ORIGINAL ARTICLE

The role of serial ^{99m}Tc-DPD scintigraphy in monitoring cardiac transthyretin amyloidosis

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ABSTRACT

Purpose: Cardiac transthyretin amyloidosis is a usually fatal form of restrictive cardiomyopathy for which clinical trials of treatments are ongoing. It is anticipated that quantitative nuclear medicine scintigraphy, which is experiencing growing interest, will soon be used to evaluate treatment efficacy. We investigated its utility for monitoring changes in disease load over a significant time period. **Methods:** Sixty-two treatment-naive patients underwent ^{99m}Tc-labelled 3,3-diphosphono-1,2propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy two to four times each over a five-year period. Quantitation of cardiac ^{99m}Tc-DPD retention was performed according to two established methods: measurement of heart-to-contralateral ratio (H/CL) in the anterior view (planar) and percentage of administered activity in the myocardium (SPECT). **Results:** In total 170 datasets were analysed. Increased myocardial retention of ^{99m}Tc-DPD was demonstrable as early as 12 months from baseline. Year-on-year progression across the cohort was observed using SPECT-based quantitation, though on 30 occasions (27.8 %) the change in our estimate was negative. **Conclusion:** The spread of our results was notably high compared to the year-on-year increases. If left unaccounted for, variance may draw fallacious conclusions about changes in disease load. We therefore urge caution in drawing conclusions solely from nuclear medicine scintigraphy on a patient-by-patient basis, particularly across a short time period.

ABBREVIATIONS

^{99m}Tc: Technetium-99m
ANOVA: Analysis of variance
ATTR: Amyloid transthyretin
ATTR$_v$: Variant ATTR
ATTR$_{wt}$: Wild-type ATTR
ATTR-CM: ATTR cardiomyopathy
CMR: Cardiac magnetic resonance
CT: Computed tomography
DPD: 3,3-diphosphono1,2-propanodicarboxylic acid
eGFR: Estimated glomerular filtration rate
GE: General Electric
H/CL: Heart-to-contralateral ratio
HDMP: Hydroxymethylene diphosphonate
HFpEF: Heart failure with preserved ejection fraction
NT-proBNP: N-terminal pro b-type natriuretic peptide
OSEM: Ordered-subset expectation maximisation
PYP: Pyrophosphate
ROI: Region of interest
SPECT: Single-photon emission computed tomography
TTR: Transthyretin
UK: United Kingdom

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1. Introduction

Cardiac transthyretin (ATTR) amyloidosis is a restrictive cardiomyopathy (ATTR-CM) which results from the deposition and accumulation of amyloid into the myocardium. Plasma transthyretin (TTR) proteins misfold and form insoluble amyloid fibrils in extracellular space. As cardiac amyloid burden increases the heart stiffens, causing a loss of diastolic function and culminating in congestive heart failure. The natural history of ATTR-CM is of gradually increasing cardiac dysfunction until death.

Diversity underlies ATTR-CM [1]. Wild-type ATTR (ATTR$_{wt}$) predominantly affects elderly males [2] and has been documented as a cause or common comorbidity in patients with heart failure with preserved ejection fraction (HFpEF) [3,4], aortic stenosis [5,6], carpal tunnel syndrome [7,8], and lumbar spine canal stenosis [9] and is widely accepted to be an underdiagnosed condition for which the true incidence is unknown. Variant ATTR (ATTR$_v$) amyloidosis is dominantly inherited [10,11] and presents with a greater heterogeneity of manifestations, which include progressive peripheral and autonomic neuropathy due to nerve involvement as well as cardiac failure [12,13]. Variants most commonly associated with ATTR$_v$ cardiomyopathy in the United Kingdom (UK) population include Val122Ile (p.Val142Ile) and Thr60Ala (p.Thr80Ala), which originate in individuals of African-American [14] or North West Irish [15] descent respectively. Both forms of cardiac ATTR amyloidosis are increasingly diagnosed, life-limiting diseases [13,16,17]. Approximately 40 % of patients with ATTR-CM are misdiagnosed and, on average, it takes six months for the correct diagnosis to be made [13].

