Optimising ^{99m}Tc-Pyrophosphate Scintigraphy Acquisition Protocols for Transthyretin Cardiac Amyloidosis Imaging

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A thesis submitted in fulfilment of the requirements for the degree of

Master of Philosophy

Sydney School of Health Sciences

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June 2025

Statement of originality

This thesis is original and the work which is presented is my own work. Unless explicitly referenced within this dissertation, all work submitted for examination is the result of my independent research, and the opinions expressed are solely mine.

No part of the work presented in this dissertation has been submitted, in whole or in part, for any other degree or award at this or any other university, nor is it being submitted simultaneously for any other degree or award.

Signed: Date:

Author contribution statement

W.N developed and performed, analysed data and wrote manuscript.

P.K & S.M contributed to the systematic review, supervised the research and provided critical feedback on the manuscript.

N.K, R.B & A.P were the readers for the reader study. S.G helped with the statistical analysis.

K.P supervised the research and provided feedback on site.

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Acknowledgement

I would like to express my heartfelt gratitude to Kunthi Pathmaraj for her unwavering support, guidance, and mentorship. Her invaluable contributions were essential to the success of this project.

I am also deeply thankful to Sylvia Gong, whose expertise was crucial in assisting me with statistical analysis.

My sincere thanks go to my supervisors, Professor Peter Kench and Professor Steven Meikle. This journey has proven to be more challenging than I initially anticipated. Over the past four years, they have taught me a tremendous amount. Transitioning into high-level research after more than a decade in the clinical field meant I had a lot to learn, and both of them showed incredible patience throughout this process. I want to thank them for their time, support, and guidance during the development and execution of this project.

I am extremely grateful to the team of technologists and to Drs. Natalia Kovaleva, Aurora Poon, and Raef Boktor from the Molecular Imaging and Therapy Department at Austin Hospital for their efforts in acquiring and reporting the scans. Special thanks are due to Associate Professor Sze Ting Lee and Professor Andrew Scott for their generous advice and thoughtful revisions to the manuscript.

Lastly, I will always be indebted to my parents, siblings, wife, and children for their constant love, guidance, and encouragement, which have been the foundation of my journey.

Attribution Statement

Chapter 4 of this thesis has been published as: Single- Versus Dual-Time-Point imaging for Transthyretin Cardiac Amyloid Using [99mTc] Tc-Pyrophosphate. *Journal of Nuclear Medicine Technology,* 53:1-6. DOI: 10.2967/jnmt.124.269395. I designed the study, analysed the data and wrote the drafts of the manuscript.

Artificial Intelligence

Grammarly Pro was used to enhance clarity, grammar, and spelling. The suggestions and improvements made through Grammarly were carefully considered and incorporated to improve the overall quality of writing, while maintaining the integrity of research.

Australian Government Support

This research was supported by an Australian Government Research Training Program (RTP) fee offset.

Abstract

The use of nuclear imaging in the detection of cardiac amyloidosis, particularly transthyretin (ATTR) amyloidosis, has become an essential aspect of its diagnosis. Current imaging protocols often recommend both early (1-hour) and delayed (3-hour) imaging time points for high diagnostic accuracy. However, the necessity of imaging at both time points and the optimal time point remains a subject of debate.

This thesis investigates whether SPECT/CT in addition to planar imaging at one hour post injection provides sufficient diagnostic information to potentially eliminate the need for delayed imaging. The project begins with a review of cardiac amyloidosis, its subtypes, diagnostic approaches, and imaging protocols. A systematic literature review explores the diagnostic performance of early versus delayed imaging, focusing on sensitivity, specificity and clinical utility. One of the inclusion criteria required that the cohort, or a subset thereof, undergo endomyocardial biopsy, the gold standard for diagnosing cardiac amyloidosis. However, the invasive nature of this procedure posed a significant challenge, limiting the number of studies eligible for inclusion.

Finally, a reader study was conducted in which nuclear medicine physicians independently interpreted single-time-point scans, blinded to the timing of the imaging. Their interpretations were then compared with the original reports which had been generated using both imaging time points. ATTR cardiac amyloidosis was diagnosed based on clinical history, diagnostic test results, electronic medical records, and dual-time-point PYP scan reports. A case was

considered positive if the diagnosis or treatment of ATTR cardiac amyloidosis was documented in the medical records.

The findings suggest that an imaging protocol which includes SPECT/CT at the early time point may offer comparable diagnostic accuracy to imaging acquired at both time points. The information provided by the acquisition of SPECT/CT, such as blood pooling and anatomical correlation, is essential for high diagnostic accuracy. The thesis concludes by discussing the implications for clinical practice, highlighting protocol simplification, reduced patient burden, reduced radiation exposure to staff members, and increased departmental efficiency. It also addresses the limitations of this project and proposes directions for future research, including quantitative imaging and the response assessment for post therapy.

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Abbreviations	Full form
AL	Immunoglobulin Light Chain
AAN	Australian Amyloidosis Network
ASNC	American Society of Nuclear Cardiology
ATTR	Transthyretin Amyloid
ATTRv	Transthyretin hereditary variant
ATTRwt	Transthyretin wild-type variant
BNP	Brain Natriuretic Peptide
DPD	3,3-Diphosphono-1,2-PropanoDicarboxylic acid
EANM	European Association of Nuclear Medicine
ECG	Electrocardiogram
EGFR	Estimated Glomerular Filtration Rate
HMDP	Hydroxymethylene Diphosphonate
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
MDP	Methylene Diphosphonate
NT-proBNP	N-terminal pro-B-type Natriuretic Peptide
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PYP	Pyrophosphate
SPECT/CT	Single Photon Emission Computed Tomography/Computer Tomography
^{99m}Tc	Technetium-99m

Chapter 1 - Introduction

1.1 Background and motivation

Cardiac nuclear imaging encompasses myocardial perfusion scans, cardiac viability assessments, gated blood pool scans, and cardiac amyloid studies, all of which are used to monitor various heart conditions, including coronary artery disease and protein folding disorders.

There has been a significant surge in interest in cardiac amyloid imaging in recent times, as evidenced by several bibliographic medical databases. Figure 1.1 shows the increasing number of transthyretin (ATTR) cardiac amyloidosis publications from PubMed over the last ten years.

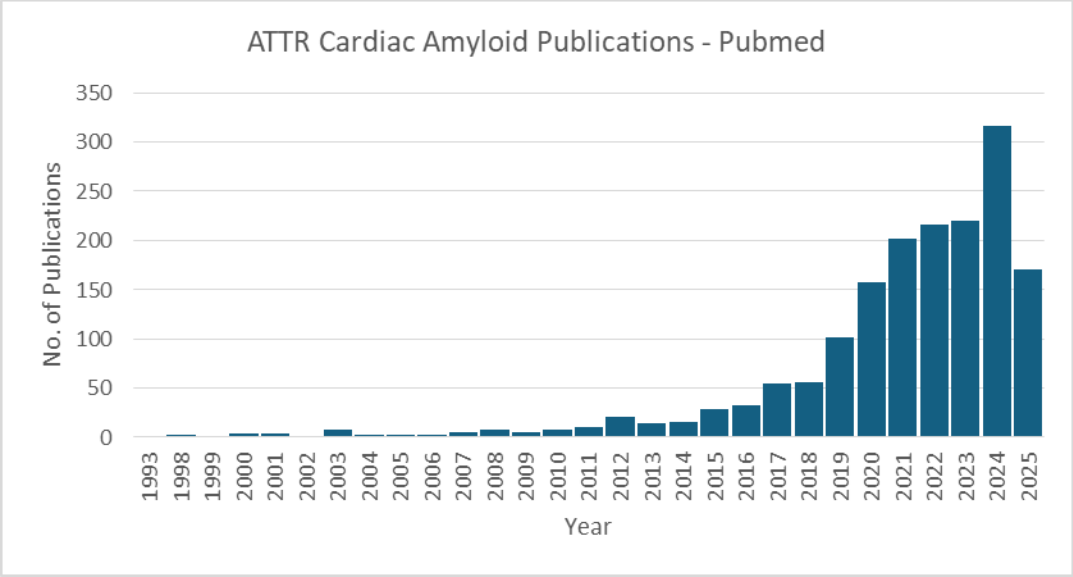


Figure 1.1 Number of transthyretin (ATTR) cardiac Amyloidosis publications from 1993 to June 2025 from PubMed.

The clinical manifestations of cardiac amyloidosis are often nonspecific, overlapping with other more common cardiovascular conditions. With common signs and symptoms such as thickened ventricular walls, heart failure, dyspnoea, fatigue, hypotension, and peripheral oedema, among

others, the diagnosis of cardiac amyloid can be challenging (Merlini, 1996; Kittleson et al., 2021). Histological confirmation through endomyocardial biopsy has long been considered the diagnostic gold standard; however, due to its invasive nature and inaccessibility, it is rarely used (González-López et al., 2017). In recent years, advances in imaging techniques have enabled more accurate detection of cardiac amyloidosis. These non-invasive imaging modalities have become essential in the diagnosis and management of cardiac amyloidosis. Echocardiography, cardiac magnetic resonance imaging, radionuclide scintigraphy and positron emission tomography play a crucial role in the diagnostic pathway, allowing for definitive diagnosis without the need for cardiac biopsies.

There are two primary types of cardiac amyloidosis: immunoglobulin light chain (AL) and transthyretin (ATTR) amyloidosis. This thesis focuses on ^{99m}Tc-labelled pyrophosphate (^{99m}Tc-PYP) scintigraphy in the detection of ATTR cardiac amyloidosis.

Many Australian imaging centres use ^{99m}Technetium labelled Pyrophosphate (^{99m}Tc-PYP) or ^{99m}Technetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) as radiopharmaceuticals to detect the presence of cardiac amyloid (Australian Amyloidosis Network, 2025). These radiopharmaceuticals, initially used for bone imaging, have evolved to become crucial in cardiac imaging. ^{99m}Technetium pyrophosphate (^{99m}Tc-PYP) is a prime example of this evolution. In the 1970s, it was widely used for detecting acute myocardial infarction due to its affinity for damaged myocardial tissue. However, as newer imaging modalities emerged, the use of ^{99m}Tc-PYP scans for this indication declined. In the past decade, ^{99m}Tc-PYP has regained clinical relevance, this time as a non-invasive diagnostic tool for

transthyretin cardiac amyloidosis. Its high specificity has made it a valuable and widely used imaging agent in contemporary cardiology practice (Casey, 2018). This evolution underscores the dynamic nature of nuclear medicine and the continuous improvement in diagnostic tools.

In 2016, the American Society of Nuclear Cardiology (ASNC) released a practice point document (Appendix A) for the imaging of transthyretin cardiac amyloidosis using ^{99m}Tc-PYP. This document, updated in 2019 and again in 2021, reflects the continuous evolution in the field, with the intention of improving diagnostic accuracy. One of the key points in the original 2016 guideline was to scan the patient one-hour post-injection of the radiopharmaceutical. A second optional imaging time point, at three hours post-injection, can be performed if blood pooling is suspected on the initial scan. In October 2019, the American Society of Nuclear Cardiology (ASNC), together with the European Association of Nuclear Medicine (EANM), released a cardiac amyloidosis practice point for ^{99m}Technetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) and ^{99m}Technetium labelled hydroxymethylene diphosphonate (^{99m}Tc-HMDP), which is mainly used in Europe (ASNC & EANM, 2019). It mentions that DPD, HMDP and PYP can all be used interchangeably. The difference between the original "^{99m}Tc-PYP practice points" and "the ^{99m}Tc-DPD and ^{99m}Tc-HMDP practice points" is the recommended imaging time.

In contrast, the recommended imaging time for ^{99m}Tc-DPD/HMDP is two to three hours post-injection, with an optional one-hour post-injection imaging time (ASNC & EANM, 2019). SPECT/CT is recommended at least once, as it provides valuable information essential for accurate reporting and diagnosis. Furthermore, the release of a "Interpretation and Reporting

of Cardiac Scintigraphy with Bone-Avid Tracers in Suspected Transthyretin Cardiac Amyloidosis (ATTR-CA)" document in 2023 mentioned similar diagnostic accuracy for both imaging time points (American Society of Nuclear Cardiology, 2024). Anecdotally, many nuclear medicine departments opt for both imaging time points in the hope of increasing the confidence levels of nuclear medicine physicians in their reporting.

Current cardiac amyloid imaging protocols can take up to 4 hours, a considerable amount of time to spend in an imaging department, particularly for older individuals who often require assistance. Patients can leave the department between the different imaging time points, but most prefer not to due to mobility and dependence issues. Lengthy medical appointments can be tedious for elderly patients and their carers. Optimising the imaging protocol workflow could result in shorter examination times for the same diagnostic accuracy, thereby improving the patient experience.

1.2 Reporting Nuclear Medicine Physicians' View

While a second imaging time point is optional, some departments routinely conduct imaging at both time points. With the advent of improved imaging systems and the widespread availability of SPECT/CT, which provides three-dimensional data, imaging departments are equipped with a valuable tool to aid in differentiating myocardial uptake from blood pooling within the heart chambers. New-generation gamma cameras offering Single Photon Emission Computed Tomography/Computed Tomography (SPECT/CT) with high sensitivity and improved

efficiency, combined with a better understanding of imaging requirements for accurate diagnosis, present an opportunity to streamline the process and enhance the patient journey.

Image quality is paramount in medical imaging. Nuclear medicine physicians require high-quality studies to issue highly accurate reports confidently. Do nuclear medicine physicians use both early and delayed imaging time points to confidently report on a study? This project aims to investigate the various scintigraphic imaging protocols and techniques used for diagnosing ATTR cardiac amyloidosis. Based on the findings, the project will assess the potential for streamlining and optimising the protocol to enhance patient experience and improve workflow.

1.3 Hypothesis:

SPECT/CT imaging using ^{99m}Tc-PYP at one-hour post-injection provides sufficient diagnostic information for ATTR cardiac amyloidosis, with no added value from delayed imaging.

1.4 Outline

The following outlines the chapters within this thesis:

Chapter one is the introduction and describes the role of nuclear imaging in the diagnosis of transthyretin cardiac amyloidosis. It demonstrates the continuous evolution of several practice points documents by ASNC and EANM. The chapter addresses the requirements for accurate reporting and updating current imaging protocols as mandated by reporting physicians and also includes the hypothesis and outlines the structure of this thesis.

Chapter two defines cardiac amyloidosis and its distinct types. It discusses the occurrence, diagnostic tools, treatment plans, and life expectancy. This chapter delves into the available diagnostic tools, their accessibility, and the associated challenges. It focuses on ^{99m}Tc-PYP as the radiopharmaceutical, including its reconstitution, patient preparation and imaging protocol for cardiac amyloid scans and finally presents several analysis methods used by nuclear medicine physicians for reporting.

In Chapter three, a systematic review is presented. The systematic literature review examines the various imaging time points employed in publications on cardiac amyloid imaging using biphosphonate-based tracers. The chapter examines various analysis methods and the sensitivities and specificities of early versus delayed imaging time points.

Chapter four is a reader study examining the diagnostic accuracy of single versus dual-time-point imaging. Cardiac amyloid studies, which included planar and SPECT/CT for both early and delayed time points, were retrieved from the hospital's Picture Archiving and Communication System to be analysed. Data from single-time-point imaging, either early or delayed, were provided to three nuclear medicine physicians for reporting. The results were then compared to the original report for diagnostic accuracy.

Chapter five concludes this project with a summary of the key findings and a discussion of their clinical implications. The project's limitations are also discussed. With ongoing advancements in the field, the chapter also explores the next phase in cardiac amyloid imaging, focusing on quantifying the degree of tracer accumulation in the myocardium, as well as assessing cardiac tracer uptake before and after treatment.

This chapter highlights the growing interest in ATTR cardiac amyloidosis over the past decade and introduces the non-invasive scintigraphic methods used for its detection and diagnosis. The release of several practice point documents from ASNC aimed to guide imaging departments shows a constantly evolving area. The topic of imaging time points post-injection is currently the subject of a heated debate. The proposed hypothesis of this manuscript is to assess whether SPECT/CT acquisition at a single time point provides sufficient information for an accurate report and allows for better patient experience and workflow. The next chapter aims to consolidate our understanding of cardiac amyloidosis. It will focus on the different types and variants of cardiac amyloidosis. Various diagnostic tools and treatments will be discussed. The final topic of chapter two will discuss the cardiac amyloid imaging protocol and its challenges.

Chapter 2 - Cardiac Amyloidosis

This chapter provides a comprehensive literature review on cardiac amyloidosis, examining its pathogenesis, clinical manifestations, diagnostic approaches and treatment strategies. It outlines the core evidence supporting current knowledge and highlights advances in diagnostic imaging and therapeutic management, thereby providing the basis for the subsequent review of sensitivity and specificity at different imaging time points. To contextualise the discussion of cardiac involvement, it is first necessary to outline the underlying process of amyloid formation. Amyloidosis is a condition caused by the deposition of misfolded protein fibrils, serum proteins and proteoglycans as amyloid (Osborne et al., 2015). A normal protein can misfold and adopt an abnormal shape. When several misfolded proteins stick together, they form soluble clusters known as oligomers. As these oligomers continue to bind together, they can create longer protein structures called protofibrils. With the addition of more misfolded proteins, amyloid fibrils are formed, which are stable and insoluble structures. Ultimately, this process leads to the development of longer proteins, protofibrils, and amyloid fibrils, all of which are characterised by their high stability and insolubility (Kaminski & Kaminski, 2016).

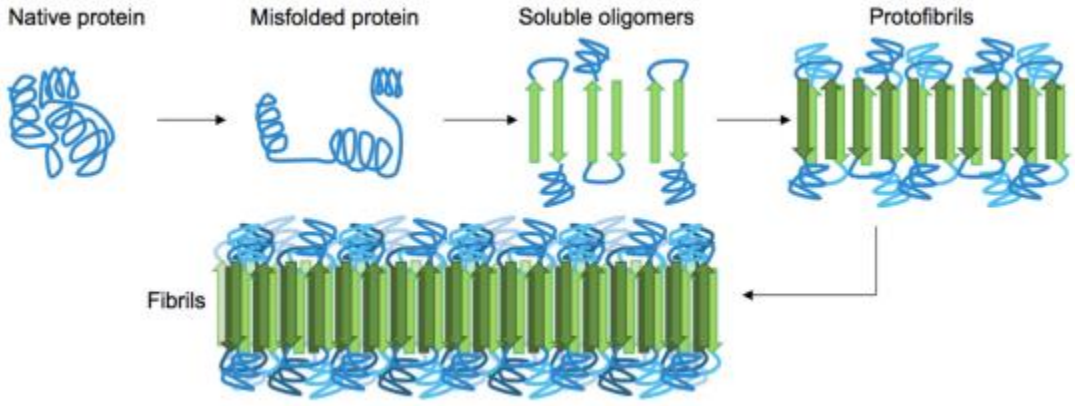


Figure 2.1 Simplified pathway toward amyloid fibril formation. Reproduced from Kamiski & Kamiski , "Probing amyloid protein aggregation with optical superresolution methods: from the test tube to models of disease." Neurophotonics, 2016, under the Creative Commons Attribution (CC BY) license.

The accumulation of these deposits can affect the function of several organs, such as the heart, brain, gastrointestinal tract, nervous system, liver, and kidneys. The accumulation of amyloid plaque is responsible for this cognitive decline in Alzheimer's Disease (Lane et al. 2019). This mechanism seems to work similarly in the case of the heart. The elderly demographic is most affected by amyloid disease. An accumulation of amyloid fibrils can occur over a prolonged period. It can lead to tissue damage and, in more severe cases, to organ failure. The infiltrative and restrictive nature of this condition can be fatal if undiagnosed and untreated (Wechalekar, Gillmore & Hawkins, 2016). At least 30 different types of proteins have been identified as the cause of amyloidosis (Dogan, 2017). Depending on the type of amyloidosis, this condition can be classified as systemic or localised disease and can be acquired or hereditary (Dogan, 2017).

There are two primary types of cardiac amyloidosis: Immunoglobulin light chain (AL) and transthyretin (ATTR) amyloidosis. Transthyretin cardiac amyloidosis can be further classified into two groups: a hereditary variant (ATTRv), which is often accompanied by systemic disease, and a wild-type variant (ATTRwt), which primarily affects the heart (Australian Amyloidosis

Network, 2025). Patients with cardiac amyloidosis may present with thickened ventricular walls, heart failure, dyspnoea, fatigue, hypotension, and peripheral oedema, among other signs and symptoms (Australian Amyloidosis Network, 2025). The detection of cardiac amyloid can be challenging, as the signs and symptoms may be attributed to other underlying conditions (Merlini, 1996; Kittleson et al., 2021). One clinical syndrome commonly seen in elderly patients with cardiac amyloid is heart failure with preserved ejection fraction (HFpEF), for which it can be particularly challenging to find a cause and can lead to a reduced life expectancy (González-López, 2015). AL and ATTR are two different types of diseases which progress at different rates and require different therapies. AL cardiac amyloidosis progresses at a much faster rate, and therefore, the correct diagnosis and treatment is critical (Soman, 2025).

2.1 Immunoglobilin Light Chain (AL) Cardiac Amyloid

Light-chain (AL) amyloidosis is the most commonly diagnosed type of systemic amyloidosis. The heart is often affected more than 50 per cent of cases. The cloning of plasma cells causes light chain amyloidosis (Stelmach-Gołdyś, 2022). These cells produce monoclonal free light chains, which ultimately form amyloid fibrils. The amyloid fibrils can infiltrate several organs and tissues, compromising their function (Comenzo, 2009). Diagnosis is a challenge due to the non-specific nature of the patient's symptoms. Patients are usually fatigued, lose weight, and have oedema, proteinuria, and peripheral neuropathy. Monoclonal immunoglobulin can also be detected in blood serum and urine using highly sensitive assays (Martinez-Naharro, Hawkins & Fontana, 2018).

2.2 Transthyrettin (ATTR) Cardiac Amyloid

Transthyretin is a plasma protein derived from the liver. Transthyretin cardiac amyloidosis can be further classified into two groups: a hereditary variant (ATTRv), which is often accompanied by systemic disease, and a wild-type variant (ATTRwt), primarily affecting the heart (Australian Amyloidosis Network, 2025). Although it is hard to accurately determine the prevalence as many cases go undetected, patients affected by ATTRwt cardiac amyloidosis tend to be predominantly older males (Martinez-Naharro, Hawkins & Fontana, 2018). ATTRv tends to affect younger patients, usually in their 50s, with peripheral and autonomic neuropathy at an earlier stage, with cardiac involvement at a later stage, as the disease progresses (Martinez-Naharro, Hawkins & Fontana, 2018).

