed.

Therefore, radiological investigations such as x-rays appear to play more of a role in deciding whether additional tests are necessary, and could influence both the choice, as well as the interpretation of subsequent tests, as elaborated extensively in the literature (Palestro, 2016; Palestro *et al.*, 2006; Simpfendorfer, 2017). The pragmatic physician may add these crucial tests to his armamentarium of investigations in the efficient workup and management of these patients.

The demographics and other characteristics of the paediatric population has been mentioned previously. We now briefly discuss the characteristics and a comparison of the two age categories of adult patients (see table 3.29).

There were roughly an equal number of patients falling into these two categories (57 patients in G1, and 51 patients in G2). In G1 the males and females were equally distributed, while in G2 there was a preponderance of females. There were more patients from Charlotte Maxeke Johannesburg Academic Hospital in both these age categories. As expected, pain was the most common presenting symptom in both these age groups, affecting over 65% of the patients (37/57 patients in G1 and 35/51 patients in G2). Mal-union or non-union of fractures appeared to predominate in G1

as this was the younger age group, while ulcers, sepsis and swelling appeared to predominate in G2, the older age group.

Many more patients had biomarker levels tested in the older age group as compared to the younger age group. These included levels of ESR, CRP and WCC. In the younger age group that had biomarker levels tested, in all of them at least one biomarker was found to be abnormally elevated. In contrast with the older age group, approximately half of the patients (3/7 patients) had normal biomarker levels. In two of these patients there was no evidence of infection, whilst the third patient's results remained inconclusive as she defaulted her appointment for the follow-up labelled leukocyte imaging. This is in keeping with findings of many authors that mention the levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) being invariably raised in the setting of infection, or being within the normal range in the absence of infection (Ouyang *et al.*, 2014; Goldsmith & Vallabhajosula, 2009; Love & Palestro, 2016; Montgomery & Epps, 2017; Schmitt, 2017).

In terms of the risk factors, many more patients had a history of trauma or fracture in the younger age group (25 patients versus 7 patients in the older age group). This concurs with findings in the literature (Schmitt, 2017; Sia & Berbari, 2006). Similarly, more patients in the younger age group were found to be HIV reactive. What we found surprising was that many more patients in the younger age group had pre-existing medical conditions or a history of malignancy (11 patients versus only 2 patients in the older age group). These two patients had concomitant diabetes mellitus and cellulitis.

CHAPTER 5

CONCLUSION

We analysed the results of 120 three-phase bone scintigraphy scans performed over a period of 36 months. Firstly, the results of the flow and blood pool phases were recorded and analysed. These results were then compared to determine if the findings were congruent and if the flow phase offered any real added advantage *(see Appendices B1 & B2)*. Any follow-up studies performed were also analysed to finally determine the presence or absence of infection in these patients.

We discovered that the findings on the flow phase were congruent with findings on the blood pool phase in 94/120 patients (78%). In these patients, the flow and blood pool phases were BOTH positive in 64/94 patients (68%). Similarly, the flow and blood pool phases were BOTH negative in 30/94 patients (32%). Since the findings were congruent – either positively congruent or negatively congruent – in all these 94 patients the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

The balance of the 26/120 patients (22%) demonstrated INCONGRUENT findings between the flow and blood pool phases. This translated to findings being negative on the flow phase, yet being positive on the blood pool phase – thus the incongruency. It is a crucial point that in each and every one of these cases, the clinician still regarded the combined phases as positive as per the blood pool phase. This gives rise to the notion that three-phase bone scintigraphy reporting and management decisions were based more on the findings of the blood pool phase. Part of the reason may be the better delineation that is possible on the blood pool phase, which allows the reporting nuclear physician to provide a better description of uptake with a greater deal of confidence. Again, in this group of patients, the flow phase could have been easily omitted as it provided no REAL ADDED advantage over the blood pool phase.

Upon closer scrutiny and comparing the findings on the flow phase to the final diagnoses in the paediatric population, we found the true positive rate to be 100%. Thus, it may appear that it is imperative to perform the flow phase imaging in the paediatric population. However, it must be noted that the blood pool phase was also positive in all these patients; thus, excluding the flow phase would not result in loss of crucial information. It must be borne in mind that these findings are based on a very small number of patients and need to be confirmed in a larger study prior to definitive recommendations being implemented.

In the adult population, on analysis of the findings on the flow phase, the false positive rate proved to be quite high (40%); suggesting that findings on the flow phase do not reliably predict the presence of infection. This high false positive rate appeared to be consistent (40%) even in symptomatic patients presenting with significant amount of pain. Thus, the flow phase could also be omitted in these patients without loss of crucial information.

What was striking in the analysis of the adult patients was the high negative predictive value of the flow phase images – suggesting that if the flow phase is negative, the possibility of infection is very low. However, these numbers are not sufficient to advocate a firm recommendation, and these findings need to be verified in a study with a larger sample or a multi-centre study.