Disease progression is inexorable in the absence of effective treatment [13]. While liver [18] and heart [19] transplantations, often combined into one intervention, have been shown to improve survival outcomes for ATTR$_v$ patients, they do not treat ATTR amyloidosis itself. Novel therapies, however, are showing promise in clinical trials. These include TTR-lowering drugs patisiran [20], a gene-silencing drug which interferes with TTR production by targeting RNA, and inotersen [21], an antisense oligonucleotide which inhibits all TTR production; and TTR

'stabilisers' such as tafamidis [22], an anti-inflammatory drug which stabilises TTR and slows TTR tetramer disassociation.

Imaging plays a vital role in the detection of ATTR-CM [23,24] and can negate the need for endomyocardial biopsy in diagnosis [25,26]. It was first observed that bone-seeking radiotracers used in skeletal scintigraphy—[99m]Tc-labelled 3,3-diphosphono1,2-propanodicarboxylic acid (DPD), hydroxymethylene diphosphonate (HMDP) and pyrophosphate (PYP)—could be repurposed to identify soft-tissue amyloid deposits over 45 years ago [27]. Numerous reports have now highlighted their utility in identifying cardiac ATTR amyloidosis [1,28,29].

Over the last decade an increasing amount of attention has been paid to quantitative imaging, which has offered hope to the prospect of accurately staging the disease, tracking its progression, and evaluating its regression in response to treatment [29–37]. But while it has been demonstrated that scintigraphy is a remarkably sensitive tool for *diagnosing* cardiac ATTR amyloidosis [26] whose quantitative results correlate strongly with an independent parameter [33], no quantitative approach has been effectively validated as an accurate and reliable tool for monitoring *changes* in disease load. Given unknown natural changes and technological limitations which limit quantitative precision [38], data are urgently required to assess the reliability of quantitative scintigraphy to help prevent spurious reporting.

Thus our objective was to quantify natural disease progression with quantitative ^{99m}Tc-DPD scintigraphy in relation to biomarkers and survival data to determine quantitative scintigraphy's role in patient management. We also sought to compare the utility of planar- and single-photon emission computed tomography (SPECT)-based methods.

2. Methods

2.1. Setting and study design

We retrospectively identified patients with confirmed cardiac ATTR amyloidosis who attended the UK National Amyloidosis Centre multiple times for ^{99m}Tc-DPD scintigraphy over a five-year period between November 2011 and November 2016. To minimise the influence of exogenous causes of increased and decreased uptake we excluded data if patients had undergone disease-modifying interventions prior to or during their imaging investigations, as identified by an experienced clinician. To facilitate serial analysis we separated data into year groups that reflected the time periods between patients' baseline and follow-up scans as follows: year 0 (baseline), year 1 (0.50 − 1.49 years), year 2 (1.50 − 2.49 years), year 3 (2.50 − 3.49 years), year 4 (3.50 − 4.49 years), year 5 (4.50 − 5.49 years). Patients with AL amyloidosis were excluded on the basis of biopsy results for a variety of tissues, including heart, bladder, gastrointestinal, bone marrow, fat and nerve, confirming ATTR amyloid deposits or on the basis of non-invasive diagnostic criteria [26].

2.2. ^{99m}Tc-DPD scintigraphy

Patients were scanned, as previously described [29], using two General Electric (GE) hybrid SPECT-CT gamma cameras, a Discovery NM/CT 670 and an Infinia Hawkeye 4, following the intravenous administration of ^{99m}Tc-DPD (GE Healthcare, Chicago, IL, USA). Accounting for residual radioactivity, the median(Q_1,Q_3) administered radioactivity was 655(645,672) MBq. Whole-body anterior and posterior images were acquired approximately three hours subsequent to administration onto a 256×1024 matrix at a speed of 10 cm min^{-1} with the patient in the supine position, followed immediately by a SPECT acquisition of the chest region with low-energy, high-resolution collimators fitted and accompanied by low-dose, non-contrast computed tomography (CT) at 3:31(3:24,3:40) hours. Raw SPECT data were reconstructed onto 256×256 matrices using

an ordered-subset expectation maximisation (OSEM) algorithm (6i10s) on a GE Xeleris workstation and corrected for attenuation, decay, and scatter. The systems' collimator-detector responses were compensated for by GE's resolution recovery algorithm. Each gamma camera's empirically measured sensitivity (in cps MBq^{-1}) was used in the calculations.