2.3 Epidemiology of Cardiac Amyloidosis

Connors et al. (2016) predict that the most common form of amyloidosis in the USA will be the wild-type variant of transthyretin cardiac amyloidosis (ATTRwt). It is reported that there are more than 120 different mutations in the transthyretin variant. Although amyloidosis is considered a systemic disease, certain mutations (Thr60Ala, Ile68Leu, Leu111Met, and Val122Ile) can specifically target and infiltrate the myocardium (Yamamoto & Yokochi, 2019). Val122Ile and Thr60Ala are the most common types of ATTR amyloid in the USA (Jacobson et al., 2015; Reilly, Staunton & Harding, 1995). The Ile68Leu variant was only detected in Central and Northern Italy, primarily affecting older males (Rapezzi et al., 2013). The ATTR Leu111Met

found in Denmark seems to have an earlier onset without being sex dependent (Suhr et al., 2003).

Mariani et al. (2015) investigated the course of transthyretin familial amyloid polyneuropathies in France and compared French patients to a control group of Portuguese patients living in France. Val30Met was the most common mutation within both cohorts. The Portuguese cohort had a mean age of 32 years at onset, whereas the Val30Met French cohort had a mean age of onset of 64 years. The study also found that early onset tended to result in polyneuropathies, while late onset affected the cardiac muscles. The late onset also had a shorter survival median of 7.6 years (Mariani et al., 2015).

Caponetti et al. (2021) investigated the link between sex and the risk of cardiac involvement in hereditary transthyretin amyloidosis. They concluded that males are more susceptible to amyloid infiltration in the heart compared to women and that the different biological characteristics of the two sexes may be the underlying explanation for this phenomenon.

Gilstrap et al. (2019) investigated hospitalisation due to heart failure associated with cardiac amyloidosis using the Medicare database in the United States between the years 2000 and 2012. One of the inclusion criteria was the patient's age being above or equal to 65 years old. The incidence of patients newly diagnosed with cardiac amyloidosis more than doubled during this period. The reason for this increase may be attributed to a change in awareness and the rise of precise and sensitive non-invasive cardiac amyloid imaging. There was no statistically significant difference between males and females. The study reported the difference in

diagnosis between white and black people to be statistically significant, with white people having a much higher incidence (Gilstrap et al., 2019).

Choi et al. (2022) investigated Transthyretin Cardiac Amyloidosis in an Australian context. There was a 440% increase in patients diagnosed with ATTR cardiac amyloidosis from 2014 to 2021. Given that nuclear cardiac scintigraphy is the most used diagnostic tool, it is thought that increased awareness and availability of this sensitive, non-invasive modality may be contributing to the significant rise in diagnosed cases. Most patients were male, and the median age was 80 years, and the survival rate was significantly higher for patients diagnosed with lower-grade amyloidosis.

With a rapidly ageing population in Australia, Dr Nikki Bart from the Victor Chang Cardiac Research Institute estimates that the number of undiagnosed patients with cardiac amyloidosis is more than 20,000 (Victor Chang Cardiac Research Institute, 2023).

To summarise, the incidence of hereditary cardiac amyloid disease seems to be independent of sex. On the other hand, the wild-type variant affects more males than females. ATTRwt is the most common type of amyloidosis diagnosed in Australian Amyloidosis Network (AAN) centres (Australian Amyloidosis Network, 2025). Another critical point is the increased risk of cardiac amyloidosis in an aging population. Early diagnosis is paramount, with several management therapies now available.

2.4 Treatment & Prognosis

For years, patients with ATTR cardiac amyloid were only managed by dealing with their different symptoms. Oedema due to heart failure is managed with loop diuretics. Beta blockers can be used in patients with an increased heart rate due to a reduced ejection fraction. Anticoagulants are often used in patients with atrial fibrillation associated with cardiac amyloidosis, as patients with atrial fibrillation are at higher risk of thrombosis due to irregular heart rhythms (Ruberg & Maurer, 2024).

Recent developments in this field have led to several treatment options, including chemotherapy, stem cell transplant, immunotherapy, gene silencer therapy, and stabilisers such as Tafamidis, some of which have recently been approved in the United States of America (Stern & Patel, 2022). Tafamidis is a stabiliser that binds to the TTR protein and prevents it from spreading. Slowing down amyloid deposition allows for the disease to remain stable. Other agents, such as Patisiran and Vutrisiran, act directly on the liver, suppressing the production of the TTR protein (Ruberg & Maurer, 2024).

In Australia, there are no curative treatments; however, several supportive treatments are being used to slow the progression of the disease and alleviate symptoms. These treatments include green tea capsules, Doxycycline and Diflunisal, which is a non-steroidal anti-inflammatory drug where clinically appropriate (Australian Amyloidosis Network, 2025).

Early diagnosis remains the best prognosis for ATTR cardiac amyloidosis. Patients diagnosed at an early stage have better survival rates (Ruberg & Maurer, 2024). Tafamidis is highly effective

in slowing down disease progression and significantly improving survival rates (Hoffman et al., 2022).

2.5 Diagnosis

Elderly patients are the most affected by this fatal condition. Due to this demographic and clinical manifestation of cardiac amyloidosis, it is often misdiagnosed or overlooked (Takashio et al., 2022). Unfortunately, most patients show very few symptoms or are asymptomatic in the early stages of this disease and are in an advanced stage at the time of diagnosis (Martinez-Naharro, Hawkins & Fontana, 2018). Endomyocardial biopsy remains the gold standard for diagnosing cardiac amyloidosis (González-López et al., 2017). The invasive nature of endomyocardial biopsy means that it must be carefully planned, and only a select number of patients consent to the high-risk procedure. Recently, a combination of tests has been used to diagnose cardiac amyloidosis confidently. The tests include echocardiography, electrocardiogram, blood biomarkers, cardiac magnetic resonance imaging, urine samples, and radionuclide scintigraphy using ^{99m}Tc-labelled biphosphonate agents (Martinez-Naharro, Hawkins & Fontana, 2018).

Echocardiography is often the first investigative tool used in suspected cardiac amyloidosis due to its non-invasive, easily accessible and affordable nature. In patients with positive findings, left ventricular thickening is a common observation. Although non-specific, this is usually a crucial first step in the investigation (Melero Polo et al., 2023).

Electrocardiography (ECG), like echocardiography, is used as a primary diagnostic tool for investigating suspected cardiac diseases. Low signal amplitude on ECG is typically observed in patients with cardiac amyloidosis due to amyloid infiltration of the myocardium (Ng et al., 2022).

Several cardiac biomarkers have been linked to cardiac amyloidosis, including N-terminal pro-B-type natriuretic peptide (NT-proBNP), brain natriuretic peptide (BNP), eGFR, Troponin I, and Troponin II. Pregenzer-Wenzler et al. (2020) suggest that biomarkers can help in diagnosis, staging, prognosis, and response to treatment.

Cardiac magnetic resonance imaging provides information on the myocardium's structure, enabling the detection of infiltrative diseases such as cardiac amyloidosis or sarcoidosis. It is also able to give more information about disease burden when compared to echocardiography (Blankstein, Shaw, & Chandrashekhar, 2020). Unfortunately, the waitlist for MRI scans is long, and having a non-MRI-compliant implant is a contraindication for the scan.

The presence of transthyretin in urine can be a useful biomarker. A study conducted by Matsushita et al. (2022) found that the concentration of the transthyretin protein in urine increased as the disease progressed. Interestingly, there was no correlation between the concentration of transthyretin in urine and serum (Matsushita et al., 2022).

The availability of more accessible, highly sensitive, and specific diagnostic tests has resulted in a considerable increase in cardiac amyloid cases (Gilstrap et al., 2019). Lane et al. (2019) defined the rise of ATTR cardiac amyloid as exponential between 2000 and 2017 in the UK. Similar trends were observed in Japan, the United States, and Germany, as ^{99m}Tc-labelled

radioactive tracers were approved by their healthcare systems, and cardiologists became aware of non-invasive imaging diagnostic tools (Gilstrap et al., 2019; Ney et al., 2023; Naiki et al., 2023). The availability of tafamidis treatment may also have contributed to this surge in Japan (Naiki et al., 2023).

2.6 ^{99m}Tc-Pyrophosphate (PYP) Cardiac Amyloid Imaging

Biphosphonate-based ^{99m}Technetium labelled tracers have been used as bone agents for decades. Initially, ^{99m}Tc-PYP was employed as a bone tracer but was later found to be particularly useful in imaging myocardial infarction, with the highest sensitivity observed 3 to 7 days after the event. As research into biomarkers progressed, Cardiac Troponin I (cTnI) levels in the serum became the gold standard for diagnosing acute myocardial infarction (Hasić et al., 2003). With the rise in the availability of tests for various biomarkers and the increasing use of Cardiac magnetic resonance imaging, the demand for ^{99m}Tc-PYP imaging in myocardial infarction diagnosis saw a significant decline.

However, over the last decade, there has been a steady increase in the use of ^{99m}Tc-PYP scans, not for myocardial infarction, but for diagnosing ATTR cardiac amyloid (Choi et al., 2022). While the exact mechanism of ^{99m}Tc-PYP uptake in the myocardium where amyloid is present remains unclear, its sensitivity and specificity are exceptional. The binding process is thought to be related to elevated calcium levels in amyloid-deposited tissues and the microcalcification present in amyloid plaques (Embry-Dierson et al., 2023).

As a relatively new tool in the diagnosis of ATTR cardiac amyloid, there is a lack of standardisation in imaging protocols. It is assumed that the ASNC PYP practice points published in 2019 are the most widely referenced, as they are often cited in the literature. The following table reflects the imaging protocol recommended by the ASNC (Appendix B):

Table 2.1 Imaging protocol for 99mTc-PYP cardiac amyloid.

Imaging Procedures	Parameters
Patient Preparation	No Specific preparation. No fasting required.
Scan	Rest Scan
Dose of ^{99m}Tc-PYP	370 – 740MBq intravenously
Time between injection and acquisition	Recommended: 1-hour SPECT and planar Optional: 3-hour SPECT or planar
Imaging Parameters	
Field of View	Recommended: Cardiac or Chest; Optional: Whole Body Planar
Image Type	Recommended: Cardiac or Chest SPECT and planar imaging
Position	Supine
Energy Window	140keV, 15-20%
Collimators	Low energy High Resolution
Matrix	Planar: 256 x 256, at least 64 x 64 is required SPECT: 128 x 128, at least 64 x 64 is required
Pixel size	3.5 - 6.5 mm
Planar Imaging Specific Parameters	
Number of views*	Anterior, Lateral, and Left Anterior Oblique
Detector configuration	90 degrees
Image duration (count based)	750,000 counts
Magnification	1.46
SPECT imaging specific parameters	
Angular range	Recommended: 180 degrees; Optional: 360 degrees
Detector configuration	Recommended 90 degrees;

	Optional 180 degrees
ECG gating	Off; Non-gated imaging
Number of views/detector	40
Time per stop	20 seconds
Magnification	1.0

Cardiac amyloid imaging with ^{99m}Tc-PYP is highly sensitive and specific (Nishi et al., 2022; Castano et al., 2016; Poterucha et al., 2021; Gillmore et al., 2016). This scan is inexpensive and included in the Medicare benefits schedule in Australia; therefore, it is provided at no cost to patients in a public hospital setting.

2.7 Reconstitution of ^{99m}Tc-PYP

Pyrophosphate cold kits can be purchased from radiopharmacy companies or prepared in-house by radiopharmacists in nuclear medicine departments. The reconstitution of the kit is remarkably simple and quick. The vials are stored in a freezer at -20°C. The reagent in the vial is allowed to thaw. Up to 1,500 MBq of ^{99m}Tc-sodium pertechnetate is added to the vial, which is then mixed for 15 seconds. Quality control is performed using thin layer chromatography and pH tests. The reconstituted kit has a shelf life of 8 hours and can be stored at room temperature.

2.8 Patient preparation and administration of ^{99m}Tc-PYP

No patient preparation is required for this procedure. There is no need to fast or be hydrated. As for all nuclear medicine studies, the patient is asked to refrain from spending time with or

near pregnant women or young children to minimise or prevent any unnecessary radiation exposure. The whole procedure is explained to the patient. An intravenous cannula is inserted, usually in one of the patient's antecubital fossae, forearms or hands. Cannulas are preferred because there is a lower risk of extravasation. The radioactive tracer is injected, and the patient is then allowed to leave the department. They are asked to return one hour after the injection. One-hour post-injection, a 5-minute chest static image is followed by a 200cm whole-body image, acquired at a speed of 12cm/min, and a SPECT/CT of the chest is acquired. Whole-body imaging is usually acquired to assess for extracardiac amyloid deposits as it is a systemic disease. On average, the time taken to acquire a whole-body image is 19 minutes, and for SPECT/CT, is 15 minutes. Three hours post-injection, a 5-minute chest static image and an optional SPECT/CT, depending on the reporting physician, are acquired. The duration of the whole procedure is approximately 4 hours from the time of radiopharmaceutical injection.

2.9 Image Processing, Grading and SPECT/CT

According to the ASNC PYP practice points, a semi-quantitative analysis is performed on the static chest images. A region of interest is drawn over the heart, and another is drawn over the contralateral lung. A ratio of the two regions is calculated, with a result of 1.5 or more considered a positive study (American Society of Nuclear Cardiology, 2021). The ratio is dependent on the size of the regions of interest and on their position on the image and therefore can vary subject to the operator. To minimise operator variability, a clinical application (Aladdin) was created using the GE Xeleris^{TM} station, where the size of the regions

of interest was predefined and of the same size. The operator is only asked to position the regions of interest in the correct spot.

The limitation of the semi-quantitative method is that it cannot distinguish between radioactivity in the myocardium or the heart chambers. In some cases, at the one-hour time point, poor clearance of the radiopharmaceutical results in blood pooling in the heart, which can mimic a positive study. To differentiate between blood pooling and myocardial uptake, SPECT/CT can be acquired to provide three-dimensional information of the heart, allowing for precise localisation of the tracer. Alternatively, delayed imaging can be acquired 3 hours post-injection, allowing for clearance of the radiopharmaceutical from the blood through renal filtration and its uptake by the bone. Fractured ribs or any abnormal rib uptake may cause the calculated ratio to be inaccurate. However, SPECT/CT can be acquired to provide more information.

A CT is usually acquired with the SPECT and is used for attenuation correction and anatomical correlation. The ability to examine the heart in transverse, coronal, and sagittal planes enables the differentiation of radiopharmaceutical uptake in the myocardium from blood pooling in the heart chambers and bone uptake in rib fractures and diseases. SPECT/CT also allows for the grading of the scan and disease. The uptake of the radiopharmaceutical in the heart is compared to the uptake in the ribs, as described in Table 2.2 and illustrated in Figure 2.2 and graded accordingly. This qualitative analysis grading method is known as the Perugini score (Perugini, 2005).

Table 2.2 Visual grading of myocardial uptake of 99mTc-PYP versus rib uptake (American Society of Nuclear Cardiology, 2021).

Grade	99mTc-PYP Uptake in the myocardium
Grade 0	No uptake and normal rib uptake
Grade 1	Uptake less than rib uptake
Grade 2	Uptake equal to rib uptake
Grade 3	Uptake greater than rib uptake with mild/absent rib uptake

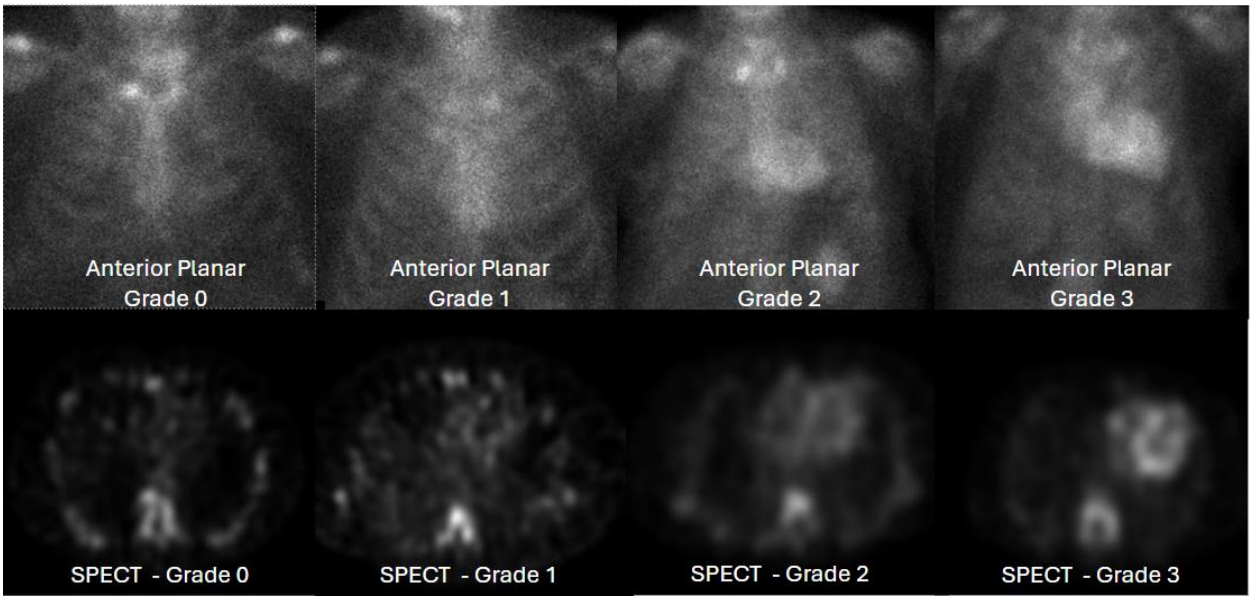


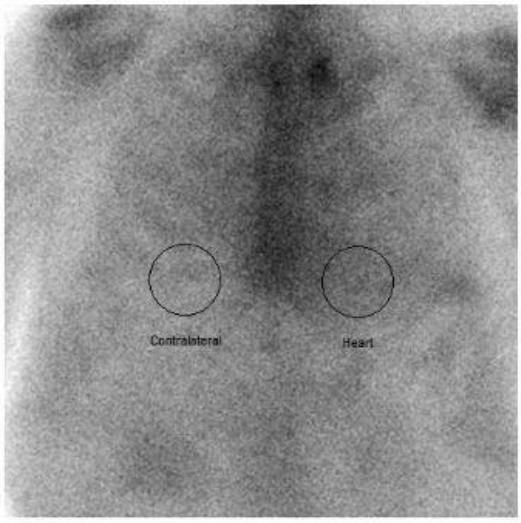
Figure 2.2 Examples of cardiac amyloid grading.

2.10 Challenges involved in ^{99m}Tc-PYP Cardiac Amyloid imaging

Although highly sensitive and specific, ^{99m}Tc-PYP cardiac amyloid scans can be challenging for patients and reporting physicians. Currently, several nuclear medicine departments prefer to image at both time points, one hour and three hours post-injection. Patients have the option to leave the imaging department between the two imaging time points, but most prefer to stay.

Most of the patients are elderly with limited mobility and find it challenging to leave the department and come back for the delayed time point. The procedure can last up to 4 hours, which is a considerable amount of time for patients to spend in an imaging department. As mentioned in the introduction, this project aims to minimise patients' time spent in the imaging department without compromising the accuracy of the diagnostic report. Single-time point imaging can enhance the patient experience and improve departmental workflow.

Blood pooling of radiopharmaceuticals within the chambers of the heart has been a significant challenge in the reporting of cardiac amyloid studies. Blood pooling on a planar image can be easily misinterpreted as a positive or equivocal study. SPECT/CT or delayed imaging are both useful for addressing blood pooling. The images below are of a single patient and were acquired on the same day. Figure 2.3 shows noticeable uptake in the heart on the early planar image. Figure 2.4 demonstrates blood pooling in the left ventricle, which is easily denoted on SPECT/CT images. Figure 2.5 shows a decrease in uptake in the heart on the delayed image, supported by a smaller ratio as the radiopharmaceutical clears from the bloodstream over time. Figures 2.3, 2.4 and 2.5 are of the same patient.



CONTRALATERAL LUNG ROI

Total Counts = 31689
Area = 1074

HEART ROI

Total Counts = 41626
Area = 1057

Heart / Contralateral Ratio = 1.31

Figure 2.3 Early planar image with regions of interest over the heart and contralateral lung showing uptake in the area of the heart.

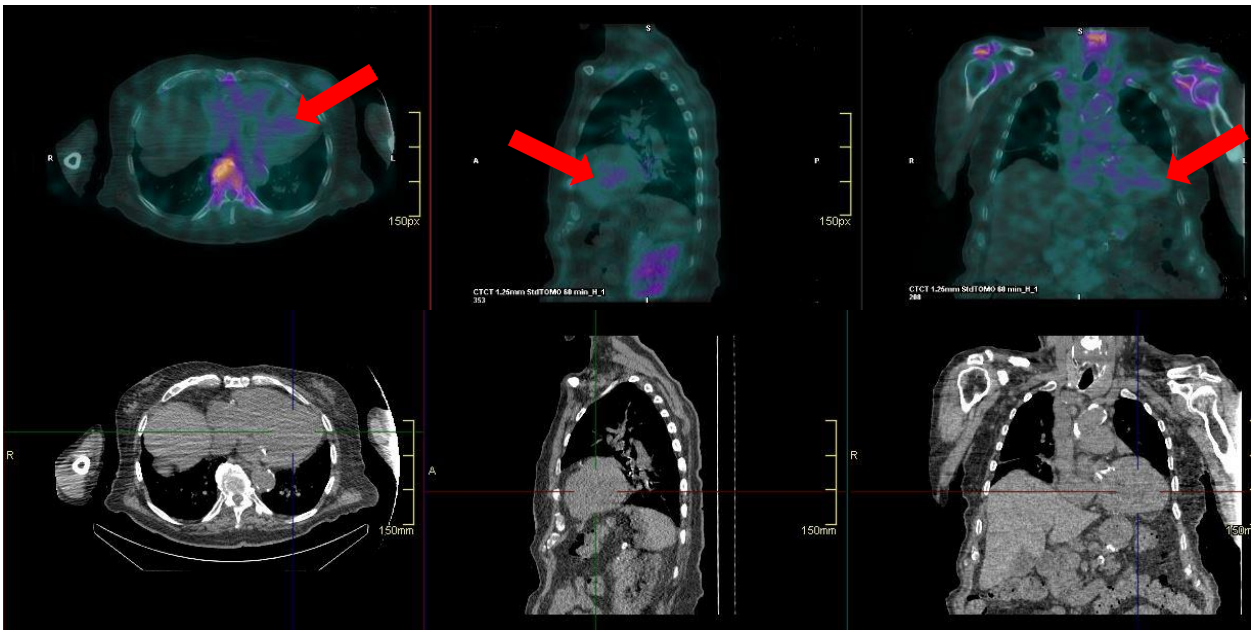
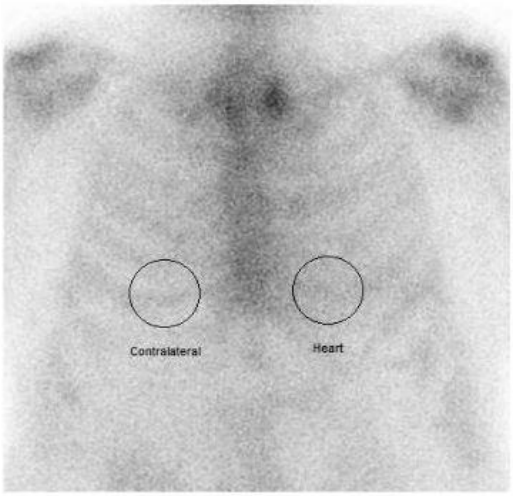


Figure 2.4 Early SPECT/CT with the red arrow demonstrating blood pooling in the left ventricle.



