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Utility and limitations of 3,3-diphosphono-1, 2-propanodicarboxylic acid scintigraphy in systemic amyloidosis

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Aims Technetium-99m-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) is a sensitive method for imaging cardiac transthyretin (ATTR) amyloid. We report utility and limitations of ^{99m}Tc-DPD scintigraphy in 321 patients with suspected cardiac amyloidosis.

Methods and results The cohort included wild-type ATTR ($ATTR_{wt}$) amyloidosis in 94 (29%), ATTR-Val122Ile amyloidosis in 38 (12%), hereditary ATTR ($ATTR_{mt}$) amyloidosis in 46 (14%), primary light-chain (AL) amyloidosis in 44 (14%), secondary (AA) amyloidosis in three (1%), other hereditary amyloidosis types in nine (3%), undetermined types in two (0.5%), and 85 (26.5%) patients in whom systemic amyloidosis was ultimately excluded. All 158 patients with ATTR amyloidosis with clinical cardiac involvement had cardiac ^{99m}Tc-DPD uptake, with median Grade 2 intensity. Thirteen further ATTR amyloidosis patients without clinical evidence of cardiac involvement also demonstrated ^{99m}Tc-DPD cardiac uptake. Eighteen of 35 (51%) AL patients with cardiac involvement had ^{99m}Tc-DPD cardiac uptake (median Grade 1 intensity). SPECT imaging indicates that the apparent reciprocal reduction in bone uptake is due to masking of bone uptake by extensive soft-tissue uptake in ATTR amyloidosis, especially $ATTR_{wt}$, and ATTR-Val122Ile types.

Conclusion ^{99m}Tc-DPD scintigraphy is a highly sensitive technique for imaging cardiac ATTR amyloidosis and is an important investigation in the diagnostic pathway of patients with cardiac amyloidosis. It is not specific for ATTR in isolation but must be interpreted in a broad clinical context to avoid dangerous diagnostic errors. Diffuse skeletal muscle uptake identifies muscle as a hitherto unrecognized site that merits investigation as a target organ in ATTR amyloidosis.

Keywords Cardiac amyloid • ^{99m}Tc DPD scintigraphy • Transthyretin • ATTR • Soft tissue

Introduction

Amyloidosis is a protein deposition disease caused by misfolding and highly ordered aggregation of proteins that accumulates in tissues.[1] Transthyretin (TTR) and acquired monoclonal immunoglobulin light-chain (AL) amyloidosis are the two commonest types of systemic amyloidosis. Wild-type non-hereditary TTR amyloidosis ($ATTR_{wt}$, senile systemic amyloidosis) occurs in the elderly and presents clinically as isolated cardiomyopathy. A clinically indistinguishable syndrome in Afro-Caribbean individuals is usually associated with the V122I variant of TTR, which is present in ~4% of this population.[2,3] In contrast, rare mutations in the TTR gene are associated with the dominantly inherited syndrome of familial amyloid polyneuropathy ($ATTR_{mt}$ amyloidosis), which involves peripheral and autonomic nerves and often the heart.[4] The true incidence of cardiac $ATTR_{wt}$ or ATTR-V122I amyloidosis is unknown. Cardiac $ATTR_{wt}$ amyloidosis may be much more frequent since $ATTR_{wt}$ amyloid deposits can be identified at autopsy in up to a quarter of individuals over 80 years of age.[5] The commonest type of cardiac amyloidosis currently diagnosed is AL type, which involves the heart in about

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half of cases;[6] cardiac AL amyloidosis is usually more rapidly progressive than ATTR type, and unlike the latter may benefit from cytotoxic chemotherapy.[7]