Cardiac uptake was visually graded from 1 to 3 by an experienced clinician according to a modified version of the Perugini grading system [30,33] which utilises image information from planar *and* SPECT data. These grades were used to separate data such that advances or retreats in the disease could be highlighted in grade-divided subgroups.

Quantitative analyses were performed using two methods: (i) planar heart-to-contralateral ratio (H/CL) [32] was calculated from the anterior whole-body image, whereby a circular region of interest (ROI) was positioned over the imaged heart and the counts within it were divided by counts from a same-sized ROI positioned on the contralateral side of the chest; (ii) percentage of administered activity was calculated from SPECT data [33] utilising GE's Q.Metrix image analysis software, whereby a volume of interest over the heart was outlined according to CT data and then transposed onto SPECT data for quantitation of myocardial radiotracer retention. An experienced nuclear medicine technologist performed the analysis for each method. Results were expressed as absolute differences from baseline data.

2.3. Biomarkers

Correlations with and progression of two independent biomarkers were investigated for comparison. These are N-terminal pro b-type natriuretic peptide (NT-proBNP) concentration and estimated glomerular filtration rate (eGFR), both of which have been shown to be associated with survival outcomes for patients with cardiac ATTR amyloidosis at the time of diagnosis and when measured serially [39,40]. Serial measurements were obtained at the same time as imaging such that all quantitative data were time-matched.

2.4. Survival

Given the relatively high average age and low median survival time of ATTR-CM patients, it was anticipated that a number of patients would pass away during our long-term investigation. We therefore established dates of death and last dates of contact, as of March 2020, to facilitate survival analysis and account for the effect of mortality on our conclusions. We hypothesised that mortality amongst patients with greater cardiac uptake would be higher.

Kaplan-Meier analyses were performed from dates of diagnosis. Data were stratified into groups based on baseline Perugini grade, H/CL (1.6 [41]), and myocardial radiotracer retention (5 % of administered activity based on optimal separation of survival curves).

2.5. Statistical analysis

All our datasets were analysed on IBM SPSS Statistics 26 software. Paired tests were performed to calculate whether statistically significant increases or decreases in uptake had occurred between years. Depending on the normality of the distribution, as assessed by a Kolmogorov-Smirnov goodness-of-fit test, a two-tailed paired t-test (for approximately Gaussian distributions) or a Wilcoxon signed-rank test (for nonparametric distributions) was performed between means and medians respectively. The null hypothesis in either case was that there is no difference between our estimates of disease load for a particular year compared to our estimates at baseline. Hypothesis-testing was not performed if there were fewer than five datapoints in one group of a pair. We also ran one-way analysis of variance (ANOVA).

Results on graphs are expressed as absolute changes from baseline. Means and 95-% confidence intervals are displayed on error-bar plots. Medians and interquartile ranges are displayed on boxplots. Entire distributions are displayed where linear regression analysis was computed. Where linear relationships were hypothesised correlations are expressed quantitatively with coefficients of determination, R^2, to evaluate goodness of fit of linear regression lines. Data are not displayed on graphs if there are fewer than three datapoints in a group. Survival

distributions are represented graphically with the Kaplan-Meier method and compared statistically with log-ranks tests.

In all cases statistical significance was assumed if $p<0.05$.

2.6. Ethical approval

All procedures performed in studies involving human participants were carried out in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

3. Results

3.1. Patients

Treatment-modifying interventions resulting in patient exclusion included diflunisal therapy [42], revusiran therapy [43], and liver transplantation. The remaining 62 treatment-naive patients had undergone two to four sets of scans and were included in our analysis. The vast majority of these patients were on some form of traditional heart failure treatment (e.g. restricted fluid intake, reduced-salt diet, diuretics).