CONTRALATERAL LUNG ROI

Total Counts = 24279
Area = 1069

HEART ROI

Total Counts = 30357
Area = 1073

Heart / Contralateral Ratio = 1.25

Figure 2.5 This image demonstrates clearance of the radiopharmaceutical from the blood stream, confirmed by the smaller ratio.

Pilebro et al. (2016) found that patients with hereditary transthyretin (ATTR) cardiac amyloidosis associated with specific genetic mutations exhibited negative results on cardiac scintigraphy using ^{99m} Technetium-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD). The differing affinity for ^{99m}Tc-DPD is believed to be linked to the types of amyloid fibrils present, specifically Type A and Type B fibrils. Notably, Type B fibrils did not demonstrate any tracer uptake, as illustrated in Figure 2.6. Therefore, gene testing is crucial in these cases.

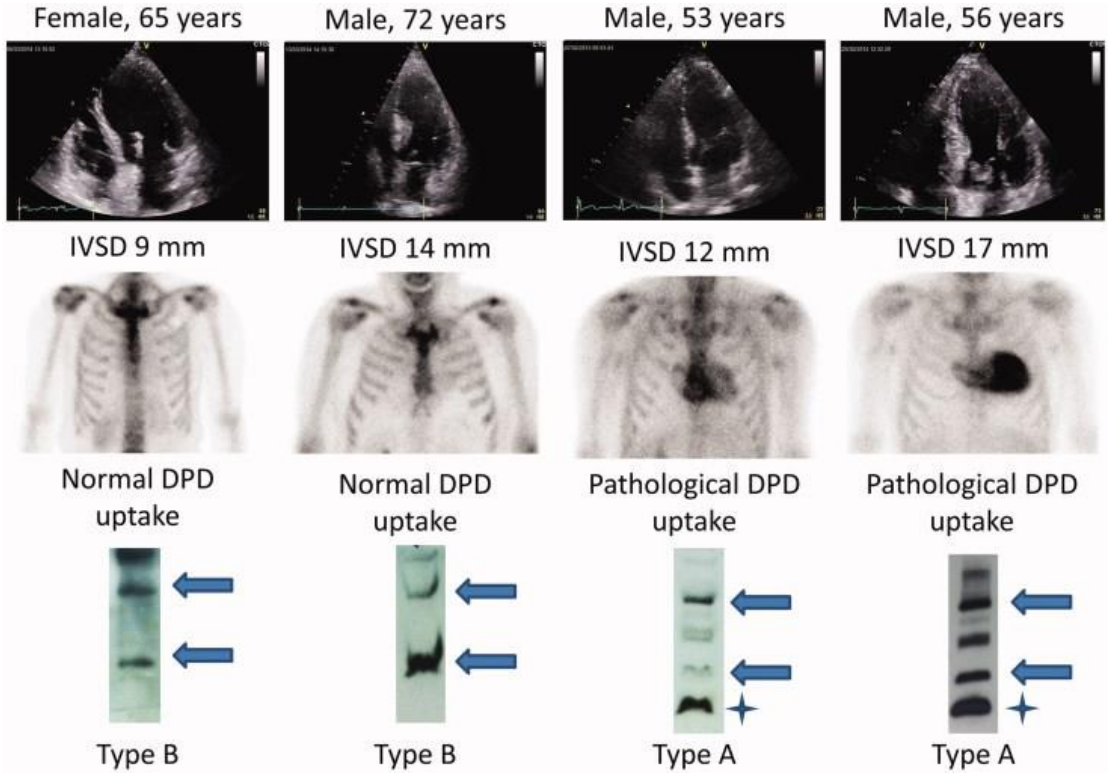


Figure 2.6 No cardiac uptake in ATTR cardiac amyloid patients with Type B fibrils, and pathological uptake in patients with Type A fibrils (Pilebro et al., 2016).

After providing a comprehensive definition and overview of cardiac amyloidosis, including its pathophysiology, subtypes, diagnostic pathways and treatment options, the next chapter focuses on the evaluation of one important diagnostic tool: biphosphonate-based radiotracer

scintigraphy. ^{99m}Tc-PYP cardiac amyloid imaging has emerged as a crucial tool in the non-invasive diagnosis of ATTR cardiac amyloidosis.

Despite its increasing popularity, uncertainties remain surrounding the optimal imaging protocols, particularly the ideal timing for imaging after injection. The following chapter presents a systematic review of the current literature to investigate the sensitivity and specificity of different imaging time points while also considering various biphosphonate-based radiotracers, doses, and analysis methods.

Chapter 3 - A systematic literature review

This chapter is presented in manuscript format, with the intent of publication. As a result, certain sections of the introduction may repeat content from previous chapters. The title of the intended article is “Imaging time points for transthyretin cardiac amyloidosis using phosphate based technetium-99m labelled radiopharmaceuticals: A Systematic Review.”

3.1 Introduction

Amyloidosis is a systemic disease that can affect several organs, such as the heart, brain, gastrointestinal tract, nervous system, liver, and kidneys. It is caused by the deposition of misfolded protein fibrils, serum proteins and proteoglycans as amyloid and can be fatal if undiagnosed (Wechalekar, Gillmore & Hawkins, 2016). Recent developments in this field have led to several treatment options, including chemotherapy, stem cell transplant, immunotherapy, gene silencer therapy, and stabilisers such as Tafamidis, some of which have recently been approved in the United States of America (Stern & Patel, 2022). The detection of cardiac amyloid can be challenging as the signs and symptoms may be the result of other underlying conditions (Merlini, 1997).

The gold standard for diagnosing cardiac amyloid is endomyocardial biopsy which is invasive and can lead to severe complications (González-López et al., 2017). The search for less invasive, highly sensitive and specific diagnostic tool in the detection of cardiac amyloidosis has led to the use of several tests such as electrocardiogram, cardiac magnetic resonance and cardiac biomarkers, none of which have been convincing on their own. Over the last decade, nuclear medicine scan using ^{99m}Technetium pyrophosphate (^{99m}Tc-PYP) has shown very high sensitivity and specificity in the diagnosis of cardiac amyloid (Dower et al., 2022). Imaging guidelines from the American Society of Nuclear Cardiology (ASNC) and the European Association of Nuclear Medicine (EANM) are widely used by imaging departments as key references. However, multiple revisions to the recommendations over the past decade, including changes to imaging

time points, have led to some confusion. This systematic literature review examines the various nuclear medicine protocols for the diagnosis of ATTR cardiac amyloid and compares the diagnostic accuracy of early and delayed imaging.

Biphosphonate-based tracers, the most used tracers in the detection of cardiac ATTR amyloid, are the focus of this systematic review (Gillmore et al., 2016). The inclusion of endomyocardial biopsies in this review was crucial, enabling the assessment of the sensitivity and specificity of different imaging protocols, analysis methods, and time points. A quality assessment tool was used to ensure the high quality of the articles included.

3.2 Materials and Methods

3.2.1 Search Strategy

This systematic review follows the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) (Page et al., 2021). The databases PubMed, Medline, Embase, Scopus, Web of Science, and CINAHL were used. The search was conducted on January 4, 2023 and limited to the last 10 years. Additionally, we examined the reference lists of eligible articles to identify any that were not captured during the database search. The search terms used to investigate the literature on Nuclear Medicine ATTR cardiac amyloid imaging are shown in Table 3.1. Article abstracts were screened based on the selection criteria detailed in Table 3.2.

Table 3.1 Electronic databases and search terms.

Electronic Database	**Search Terms**						
PubMed, Medline, Embase, Scopus, Web of Science, Cinahl	Technetium	AND	Cardiac	AND	Planar	AND	Biopsy
	OR		OR		OR		OR
	Tc-99m		Heart		SPECT		Biopsies
	OR		OR		OR		
	99m-Tc		Myocardium		Scintigraphy		
					OR		
					Nuclear		
					OR		
					Imaging		

Table 3.2 Selection Criteria

Inclusion Criteria	• Published within the last 10 years from 4th of January 2023 • Full Text • Original Studies • Published in English • Peer reviewed • Stated imaging time point • Contained human subjects
Exclusion Criteria	• Positron Emission Tomography (PET) • Reviews • Non-English articles • Non-Human studies • Case Studies • Non-Nuclear Medicine Related • Number of patients who had Reference Standard of at least 20 patients

3.2.2 Quality Assessment

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool was used to evaluate the potential for bias and the overall quality of the included articles (Whiting et al., 2003). It helps confirm that the results are valid and reliable and that the research was conducted to high standards. QUADAS includes 14 questions to which the answers can be “Yes”, “No”, or “Unclear”. Quality assessment was carried out by two independent reviewers (WN, PK).

3.2.3 Data Extraction

The following information was extracted from the articles for comparison purposes: author, year of publication, radiopharmaceutical used, number of participants, imaging time points, number of participants who underwent endomyocardial biopsy, analysis method, as well as sensitivity and specificity.

3.2.4 Statistics

Descriptive statistics were meticulously conducted on both sensitivity and specificity at each time point using the Analysis ToolPak in Microsoft Excel 2010. To effectively compare two imaging time points from the same dataset, a paired t-test was employed for both sensitivity and specificity measures. Furthermore, we calculated the p-value for each comparison to underscore the significance of our findings.

3.3 Results

On January 4, 2023, a search conducted using six medical databases resulted in 1,072 articles for screening (see Figure 3.1). After excluding 389 duplicates, 683 articles remained for screening. From these, 31 articles were selected for closer analysis. Fourteen of these articles were found unsuitable for this systematic review and were excluded.

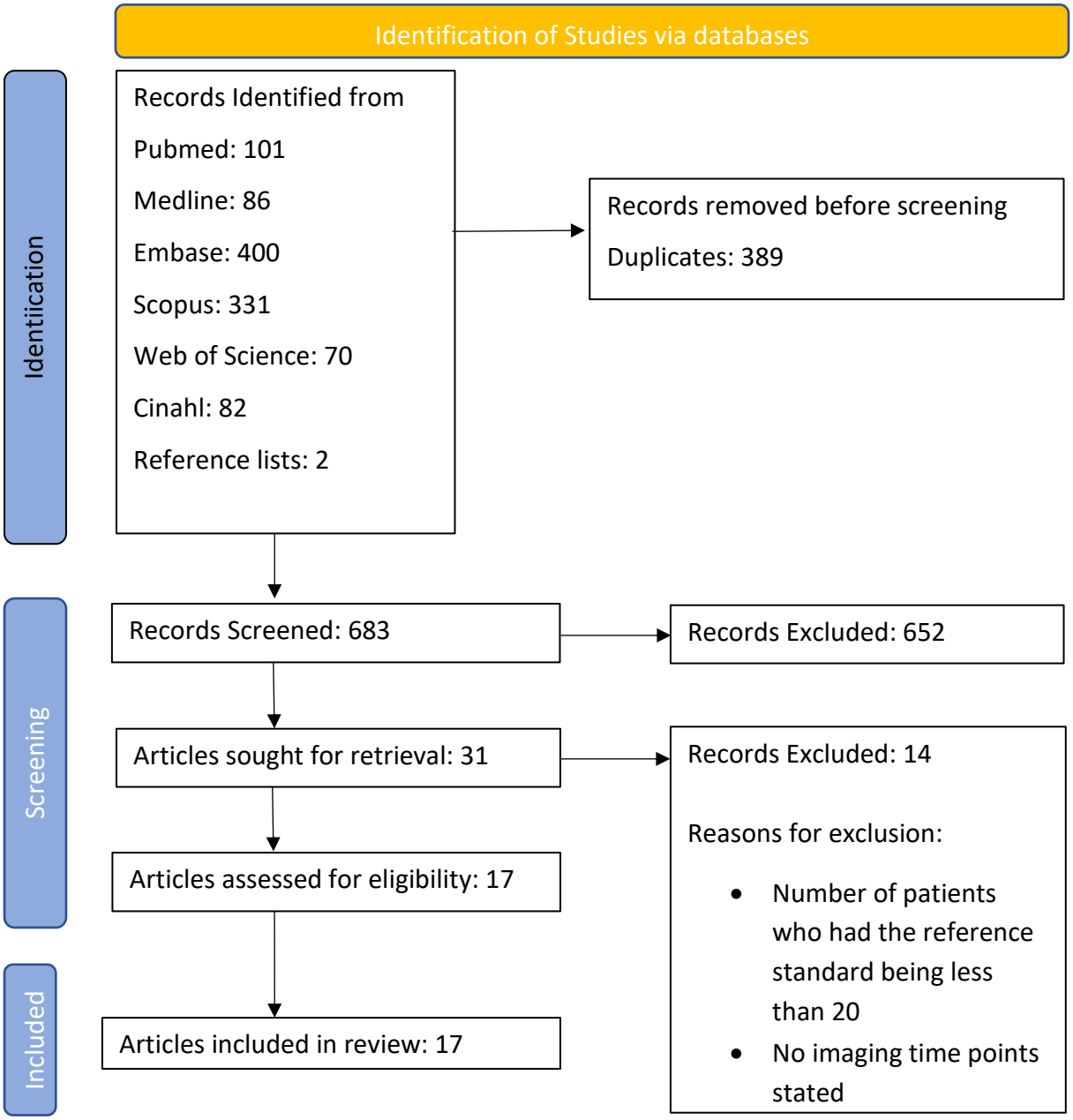


Figure 3.1 Identification and inclusion of articles using Pubmed, Medline, Embase, Scopus, Web of Science and Cinahl.

As a result, 17 articles met all the criteria and were included in the review. The quality assessment results, as determined using the QUADAS tool, are presented in Table 3.3, while the data extraction results are outlined in Table 3.4.

3.3.1 Quality Assessment

QUADAS, which are utilised in systematic reviews to evaluate the potential for bias and the overall quality of the studies included. The results of the quality assessment confirm a relatively high standard of the included articles. Across the included studies, most domains demonstrate a low risk of bias. The spectrum of patients, reference standard validity and the execution of both index and reference tests are well described. However, several areas show limitations in the methodology. The time interval between the index and reference tests were often unclear, and not all patients received uniform or independent reference standards. The blinding between the index and reference were also inconsistently applied. While the reporting quality was satisfactory in most domains, variability in the reference standard application and blinding reduced methodological consistency.

Given the relatively scarce use of endomyocardial biopsies as a reference standard in the majority of studies, the following questions warrant further investigations.

Question 4, 'Is the period between the reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?' (Whiting et al., 2003). The QUADAS tool for quality assessment, interestingly, resulted in "unclear" ratings for all articles. There is no mention of the period between the imaging date and the reference endomyocardial biopsy date in any of the studies. Due to the high-risk nature of endomyocardial biopsy, only four studies (Castano et al. 2016, Dower et al. 2022, Nishi et al. 2022 & Ogasawara et al. 2021) satisfied Question 5, 'Did the whole sample or a random selection of the sample receive verification using a reference standard?' (Whiting et al., 2003).

For the studies that compared their imaging results with a reference standard, five articles (Bokhari et al. 2018; Capelli et al. 2017; Castano et al. 2016; González-López et al., 2017 & Nitsche et al. 2020) mentioned a different reference standard than the gold standard of endomyocardial biopsy. Genetic testing, electrocardiography, cardiac echocardiography, cardiac magnetic resonance imaging, plasma assay, subcutaneous adipose tissue biopsy from the abdomen and non-cardiac biopsy of an involved organ were used by some studies as the reference standard. For Question 11, 'Were the reference standard results interpreted without knowledge of the results of the index test?' (Whiting et al., 2003), eight of the included articles (Bokhari et al. 2018; Cappelli et al. 2017; Gillmore et al. 2016; González-López et al., 2017; Hutt et al. 2014; Ogasawara et al. 2021; Schatka et al. 2021 & Tsutsui et al. 2019), the nuclear medicine cardiac amyloid imaging was performed and analysed independently of the reference standard. For the remaining articles, it was unclear for two articles whether the imaging results were independent of the reference standard, while seven articles stated that an endomyocardial biopsy was performed only if the cardiac amyloid imaging was positive.

Table 3.3 Quality assessment of articles using the QUADAS tool.

Author, Year	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14
Alreshq et al., 2022	Y	Y	Y	U	N	N	N	Y	Y	Y	N	Y	Y	Y
Bokhari et al., 2018	Y	Y	Y	U	N	N	Y	Y	Y	N	Y	Y	Y	Y
Quarta et al., 2012	Y	Y	Y	U	N	Y	Y	Y	Y	Y	N	Y	Y	Y
Cappelli et al., 2017	Y	Y	Y	U	N	N	Y	Y	Y	N	Y	Y	N	N
Castano et al., 2016	Y	Y	Y	U	Y	N	Y	Y	Y	Y	U	Y	Y	Y
Dower et al., 2022	Y	Y	Y	U	Y	N	N	Y	Y	Y	N	Y	Y	Y
Gillmore et al., 2016	Y	Y	Y	U	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
Gonzalez-Lopez et al., 2017	Y	Y	Y	U	N	N	Y	Y	Y	N	Y	Y	Y	Y
Hutt et al., 2014	Y	Y	Y	U	U	N	Y	Y	Y	N	Y	Y	Y	Y
Ikoma et al., 2022	Y	Y	Y	U	N	Y	N	Y	Y	Y	N	Y	Y	Y
Morioka et al., 2022	Y	Y	Y	U	N	N	N	Y	Y	Y	N	Y	Y	Y
Nishi et al., 2022	Y	Y	Y	U	Y	N	N	Y	Y	Y	N	Y	Y	Y
Nitsche et al., 2022	N	Y	Y	U	N	N	N	Y	Y	Y	U	Y	Y	Y
Ogasawara et al., 2021	Y	Y	Y	U	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Poterucha et al., 2021	Y	Y	Y	U	U	Y	N	Y	Y	Y	N	Y	Y	Y
Schatka et al., 2021	Y	Y	Y	U	N	N	Y	N	Y	N	Y	Y	Y	Y
Tsutsui et al., 2019	Y	Y	Y	U	N	Y	Y	Y	Y	Y	Y	Y	Y	Y

Y = Yes; N = No and U = Unclear

Methodological quality was assessed using quality assessment of diagnostic accuracy studies criteria: (Whiting et al., 2003).

Quality item 1: Was the spectrum of patients representative of the patients who will receive the test in practice?

Quality item 2: Were selection criteria clearly described?

Quality item 3: Is the reference standard likely to correctly classify the target condition?

Quality item 4: Is the time period between reference standard and index test short enough to be sure that the target condition did not change between the two tests?.
Quality item 5: Did the whole sample, or a random selection of the sample, receive verification using a reference standard of diagnosis?
Quality item 6: Did patients receive the same reference standard regardless of the index test result?
Quality item 7: Was the reference standard independent of the index test (i.e., the index test did not form part of the reference standard?).
Quality item 8: Was the execution of the index test described in sufficient detail to permit replication of the test?
Quality item 9: Was the execution of the reference standard described in sufficient detail to permit replication?
Quality item 10: Were the index test results interpreted without knowledge of the results of the reference standard?
Quality item 11: Were the reference standard results interpreted without knowledge of the results of the index test?
Quality item 12: Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?
Quality item 13: Were uninterpretable/intermediate test results reported?
Quality item 14: Were withdrawals from the study explained?

3.3.2 Data Extraction

The radioactive tracers used in the studies included in this systematic review were all biphosphonate-based, namely ^{99m}Technetium-3,3-diphosphono-1,2-2 propanodicarboxylic acid (^{99m}Tc-DPD), ^{99m}Technetium- Pyrophosphate (^{99m}Tc-PYP) and ^{99m}Technetium- hydroxymethylene diphosphonate (^{99m}Tc-HMDP). The number of subjects ranged from 45 to 11,527. Early imaging time ranged from 5 minutes to 1-hour post-injection, and delayed imaging time ranged from 2 hours to 4 hours. Alreshq et al. (2022) performed early imaging only, whereas Cappelli et al. (2017), Ikoma et al. (2022), Morioka et al. (2022), Nishi et al. (2022), Nitsche et al. (2020), Ogasawara et al. (2021) and Tsutsui et al. (2019) performed delayed imaging only. The remaining studies had a combination of early and delayed imaging. The analysis methods used were visual grading, semi-quantitative and quantitative analyses. The sensitivity and specificity for early imaging ranged from 92% to 100% and 79% to 100%, respectively. The sensitivity and specificity for delayed imaging ranged from 58% to 100% and 86% to 100%, respectively. Statistical analysis indicates that the difference in sensitivity and specificity between the early and delayed time points is not statistically significant. The difference in sensitivity between the two-time points is not statistically significant ($p=0.38$). The difference in specificity between the two-time points is also not statistically significant ($p=0.57$). (Table 3.5)

Table 3.4 Study characteristics for studies included in the review.