Identification of cardiac amyloid deposits, a major determinant of morbidity and mortality,[8] is important for prognosis and planning therapy. Echocardiography, the current gold standard, is neither specific nor sensitive.[8] Cardiac MRI (CMR) is a more discriminating method for cardiac amyloidosis, but does not differentiate types of amyloidosis and cannot be performed in the presence of implanted devices.[9] Bisphosphonate bone scintigraphy agents have long been reported to localize to cardiac amyloidosis in certain cases.[9] Recent more systematic evaluation of this approach suggests that two such tracers, ^{99m}Tc-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD)[10–12] and ^{99m}Tc-labelled pyrophosphate (PYP),[9,13–15] may have particular applicability for imaging cardiac amyloidosis.[10,16] The low cost and accessibility of bone scintigraphy encourages the use and further evaluation of DPD and PYP imaging of cardiac amyloid, although the basis for its localization in the heart and its limitations in clinical practice have not been determined. Recent reports suggest positron emission tomography using *N*-[methyl-^{11}C]2-(4′-methylamino-phenyl)-6-hydroxybenzothiazole potentially offers another way to visualize cardiac amyloid deposits[17] and other such agents are also under investigation.

We report our experience with using ^{99m}Tc-DPD scintigraphy in a large cohort of patients with the aim of assessing cardiac uptake, reporting limitations and uncertainties with this imaging modality and describing a novel finding of substantial and characteristic soft-tissue (mainly muscle) uptake of this tracer in patients with ATTR amyloidosis.

Methods

Patients

The study was performed at the UK National Amyloidosis Centre (NAC) and included all 321 patients who underwent ^{99m}Tc-DPD scintigraphy for suspected or proven cardiac amyloidosis between June 2010 and July 2012. This included all patients with ATTR amyloidosis or suspected ATTR amyloidosis seen during this period. After an initial 35 patients showing limited and random uptake in AL amyloidosis, patients with proven AL amyloidosis were not subject to ^{99m}Tc-DPD scintigraphy. All patients underwent standard clinical assessment for amyloidosis including haematology, biochemistry including cardiac biomarkers, echocardiography, electrocardiography and, in most cases, iodine-123-labelled serum amyloid P component (^{123}I-SAP) scintigraphy.[18] The presence of amyloid was confirmed histologically where possible by the presence of characteristic staining with Congo Red under crossed polarized light.[19] Amyloid type was confirmed by immunohistochemistry and corroborated by genetic sequencing for hereditary amyloidosis. Organ involvement was defined according to the international amyloidosis consensus criteria (ICC).[20] Additionally, patients with characteristic CMR findings after gadolinium contrast were considered to have cardiac amyloidosis (together referred to as ICC/CMR). All patients provided written informed consent in accordance with the Declaration of Helsinki for retrospective study approved by the Royal Free Hospital ethics committee.

^{99m}Tc-DPD scintigraphy

Patients were scanned using two General Electric (GE) Medical Systems hybrid SPECT-CT gamma cameras (Infinia Hawkeye 4 and Discovery 670) after intravenous injection of 700 MBq of ^{99m}Tc-DPD. Whole body planar images were acquired 3 h post-injection followed by cardiac SPECT-CT (single photon emission computed tomography with a low-dose, non-contrast CT scan). The whole body sweep images were acquired using low energy, high-resolution collimators and a scan speed of 10 cm/min. SPECT-CT reconstruction and image fusion were performed on the GE Xeleris workstation. The CT raw data were reconstructed three times using soft tissue, lung, and bone settings with a 512 matrix and 3.75 mm slice thickness. The soft-tissue reconstruction was loaded into the Myovation programme on the Xeleris to perform the attenuation correction on the SPECT data. The SPECT data were reconstructed using filtered back projection. Data were prefiltered using a Butterworth filter with a critical frequency of 0.4 cycles/cm and a power of 10. It was then reconstructed with a quantitative ramp filter. Four patients underwent whole body sweeps performed at 5 min, 1 h, and 2 h (scan speed 20 cm/min) and 3 h (10 cm/min) post-injection. The images were corrected for scan speed and radioactive decay.

Cardiac retention of ^{99m}Tc-DPD was visually scored using a modification of the grading devised by Perugini *et al.* [10] the modification being that cardiac uptake visualized on SPECT-CT but not on planar imaging was also classified as Grade 1. All scans were reported by two experienced clinicians who were blinded to all clinical data.