The median(range) age of these patients was 74(41–88) years old at baseline. Eighty-five percent were male. Forty-two patients were diagnosed as having $ATTR_{wt}$ amyloidosis (biopsy-proven in 28) and 20 as having $ATTR_v$ (biopsy-proven in 15).

TTR mutations in the $ATTR_v$ population were as follows: Thr60Ala ($n=5$), Val122Ile ($n=8$), Gly47Val ($n=1$), Val30Met ($n=2$), Glu54Gly ($n=2$), Glu89Lys ($n=1$), and Ile107Phe ($n=1$). Of the 19 patients across both ATTR groups for whom biopsy proof of amyloid could not be obtained 18 were diagnosed on the basis of established non-biopsy diagnostic criteria [26]. The remaining patient, who had a Val30Met mutation, exhibited early neuropathy, evidence of early cardiac

amyloidosis on cardiac magnetic resonance (CMR) images, and grade-1 cardiac uptake on ^{99m}Tc-DPD images.

In total 170 datasets were available for analysis with each quantitative method: 62 (year 0), 43 (year 1), 38 (year 2), 19 (year 3), 5 (year 4), and 3 (year 5). Myocardial uptake was visually scored as Perugini grade 1, 2, or 3 at baseline for 15, 30, and 17 patients respectively.

3.2. ^{99m}Tc-DPD scintigraphy

Serial results for each quantitative method are displayed graphically in Figure 1 and Figure 2.

No statistically significant differences in H/CL were demonstrated for comparisons between baseline and any other year. On 40 occasions (37.0 %) H/CL fell with respect to baseline. Although the trend from baseline to year 5 was upwards with time, the mean change in year 2 was lower than the change in year 1, albeit probably within the margin for error. ANOVA revealed no statistically significant differences between years (p=0.145). No signs of linear progression were inferred from regression analysis (R^2=0.020). Variances were high. The large spreads, which extend to negative values (implying disease regression), are appreciable in Figure 1c.

In contrast, statistically significant differences in percentage of administered activity were demonstrated for years 1 to 4, with year-on-year progression being observed. On 30 occasions (27.8 %) the percentage of administered activity fell with respect to baseline. ANOVA revealed statistically significant differences between years (p=0.049). No sign of linear progression was inferred from regression analysis (R^2=0.036). Variances were also high. The large spreads, which extend to negative values (implying disease regression), are appreciable in Figure 2c.

When data are broken down by baseline Perugini grade, as shown in Figure 1b and Figure 2b, progression is visually more pronounced for lower grades with either quantitative method. Statistically significant differences in H/CL were demonstrated for grade-1 patients in years 1 (p=0.007) and years 2 (p=0.011). For percentage of administered activity statistically significant

differences were demonstrated for grade1 (p=0.015) and grade-2 (p=0.010) patients in year 2 only.

3.3. Biomarkers

Serial results for NT-proBNP concentration are displayed graphically in Figure 3. Regression analysis is shown with respect to percentage of administered activity at baseline. A power model fitted between NT-proBNP concentration and percentage of administered activity using natural logarithms (R^2=0.636) was shown to exhibit a stronger relationship than a linear model (R^2=0.498). The strength of both models demonstrates adequate evidence of a relationship between increased risk of heart failure, since NT-proBNP concentration is associated with BNP release and therefore increased pressure on the heart, and ^{99m}Tc-DPD bound to amyloid. For H/CL, however, neither a power (R^2=0.283) nor a linear (R^2=0.123) model was convincingly proven.

No evidence of a linear relationship with eGFR was shown for either nuclear medicine parameter (H/CL: R^2=0.018; percentage of administered activity: R^2=0.066).

NT-proBNP concentration rose each year until year 4, when it dropped. Differences were only statistically significant for years 2 (p=0.024) and 3 (p=0.027). eGFR decreased with each year except in year 3. The only statistically significant difference occurred in year 2 (p=0.008).

3.4. Survival

Boxplots demonstrating how data were stratified for survival analysis are displayed in Figure 4. Survival curves are shown in Figure 5.