Author, Year	Radiopharm-aceutical	Dose	No. of patients	Early Imaging (hours)	Delayed Imaging (hours)	No. of patients - Standard Ref	SPECT/CT acquisition	Analysis Method	Sensitivity early (%)	Specificity early (%)	Sensitivity delayed (%)	Specificity delayed (%)
Alreshq et al., 2022	PYP	-	378	1	No	25	Yes	Visual Grading and semi quantitative	*96.9	*100		
Bokhari et al., 2018	PYP	370 – 925 MBq	45	1	2 - 4	37	No	Visual Grading and semi quantitative	97	100	97	100
Quarta et al., 2012	DPD	740MBq	67	5 min	3	46	No	Visual Grading			100	100
Cappelli et al., 2017	HMDP	700 - 740MBq	131	No	3	20	No	Visual Grading and semi quantitative			96	100
Castano et al., 2016	PYP	370 – 925 MBq	171	1	3	88	Yes	Visual Grading	95	79	58	100
								Semiquantitative	92	97	88	86
Dower et al., 2022	PYP	555 MBq	273	1	3hr done only if blood pooling seen on early images	27	Yes	Visual Grading and semi quantitative			*83.3	*88.9
Gillmore et al., 2016	PYP/DPD/HMDP	700 MBq	1217	1 (for some PYP pts)	3	374	Yes	Visual Grading			88	87
Gonzalez-Lopez et al., 2017	DPD	-	108	5 min	3	65	Yes	Visual Grading			100	100
Hutt et al., 2014	DPD	700 Mbq	321	Yes (for 4	3	53	Yes	Visual Grading			91	82

Author, Year	Radiopharm-aceutical	Dose	No. of patients	Early Imaging (hours)	Delayed Imaging (hours)	No. of patients - Standard Ref	SPECT/CT acquisition	Analysis Method	Sensitivity early (%)	Specificity early (%)	Sensitivity delayed (%)	Specificity delayed (%)
				pts)								
Ikoma et al., 2022	PYP	740 MBq	164	No	3	30	Yes	Visual Grading and semi quantitative			100	93.3
Morioka et al., 2022	PYP	-	88	No	3	69	Yes	Visual Grading and semi quantitative			*100	*100
Nishi et al., 2022	PYP	555 – 740 MBq	231	No	3	109	Yes	Visual Grading and semi quantitative			99	X
Nitsche et al., 2022	DPD/MDP	700 MBq	11527	No	3 hour	33	Yes	Visual Grading			*100	*100
Ogasawara et al., 2021	PYP	555 – 740 MBq	68	No	3 hour	68	Yes	Quantitative			X	X
Poterucha et al., 2021	PYP	-	753	1	< 10% of scans, images were acquired at 2hr and 3hr	104	Yes	Visual Grading and semi quantitative	94	89		
Schatka et al., 2021	DPD	500 MBq	63	1	3 hour	21	Yes	Visual Grading	100	89	96	95
								Semiquantitative	96	97	96	95
Tsutsui et al., 2019	PYP	740 MBq	98	No	2.5 hour	29	Yes	Visual Grading and semi quantitative			100	97.50

* Author calculated sensitivity and specificity based on raw data provided in articles.

X Sensitivity and specificity were not mentioned and could not be calculated.

Table 3.5 Descriptive and t-Test: two-sample assuming unequal variances statistics for the sensitivity and specificity of early and delayed time points.

	Sensitivity		**Specificity**	
	Early	Delayed	Early	Delayed
Mean	95.84	93.27	93.00	94.98
Standard Error	0.96	2.70	2.92	1.60
Median	96	96.5	97	97.5
Mode	#N/A	100	100	100
Standard Deviation	2.53	10.79	7.72	6.18
Sample Variance	6.44	116.33	59.67	38.24
Kurtosis	0.59	7.87	0.40	-0.40
Skewness	0.16	-2.61	-1.04	-0.96
Range	8	42	21	18
Minimum - Maximum	92 - 100	58 - 100	79 - 100	82 - 100
Count	7	16	7	15
Confidence Level (95.0%)	2.35	5.75	7.14	3.42
t-Test: Hypothesized Mean Difference	0		0	
df	18		10	
t Stat	0.90		-0.60	
P(T<=t) one-tail	0.19		0.28	
t Critical one-tail	1.73		1.81	
P(T<=t) two-tail	0.38		0.57	
t Critical two-tail	2.10		2.23	

The sensitivity and specificity of early versus delayed images, as reported by the included studies, are presented in Figure 3.2. The boxplots show very high sensitivity and specificity across all time points, with medians exceeding 95%.

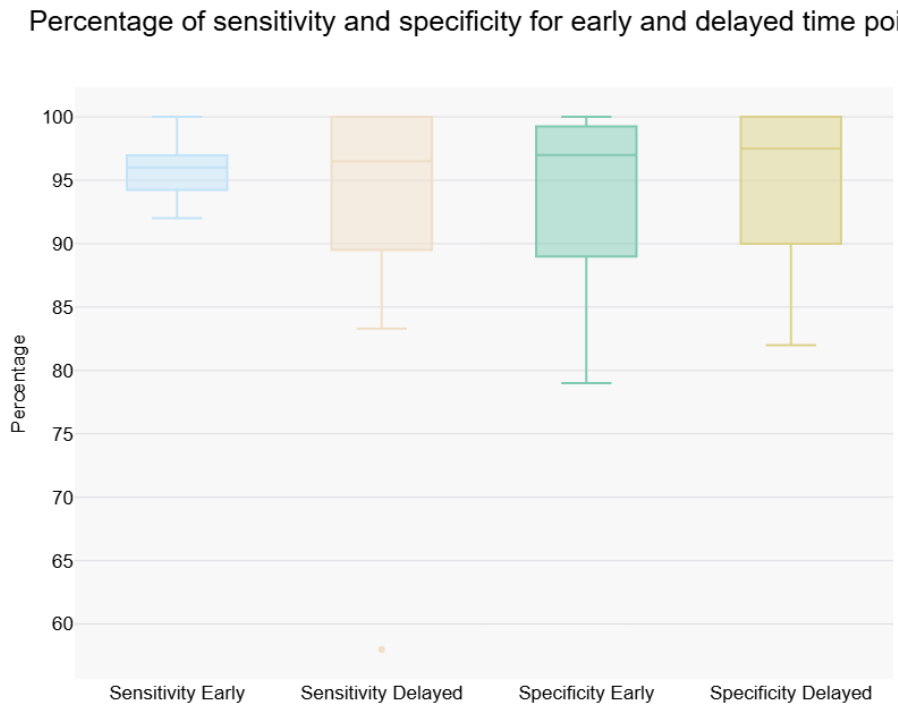


Figure 3.2 Boxplot demonstrates the difference in sensitivity and specificity between early and delayed time points.

To evaluate the effects of different radiopharmaceuticals in the included studies, the mean sensitivity and specificity for early and delayed imaging using ^{99m}Tc-PYP and ^{99m}Tc-DPD were calculated. The differences in means for sensitivity and specificity at various time points were not statistically significant, as indicated by the p-values in Figure 3.3. Three studies were

excluded from this analysis. Cappelli et al. (2017) was the only study to use ^{99m}Tc-Hydromethylene diphosphonate (HMDP) on its own. Gilmore et al. (2016) utilized a combination of ^{99m}Tc-PYP, ^{99m}Tc-DPD, and ^{99m}Tc-HMDP, while Nitsche et al. (2022) used a combination of ^{99m}Tc-DPD and ^{99m}Tc-MDP. The range of injected activity is presented in Figure 3.4. Several studies used a range of injected doses, while others administered fixed doses. With no more than three studies in each group, it was difficult to conduct any meaningful statistical analysis. Additionally, three studies did not specify the injected dose.

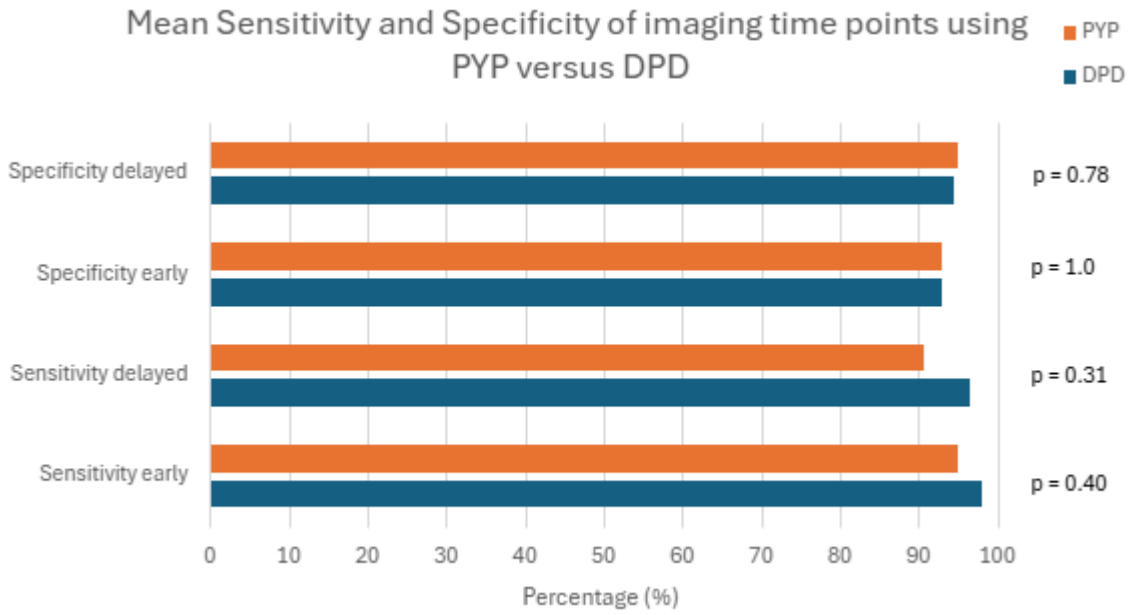


Figure 3.3 Mean Sensitivity and Specificity of imaging time points using PYP versus DPD.

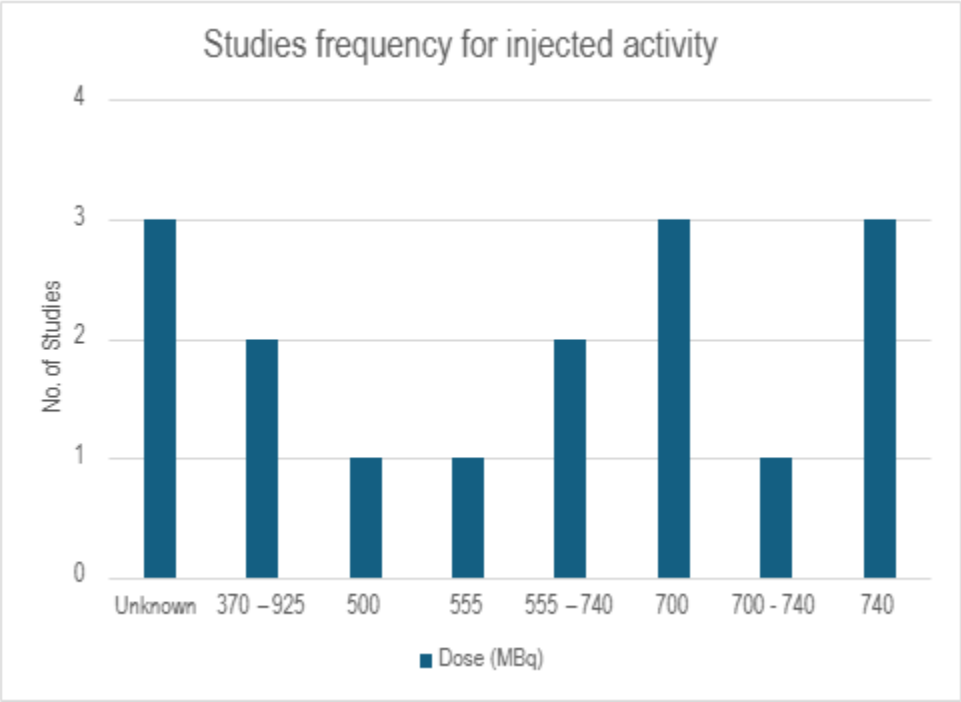


Figure 3.4 Activity of radiopharmaceutical injected for the included studies.

The sensitivity and specificity ranges using the different analysis methods, visual grading, semi-quantitative and a combination of both visual grading and semi-quantitative analysis, are shown in Figures 3.5 and 3.6, respectively. The boxplots show high sensitivity and specificity across all time points, with medians exceeding 90% except for the early visual grading method with a specificity of 84%.

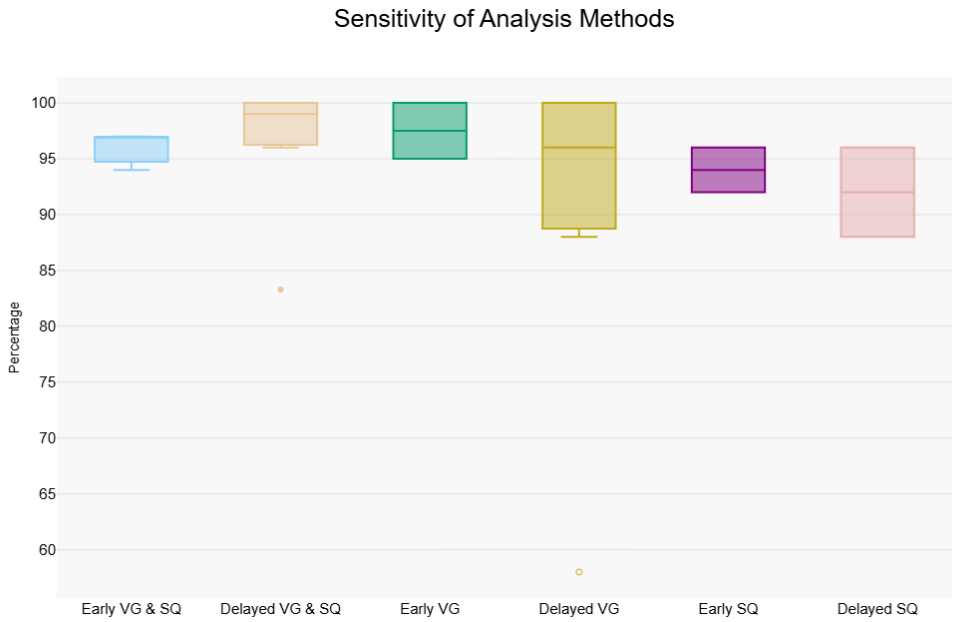


Figure 3.5 Sensitivity using different analysis methods. (VG = Visual Grading, SQ = Semi Quantitative).

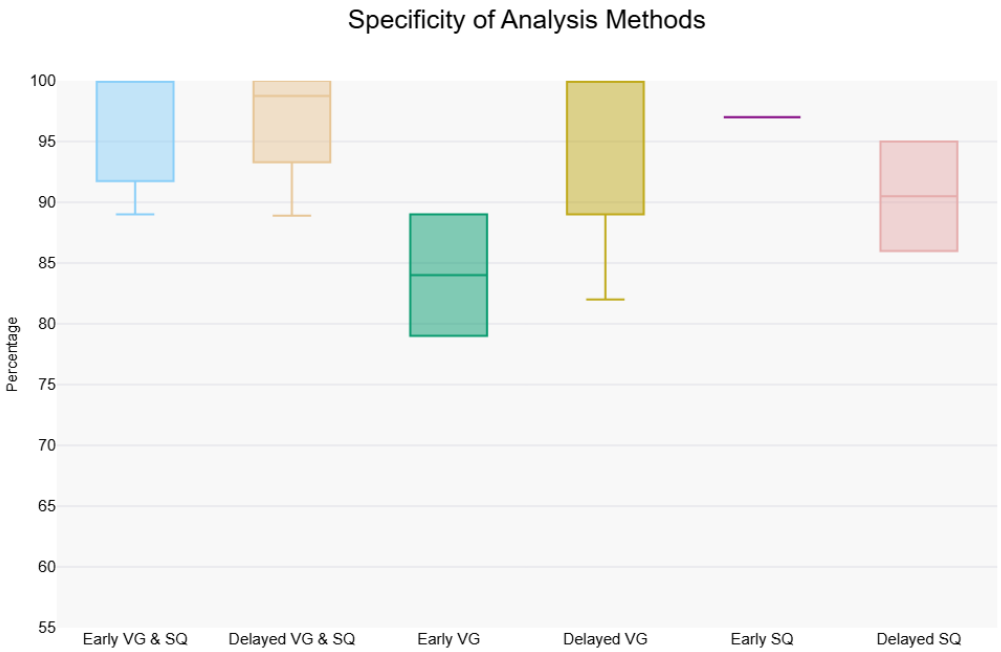


Figure 3.6 Specificity using different analysis methods. (VG = Visual Grading, SQ = Semi Quantitative).

3.4 Discussion

The QUADAS tool used in the quality assessment of the included studies does not provide an overall quality score; however, the quality of the articles can be inferred from the number of "Yes" responses reported for each question in the assessment. This demonstrates a relatively high standard of the articles included, with an average of 9.5 "Yes" out of 14 questions. Most of the articles reported negatively on questions 5, 6 and 7, all of which are related to the reference standard. Endomyocardial biopsy is the gold standard diagnostic tool for detecting

cardiac amyloidosis. Due to the risks and costs associated with endomyocardial biopsy, it was only performed on all 68 participants in Ogasawara et al.'s (2021) study. The remaining studies had limited participants endure endomyocardial biopsies. Although all 68 participants in Ogasawara's study underwent endomyocardial biopsies, the sensitivity and specificity of ^{99m}Tc-PYP nuclear medicine scintigraphy were not reported and could not be calculated.

Although the timing between the nuclear medicine scan and the reference standard was not reported in any of the included studies, this is unlikely to have affected the outcomes. Cardiac amyloidosis, due to its infiltrative nature, is a progressive and relatively stable condition. Therefore, if a participant tested positive, the disease status is unlikely to have changed significantly in the interim. Conversely, if a participant was negative, it is improbable that they would have developed detectable amyloid deposition in a short period (Shah, Inoue, & Mehra, 2006).

The analysis of various radioactive tracers revealed no significant differences in sensitivity and specificity between early and delayed time points. ^{99m}Tc-HMDP was excluded from this analysis because one article utilised it as the only tracer, while another article employed a combination of ^{99m}Tc-DPD, ^{99m}Tc-PYP, and ^{99m}Tc-HMDP. The analysis of dosage proved unfeasible due to the extensive variability in doses utilized, compounded by the lack of dosage specification in three of the reviewed articles.

This systematic review suggests that the combination of visual grading and semi-quantitative analysis techniques provides the best outcome. All analysis methods demonstrate high sensitivity at both imaging time points. The combined visual grading and semi-quantitative analysis method has a higher specificity for both imaging time points compared to the other methods. Due to the low sample size of the sensitivity and specificity of some analysis methods, the statistical significance of the difference could not be calculated. The study by Ogasawara et al. (2021) employed a quantitative analysis method using SPECT/CT, but sensitivity and specificity were not provided, nor could they be calculated. SPECT/CT was used in 14 studies, while the remaining studies employed planar acquisitions only. High sensitivity and specificity can be achieved using planar images only. Still, the three-dimensional information provided by SPECT/CT allows differentiation between the presence of cardiac amyloid and blood pooling in the heart chambers (Régis et al., 2020; Poterucha et al., 2021). SPECT/CT may increase the reporting physicians' confidence, regardless of the imaging time point.

This systematic review demonstrates that both early and delayed imaging time points exhibit high sensitivity and specificity. There are more advantages for the early imaging time points compared to delayed or both imaging time points. After the 1-hour mark, 10% of the injected ^{99m}Tc-PYP remains in the myocardium, which is, therefore, suitable for imaging (Bokhari et al.,

2018). Delayed imaging has the advantage of minimal blood pooling, but it also has more rib uptake that can affect the heart-to-contra-lateral side ratio.

The early imaging time point enables the patient to complete the procedure at least 2 hours earlier than the delayed imaging protocol, resulting in less time spent at the hospital or imaging facility. Most candidates for cardiac amyloid are typically elderly and may require the assistance of family members to attend such imaging. Reducing the length of the imaging procedure benefits the patient and may also alleviate the burden on family members. Furthermore, with a shorter duration of imaging the radiation exposure is minimised to staff, and the nuclear medicine department resources become available for other imaging procedures.

3.5 Limitations

Only three studies included in the systematic review used both early and delayed imaging time points. In the studies by Bokhari et al. (2018) and Castano et al. (2016), the readers were blinded to the biopsy status; however, it is unclear whether they were aware of the imaging acquisition time points. In the study conducted by Schatka et al. (2021), the readers were not blinded by the imaging time points, rather they were first shown an early set of images and were kept blinded from the delayed images. They were then shown the delayed images and their reports may have been influenced by the early set of images.

Four studies did not provide the sensitivity and specificity for any of the imaging time points. Based on the raw data from these articles, sensitivity and specificity were subsequently calculated (see Table 3.4).

3.6 Conclusion

The systematic review demonstrated very similar sensitivity and specificity for the two imaging time points for cardiac amyloid. SPECT/CT is crucial in the imaging protocol as it helps differentiate blood pooling in the cardiac chambers and radioactive tracer uptake in the myocardium. Imaging early is more favourable for both patients and imaging sites, but delayed imaging can be acquired for difficult or intermediate cases.

3.7 Summary

The preceding systematic review has provided an elaborate evaluation of the diagnostic performance of early versus delayed cardiac amyloid imaging using technetium labelled phosphate-based radiopharmaceuticals in the detection of ATTR cardiac amyloidosis. The findings highlight the high sensitivity and specificity of cardiac scintigraphy, for each imaging time point. Although most studies used the delayed imaging time point, the review suggests

that early imaging may offer comparable results with the added benefit of improved patient experience and workflow.

Considering this, there remains an essential need to assess whether a more streamlined imaging protocol using a single time point, preferably the early one, can provide high diagnostic accuracy in the clinical setting. The next chapter presents a reader study aimed at evaluating the feasibility of reporting ATTR cardiac amyloid scan with data provided from a single time point. It focusses on inter-reader agreement and diagnostic accuracy. Building on the findings of the systematic review, this study seeks to transfer the theoretical potential into practical evidence which can help improve imaging guidelines.

Chapter 4 - Single- Versus Dual-Time-Point Imaging for Transthyretin Cardiac Amyloid Using ^{99m}Tc-Pyrophosphate

This chapter is based on the following article:

Wesley Ng, Kunthi Pathmaraj, Natalia Kovaleva, Aurora Poon, Peter Kench, Steven Meikle, Andrew Scott and Raef Boktor. (2025). Single- Versus Dual-Time-Point imaging for Transthyretin Cardiac Amyloid Using [99mTc] Tc-Pyrophosphate. Journal of Nuclear Medicine Technology, 53:1-6. DOI: 10.2967/jnmt.124.269395

4.1 Introduction

Amyloidosis is a systemic condition which can affect several organs such as the kidneys, brain, liver, and heart, and can be fatal if untreated (Wechalekar, Gillmore & Hawkins, 2016). There are two main types of cardiac amyloidosis, transthyretin (ATTR) and Light Chain. The diagnosis of cardiac amyloidosis can be challenging due to the wide range of symptoms similar to those of other conditions and the requirement of several tests to confirm the presence of amyloidosis (Jerome et al., 2023). This study focuses on transthyretin cardiac amyloidosis. The gold standard for the diagnosis of cardiac amyloidosis is endomyocardial biopsy (Vogelsberg et al., 2008). The invasive nature of this procedure, which requires highly skilled medical professionals, can lead to severe complications such as bleeding, arrhythmias, infection, perforation of the heart and damage to heart valves or blood vessels (González-López et al., 2017). An array of diagnostic tools is being used where possible to accurately diagnose cardiac amyloidosis without endomyocardial biopsies. These tools include but are not limited to cardiac MRI, echocardiography, electrocardiography , serum biomarkers, and nuclear medicine scintigraphy (Ochi et al., 2020; Ochi et al., 2021; Khor et al., 2020). [^{99m}Tc]Tc-Pyrophosphate ([^{99m}Tc]Tc-PYP) scintigraphy has proven valuable in the diagnosis of ATTR cardiac amyloid in recent years with

high sensitivity and specificity ranging between 97% to 100% and 93.3% to 100% respectively (Bokhari et al., 2018; Ikoma et al., 2023; Tsutsui et al., 2019).