Results

Patient population

Table 1 summarizes baseline characteristics of all 321 patients. Mutations within the ATTR$_{mt}$ cohort were as follows: Thr60Ala ($n = 19$), Val30Met ($n = 12$), Gly47Val ($n = 4$), Glu54Gly ($n = 3$), Ser77Tyr ($n = 2$), Gly47Arg ($n = 1$), Glu89Lys ($n = 1$), Ile84Ser ($n = 1$), Ile107Phe ($n = 1$), Phe33Val ($n = 1$), and Ser77Phe ($n = 1$). One patient with ATTR-V122I was excluded from analysis because he had an orthotropic heart transplant for amyloid cardiomyopathy. Amyloid type could not be confirmed in two (0.5%) cases and 85 (26%) patients were subsequently found not to have systemic amyloidosis.

Cardiac uptake of ^{99m}Tc-DPD in ATTR and AL amyloidosis

Table 2 summarizes ^{99m}Tc-DPD scintigraphic findings for cardiac uptake. One hundred seventy-one of 178 (96%) patients with ATTR amyloidosis (105 with biopsy-proven amyloidosis of which 37 had endomyocardial biopsy-proven cardiac amyloidosis) showed cardiac uptake on ^{99m}Tc-DPD scintigraphy—159 with and 12 without evidence of cardiac involvement by ICC/CMR criteria. Seven patients had no cardiac uptake (six with ATTR-V30M and one ATTR$_{wt}$) and no evidence of cardiac involvement by ICC/CMR. The sensitivity and specificity of ^{99m}Tc-DPD scintigraphy, respectively, for 321 patients for cardiac ATTR amyloidosis was 91 and 82% with a positive and negative predictive value of 88 and 87%, respectively (*Table 3*). However, when patients with only suspected ATTR amyloidosis are included, there were no false-positive or -negative scans. Of the 12 patients without cardiac involvement by ICC but with cardiac ^{99m}Tc-DPD uptake, the amyloid type was ATTR$_{wt}$ in four and eight with ATTR$_{mt}$ (two with Gly47Val and one each with Thr60Ala, Val30Met, Ile84Ser, Glu54Gly, Ile107Phe and Gly47Arg). Echocardiography was normal in all 12 cases and CMR was normal in six. ATTR amyloid deposition was confirmed

Table 1 Patient characteristics

Amyloid type	*n* (%)	Age median years (range)	Male gender *n* (%)	Cardiac involvement *n* (%)
ATTR	178 (55)	75 (24–92)	147 (83)	159 (89)
AL	44 (14)	68 (43–82)	31 (70)	35 (80)
Apolipoprotein A1[a]	5 (2)	53 (45–71)	3 (60)	4 (80)
AA	3 (1)	64 (48–70)	2 (67)	1 (33)
Fibrinogen[b]	2 (0.5)	70 (69–71)	2 (100)	0 (0)
Gelsolin[c]	1 (0.5)	55	0 (0)	0 (0)
Lysozyme[d]	1 (0.5)	45	1 (100)	0 (0)
Unknown	2 (0.5)	77 (76–77)	2 (100)	1 (50)
No amyloidosis	85 (26)	68 (40–89)	50 (59)	10 (12)
ATTR—subtypes				
$ATTR_{wt}$	94 (53)	76 (57–92)	86 (91)	89 (95)
ATTR-V122I	38 (21)	77 (58–88)	30 (79)	38 (100)
ATTRwt + ATTR-V122I	132	77 (57–92)	116 (88)	127 (96)
$ATTR_{mt}$ (total)	46 (26)	63 (24–82)	31 (67)	32 (70)
ATTR-V30M	12	49 (24–75)	8 (67)	5 (42)

[a]Apolipoprotein A1—(Gln172Pro) in 1 patient, (Leu60Arg) and (Arg173Pro) in two patients, respectively.
[b]Fibrinogen Aα-chain—(Glu526Val).
[c]Gelsolin—(Asp214Asn).
[d]Lysozyme— (Asp67His).

on biopsy in 12 cases (cardiac biopsy in two and other tissue biopsy in 10 cases).

Thirty-five of 44 (80%) patients with AL amyloidosis (41 with biopsy-proven amyloidosis) had cardiac involvement by ICC. Eighteen (51% of those with cardiac involvement) had cardiac uptake on ^{99m}Tc-DPD scintigraphy and cardiac amyloid deposition was confirmed on cardiac biopsy in 13 cases. No patient with AL amyloidosis without evidence of cardiac involvement by ICC had cardiac uptake on ^{99m}Tc-DPD scintigraphy.