For data separated by visual assessment of cardiac uptake statistically significant differences were demonstrated between grades 1 and 2 (p=0.004) and 1 and 3 (p=0.003) but not grades 2 and 3 (p=0.910). For data separated by quantitative assessments of cardiac uptake statistically

significant differences were demonstrated between groups for our planar- (p=0.005) and SPECT-based (p=0.011) methods.

4. Discussion

4.1. Findings

We sought an appreciation of natural changes in cardiac amyloid burden over a significant period of time using quantitative scintigraphy. We hoped to capture how quickly disease load increased over time due to ongoing natural progression. An understanding of this phenomenon would improve our ability to assess disease response to treatment.

We evaluated two methods of quantification, having retrospectively recruited a cohort of 62 treatment-naive patients with ATTR-CM. It was anticipated that SPECT-based quantitation of myocardial radiotracer retention would be the superior marker of disease load since the method involves three-dimensional anatomical outlining and compensates for losses in signal due to photon attenuation and scatter and collimator-detector response. Indeed, the results were more-strongly correlated with NT-proBNP concentration than H/CL results were, while a strong correlation with extracellular volume fraction, as measured by CMR, has been previously demonstrated [33], as has a correlation with findings from strain echocardiography [44]. Conversely, changes in eGFR over time did not correlate strongly with either set of results. eGFR is a marker of kidney function, not cardiac amyloid burden, but an impact on radiotracer retention was suspected. The results did not suggest a steady decline in kidney performance nor an adverse effect on quantitative results.

For our SPECT data statistically significant differences were computed for four out of the five years (there were insufficient data to perform hypothesis-testing for year 5). However, the degree of progression over five years was somewhat low. From year 0 to year 5 the mean increase in radiotracer uptake was 1.10 %, which, assuming a median administered activity of 655 MBq,

amounts to an increase in uptake of 6.6 MBq. Nonetheless, these findings support our hypothesis that cardiac amyloid burden increases gradually over time, something which was particularly true for low-grade patients. Linearity of natural disease progression was not proven with linear regression analysis.

Over the same time period NT-proBNP concentration increased from year 0 to year 3, providing more evidential weight for our hypothesis that cardiac amyloid burden increases over time, given that higher NT-proBNP concentrations have been associated with lower survival probabilities [39]. Interestingly, however, grade-2 and grade-3 patients exhibited similar NT-proBNP concentrations at baseline (Figure 3). Furthermore, in our study NT-proBNP concentration dropped in year 4, suggesting that the risk of heart failure fell during this year. While this latter result was surprising, the number of patients who had survived until and been scanned in year 4 was five, meaning the statistical power of our test was low. In addition, there was likely selection bias for 'prolonged survivors' at this timepoint.

Val30Met patients in the Type B subgroup can trigger false-negative imaging results but none were included in our study. Moreover, in our experience of patients with V30M-associated hereditary ATTR amyloidosis, including 67 of whom have undergone ^{99m}Tc-DPD scintigraphy, there has to date been a 100-% correlation between findings on CMR and ^{99m}Tc-DPD scintigraphy, with the expected grade of uptake on ^{99m}Tc-DPD scintigraphy in relation to the CMR findings in every case.

Our findings are significant in that, for the first time, long-term natural changes in cardiac amyloid burden were observed with quantitative scintigraphy. Statistically significant differences and strong correlations with NT-proBNP concentration across a substantial cohort provide the weight to this claim. The results were compared to relevant independent measures and expressed in terms of survival outcome. While serial imaging of cardiac ATTR amyloidosis patients has been investigated by other authors [45], the follow-up period was only 1.5 years, a different radiotracer (^{99m}Tc-PYP) was used, quantitative SPECT was not evaluated, and the authors did

not have access to a large treatment-naive cohort. Their conclusion was that quantitative scintigraphy does not show disease progression where other markers do. In contrast, we showed that cohort-wide progression *is* demonstrable using quantitative scintigraphy.