Thus, looking at the congruent and incongruent scans combined, it can be concluded that not a single patient of the 120 patients analysed benefitted additionally from the flow phase. Thus, our recommendation is that the flow phase can be safely omitted in patients being worked up for active infection involving the musculoskeletal system without fear of losing crucial information.

A further prospective study with a larger patient base may yield more conclusive results. Such a study should consider analysing three-phase bone scintigraphy performed for other indications as well. It would also be prudent to include scans with negative findings for a more thorough analysis.

If such a study were to concur with our findings, this may change the practice of medicine in many centres in future, specifically in patients being worked up for active infection involving the musculoskeletal system.

We noticed a very prolonged time delay to obtain additional imaging studies, in some cases over six months. We suggest that patients be counselled about defaulting appointments and future studies analyse the various reasons for patients defaulting appointments and define appropriate recommendations.

We also observed that three-phase bone scintigraphy alone yielded conclusive results in only 10% of the patients, and most of the patients with violated bone required additional infection imaging. We propose a large multi-centre study in which infection imaging only would be performed in such patients, omitting the three-phase bone scintigraphy. If the results concur with our study, we would then suggest that three-phase bone scintigraphy still play a crucial role in patients with non-violated bone only. However, in patients with previous surgery, previous trauma or fractures, these patients can have labelled leukocyte imaging directly, and subsequent colloid imaging if required. This would go a long way in reducing the very prolonged delays noted in obtaining the required additional imaging – in some cases this delay was over six months.

We were unable to analyse some of the parameters, such as correlation of biomarkers with three-phase bone scintigraphy findings etc. This was largely a result of incomplete patient records. We recommend that physicians take utmost care during the history taking phase of the patient consultation and document all subsequent results accurately and completely.

The limited results we managed to obtain suggest that in our setting, biochemical markers are not a reliable indicator in confirming or excluding infection, and often give a mixed confusing picture. However, this is based on a very small number of patients. Thus, larger studies addressing this specific question need to be performed prior to making firm recommendations in this regard.

Only three patients (2.5%) in our sample population had undergone HIV testing, and two were found to be reactive. This was very surprising given the prevalence of HIV in our country. We believe that HIV testing should have been promoted more extensively in these patients, as it is often an important cause of non-specific sepsis.

We also suggest that patient records at Charlotte Maxeke Johannesburg Academic Hospital be labelled according to the nature of the study, rather than the name of the patient, as this makes data collection more efficient.

LIMITATIONS OF THE STUDY

Some of the limitations of the study include the following:

1. The number of patients studied was relatively small. Thus, the conclusions drawn from this pilot study may not be entirely accurate.
2. Selection bias. Since the study concentrated only on inpatients presenting to one of the two largest University Hospitals, the patient sample is not entirely representative of the South African cross-sectional population.
3. Sampling bias, due to exclusion of negative scans.
4. The time delay between the three-phase bone scintigraphy and additional scans ranged from as early as three days to a prolonged 183 days. Although this may influence the results and skew the analysis, it must be noted that the average delay was only approximately three weeks. Similarly, although the time delay between the labelled leukocyte imaging and the colloid scan ranged from as little as two days to 36 days; on average, the colloid scan was performed within a week of the labelled leukocyte imaging. Thus, the prolonged delays occurred in only a few patients and are thus unlikely to significantly skew the analysis.
5. The number of paediatric patients was small. Thus, we are unable to make firm recommendations for this sub-group.
6. The study also did not include patients that might have been diagnosed clinically or by other investigations and were not referred for the three-phase bone scintigraphy.
7. As this was a retrospective analysis, there were no pre-defined criteria in place for the clinicians to refer patients for the three-phase bone scintigraphy.
8. We further acknowledge that the information was obtained from patient records. Thus, incomplete or incorrect data may skew the results.
9. Intricate details regarding processing and displaying of images have not been mentioned as our study did not involve the reprocessing of the images, but only analysed the results. Thus, the interpretation of the images may still be user (interpreter) dependent.

RECOMMENDATIONS

- For patients who find it difficult to lie still for the entire duration of the three-phase bone scintigraphy, the first phase or the flow phase can be safely omitted without losing crucial information.
- Due to the time constraints applicable to the flow phase, it would be more prudent to pay more attention in obtaining good quality blood pool images and omit the flow phase.
- This would reduce the overall time required for a patient to be lying motionless on the camera, which is especially difficult in the paediatric population.
- Ultimately, this would also make management of the camera time more efficient in a busy department with limited capacity.
- A further prospective study with a larger patient base may yield more conclusive results. Such a study should consider analysing three-phase bone scintigraphy performed for other indications as well. It would also be prudent to include scans with negative findings – as well as a larger portion of paediatric patients – for a more thorough analysis.

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