The median grade of DPD uptake was two in patients with ATTR amyloidosis and one in AL patients (*Figure 1*). Only one patient with cardiac biopsy-proven AL amyloidosis had Grade 2 cardiac uptake. Twelve patients with ATTR amyloidosis had Grade 1 cardiac uptake. Six of these had a mean LV wall thickness $\geq$12 mm and included $ATTR_{wt}$—three and $ATTR_{mt}$—three (S77Y—two; S77F—one). Seven patients (five with AL, and one each with ATTR-Val30Met and apolipoprotein A1 Leu60Arg amyloidosis), with a normal planar scan, demonstrated cardiac ^{99m}Tc-DPD uptake on SPECT-CT. The reproducibility in reporting a scan as positive or negative for myocardial ^{99m}Tc-DPD uptake was 100% between the two observers. In patients with cardiac uptake, SPECT-CT imaging demonstrated that ^{99m}Tc-DPD uptake was seen in the septum in all cases. ^{99m}Tc-DPD was present in all walls in patients with Grades 2 and 3 scans but this was very patchy, often seen only in a few slices with much greater uptake in the base with relative sparing of the apex of the left ventricle (see Supplementary data online, *Figure*).

Cardiac uptake of ^{99m}Tc-DPD in other types of amyloidosis

Grade 1 cardiac uptake of ^{99m}Tc-DPD was seen in four patients with apolipoprotein A1, and one with AA amyloidosis—all with cardiac involvement by ICC. ^{99m}Tc-DPD cardiac uptake was not seen in any of the other hereditary amyloid types.

Extra-cardiac visceral ^{99m}Tc-DPD uptake

Extra-cardiac organ uptake was noted in the liver of seven patients (three AL, two ATTR-V122I and one each with apolipoprotein A1-Leu60Arg and AA amyloidosis) and the spleen of six patients (three AL, one ATTR Gly47Arg and two apolipoprotein A1—both Leu60Arg). In all cases, the intensity of tracer uptake was much less by ^{99m}Tc-DPD scintigraphy compared with ^{123}I-SAP scintigraphy (*Figure 2*). Twenty-one other patients had visceral uptake on ^{123}I-SAP scintigraphy but did not show any extra-cardiac visceral uptake on their ^{99m}Tc-DPD scan.

Identification of soft-tissue uptake

During analysis of patient scans with ATTR amyloidosis, particularly patients with $ATTR_{wt}$ and ATTR-V122I amyloidosis, we noted a consistent and unusual pattern of ^{99m}Tc-DPD uptake involving the gluteal, shoulder, chest, and abdominal wall regions (*Figure 1C* and *D*). SPECT-CT confirmed that uptake was within soft tissue, predominantly muscle (*Figure 3D*). The muscle uptake was similar in all patients with ATTR amyloidosis but more pronounced and characteristic in $ATTR_{wt}$ and ATTR-V122I amyloidosis. Autopsy muscle samples (deltoid, gluteal, and abdominal wall) obtained from a patient who died of $ATTR_{wt}$ cardiac amyloidosis demonstrated significant ATTR amyloid deposition (*Figure 4*). Given the unusual muscle uptake, we re-examined the first 171 scans to define the uptake pattern. The first cohort of 171 patients included 91 with ATTR amyloidosis (77 with cardiac involvement). Seventy of 77 patients with cardiac ATTR amyloidosis showed muscle uptake including all 53 cases of $ATTR_{wt}$ ($n = 36$) and ATTR-V122I ($n = 17$)