However, one of the most striking features of our results is high variance, whose obfuscation of true change will need to be accounted for when assessing treatment efficacy. Our ranges are large and come with a comparatively high degree of year-by-year overlap, suggesting potentially limited benefits of using quantitative scintigraphy for tracking natural disease progression or for assessing treatment response on a patient-by-patient basis, particular across a time short time period. This large variance illustrated should elicit caution, which is clearly required in commenting on numerical changes. Rather than referring to quantitative results in isolation, conclusions should be drawn in conjunction with results from other established clinical tests and biomarkers.

The large variance, in part, may be attributed to the lack of technological precision currently associated with nuclear medicine imaging. Additionally, a number of causal factors specific to our study are hypothesised to have added uncertainty: a lack of data (and statistical power), intra-operator variability, our placing of patients into five discrete temporal groups (years 0 to 5) despite a continuous range of scan dates, and patient mortality. On the last point, we cannot say *how* patient mortality affected our results. We know that the median survival time across our cohort was less than five years from baseline; and, according to another study [39], median survival time from diagnosis is approximately two to five years, depending on disease stage. So an effect of patient mortality, possibly more so for those with greater uptake, is likely. But while mortality was higher in patients with greater uptake after they were dichotomised into groups (H/CL > 1.6 and percentage of administered activity > 5 %), the prognostic significance of greater radiotracer uptake across a continuous range of values, adjusted for confounding factors such as age and kidney function, is unknown.

These potential sources of uncertainty across the study were likely compounded by physiological sources of uncertainty in radiotracer retention. A current lack of evidence indicates a poor understanding of imaging cardiac ATTR amyloidosis. While it has been put forward that radiotracer is taken up into the heart, muscle, and bone according to a three-way model, whereby each compartment competes for radiotracer [29], such a model is yet to be integrated into quantitative analysis. This means, as cardiac amyloidosis advances—particularly in higher-grade patients, for whom more soft-tissue uptake is expected [33]—quantitative results may not reflect true changes in cardiac amyloid burden. This presents a major challenge for assuming cardiac radiotracer uptake is a reliable surrogate of disease load.

Our investigation was a timely one. With promising new therapeutic options entering the market, it is expected that quantitation may soon be widely adopted by centres as a method of assessing treatment efficacy. Moreover, cardiac ATTR amyloidosis is being increasingly diagnosed [13,16]. Estimates of incidence depend on patient population and amyloid detection methodology. González-López et al [4] calculated an incidence of ~13% for HFpEF patients. In an Australian subpopulation of bone-scan patients Cuscaden *et al* [46] calculated a figure of ~6% for males aged 85 and ~1 % for other groups. Based on autopsies, Buerger and Braunstein [47] estimated an incidence between 0 % and 25 % as a function of age; Lie and Hammond [48] detected amyloidosis in 65 % of patients between 90 and 105 years old; and, more recently, Tanskanen *et al* [49] identified deposits in up to 25 % of individuals over 80 years of age. But while estimations of incidence vary, together they suggest that many diagnoses are overlooked.

Quantitative SPECT produced results which correlated more strongly with an independent biomarker, NT-proBNP concentration, than quantitative planar imaging; and only for this method was year-on-year natural progression observed across our cohort. However, the frequency of imaging the benefits of using SPECT should be carefully weighed against the additional patient imaging time and radiation dose from CT. It is interpretable from Figure 4 and

Figure 5a that there is fidelity between visual assessment and quantitative findings, at least in terms of separating grade-1 patients from grade-2 and grade-3 patients and survival outcomes.

Based on our SPECT data, a fall in cardiac radiotracer retention is expected in 27.8 % of results. A fall could give clinicians a false sense of confidence in a treatment if they conflate variance with treatment efficacy. The present challenge, then, is to further evaluate quantitative scintigraphy as an accurate and reliable means of measuring cardiac amyloid burden for more patients across more centres, such that we can better understand and remove variance. Doing so will help us disambiguate true changes in cardiac amyloid burden from variance, which could be better accounted for, for example, by improving technological precision; by recruiting bigger cohorts and expanding on the results of our study; by compensating for intra- and inter-operator variability; by scanning patients at the same time intervals; and by investigating, modelling, and correcting for competition for radiotracer.