The timing of the nuclear medicine scans after the administration of [^{99m}Tc]Tc-PYP is not a standardized practice across nuclear medicine departments. The uptake mechanism of pyrophosphate in ATTR amyloidosis is not very well understood but it is believed that it is related to the presence of elevated calcium levels in amyloid-deposited tissues (Willerson et al., 1980). The American Society of Nuclear Cardiology (ASNC) originally released a [^{99m}Tc]Tc-PYP imaging practice points in 2016, with an updated version in February 2019. The 2019 version recommends images to be acquired at one-hour post injection and if more information is needed, additional images to be acquired 3-hours post injection (ASNC, 2022). In September 2019, an ASNC and European Association of Nuclear Medicine (EANM) Cardiac Amyloidosis DPD (Diphosphono-1,2-Propanodicarboxylic Acid) Practice Points adapted from the February 2019 version was released. This adaptation focused on the different radiopharmaceuticals that are more widely used in Europe, [^{99m}Tc]Tc-Diphosphono-1,2-propanodicarboxylic acid ([^{99m}Tc]Tc-DPD) and [^{99m}Tc]Tc-Hydroxymethylene Diphosphonate ([^{99m}Tc]Tc-HMDP). The recommended imaging time points were two or three hours post injection with both planar and SPECT imaging and the one-hour time point being optional (ASNC & EANM, 2019). Most recently, in 2023, ASNC released an interpretation and reporting document “Interpretation and Reporting of

Cardiac Scintigraphy with Bone-Avid Tracers in Suspected Transthyretin Cardiac Amyloidosis (ATTR-CA)" which found similar diagnostic accuracy for both imaging time points, 1-hour and 3-hour. SPECT/CT was found to be essential for the accurate reporting of cardiac amyloidosis regardless of the time point, whilst semi-quantitative analysis is optional (ASNC, 2024).

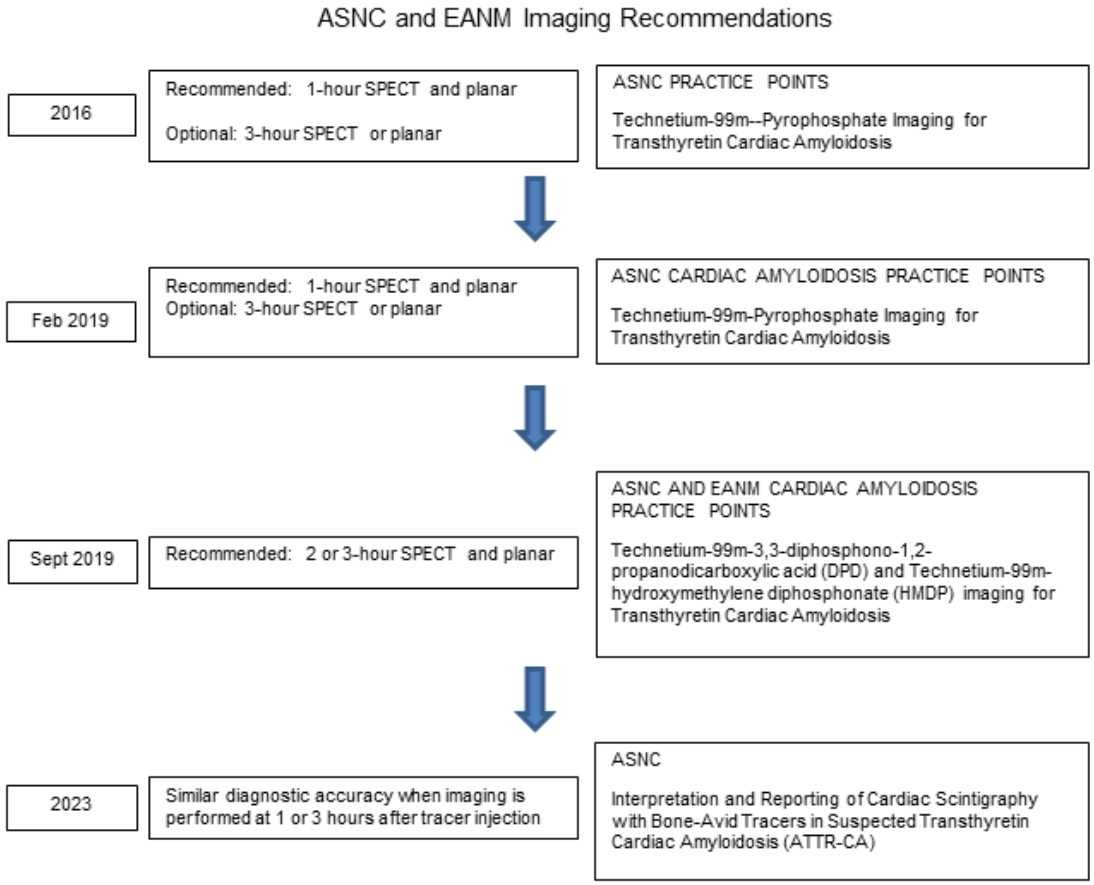


Figure 4.1 Timeline of recommendation from ASNC and EANM.

We aimed to determine if single or dual time point imaging is required for reporting purposes and which time point would be the most appropriate if a single time point was to be considered.

4.2 Materials and Methods

The current protocol in our department is to image the patient at two time points, at one hour post injection and at three hours post injection, acquiring planar images and at least one SPECT/CT. A definitive diagnosis of cardiac amyloidosis is often established by endomyocardial biopsy, however this is not always performed. For our study, a diagnosis of ATTR cardiac amyloidosis was established by reviewing the clinical history, diagnostic tests, results from patients' electronic medical records, and original clinical reports of the PYP scans, which used both imaging time points. Most patients had diagnostic tests including echocardiogram, blood tests, cardiac MRI, and PYP scan to investigate the presence of ATTR cardiac amyloidosis. Ethics approval for this retrospective study was granted by Austin Health Human Research Ethics Committee and the requirement for obtaining informed consent was waived.

There were 207 consecutive patients who had [^{99m}Tc]Tc-PYP cardiac amyloid studies performed on a GE Discovery 670DR (Wisconsin, U.S) reviewed. The [^{99m}Tc]Tc-PYP scans were retrieved from the hospital's AGFA Picture Archiving and Communication System. Only patients who had static chest planar and SPECT/CT of the chest at both imaging time points were included in this review. 70 patients met these criteria and were thus included. Each study was split into two separate datasets, early and delayed datasets. All the datasets were anonymised, time stamps

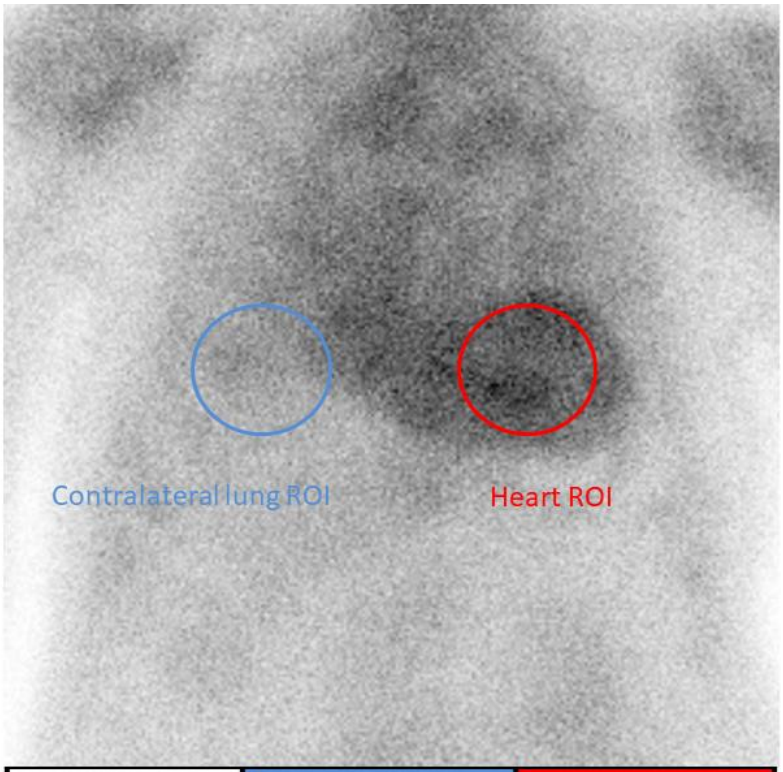
erased, processed, and reviewed by the three readers two qualified Nuclear Medicine Physicians and one Nuclear Medicine Trainee), blinded to the patients' clinical history and imaging time points.

Table 4.1 Acquisition parameters for [99mTc]Tc-PYP scans.

	Parameters	Early Image (T0 + 60min)	Delayed Image (T0 + 180min)
Static	**Acquisition Time**	300 sec	300 sec
	Matrix	256 x 256	256 x 256
	zoom	1.5	1.5
	Energy	140.5kV ± 10%	140.5kV ± 10%
SPECT/CT	**Acquisition Time**	20 sec/frame	20 sec/frame
	No. Of views	60	60
	Matrix	128 x 128	128 x 128
	Body Contour	Yes	Yes
	Energy	140.5kV ± 10%	140.5kV ± 10%

A semi-quantitative analysis was performed on the static chest planar image. Two regions of interest (ROIs) of the same size were drawn over the heart (H) and the contralateral (CL) right lung. A ratio was calculated by dividing the counts from the heart ROI by the counts from the contralateral lung (ROI). The ratio was noted on the saved image for the Specialist to review. At our Centre, as per the 2019 ASNC practice points, a ratio of 1.5 or more on the early static image and uptake within the myocardium on SPECT/CT was considered a positive study (ASNC, 2022). The ASNC PYP Practice Points (ASNC, 2022) does not stipulate a cut-off ratio value for

delayed imaging, but Scully et al. (2020) suggest a cut-off value of 1.3, with a ratio of 1.3 or more indicative of a positive study for the delayed time point.



	Contralateral lung ROI	Heart ROI
Total counts	37,434	61,119
Area	1,067	1,074
Heart vs Contralateral Lung Ratio: 1.63%		

Figure 4.2 An example of a positive study, with a ratio of the heart ROIvs the Contralateral Lung ROI being more than 1.5.

All image processing was done by a single experienced nuclear medicine technologist. The processed data was independently reviewed by each reader who reported the datasets as

positive, negative, or equivocal. A case was recorded as positive if there was a mention of diagnosis or treatment of ATTR Cardiac Amyloidosis in the patient's medical records. No hospital medical records could be found for two patients, who had external follow up. Excluding these two cases, the data was used to calculate the specificity of a single time point of [^{99m}Tc]Tc-PYP cardiac amyloid scans.

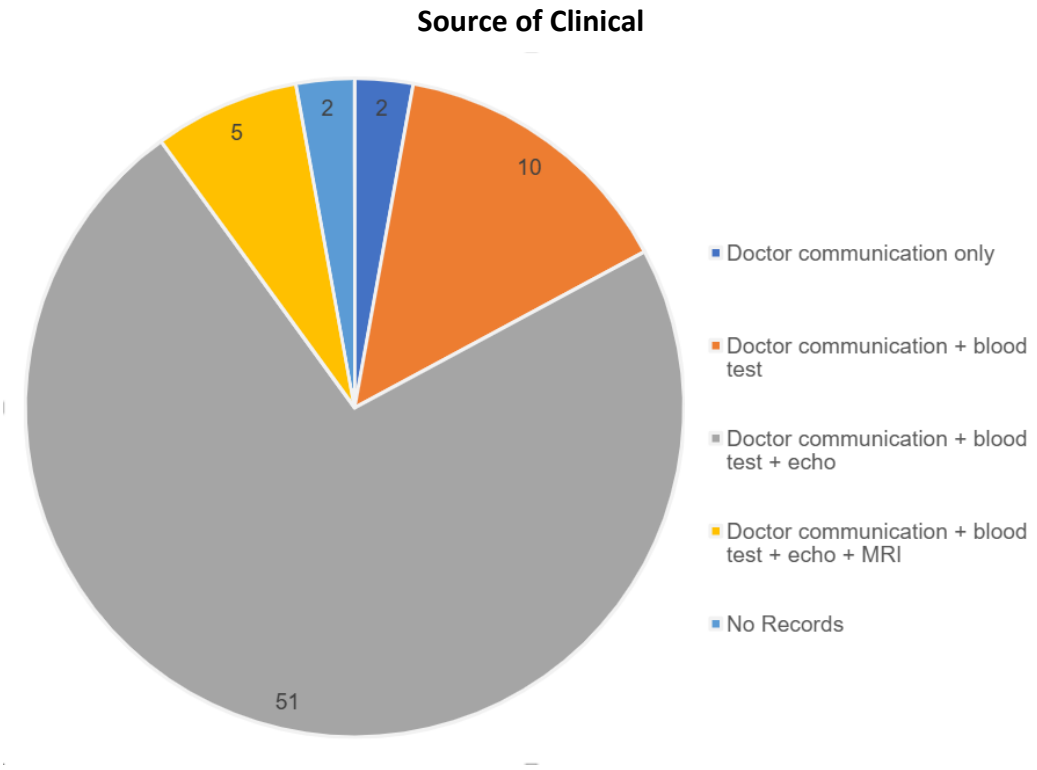


Figure 4.3 Source of clinical data for diagnosis of possible cardiac amyloidosis.

4.2.1 Statistics

The percentage agreement between all three readers and the two experienced readers was calculated. The interobserver variability was corrected for chance between any two readers and assessed using the Cohen's kappa coefficient at the 95% confidence interval. The interobserver variability between all three readers was evaluated using the weighted Fleiss' kappa coefficient (Landis & Koch, 1977). A kappa value less than 0.01 would be considered no agreement, 0.01 to 0.2 poor, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial agreement and 0.81 to 1.00 good agreement.

Statistical analysis of the datasets was performed using the Statistical Package for the Social Sciences (SPSS).

4.3 Results

4.3.1 Population Cohort

Twelve patients had confirmed diagnosis of cardiac amyloidosis. . The diagnosis of 10 patients was confirmed via doctor communication, blood tests and echocardiograms, one patient diagnosis was confirmed via doctor communication, blood tests, echocardiograms and cardiac MR and one patient diagnosis was confirmed via doctor communication and blood test only.

Demographics and indications for the cardiac amyloid scans for patients who had SPECT/CT chest scan at both time points are shown in Table 2.

Table 4.2 Characteristics of patients in cohort.

Total number of patients	70	
Age Range	26 - 90 years	
Injected Dose (99mTc-PYP) Range	567 -759 MBq	
Sex		
Male	52	
Female	18	
Indication for Scan	N	%
Left Ventricular Hypertrophy (LVH)	26	37.1
LVH and (Aortic Stenosis or Atrial Fibrillation or Heart Failure or Diastolic Dysfunction or Pulmonary Hypertension or Carpel Tunnel)	28	40.0
Heart failure	10	14.3
Aortic stenosis	3	4.3
Other	3	4.3

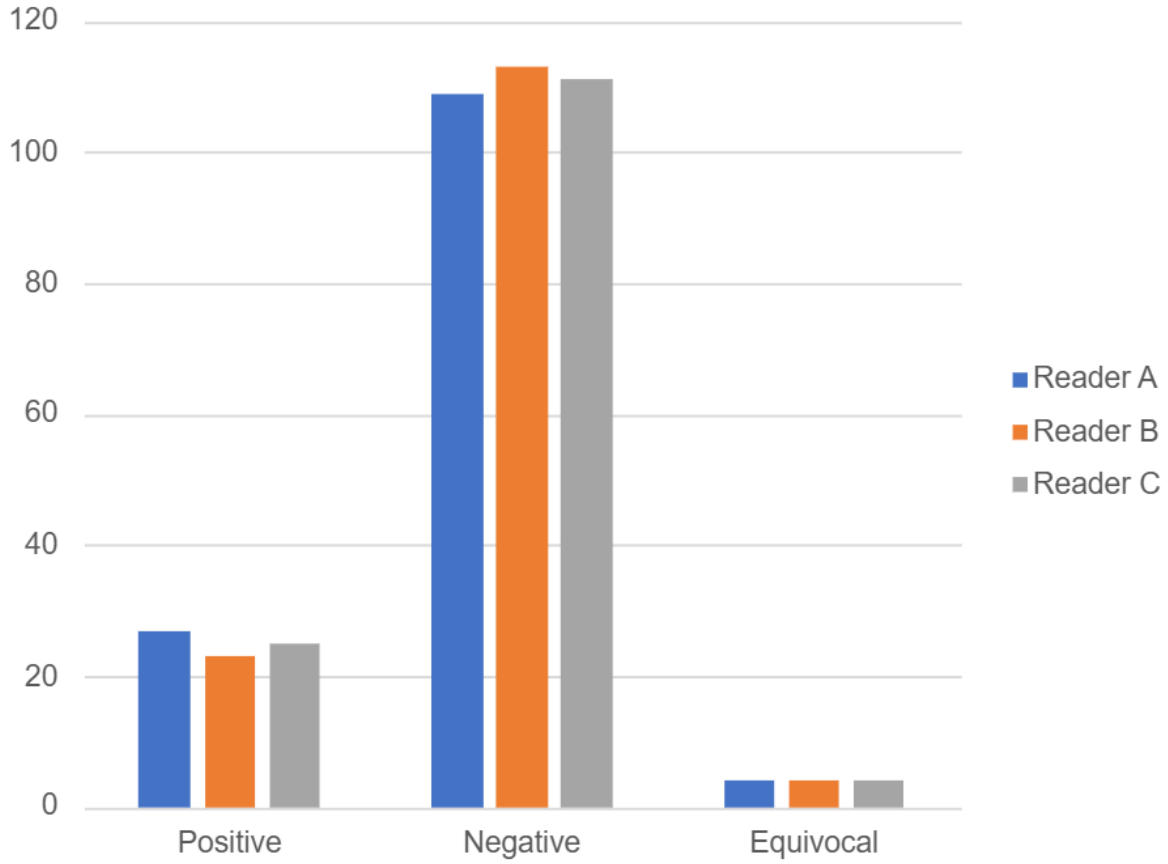


Figure 4.4 Scans results reported by each reader (Reader A and C were senior physicians).

All three readers had 4 equivocal datasets from out of the 140 datasets (70 for the early imaging time point and 70 for the delayed imaging time point). The equivocal datasets were not necessarily the same for all readers. The sensitivity of reporting using the early imaging point was 100% for all three readers. The sensitivity of reporting using the delayed dataset only was 100%, 90.9% and 100% for reader A, reader B and reader C respectively. The accuracy of reporting using the early dataset only was 95.7%, 97.1% and 95.7% for reader A, reader B and

reader C, respectively. The accuracy of the report using the delayed dataset only was 95.7%, 94.2% and 97.1% for reader A, reader B and reader C, respectively. The specificity of the reporting using the early dataset was 96.4%, 98.2% and 98.2% for reader A, reader B and reader C respectively, the specificity of reporting using the delayed dataset was 98.2% for reader A and reader B and 98.3% for reader C. The agreement between all three readers was 95.7% and 92.8% for early dataset, and delayed dataset, respectively. The agreement between the two senior readers, reader A and reader C was 95.7% for both datasets. The Fleiss' Kappa at 95% confidence interval for all three readers was 0.91 for the early time point and 0.83 for the delayed time point, which is categorised as good agreement. The Cohen's Kappa at 95% confidence interval for the two senior readers was 0.88 and 0.87 for the early and delayed time points respectively, which is again categorised as good agreement.

The difference of the sensitivity, accuracy and specificity between early and delayed imaging was not statistically significant, p value of 0.422, 0.741 and 0.400 respectively.

Table 4.3 Sensitivity, Accuracy and Specificity of using a single dataset for reporting 99mTc-PYP Cardiac Amyloid.

		Reader A	Reader B	Reader C
Early dataset only	Sensitivity	100.0%	100.0%	100.0%
	Accuracy	95.7%	97.1%	95.7%
	Specificity	96.4%	98.2%	98.2%
Delayed dataset only	Sensitivity	100.0%	90.9%	100.0%
	Accuracy	95.7%	94.2%	97.1%
	Specificity	98.2%	98.2%	98.3%

4.4 Discussion

One of the challenges of reporting [^{99m}Tc]Tc-PYP cardiac amyloid scans is the accumulation of the radiopharmaceutical in the chambers of the heart on early images and it is challenging to distinguish between myocardial uptake and blood pooling on static planar images only. In cases where blood pooling is seen and suspected on early images, delayed images can be acquired, as blood pooling is expected to be cleared by 3-hours, or an early SPECT/CT can be acquired to distinguish between myocardial uptake and activity in the heart chambers (Willerson et al., 1980). There appears to be varying approaches regarding optimal imaging time in the current literature. Many nuclear medicine departments are using the 3 – hour time point for reporting (Ikoma et al., 2023; Tsutsui et al., 2019; Morioka et al., 2022; Nishi et al., 2022; Ogasawara et al., 2022) but some recent studies have demonstrated that scans can be reported at the 1-hour time point with equal confidence (Bokhari et al., 2018; Masri et al., 2020).

The results of our study show that the difference in sensitivity and specificity of reporting of any single imaging time point is not statistically significant when compared to using both imaging time points for reporting ($p > 0.05$). Furthermore, the difference in accuracy of reporting early images only versus delayed images only is not statistically significant ($p > 0.05$). The results

show that experienced readers have 100% sensitivity for both time points. The junior reader (Trainee) had 100% sensitivity for the early dataset compared to 90.9% for the delayed dataset. This drop in sensitivity was due to a single false negative case in the delayed dataset. The experience levels of readers in nuclear medicine and the strong inter-observer agreement demonstrate that both imaging time points are not necessary for all patients. Our study is in agreement with the work done by Bokhari et al. (2016) who investigated several factors affecting image quality, including matrix size, counts per image and imaging time points and chose the 1-hour time point due to its excellent image quality and lower extracardiac activity. It also supports the findings of Masri et al. (2020) which suggest that little additional information gained when images are acquired at both time points. The diagnostic accuracy for each time point is equally high.