Table 2 Uptake of ^{99m}Tc-DPD

Amyloid type (*n*)	Cardiac ^{99m}Tc-DPD uptake				Cardiac uptake (*n*)/cardiac involvement by ICC (*n*)	Cardiac uptake (*n*)/no cardiac involvement by ICC (*n*)	Extra-cardiac ^{99m}Tc-DPD uptake		
	Grade of myocardial uptake *n* (%)						Liver *n* (%)	Spleen *n* (%)	Abnormal ^{123}I-SAP scan[a] *n* (%)
	0	1	2	3					
$ATTR_{wt}$ $n = 94$	1 (1)	5 (5·5)	81 (86)	7 (7·5)	89/89	4/5	0	0	0
ATTR-Val122Ile $n = 38$	0	0	23 (61)	15 (39)	38/38	0/0	2 (5)	0	0
$ATTR_{mt}$ (total) $n = 46$	6 (13)	7 (15)	26 (57)	7 (15)	32/32	8/14	0	1 (1)	6 (7)
Val30Met $n = 12$	6 (50)	1 (8)	5 (42)	0	5/5	1/7	0	0	4 (33)
AL $n = 44$	26 (59)	17 (39)	1 (2)	0	18/35	0/9	3 (7)	3 (7)	15 (34)
AA $n = 3$	2 (67)	1 (33)	0	0	1/1	0/2	1 (33)	0	3 (100)
ApoA1 $n = 5$	1 (20)	4 (80)	0	0	4/4	0/1	1 (20)	2 (40)	2 (40)
AFib $n = 2$	2 (100)	0	0	0	0/0	0/2	0	0	2 (100)
ALys $n = 1$	1	0	0	0	0/0	0/1	0	0	1 (100)
Unknown $n = 2$	1 (50)	1 (50)	0	0	1/1	0/1	0	0	0

ICC, International Consensus Criteria (2005).[20]
[a]Defined by abnormally increased uptake of ^{123}I-SAP in the liver, spleen, or kidneys.

in which uptake was seen in the shoulder in 46 (87%), abdominal wall in 49 (92%), and gluteal region in 50 (94%). Of the 24 patients with $ATTR_{mt}$ (excluding V122I) cardiac amyloidosis, 17 (71%) had muscle uptake but this was not as pronounced as that seen in patients with $ATTR_{wt}$ or ATTR-V122I. One patient with ATTR-V122I amyloidosis who had an orthotropic heart transplant for cardiac amyloidosis showed no cardiac uptake in the transplanted heart, but demonstrated this typical muscular uptake pattern (*Figure 3C*). The appearance of a photopenic or 'cold' liver was seen in 68% of $ATTR_{wt}$/V122I patients and 50% of all other $ATTR_{mt}$ patients. This liver appearance may be due to congestive hepatomegaly secondary to long-standing heart failure.

We assessed the specificity of the muscle uptake for ATTR amyloidosis by blind analysis of the next 150 patients—AL amyloidosis—10; ATTR amyloidosis—87 —and the rest (53 patients) were eventually found to have no evidence of amyloid deposition. Eighty-five of 86 patients with ATTR amyloidosis (99%) showed the typical muscle uptake and on blind reporting, all 85 were correctly identified as ATTR amyloidosis based on the characteristic pattern of soft-tissue uptake in the presence of cardiac uptake Grade 2 or 3. Only one patient without characteristic muscle uptake had cardiac biopsy-proven ATTR amyloidosis and had low-grade cardiac uptake (Grade 1). None of the other 64 patients without ATTR amyloidosis were misidentified as ATTR based on blind reporting—indeed none of non-ATTR patients (including AL amyloidosis) had the specific muscle uptake pattern.

In this series, 66 patients had ATTR amyloidosis confirmed histologically (37 by endomyocardial biopsy). Using a combination of the following: cardiac ^{99m}Tc-DPD uptake of ≥Grade 2, typical muscle uptake, no visceral uptake on ^{123}I-labelled SAP scintigraphy, and no detectable monoclonal protein in serum with normal serum free light chains would have correctly allowed classification of all 66 cases as amyloidosis of ATTR type non-invasively.

Apparent decrease of bone uptake

Since the early reports of ^{99m}Tc-DPD scintigraphy, colleagues in Italy[10,11] developed a simple grading system (Grades 0–3) using relative changes in cardiac uptake with a reciprocal reduction in the bone uptake. We have used this system in the current study with minor modification. However, one patient (with ATTR-V122I amyloidosis) fell beyond the grades previously described due to extensive soft–tissue uptake (as per the pattern mentioned in the preceding paragraphs) which completely obscured visualization of tracer uptake in the heart on planar imaging, but the cardiac uptake was evident on 3D SPECT-CT (*Figure 3A* and *B*). This leads us to suggest that there should be a fourth grade in addition to the previously described three grades.