4.2. Study limitations

We recognise a number of limitations with our investigation, many of which arose because of the study's retrospective nature and our associated inability to account for certain factors in its design. Precision was lost by placing patients into discrete temporal groups when, actually, they were scanned across a continuous time range. Performing a prospective study would have enabled us to control time as a factor better. Further to this criticism, selection bias was likely at play since in our group survivors are overrepresented by virtue of our decision to choose patients retrospectively. However, patients passing away during the study probably moved the data in the other 'direction'. The median age at baseline in our cohort was 74 years old and the median survival time was 4.8 years, meaning many patients died during our five-year investigation, not always with cardiac amyloidosis as an identified cause of death (in many cases the cause of death was unavailable). To what degree the rate of average progression was affected by patients passing away we cannot say. Age was likely a confounding factor: older patients are more likely to have

more amyloid in their hearts but are more disposed to other causes of death. Full regression analysis (e.g. Cox) with a greater-sized cohort would be required to better quantify the clinical significance of greater cardiac uptake (expressed as a risk factor), which would be superior to separating patients by radiotracer uptake at baseline. Nevertheless, we were able to identify statistically significant differences in survival for patients with greater radiotracer uptake—something, however, which was not found in a bigger study [17].

Despite starting off with a relatively large treatment-naive cohort, when the data were further broken down into separate groups we lost statistical power with time. However, we were still able to conclude a statistically significant difference in year 4 for our SPECT data (n=5).

We included more grade-2 patients (n=30) than grade-1 (n=15) and grade-3 (n=17) patients and patients were not scanned equal numbers of times, which may have led to selection bias in the results if the true breakdown across the population was different. We also acknowledge that we may have engendered selection bias by investigating uptake in treatment-naive patients since disease progression was likely lesser in these patients. However, our selection criteria were necessary to characterise natural progression.

Lastly, the binding mechanism between ^{99m}Tc-DPD (and other radiolabelled phosphate derivatives) and amyloid is still not understood, a fact which further prevents us from modelling and correcting for competition for radiotracer between three compartments, particularly in more-advanced patients. If the extent to which ^{99m}Tc-PYP binds to skeletal muscle is, indeed, 'not significant' [50], ^{99m}Tc-PYP should be investigated as an alternative to ^{99m}Tc-DPD.

4.3. Conclusions

We demonstrated increased myocardial retention of ^{99m}Tc-DPD as soon as 12 months from baseline and year-on-year progression there and thereafter. However, variance was notably high. Monitoring changes using quantitative scintigraphy is therefore possible but should not be relied upon on a patient-by-patient basis to monitor changes in disease load.

Supplementary material

We have tabulated our results and appended them to our submission, enabling readers to scrutinise our data more thoroughly.

Serial results for each quantitative method are contained in Table 1. Statistical comparisons by year can be found in Table 2; sufficient data were only available for the first three years. Table 3 contains the statistical results from survival analysis.

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Availability of data and material (data transparency)

The data underlying this article will be shared on reasonable request to the corresponding author.

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Figure legends

Figure 1: Planar.
    (a)   Error-bar plots.
    (b)   Error-bar plots separated by baseline Perugini grade.
    (c)   Linear regression analysis on the full range of data.

Figure 2: SPECT.
    (a)   Error-bar plots.
    (b)   Error-bar plots separated by baseline Perugini grade.
    (c)   Linear regression analysis on the full range of data.

Figure 3: NT-proBNP.
    (a)   NT-proBNP concentration data plotted against SPECT data for year 0, where both datasets have been transformed using natural logarithms.
    (b)   NT-proBNP concentration data expressed serially.
    (c)   NT-proBNP concentration data expressed serially and separated by visual assessment of cardiac uptake for year 0.

Figure 4: Dashed lines show where data were stratified for survival analysis.
    (a)   Boxplots of planar data separated by visual assessment of cardiac uptake at baseline.
    (b)   Boxplots of SPECT data separated by visual assessment of cardiac uptake at baseline.
    (c)   Visual assessment of cardiac uptake.

Figure 5: Survival curves. 'Censored' denotes the time at which last contact was made with the patient where no death was recorded.
    (a)   H/CL.
    (b)   Percentage of administered activity (%AA).