Castano et al. (2016) and Schatka et al. (2021) both investigated the sensitivity of cardiac amyloid scans in nuclear medicine, using the gold standard endomyocardial biopsy (EMB) as reference and demonstrated higher sensitivity at the 1-hour time point. [^{99m}Tc]Tc-PYP clears from the blood stream quickly and it is taken up by the bone and myocardium (Bokhari & Cerqueira, 2020). The uptake of ^{99mTc}-PYP in the myocardium reaches its peak around the 1-hour mark which could be a contributing factor to the higher sensitivity mentioned above (Embry-Dierson et al., 2023).

The median age onset is commonly after the age of 40 but not usually diagnosed until geriatrics (Martinez-Naharro, Hawkins & Fontana, 2018).. At our center, it was observed that the patient population tested for cardiac amyloidosis is primarily elderly (Bashir et al., 2024), many of whom require assistance with daily activities and are typically accompanied by a family member. In this cohort of patients, tolerance for long and tedious diagnostic procedures is low. The time between a patient arriving at the Department and completing the early images is approximately two hours. If the delayed images are acquired, at a minimum, this time is doubled. Due to the decrease in mobility of patients in this age group, they are usually not willing to leave the department between the early and delayed images, and an area needs to be found to accommodate them in nuclear medicine departments. Space limitations are not uncommon in many departments. Therefore, single time point imaging is more tolerable by the patients and preferred in our imaging department.

It is important to note that in some cases, delayed imaging does play a significant role and can provide additional information and increase the confidence level of the reporting physician. In our review, two scans were classified as equivocal on the original reports. These two scans were re-examined by an independent senior Nuclear Medicine Physician (not part of the original reader team), who deemed one of the equivocal readings as negative and confirmed the other as equivocal. This example demonstrates the reporting challenges in a few cases even

when both early and delayed images were available. In our study, 9 patients (12 datasets) had a dataset reported as equivocal between the three readers. This result is very encouraging as it suggests that single imaging time point is adequate for most cardiac amyloid studies and only in a few difficult cases will need two imaging time points.

4.4.1 Limitations

Endomyocardial biopsy is the gold standard for the diagnosis of cardiac amyloidosis (Pellikka et al., 1988). Due to the invasive nature of this procedure, it is not commonly performed, and none of the patients included in this study had an endomyocardial biopsy. For this reason, information obtained from the patients' medical records was used to confirm their ATTR cardiac amyloid status which was then used to calculate the specificity for single imaging time point. This investigation was a single Centre and retrospective study.

4.5 Conclusion

The findings of our study demonstrate that single time point imaging, at either one-hour or three-hour post injection is sufficient for reporting most cardiac amyloid studies, which aligns with the recommendations of the most recent ASNC reporting and interpretation guideline (13) and the findings of recent literature (8,19). Early imaging time point is sufficient for most cases as supported by the high sensitivity, specificity, and diagnostic accuracy of our study, with the

option of delayed imaging in equivocal cases. SPECT/CT acquisition at the early imaging time point plays a critical role in the reporting of cardiac amyloid scans and can help avoid dual time point imaging.

Chapter 5 - Discussion and Conclusion

5.1 Discussion of key findings

The evolution of cardiac amyloid imaging over the last decade has led to increased popularity among cardiologists. ATTR cardiac amyloid imaging using ^{99m}Tc-PYP exhibits good diagnostic accuracy, and when combined with serum and urine studies, the sensitivity and specificity are remarkable (Gillmore et al., 2016). In cases where there are discrepancies between clinical impressions and imaging findings, endomyocardial biopsies still have a key role to play in the diagnosis or ruling out cardiac amyloidosis.

With no standardised ATTR cardiac amyloid imaging protocol, the practice points published by ASNC (ASNC, 2019, 2021 & 2024) are used as references. However, with several ASNC versions advocating for different imaging time points, several imaging departments have historically opted for dual-time-point imaging to avoid compromising the confidence level of reporting physicians. While imaging at the second point does not enhance diagnostic accuracy, sensitivity, or specificity, as demonstrated in the reader study, altering the imaging protocol can be challenging, as it may introduce uncertainties for reporting physicians. Imaging protocols are essential to achieving high-quality and standardised results across various fields, including medical diagnostics and scientific research. These protocols serve as guidelines that advise on

all technical aspects of image acquisition, from patient positioning to acquisition time and post-processing procedures. Standardised protocols are crucial for diagnostic accuracy and treatment planning.

Most of the data and reporting guidelines for ATTR cardiac amyloid imaging are based on planar images. The semi-quantitative analysis method uses the counts from regions of interest over the heart and the contralateral lung off a planar image of the chest and calculates its ratio. A ratio of 1.5 or above points towards a positive study, but blood pooling cannot be ruled out unless delayed planar images or SPECT/CT are acquired. The Perugini grading method is also based on planar images but can be applied to SPECT/CT (ASNC, 2024). The earlier chapter proves the invaluable information provided by SPECT/CT, which can differentiate between myocardial uptake and blood pooling. Employing SPECT/CT at an earlier time point decisively eliminates the need for delayed imaging. Often, equivocal scans on planar imaging can be definitive on SPECT/CT, providing confidence in the accuracy of the technique.

Due to the inherent risks and high costs of endomyocardial biopsy, most studies within the systematic review limited the number of participants who underwent this invasive procedure. The findings from the systematic literature review confirmed that the nuclear medicine cardiac amyloid imaging protocols were robust and reliable. Some centres performed early images only, between five minutes and one-hour post-injection; some performed delayed images only,

between two hours and four hours post-injection, and others a combination of early and delayed images, demonstrating the non-standardisation within the nuclear medicine community. The sensitivity and specificity of these different imaging techniques were very similar, further reinforcing our findings. The difference in sensitivity and specificity between early and delayed imaging time points was not statistically significant, providing a solid foundation for our research.

The reader study investigated the application of single-time-point imaging for nuclear medicine cardiac amyloid imaging. The difference in sensitivity and specificity between the early and delayed imaging time points, as with the systematic literature review, was of no statistical significance. More importantly, the results of diagnostic accuracy of a single imaging time point were similar to dual imaging time points. These encouraging results have the potential to significantly impact the field of nuclear medicine, inciting a review of the cardiac amyloid imaging protocol. The current practice points from the ASNC recommend imaging should occur 2 to 3 hours after injection, with optional early imaging at 1-hour post-injection. However, this thesis demonstrates that single point SPECT/CT imaging provides sufficient information for nuclear medicine physicians to generate accurate reports. While delayed imaging, as suggested by ASNC, improves the target-to-background ratio by minimising residual activity in the circulatory system, the acquisition of SPECT/CT at the early imaging time point can also

effectively address this issue. SPECT/CT offers three-dimensional data that distinguishes between blood activity and myocardial activity. This advancement significantly enhances the patient's experience by reducing the time patients spend in imaging departments.

5.2 Cases Presented

On July 17, 2024, based on the findings of the systematic review and reader study, consensus was reached among the senior medical staff of the Molecular Imaging and Therapy Department at Austin Hospital regarding a change in protocol for ATTR Cardiac Amyloid studies. Images are acquired one-hour post-injection for most patients, and for challenging cases, delayed images may be required.

The first cardiac amyloid study performed using the new protocol was on an 80-year-old male. He arrived at his appointment via taxi with his elderly wife, arriving 15 minutes early. The study commenced promptly, and he was injected with the radioactive tracer 30 minutes after arriving at our department. The patient was aware of the possibility of delayed images 3 hours post-injection. When he was told that he could leave our department and have lunch before returning for the delayed images in the slim chance that it was required, the patient informed us that he was not willing to go to the cafeteria because it was too far for him and his elderly wife. They were worried that they would get lost and tired.

This is a recurring issue with older patients unwilling to leave the department. Under the previous imaging protocol, it meant that they spent close to 4 hours within our department. Some patients are supported by adult children who attend appointments with them, helping them with anything that they require, from escorting them to a cup of coffee. The gentleman mentioned he could not stay in the department for five hours, as his children were interstate, and his wife, who was also not very well, insisted on accompanying him for the imaging test. The new protocol meant he did not have to come back for the delayed images. A planar image and a SPECT/CT of the chest were enough for the nuclear medicine physician to report the scan confidently. The patient spent a remarkably short two hours in our department, rather than the expected five hours.

Providing evidence of the adequacy of single-time point early imaging has been a lengthy and challenging exercise aimed at minimizing the time patients spend in imaging departments. However, it is rewarding to see that patients in similar situations to the one mentioned above leave the imaging departments in half the time compared to the previous protocol, with the same diagnostic accuracy.

5.3 Implementation of New Protocol

From July 17, 2024, to March 10, 2025, the Molecular Imaging and Therapy Department at Austin Hospital successfully conducted 42 cardiac amyloid studies using an enhanced protocol designed to optimise patient care based on the findings from earlier studies described in Chapters three and four. Out of these, 34 studies required only a single imaging session, one hour after injection. Notably, eight of these studies returned positive results, while 26 were negative. For the eight remaining patients, reporting physicians deemed it necessary to conduct imaging at both early and delayed time points; all of these studies were ultimately reported as negative.

The efficiency of our new protocol is evident in the average time from injection to study completion. Single-time-point imaging averaged just 87 minutes, while dual-time-point imaging took significantly longer at 203 minutes. The feedback on this protocol shift has been overwhelmingly positive from nuclear medicine technologists, reporting physicians, and patients alike. Physicians can now complete their reports shortly after acquiring images, typically within one-hour post-injection, which greatly enhances workflow efficiency. Furthermore, patients and their families benefit from reduced wait times in the imaging department, which allows us to allocate more staff and resources to meet the needs of our

patients effectively. This innovative approach not only enhances patients' experiences but also optimises our operational capabilities.

5.4 Future work

The next phase of our research on cardiac amyloidosis focuses on the ability to precisely quantify cardiac amyloid in patients who test positive using advanced SPECT/CT imaging. Accurate attenuation correction using CT and scatter correction significantly enhances the quantification and localisation capabilities of current SPECT/CT systems. Leveraging state-of-the-art digital cameras and quantification software with unparalleled accuracy for measuring the amount of radioactive tracer absorbed by specific organs and volumes of interest.

For example, to quantify technetium-99m radiopharmaceutical uptake the system uses a premeasured sensitivity of a specific isotope, e.g., 93.5 counts per second per megabecquerel (cps/MBq). The pre and post injection doses, together with their calibration times are entered to accurately determine the uptake of the tracer. Figure 5.1 demonstrates a snapshot of the quantitation showing the amount of activity in the heart and a percentage of the injected dose. The system also has the ability to create three dimensional regions of interest by tracking any voxel of similar or higher intensity as a selected voxel which allows for easy denotation of an area or organ, and reproducibility.

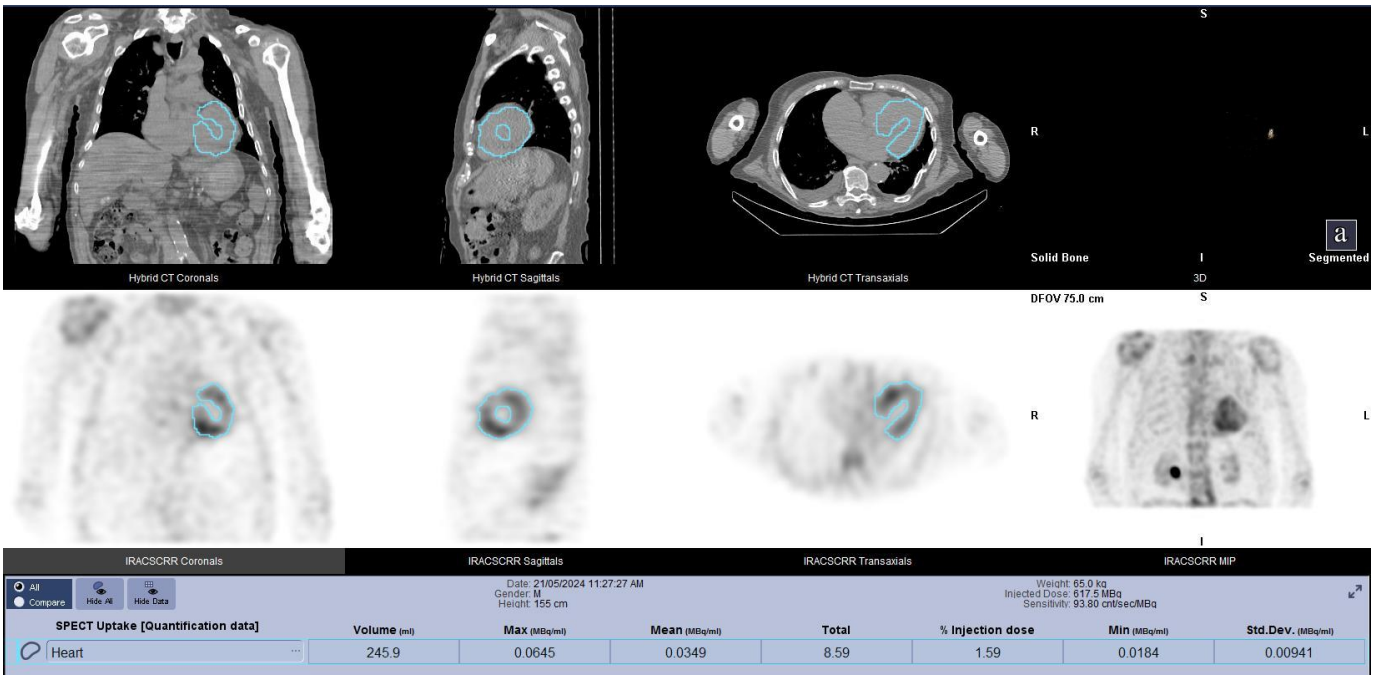


Figure 5.1 Quantitation of activity in the myocardium in MBq and percentage of injected dose.

Phantom studies will validate the precision and reliability of both the camera and the software. The goal is to quantify amyloid levels at baseline and post-treatment to assess the effectiveness of therapies. This work has the potential to significantly impact patient care and treatment strategies in cardiac amyloidosis.

Positron Emission Tomography (PET) is a nuclear medicine imaging technique that has demonstrated promising results in detecting cardiac amyloid plaques using neurological amyloid PET tracers. It offers higher spatial resolution compared to SPECT. By utilizing the

standardized uptake value (SUV), the activity in specific regions can be calculated and these values compared to previous studies, which allows for the monitoring of treatment responses.

For over a decade, researchers have been examining dementia and Alzheimer's disease using amyloid-sensitive tracers such as ^{18}F-Florbetaben, ^{18}F-Florbetapir, ^{18}F-NAV4694, and ^{11}C-Pittsburgh Compound B (Rowe & Villemagne, 2011). Now that ^{18}F-NAV4694 is clinically available for patients with suspected dementia, its application in detecting cardiac amyloidosis could have a significant impact. Amyloid PET tracers are sensitive to both AL (light-chain) and ATTR (transthyretin) cardiac amyloidosis. As a result, a combination of PET and SPECT studies can help differentiate between these two types (Soman, 2025).

5.5 Conclusion

This project aimed to investigate the sensitivity and specificity of various cardiac amyloid scintigraphic imaging protocols for ATTR reported in the literature and determine effective techniques. The analysis revealed that the difference in sensitivity and specificity between early and delayed time points was not statistically significant. The most critical factor in the imaging protocol was the inclusion of SPECT/CT, which was supported by a reader study concluding that a single time point using SPECT/CT was sufficient to achieve high diagnostic accuracy. Additionally, changing the imaging protocol from dual time points to a single time point at one-

hour post-injection has significantly improved the patient experience and enhanced workflow, thereby supporting the hypothesis of this thesis.

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Appendices

Appendix A – ASNC, Practice Points, 99mTechnetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis (2016)

ASNC

ASNC
PRACTICE POINTS

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

OVERVIEW

The purpose of this document is to identify the critical components involved in performing ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) imaging for the evaluation of cardiac transthyretin amyloidosis (ATTR).

BACKGROUND

- The majority of individuals with cardiac amyloidosis have myocardial amyloid deposits formed from misfolded light chain (AL) or transthyretin (TTR) proteins. Diagnosis of amyloidosis and differentiation between the types is important for prognosis, therapy, and genetic counseling.
- Cardiac TTR amyloidosis, the focus of this practice points document, is an under-diagnosed cause of heart failure.
- Amyloid derived from wild-type TTR results in a restrictive cardiomyopathy, most commonly presenting in men in their early 70's onwards, but occasionally seen as young as age 60. Although almost 1 in 4 males > 80 years have some TTR-derived amyloid deposits at autopsy, the clinical significance of a mild degree of deposition is unknown--generally clinical manifestations of heart failure occur once enough amyloid has been deposited to cause LV wall thickening (1).
- Approximately 3 – 4% among US African Americans have a common inherited mutation of the TTR gene (Val122Ile), which produces a restrictive cardiomyopathy in a minority, but may contribute to heart failure in a higher proportion (1).
- Cardiac amyloidosis should be suspected in individuals with heart failure and thickened ventricles with grade 2 or greater diastolic dysfunction on echocardiography or typical findings on cardiac magnetic resonance imaging (CMR; diffuse late gadolinium enhancement, ECV expansion or characteristic T-1 relaxation times); diagnosis is confirmed by endomyocardial biopsy and typing of amyloid fibrils as needed.
- Several studies confirm the high sensitivity and specificity of ^{99m}Tc-bone compound scintigraphy (^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) or PYP(2, 3)) for cardiac ATTR amyloidosis; recent studies highlight the value of DPD and/or PYP in differentiating cardiac ATTR from AL amyloidosis (4).
- A distinct advantage of ^{99m}Tc-PYP imaging, even when echocardiography and CMR are diagnostic for cardiac amyloidoisis, is its ability to specifically identify ATTR cardiac amyloidosis non-invasively and thereby guide patient management (5).

PATIENT SELECTION

- Individuals with heart failure and unexplained increase in left ventricular wall thickness.
- African-Americans over the age of 60 years with heart failure, unexplained or with increased left ventricular wall thickness (>12 mm).
- Individuals over the age of 60 years with unexplained heart failure with preserved ejection fraction.
- Individuals, especially elderly males, with unexplained neuropathy, bilateral carpal tunnel syndrome or atrial arrhythmias in the absence of usual risk factors, and signs/symptoms of heart failure.
- Evaluation of cardiac involvement in individuals with known or suspected familial amyloidosis.
- Diagnosis of cardiac ATTR in individuals with CMR or echocardiography consistent with cardiac amyloidosis.
- Patients with suspected cardiac ATTR amyloidosis and contraindications to CMR such as renal insufficiency or an implantable cardiac device (5).

ASNC

ASNC PRACTICE POINTS

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

OBTAINING THE RADIOTRACER

- ^{99m}Tc-PYP is readily available as unit doses from commercial radiopharmaceutical distributors or as kits for preparation (TechneScan PYPTM, Mallinkcrodt, St. Louis, MO).
- Kits containing 5 or 30 single-use vials are commercially available. Each 10 ml vial contains 11.9 mg of sodium pyrophosphate and 3.2 mg of stannous chloride and 4.4 mg of total tin, and this kit is approved for bone, cardiac (for the detection of myocardial infarction), and blood pool (radionuclide ventriculography and GI bleeding) imaging (see package insert for details of reconstitution of ^{99m}Tc-PYP).
- The total body effective dose from 15 mCi of ^{99m}Tc-PYP is estimated at 3.2 mSv.
- ^{99m}Tc-DPD is not available for clinical use in the United States. Although there are no large studies directly comparing the agents, the principles in this document apply similarly to ^{99m}Tc-DPD and ^{99m}Tc-PYP imaging.

TEST PREPARATION

- No specific test preparation is required.

IMAGING PROCEDURE

- Commonly used imaging procedures for ^{99m}Tc-PYP imaging are shown in **Tab**le 1. Individual centers can modify imaging procedures based on local camera capabilities and expertise.
- Cardiac or chest SPECT and planar images are obtained one hour after injection of ^{99m}Tc-PYP using the parameters listed in Table 1. If persistent blood pool activity is noted on one hour images (e.g., renal failure), delayed images may be obtained at 3 hours.
- Planar imaging is rapid, simple to perform, and useful for visual interpretation and quantification of the degree of myocardial uptake (see image interpretation) by heart to lung ratio or comparison to rib uptake.
- SPECT imaging may be helpful to
  1. avoid overlap of bone uptake
  2. distinguish blood pool activity from myocardial activity(3)
  3. assess the distribution of myocardial ^{99m}Tc-PYP uptake in individuals with positive planar scans
  4. identify ^{99m}Tc-PYP uptake in the interventricular septum (commonly involved in amyloidosis) and
  5. quantify the degree of myocardial uptake by comparison to rib uptake.
- Whole body planar imaging may be helpful to identify uptake of ^{99m}Tc-PYP in the shoulder and hip girdles (a specific sign of systemic ATTR amyloidosis) (6) and should be considered adjunctive and optional in addition to standard cardiac-centered imaging, based on local expertize.
- The value of ^{99m}Tc-PYP imaging with the newer "cardiac only" SPECT cameras needs further validation (due to inability to accurately display bone and lung ^{99m} Tc-PYP uptake with these systems; see image interpretation section).

ASNC

ASNC
PRACTICE POINTS

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

Table 1. Imaging Parameters for Cardiac ^{99m}Tc-PYP Imaging

Imaging procedures	Parameters
Preparation	No specific preparation. No fasting required.
Scan	Rest scan
Dose of ^{99m}Tc-PYP	10-20 mCi intravenously
Time between injection and acquisition	Recommended: 1-hour SPECT and planar; Optional: 3-hour SPECT or planar
Imaging parameters	
Field of view	Recommended: Cardiac or chest; Optional: Wholebody planar
Image type	Recommended: Cardiac or chest SPECT and planar imaging
Position	Supine
Energy window	140 keV, 15-20%
Collimators	Low energy, high resolution
Matrix	64 X 64 minimum
Pixel size	3.5 – 6.5 mm
Planar imaging specific parameters	
Number of views*	Anterior, Lateral, and Left Anterior Oblique
Detector configuration	90 degrees
Image duration (count based)	750,000 counts
Magnification	1.46
SPECT imaging specific parameters	
Angular range	360 degrees
Detector configuration	180 degrees
ECG gating	Off; Nongated imaging
Number of views/ detector	40
Time per stop	20 seconds
Magnification	1.0

*Anterior and lateral views can be obtained at the same time using a 90 degree detector configuration; lateral planar views or SPECT imaging may help separate sternal from myocardial uptake.