To assess this phenomenon of soft-tissue uptake further, four patients underwent planar whole body imaging at multiple time points comparing heart, bone, and soft-tissue localization. For each area of interest, the value at the 5 min scan after tracer injection was taken to be 100% and then at each subsequent time point was converted to a percentage of this initial value. A region of interest (ROI) was drawn over the heart (excluding sternum), a clear area of bone uptake (typically iliac crest), and soft-tissue. The cardiac uptake in these four patients was ≥Grade 2. Over the 3 h imaging period, the bone uptake continued to increase over time, while retention of the

Table 3 Sensitivity and predictive values of ^{99m}Tc-DPD scintigraphy

Amyloid type	*N*	Patients with cardiac uptake (*n*)	Sensitivity (%)	Positive predictive value (%)	Negative predictive value (%)
Cardiac biopsy proof of amyloid					
All patients with cardiac biopsy proof of amyloid deposition	53	47	89	100	–
Cardiac biopsy-proven AL amyloidosis	14	8	57	100	–
Cardiac biopsy-proven ATTR amyloidosis	37	37	100	100	–
Patients with ATTR amyloid					
ATTR amyloidosis including patients with cardiac involvement by ICC	159	159	100	100	–
All ATTR patients with and without cardiac involvement by ICC	178	171	96	100	–
Patients with AL amyloidosis	44	18	51	100	35

ICC, International Consensus Criteria (2005).[20]

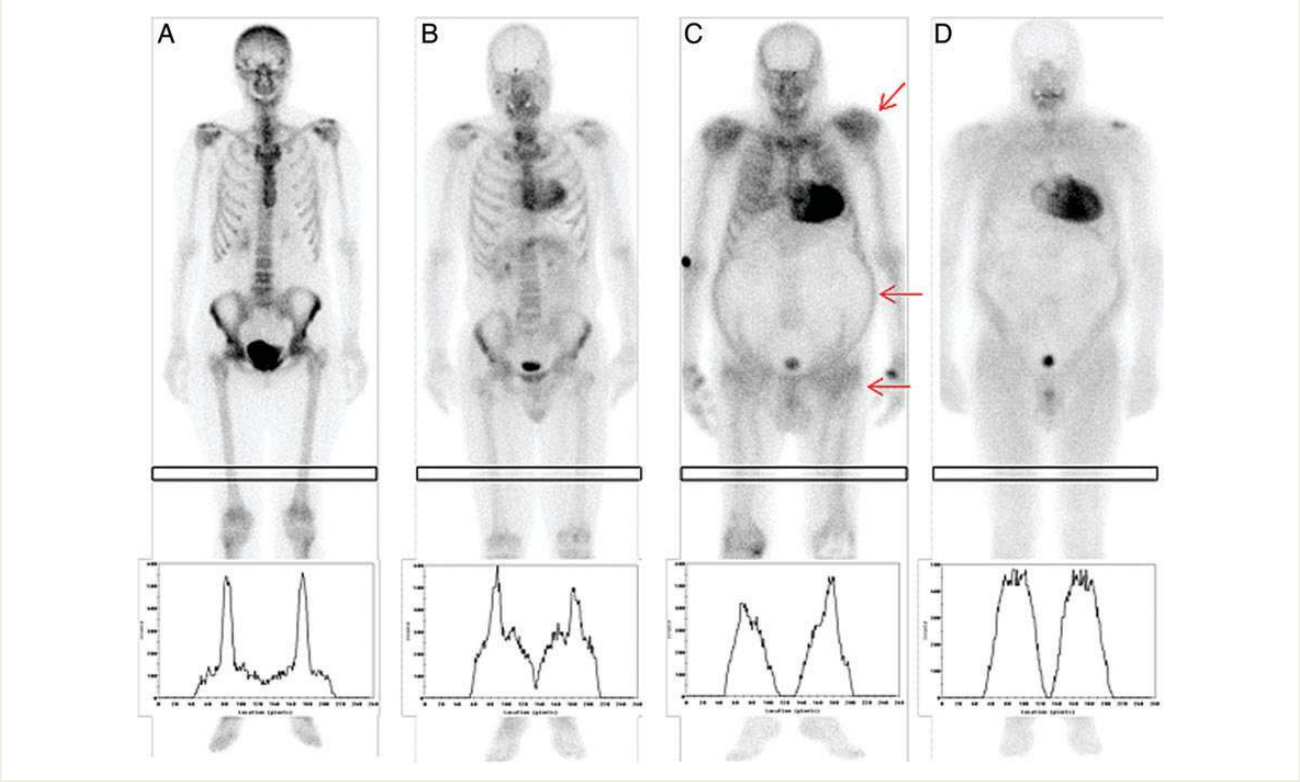

Figure 1 Cardiac and skeletal muscle uptake of ^{99m}Tc-DPD with a line count profile created by drawing an ROI across an area of thigh (4.5 cm wide) on the anterior whole body image. (*A*) A patient with no cardiac uptake with line profile showing sharply defined peaks over the femur and only background counts from adjacent soft tissue. (*B*) A patient with Grade 1 uptake with maintained femoral peak but more counts from the soft tissue on either side of the bone peak. (*C*) A patient with Grade 2 uptake with a maintained femoral peak but the soft-tissue counts have markedly increased. Deltoid, gluteal, and abdominal wall area (arrows)—a characteristic feature of ATTR amyloidosis. (*D*) A patient with Grade 3 uptake with no discernible femoral peak and just a generalized increase in counts. These figures (A–D) show that the peak bone uptake appears similar across the grades and the 'loss of bone signal' is likely due to progressive increase in soft-tissue uptake.

tracer in the heart varied among the patients, either remaining steady or slowly decreasing (*Figure 5*). The soft-tissue uptake progressively decreased over the 3 h imaging period in all four cases.

The reciprocal and relative decrease in bone uptake on planar imaging that forms the basis of the Perugini grading system, is therefore unlikely to be simply due to true competitive reduction in tracer