IMAGE INTERPRETATION

- The anterior and lateral planar images as well as the rotating projection images and reconstructed SPECT images are reviewed in standard cardiac imaging planes using commercial software.
- Myocardial ^{99m}Tc-PYP uptake patterns are categorized as absent, focal, diffuse or focal on diffuse.
- Scans with focal ^{99m}Tc-PYP uptake could represent rib fracture or previous myocardial infarction. Following a myocardial infarction, myocardial ^{99m}Tc-PYP uptake may be positive for upto 7 days and rarely may remain persistently positive.

Quantifying myocardial ^{99m}Tc-PYP Uptake

There are two approaches to quantification:

1. **Quantitative: myocardial to contralateral lung ratio of uptake at 1 hour**

- Circular target regions of interest (ROI) are drawn over the heart on the planar images and are mirrored over the contralateral chest to account for background and ribs **(see Figure 1).**
- Total and absolute mean counts are measured in each ROI. A heart to contralateral (H/CL) ratio is calculated as the fraction of heart ROI mean counts to contralateral chest ROI mean counts.
- H/CL ratios of ≥ 1.5 at one hour are classified as ATTR positive and ratios < 1.5 as ATTR negative (4).

2. **Semi-quantitative: visual comparison to bone (rib) uptake at 3 hours**

Cardiac uptake of ^{99m}Tc-PYP is evaluated using a semi-quantitative visual scoring method in relation to bone uptake (**Table 2 and Figure 2**). Based on previously published results, visual scores of greater than or equal to 2 on planar (2, 3) or SPECT images at 3 hours (6) are classified as ATTR positive, and scores of less than 2 as ATTR negative.

While grade 2-3 or H/CL >1.5 uptake is strongly suggestive of TTR amyloidosis, any degree of ^{99m}Tc-PYP uptake can also be seen in AL amyloidosis, and as such a complete evaluation is warranted to exclude this diagnosis.

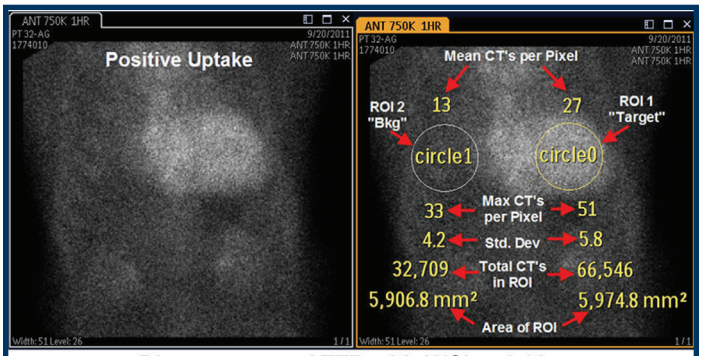
In clinical practice both semi-quantitative visual scoring and H/CL are used.

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

Table 2. Semi-quantitative Visual Grading of Myocardial ^{99m}Tc-PYP Uptake by Comparison to Bone(rib) Uptake

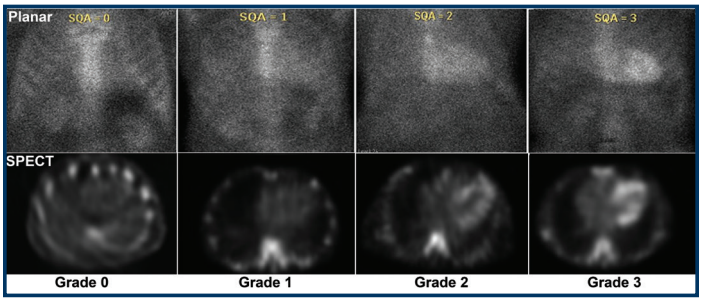
Grade	Myocardial ^{99m}Tc-PYP Uptake
Grade 0	no uptake and normal bone uptake
Grade 1	uptake less than rib uptake
Grade 2	uptake equal to rib uptake
Grade 3	uptake greater than rib uptake with mild/ absent rib uptake

Figure 1. Quantitation of Cardiac ^{99m}Tc-PYP Uptake Using Heart to Contralateral Lung (H/CL) Ratio



Biopsy proven ATTR with H/CL = 2.08

Figure 2. Grading ^{99m}Tc-PYP Uptake on Planar and SPECT Images



REPORTING

The report should include all elements of an ideal report as per standard ASNC guidelines.

Table 3. Myocardial ^{99m}Tc-PYP Imaging Guideline for Reporting

Parameters	Elements
Demographics	Patient name, age, sex, reason for the test, date of study, prior imaging procedures, biopsy results if available (required)
Methods	Imaging technique, radiotracer dose and mode of administration, interval between injection and scan, scan technique (planar and SPECT) (required)
Findings	Image quality Visual scan interpretation (required) Semi-quantitative interpretation in relation to rib uptake (required) Quantitative findings heart to contralateral lung ratio (optional; recommended for positive scans)
Ancillary findings	Whole body imaging if planar whole body images are acquired (optional) Interpret CT for attenuation correction if SPECT/CT scanners are used (recommended)
Conclusions	1. An overall interpretation of the findings into categories of 1) not suggestive of TTR amyloidosis; 2) strongly suggestive of TTR amyloidosis or 3) equivocal for TTR amyloidosis a. Not suggestive: A semi-quantitative visual score of 0 or H/CL ratio < 1. b. Strongly suggestive: A semi-quantitative visual score of 2 or 3 or H/CL ratio >1.5 c. Equivocal: A semi-quantitative visual score of 1 or H/CL ratio 1-1.5 2. Interpret the results in the context of prior evaluation a. If echo/CMR are strongly positive, and ^{99m}Tc-PYP negative, consider further evaluation including endomyocardial biopsy Of note: A negative or mildly positive PYP does not exclude AL amyloid. In addition, equivocal results could represent AL amyloid or early TTR amyloid

ASNC

ASNC PRACTICE POINTS

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

BILLING

ASNC would recommend:

- For planar with SPECT report CPT 78803 Radiopharmaceutical localization of tumor or distribution of radiopharmaceutical agent(s); tomographic (SPECT).
- When reporting CPT 78803, planar imaging of a limited area or multiple areas should be included with the SPECT.
- For the HCPCS level II code report A9538 ^{99m}Tc-pyrophosphate, diagnostic, per study dose, up to 25 millicuries.
- For a single planar imaging session alone (without a SPECT study), report CPT 78800 Radiopharmaceutical localization of tumor or distribution of radiopharmaceutical agent(s); limited area.

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Appendix B – ASNC, Cardiac Amyloidosis Practice Points, 99mTechnetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis (2019)

ASNC

American Society of Nuclear Cardiology

ASNC CARDIAC AMYLOIDOSIS **PRACTICE POINTS**

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

^{99m}Technetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis

OVERVIEW

The purpose of this document is to identify the critical components involved in performing ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) imaging for the evaluation of cardiac transthyretin amyloidosis (ATTR).

BACKGROUND

- The majority of individuals with cardiac amyloidosis have myocardial amyloid deposits formed from misfolded light chain (AL) or transthyretin (TTR) proteins. Diagnosis of amyloidosis and differentiation between the types is important for prognosis, therapy, and genetic counseling.
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- Cardiac amyloidosis should be suspected in individuals with heart failure and thickened ventricles with grade 2 or greater diastolic dysfunction on echocardiography or typical findings on cardiac magnetic resonance imaging (CMR; diffuse late gadolinium enhancement, ECV expansion or characteristic T-1 relaxation times); diagnosis is confirmed by endomyocardial biopsy and typing of amyloid fibrils as needed.
- Several studies confirm the high sensitivity and specificity of ^{99m}Tc-bone compound scintigraphy (^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) or PYP (2, 3) for cardiac ATTR

amyloidosis; recent studies highlight the value of DPD and/or PYP in differentiating cardiac ATTR from AL amyloidosis (4).

- A distinct advantage of ^{99m}Tc-PYP imaging, even when echocardiography and CMR are diagnostic for cardiac amyloidoisis, is its ability to specifically identify ATTR cardiac amyloidosis non-invasively and thereby guide patient management (5).

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- African-Americans over the age of 60 years with heart failure, unexplained or with increased left ventricular wall thickness (>12 mm).
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- Evaluation of cardiac involvement in individuals with known or suspected familial amyloidosis.
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- Patients with suspected cardiac ATTR amyloidosis and contraindications to CMR such as renal insufficiency or an implantable cardiac device (5).

OBTAINING THE RADIOTRACER

- ^{99m}Tc-PYP is readily available as unit doses from commercial radiopharmaceutical distributors or as kits for preparation.
- Kits containing 5 or 30 single-use vials are commercially available. Each 10 ml vial contains 11.9 mg of sodium pyrophosphate and 3.2 mg of stannous chloride and 4.4 mg of total tin, and this kit is approved for bone, cardiac (for the detection of myocardial infarction), and blood pool (radionuclide ventriculography and GI bleeding) imaging (see package insert for details of reconstitution of ^{99m}Tc-PYP).
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- ^{99m}Tc-DPD is not available for clinical use in the United States. Although there are no large studies directly comparing the agents, the principles in this document apply similarly to ^{99m}Tc-DPD and ^{99m}Tc-PYP imaging.

TEST PREPARATION

- No specific test preparation is required.

IMAGING PROCEDURE

- Commonly used imaging procedures for ^{99m}Tc-PYP imaging are shown in **Table 1**. Individual centers can modify imaging procedures based on local camera capabilities and expertise.
- Cardiac or chest SPECT and planar images are obtained one hour after injection of ^{99m}Tc-PYP using the parameters listed in **Table 1**. If persistent blood pool activity is noted on one hour images (e.g., renal failure), delayed images may be obtained at 3 hours.
- Planar imaging is rapid, simple to perform, and useful for visual interpretation and quantification of the degree of myocardial uptake (see image interpretation) by heart-to-lung ratio or comparison to rib uptake.
- SPECT imaging may be helpful to
    1. avoid overlap of bone uptake
    2. distinguish blood pool activity from myocardial activity (3)
    3. assess the distribution of myocardial ^{99m}Tc-PYP uptake in individuals with positive planar scans
    4. identify ^{99m}Tc-PYP uptake in the interventricular septum (commonly involved in amyloidosis) and
    5. quantify the degree of myocardial uptake by comparison to rib uptake.
- Whole body planar imaging may be helpful to identify uptake of ^{99m}Tc-PYP in the shoulder and hip girdles (a specific sign of systemic ATTR amyloidosis) (6) and should be considered adjunctive and optional in addition to standard cardiac-centered imaging, based on local expertize.
- The value of ^{99m}Tc-PYP imaging with the newer "cardiac only" SPECT cameras needs further validation (due to inability to accurately display bone and lung ^{99m} Tc-PYP uptake with these systems; see image interpretation section).

Table 1. Imaging Parameters for Cardiac ^{99m}Tc-PYP Imaging

Imaging procedures	Parameters
Patient Preparation	No specific preparation. No fasting required.
Scan	Rest scan
Dose of ^{99m}Tc-PYP	10-20 mCi intravenously
Time between injection and acquisition	Recommended: 1-hour SPECT and planar; Optional: 3-hour SPECT or planar
Imaging parameters	
Field of view	Recommended: Cardiac or chest; Optional: Whole body planar
Image type	Recommended: Cardiac or chest SPECT and planar imaging
Position	Supine
Energy window	140 keV, 15-20%
Collimators	Low energy, high resolution
Matrix	Planar: 256 by 256, at least 64 by 64 is required. SPECT: 128 by 128, at least 64 by 64 is required.
Pixel size	3.5 – 6.5 mm
Planar imaging specific parameters	
Number of views*	Anterior, Lateral, and Left Anterior Oblique
Detector configuration	90 degrees
Image duration (count based)	750,000 counts
Magnification	1.46
SPECT imaging specific parameters	
Angular range	Recommended: 180 degrees; Optional: 360 degrees
Detector configuration	Recommended 90 degrees; Optional 180 degrees
ECG gating	Off; Nongated imaging
Number of views/detector	40
Time per stop	20 seconds
Magnification	1.0

*Anterior and lateral views can be obtained at the same time using a 90-degree detector configuration; lateral planar views or SPECT imaging may help separate sternal from myocardial uptake.

IMAGE INTERPRETATION

- The anterior and lateral planar images as well as the rotating projection images and reconstructed SPECT images are reviewed in standard cardiac imaging planes using commercial software.
- Myocardial ^{99m}Tc-PYP uptake patterns are categorized as absent, focal, diffuse or focal and diffuse.
- When myocardial uptake is visually present on SPECT images H/CL ratios of ≥1.5 at one hour are classified as ATTR positive and ratios of <1.5 ATTR negative (4).

Quantifying Myocardial ^{99m}Tc-PYP Uptake

There are two approaches to quantification:

1. **Quantitative Myocardial-to-Contralateral lung uptake ratio at 1 hour**
    - Circular target regions of interest (ROI) are drawn over the heart on the planar images and are mirrored over the contralateral chest to account for background and ribs **(see Figure 1).**
    - Total and absolute mean counts are measured in each ROI. A heart-to-contralateral lung (H/CL) ratio is calculated as the fraction of heart ROI mean counts to contralateral chest ROI mean counts.
    - When myocardial uptake is visually present on SPECT images H/CL ratios of ≥1.5 at one hour are classified as ATTR positive and ratios of <1.5 ATTR negative (4).
2. **Semi-quantitative: visual comparison to bone (rib) uptake at 3 hours**

Cardiac uptake of ^{99m}Tc-PYP is evaluated using a semi-quantitative visual scoring method in relation to bone (rib) uptake (**Table 2 and Figure 2**). Based on previously published results, visual scores of greater than or equal to 2 on planar (2, 3) or SPECT images at 3 hours (6) are classified as ATTR positive, and scores of less than 2 as ATTR negative.

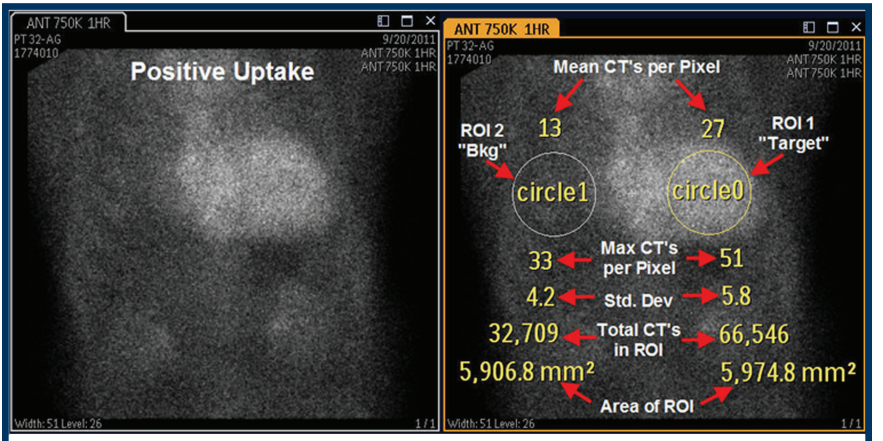
While grade 2 or 3 or H/CL >1.5 uptake is strongly suggestive of ATTR amyloidosis, any degree of ^{99m}Tc-PYP uptake can also be seen in AL amyloidosis, and as such a complete evaluation is warranted to exclude this diagnosis.

In clinical practice both semi-quantitative visual scoring and H/CL are used.

Table 2. Semi-quantitative Visual Grading of Myocardial ^{99m}Tc-PYP Uptake by Comparison to Bone (rib) Uptake

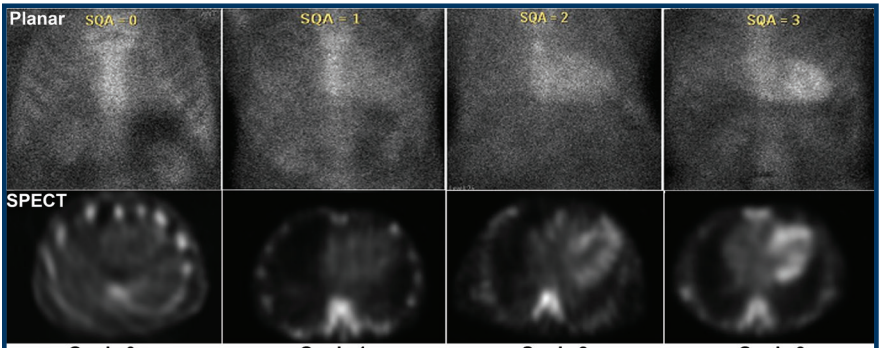
Grade	Myocardial ^{99m}Tc-PYP Uptake
Grade 0	no uptake and normal rib uptake
Grade 1	uptake less than rib uptake
Grade 2	uptake equal to rib uptake
Grade 3	uptake greater than rib uptake with mild/absent rib uptake

Figure 1. Quantitation of Cardiac ^{99m}Tc-PYP Uptake Using Heart-to-Contralateral Lung (H/CL) Ratio



Biopsy proven ATTR cardiac amyloidosis with H/CL = 2.08

Figure 2. Grading ^{99m}Tc-PYP Uptake on Planar and SPECT Images



REPORTING

The report should include all elements of an ideal report as per standard ASNC guidelines.

Table 3. Myocardial ^{99m}Tc-PYP Imaging Guideline for Reporting

Parameters	Elements
Demographics	Patient name, age, sex, reason for the test, date of study, prior imaging procedures, biopsy results if available (required)
Methods	Imaging technique, radiotracer dose and mode of administration, interval between injection and scan, scan technique (planar and SPECT) (required)
Findings	Image quality Visual scan interpretation (required) Semi-quantitative interpretation in relation to rib uptake (required) Quantitative findings heart-to-contralateral lung ratio (optional; recommended for positive scans)
Ancillary findings	Review whole body planar images if acquired Interpret CT for attenuation correction if SPECT/CT scanners are used
Conclusions	1. An overall interpretation of the findings into categories of 1) not suggestive of ATTR amyloidosis; 2) strongly suggestive of ATTR amyloidosis or 3) equivocal for ATTR amyloidosis a. Not suggestive: A semi-quantitative visual score of 0 or H/CL ratio < 1. b. Strongly suggestive: A semi-quantitative visual score of 2 or 3 or H/CL ratio >1.5 c. Equivocal: A semi-quantitative visual score of 1 or H/CL ratio 1-1.5 2. Interpret the results in the context of prior evaluation a. If echo/CMR are strongly positive, and ^{99m}Tc-PYP negative, consider further evaluation including endomyocardial biopsy b. The writing group would like to emphasize the importance of excluding a monoclonal process with serum and urine immunofixation and a serum free light chains assay in all patients with suspected amyloidosis referred for ^{99m}Tc-PYP scan irrespective of the scan results. c. Of note: equivocal results could represent AL amyloid or early ATTR cardiac amyloid

BILLING

ASNC would recommend:

- For planar with SPECT report CPT 78803 radiopharmaceutical localization of tumor or distribution of radiopharmaceutical agent(s); tomographic (SPECT).
- When reporting CPT 78803, planar imaging of a limited area or multiple areas should be included with the SPECT.
- For the HCPCS level II code report A9538 ^{99m}Tc- pyrophosphate, diagnostic, per study dose, up to 25 millicuries.
- For a single planar imaging session alone (without a SPECT study), report CPT 78800 radiopharmaceutical localization of tumor or distribution of radiopharmaceutical agent(s); limited area.

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Version History:
First Issued: February 2016
Present Version (Version 2.0): Updated February 2019

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Appendix C – Single- Versus Dual-Time-Point imaging for Transthyretin Cardiac Amyloid Using [99mTc] Tc-Pyrophosphate. Journal of Nuclear Medicine Technology, 53:1-6. DOI: 10.2967/jnmt.124.269395

Single- Versus Dual-Time-Point Imaging for Transthyretin Cardiac Amyloid Using ^{99m}Tc-Pyrophosphate

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Nuclear medicine scintigraphy using ^{99m}Tc-pyrophosphate has proven valuable in the diagnosis of cardiac transthyretin amyloidosis in recent years. However, there is still confusion over the optimal imaging time points. The American Society of Nuclear Cardiology has recommended different imaging time points over the last decade. We aimed to determine whether single- or dual-time-point imaging is required for reporting purposes and which time point would be the most appropriate if a single time point was to be considered. **Methods:** Cardiac amyloid scans using ^{99m}Tc-pyrophosphate acquired from 2017 to 2023 were retrieved from our Picture Archiving and Communications System. Scans with static views and SPECT/CT images of the chest for both imaging time points, at 1 h (early) and 3 h (delayed) after injection, were included. Each study was independently read by 3 nuclear medicine physicians. Original clinical reports using both imaging time points were used as a reference to calculate the accuracy of a single time point. **Results:** In total, 70 patients were included in this study. Reports of cardiac amyloid studies using any single-time-point imaging were highly sensitive, accurate, and specific. There was agreement among all readers. Of the 140 datasets reported by each reader, 4 scans were classified as equivocal, requiring more imaging for confident reporting. **Conclusion:** Single-time-point imaging showed an accuracy comparable to the dual-time-point imaging in diagnosing cardiac transthyretin amyloidosis. This was further validated by agreement among the 3 readers. Early time-point imaging is preferred, and additional delayed imaging can be acquired when the early result is equivocal.

Key Words: cardiology; SPECT/CT; cardiac amyloid; transthyretin; ^{99m}Tc

J Nucl Med Technol 2025; 53:140–145
DOI: 10.2967/jnmt.124.269395

Amyloidosis is a systemic condition which can affect several organs, including the kidneys, brain, liver, and heart, and can be fatal if untreated (*1*). There are 2 main types of cardiac amyloidosis, transthyretin amyloidosis (ATTR) and light chain amyloidosis. The diagnosis of cardiac amyloidosis can be challenging because of the wide range of symptoms that are similar to those of other conditions and the requirement of several tests to confirm the presence of amyloidosis (*2*). This study focuses on cardiac ATTR. The gold standard for the diagnosis of cardiac amyloidosis is endomyocardial biopsy (*3*). The invasive nature of this procedure, which requires highly skilled medical professionals, can lead to severe complications such as bleeding, arrhythmias, infection, perforation of the heart, and damage to heart valves or blood vessels (*4*). An array of diagnostic tools is being used where possible to accurately diagnose cardiac amyloidosis without endomyocardial biopsies. These tools include but are not limited to cardiac MRI, echocardiography, electrocardiography, serum biomarkers, and nuclear medicine scintigraphy (*5–7*). ^{99m}Tc-pyrophosphate (^{99m}Tc-PYP) scintigraphy has proven valuable in the diagnosis of cardiac ATTR in recent years with high sensitivity and specificity ranging between 97%–100% and 93.3%–100%, respectively (*8–10*).

The timing of the nuclear medicine scans after the administration of ^{99m}Tc-PYP is not a standardized practice across nuclear medicine departments. The uptake mechanism of pyrophosphate in ATTR is not very well understood, but it is believed that it is related to the presence of elevated calcium levels in amyloid-deposited tissues (*11*). The American Society of Nuclear Cardiology (ASNC) originally released a ^{99m}Tc-PYP imaging practice points in 2016, with an updated version in February 2019 (Fig. 1). The 2019 version recommends images to be acquired at 1 h after injection, and if more information is needed, additional images to be acquired 3 h after injection (*12*). In September 2019, an ASNC and European Association of Nuclear Medicine Cardiac Amyloidosis DPD (diphosphono-1,2-propanodicarboxylic acid) Practice Points adapted from the February 2019 version was released. This adaptation focused on the different radiopharmaceuticals that are more widely used in Europe, ^{99m}Tc-DPD and ^{99m}Tc-hydroxymethylene diphosphonate. The recommended imaging time points were 2 or 3 h after injection, with both planar and SPECT imaging and the 1-h time point being optional (*13*). Most recently, in 2023, ASNC released an interpretation and reporting document that found similar

Received Jan. 5, 2025; revision accepted Mar. 20, 2025.
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Published online Apr. 22, 2025.

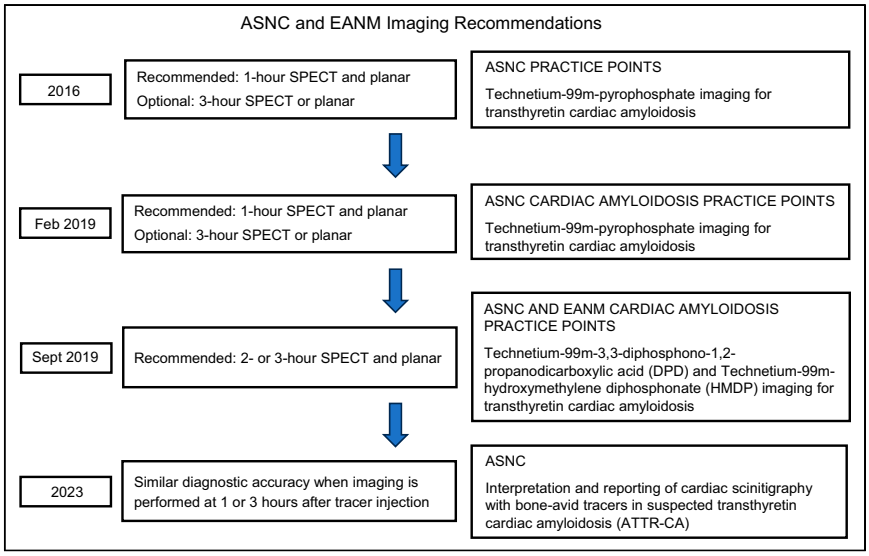


FIGURE 1. Timeline of recommendation from ASNC and EANM.

diagnostic accuracy for both imaging time points of 1 and 3 h. SPECT/CT was found to be essential for the accurate reporting of cardiac amyloidosis regardless of the time point, whereas semiquantitative analysis is optional (*14*).

We aimed to determine if single- or dual-time-point imaging is required for reporting purposes and which time point would be the most appropriate if a single time point was to be considered.

MATERIALS AND METHODS

The current protocol in our department is to image the patient at 2 time points, at 1 and 3 h after injection, acquiring planar images and at least 1 SPECT/CT image (Table 1). A definitive diagnosis of cardiac amyloidosis is often established by endomyocardial biopsy; however, this is not always performed. For our study, a diagnosis of cardiac ATTR was established by reviewing the clinical history, diagnostic tests, results from patients' electronic medical records, and original clinical reports of the PYP scans, which used both imaging time points. Most patients had diagnostic tests including echocardiogram, blood tests, cardiac MRI, and a PYP scan to investigate the presence of cardiac ATTR. Ethics approval for this retrospective study was granted by Austin Health Human Research Ethics Committee, and the requirement for obtaining informed consent was waived.

There were 207 consecutive patients who had ^{99m}Tc-PYP cardiac amyloid studies performed on a GE Discovery 670DR (GE HealthCare) and reviewed. The ^{99m}Tc-PYP scans were retrieved from the hospital's AGFA Picture Archiving and Communication System. Only patients who had static chest planar and SPECT/CT imaging of the chest at both imaging time points were included in this review. In total, 70 patients met these criteria and were thus included. Each study was split into 2 separate datasets, early and delayed datasets. All the datasets were anonymized, and time stamps were erased, processed, and reviewed by 3 readers, 2 qualified nuclear medicine physicians and 1 nuclear medicine trainee, masked to the patients' clinical history and imaging time points.

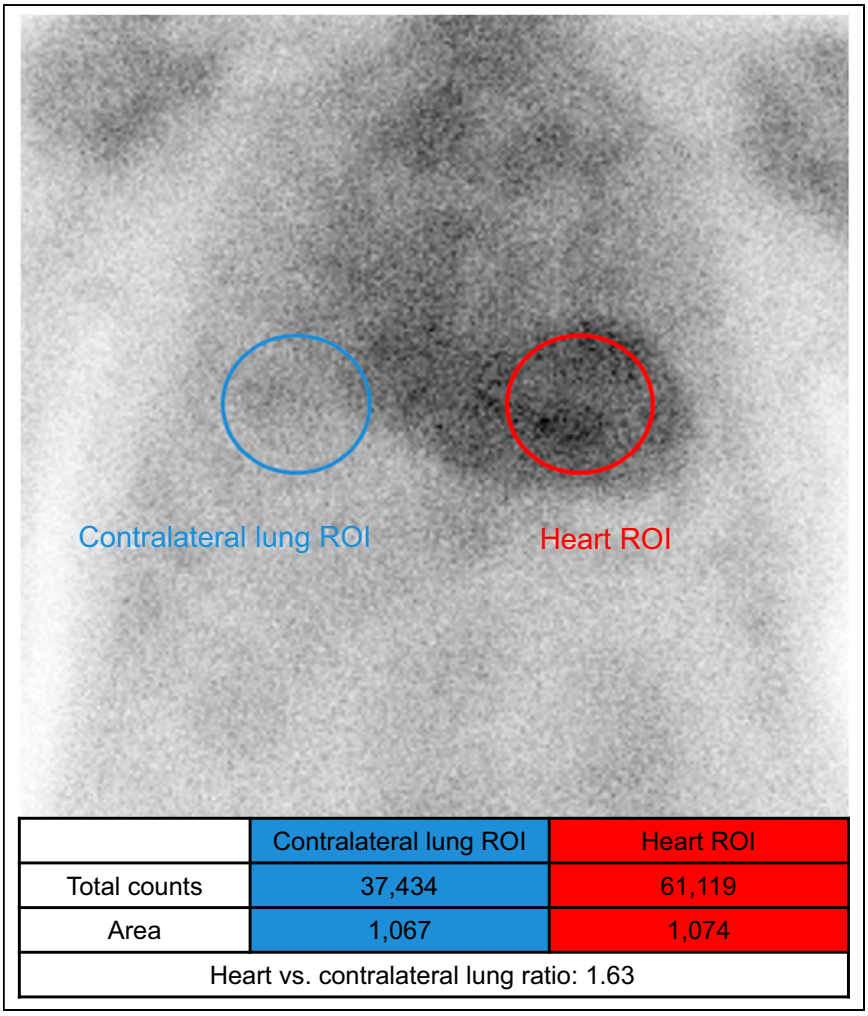
A semiquantitative analysis was performed on the static chest planar image. Two regions of interest of the same size were drawn over the heart and the contralateral right lung (Fig. 2). A ratio was calculated by dividing the counts from the heart regions of interest by the counts from the contralateral lung regions of interest. The ratio was noted on the saved image for the specialist to review. At our center, as per the 2019 ASNC practice points, a ratio of 1.5 or more on the early static image and uptake within the myocardium on SPECT/CT was considered a positive study (*12*). The ASNC PYP Practice Points (*12*) does not stipulate a cutoff ratio value for delayed imaging, but Scully et al. (*15*) suggest a cutoff value of 1.3, with a ratio of 1.3 or more indicative of a positive study for the delayed time point.

All image processing was done by a single experienced nuclear medicine technologist. The processed data were independently reviewed by each reader who reported the datasets as positive, negative, or equivocal. A case was recorded as positive if there was a mention of diagnosis or treatment of cardiac ATTR in the patient's medical records. No hospital medical records could be found for 2 patients, who had external follow-up. Excluding these

TABLE 1
Acquisition Parameters for ^{99m}Tc-PYP Scans

Scan	Parameter	Early image (T0 + 60 min)	Delayed image (T0 + 180 min)
Static	Acquisition time	300 s	300 s
	Matrix	256 × 256	256 × 256
	Zoom	1.5	1.5
	Energy	140.5 kV ± 10%	140.5 kV ± 10%
SPECT/CT	Acquisition time	20 s/frame	20 s/frame
	Number of views	60	60
	Matrix	128 × 128	128 × 128
	Body contour	Yes	Yes
	Energy	140.5 kV ± 10%	140.5 kV ± 10%

T0 = time of injection.



	Contralateral lung ROI	Heart ROI
Total counts	37,434	61,119
Area	1,067	1,074
Heart vs. contralateral lung ratio: 1.63		

FIGURE 2. Example of positive study, with ratio of heart region of interest (ROI) vs. contralateral lung region of interest being more than 1.5.

2 cases, the data were used to calculate the specificity of a single time point of ^{99m}Tc-PYP cardiac amyloid scans.

Statistics

The percentage agreement among all 3 readers and the 2 experienced readers was calculated. The interobserver variability was corrected for chance between any 2 readers and assessed using Cohen κ coefficient at the 95% CI. The interobserver variability among all 3 readers was evaluated using the weighted Fleiss κ coefficient (*16*). A κ value of less than 0.01 would be considered no agreement, 0.01 to 0.2 poor, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial agreement, and 0.81 to 1.00 good agreement.

Statistical analysis of the datasets was performed using the Statistical Package for the Social Sciences (IBM Corp.).

RESULTS

Population Cohort

Twelve patients had confirmed diagnosis of cardiac amyloidosis. The diagnosis of 10 patients was confirmed via doctor communication, blood tests, and echocardiograms; 1 patient's diagnosis was confirmed via doctor communication, blood tests, echocardiograms, and cardiac MR, and 1 patient's diagnosis was confirmed via doctor communication and blood test only (Fig. 3). Demographics and indications for cardiac amyloid scans for patients who had a SPECT/CT chest scan at both time points are shown in Table 2.

All 3 readers had 4 equivocal datasets from the 140 datasets (70 for the early imaging time point and 70 for the delayed imaging time point). The equivocal datasets were not necessarily the same for all readers (Fig. 4). The sensitivity of reporting using the early imaging point was 100% for all 3 readers. The sensitivity of reporting using the delayed dataset only was 100%, 90.9%, and 100% for reader A, reader B, and reader C, respectively. The accuracy of reporting using the early dataset only was 95.7%, 97.1%, and 95.7% for reader A, reader B, and reader C, respectively. The accuracy of the report using the delayed dataset only was 95.7%, 94.2%, and 97.1% for reader A, reader B, and reader C, respectively. The specificity of the reporting using the early dataset was 96.4%, 98.2%, and 98.2% for reader A, reader B, and reader C, respectively. The specificity of reporting using the delayed dataset was 98.2% for reader A and reader B and 98.3% for reader C (Table 3). The agreement among all 3 readers was 95.7% and 92.8% for early dataset and delayed dataset, respectively. The agreement between the 2 senior readers, reader A and reader C, was 95.7% for both datasets. The Fleiss κ at 95% CI for all 3 readers was 0.91 for the early time point and 0.83 for the delayed time point, which is categorized as good agreement. The Cohen κ at 95% CI for the 2 senior readers was 0.88 and 0.87 for the early and delayed time points, respectively, which is again categorized as good agreement.

The difference in sensitivity, accuracy, and specificity between early and delayed imaging was not statistically significant, with P values of 0.422, 0.741, and 0.400, respectively.

DISCUSSION

One of the challenges of reporting ^{99m}Tc-PYP cardiac amyloid scans is the accumulation of the radiopharmaceutical in the chambers of the heart on early images, and it is challenging to distinguish between myocardial uptake and

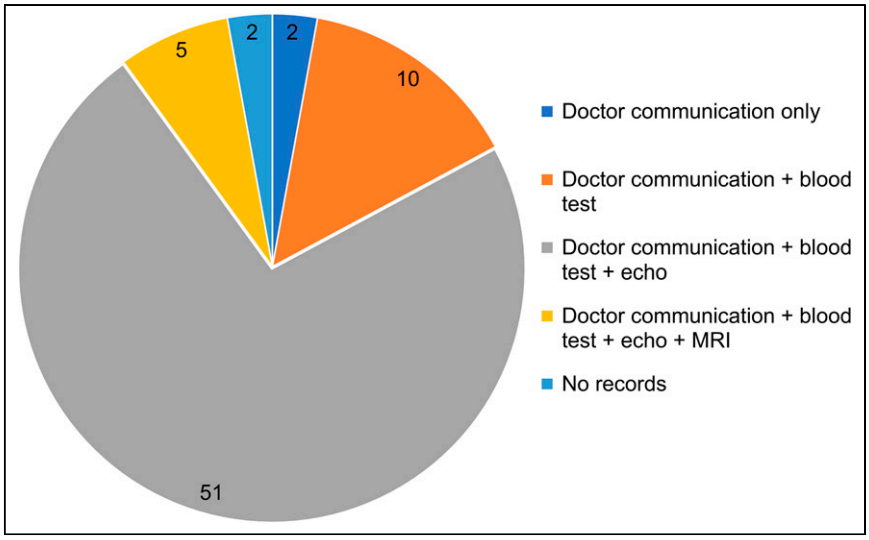


FIGURE 3. Source of clinical data for diagnosis of possible cardiac amyloidosis.

blood pooling on static planar images only. For cases in which blood pooling is seen and suspected on early images, delayed images can be acquired, as blood pooling is expected to be cleared by 3 h, or an early SPECT/CT can be acquired to distinguish between myocardial uptake and activity in the heart chambers (*11*). There appears to be varying approaches regarding optimal imaging time in the current literature. Many nuclear medicine departments are using the 3 h time point for reporting (*9*,*10*,*17*–*19*), but some recent studies have demonstrated that scans can be reported at the 1-h time point with equal confidence (*8*,*20*).

The results of our study show that the difference in sensitivity and specificity of reporting of any single imaging time point is not statistically significant when compared with using both imaging time points for reporting ($P > 0.05$). Furthermore, the difference in accuracy of reporting early images only versus delayed images only is not statistically significant ($P > 0.05$). The results show that experienced readers have 100% sensitivity for both time points. The junior reader (trainee) had 100% sensitivity for the early dataset compared with 90.9% for the delayed dataset. This drop in sensitivity was due to a single false-negative case in the delayed dataset. The experience levels of readers in nuclear medicine and the strong interobserver agreement demonstrate that both imaging time points are not necessary for all patients. Our study is in agreement with the work done by Bokhari et al. (*8*) who investigated several factors affecting image quality, including matrix size, counts per image, and imaging time points and chose the 1-h time point because of its excellent image quality and lower extracardiac activity. It also supports the findings of Masri et al. (*20*) who demonstrated that little additional information was gained when images were acquired at both time points. The diagnostic accuracy for each time point is equally high.

Castano et al. (*21*) and Schatka et al. (*22*) both investigated the sensitivity of cardiac amyloid scans in nuclear medicine, using the gold standard endomyocardial biopsy as reference and demonstrated higher sensitivity at the 1-h time point. ^{99m}Tc-PYP clears from the bloodstream quickly, and it is taken up by the bone and myocardium (*23*). The uptake of ^{99m}Tc-PYP in the myocardium reaches its peak around the 1-h mark, which could be a contributing factor to the higher sensitivity mentioned above (*24*).

The median age onset is commonly after the age of 40 but not usually diagnosed until a geriatric age (*25*). At our center, it was observed that the patient population tested for cardiac amyloidosis is primarily elderly (*26*), many of whom require assistance with daily activities and are typically accompanied by a family member. In this cohort of patients, tolerance for long and tedious diagnostic procedures is low. The time between a patient arriving at the department and completing the early images is approximately 2 h. If the delayed images are acquired, at a minimum, this time is doubled. Because of the decrease in mobility of patients in this age group, they are usually not willing to leave the department between the early and delayed images, and an area needs to be found to accommodate them in nuclear medicine departments. Space limitations are not uncommon in many departments. Therefore, single-time-point imaging is more tolerable for the patients and preferred in our imaging department.

TABLE 2
Characteristics of Patients in Cohort

Characteristic	Data
Total number of patients	70
Age (y)	26 – 90
Injected dose of ^{99m}Tc-PYP (MBq)	567 – 759
Sex	
Male	52
Female	18
Indication for scan	
LVH	26 (37.1)
LVH and aortic stenosis or atrial fibrillation or heart failure or diastolic dysfunction or pulmonary hypertension or carpel tunnel	28 (40.0)
Heart failure	10 (14.3)
Aortic stenosis	3 (4.3)
Other	3 (4.3)

Data are number and percentage in parentheses; continuous data are ranges.
LVH = left ventricular hypertrophy.

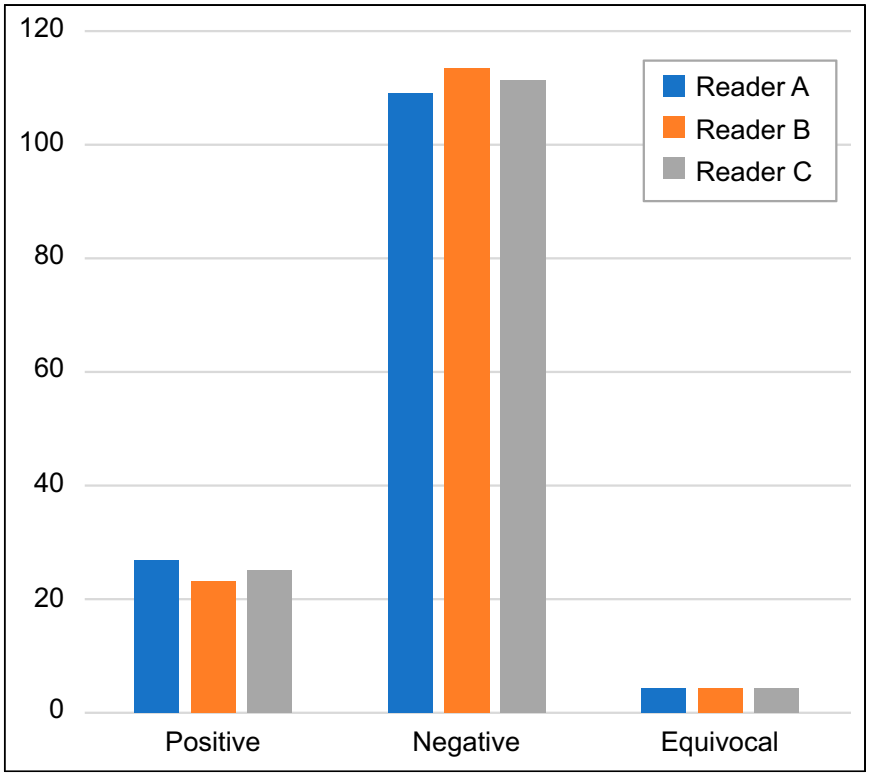


FIGURE 4. Scans results reported by each reader (readers A and C were senior physicians).

It is important to note that, in some cases, delayed imaging does play a significant role and can provide additional information and increase the confidence level of the reporting physician. In our review, 2 scans were classified as equivocal on the original reports. These 2 scans were reexamined by an independent senior nuclear medicine physician (not part of the original reader team), who deemed one of the equivocal readings as negative and confirmed the other as equivocal. This example demonstrates the reporting challenges in a few cases even when both early and delayed images were available. In our study, 9 patients (12 datasets) had a dataset reported as equivocal among the 3 readers. This result is very encouraging as it suggests that single-time-point imaging is adequate for most cardiac amyloid studies and only in a few difficult cases will need 2 imaging time points.

TABLE 3
Sensitivity, Accuracy, and Specificity of Using Single Dataset for Reporting ^{99m}Tc-PYP Cardiac Amyloidosis

Characteristic	Parameter	Reader A	Reader B	Reader C
Early dataset only	Sensitivity	100.0%	100.0%	100.0%
	Accuracy	95.7%	97.1%	95.7%
	Specificity	96.4%	98.2%	98.2%
Delayed dataset only	Sensitivity	100.0%	90.9%	100.0%
	Accuracy	95.7%	94.2%	97.1%
	Specificity	98.2%	98.2%	98.3%

Limitations

Endomyocardial biopsy is the gold standard for the diagnosis of cardiac amyloidosis (*27*). Because of the invasive nature of this procedure, it is not commonly performed, and none of the patients included in this study had an endomyocardial biopsy. For this reason, information obtained from the patients' medical records was used to confirm their cardiac ATTR status which was then used to calculate the specificity for single-time-point imaging. This investigation was a single-center, retrospective study.

CONCLUSION

The findings of our study demonstrate that single-time-point imaging, at either 1 or 3 h after injection, is sufficient for reporting most cardiac amyloid studies, which aligns with the recommendations of the most recent ASNC reporting and interpretation guideline (*13*) and the findings of recent literature (*8*,*19*). Early time-point imaging is sufficient for most cases, as supported by the high sensitivity, specificity, and diagnostic accuracy of our study, with the option of delayed imaging in equivocal cases. SPECT/CT acquisition at the early imaging time point plays a critical role in the reporting of cardiac amyloid scans and can help avoid the need for dual-time-point imaging.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

KEY POINTS

QUESTION: Are 2 imaging time points necessary for cardiac ATTR imaging, and if so, which is the preferred time point?

PERTINENT FINDINGS: This retrospective analysis demonstrates that the difference in sensitivity, specificity, and accuracy between single- and dual-time-point imaging is not statistically significant. The early and delayed imaging time points also revealed similar sensitivity, specificity, and accuracy.

IMPLICATIONS FOR PATIENT CARE: Most patients referred for cardiac amyloid imaging are elderly and require a family member or a caregiver to accompany them to their appointments. The length of the imaging study has more than halved with single-time-point imaging at the 1 h mark.

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