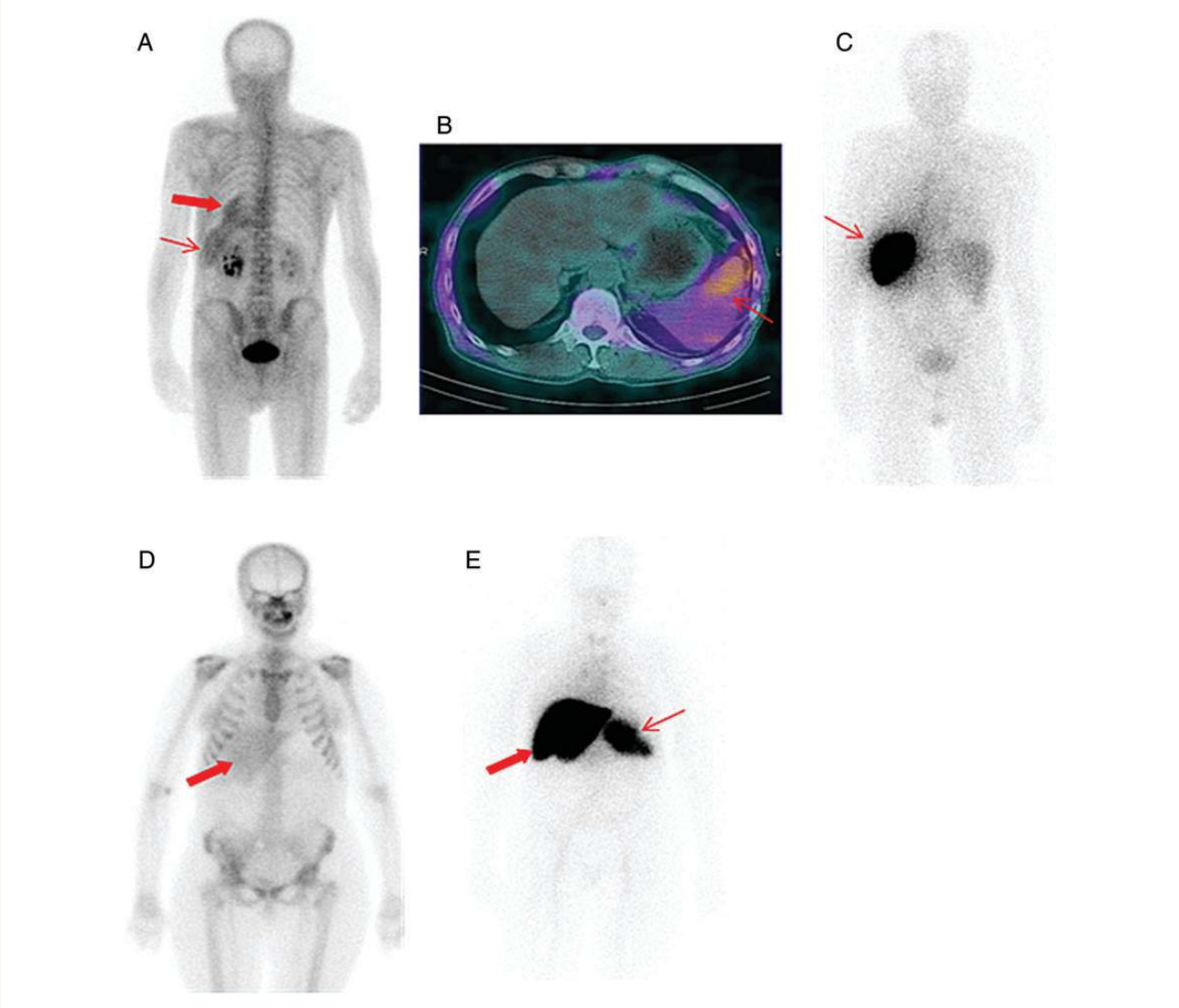

Figure 2 (*A*–*C*) Visceral uptake: ^{99m}Tc-DPD (A) and ^{123}I-SAP (C) scintigraphy in a patient with ATTR amyloidosis with spleen involvement. The planar ^{99m}Tc-DPD scan (posterior view) demonstrates cardiac uptake (large arrow) and splenic uptake (small arrow) confirmed by fused SPECT-CT imaging (B) but splenic uptake is much less than seen on ^{123}I-SAP scan. (*D* and *E*) Anterior ^{99m}Tc-DPD (D) and ^{123}I-SAP (E) scans on a patient with AL amyloidosis showing low intensity tracer uptake in the liver (large arrow) on ^{99m}Tc-DPD scintigraphy but a large amyloid load in the liver (large arrow) and spleen (small arrow) on ^{123}I-SAP scintigraphy.

uptake in bone, but due to the tracer uptake in bone being obscured by tracer in overlying skeletal muscle.

Discussion

This report confirms the high sensitivity of ^{99m}Tc-DPD scintigraphy for imaging cardiac ATTR amyloid deposits. ^{99m}Tc-DPD has limited utility in amyloid types other than AL and ATTR amyloidosis. The novel finding of this study is identification of extensive soft-tissue uptake (mainly in the muscles) in most patients with ATTR amyloidosis, especially ATTR$_{wt}$ and ATTR-V122I. One patient with advanced ATTR amyloidosis in our study had such intense soft-tissue uptake that the tracer uptake within the myocardium (clearly visualized on the SPECT images), was completely obscured by the soft-tissue uptake on the planar study. One other patient who had had a heart transplant for ATTR-V122I cardiomyopathy showed only soft-tissue uptake on ^{99m}Tc-DPD scintigraphy. This extensive uptake in ATTR$_{wt}$ and ATTR-V122I amyloidosis, currently considered to cause mainly cardiac disease, may have a wider phenotypic spectrum than previously recognized. Amyloid myopathy, well described in AL amyloidosis, may also be occurring in such cases of ATTR amyloidosis and contribute to patient symptoms such as lethargy and fatigue. Studies are underway at our centre to assess the clinical importance of ATTR amyloid deposition in the muscles as a contributor to morbidity.

The group at Bologna, Italy, pioneered the use of ^{99m}Tc-DPD in cardiac amyloid imaging[10–12] reporting a high sensitivity for ATTR amyloidosis—^{99m}Tc-PYP appears to be of similar value.[13] In the

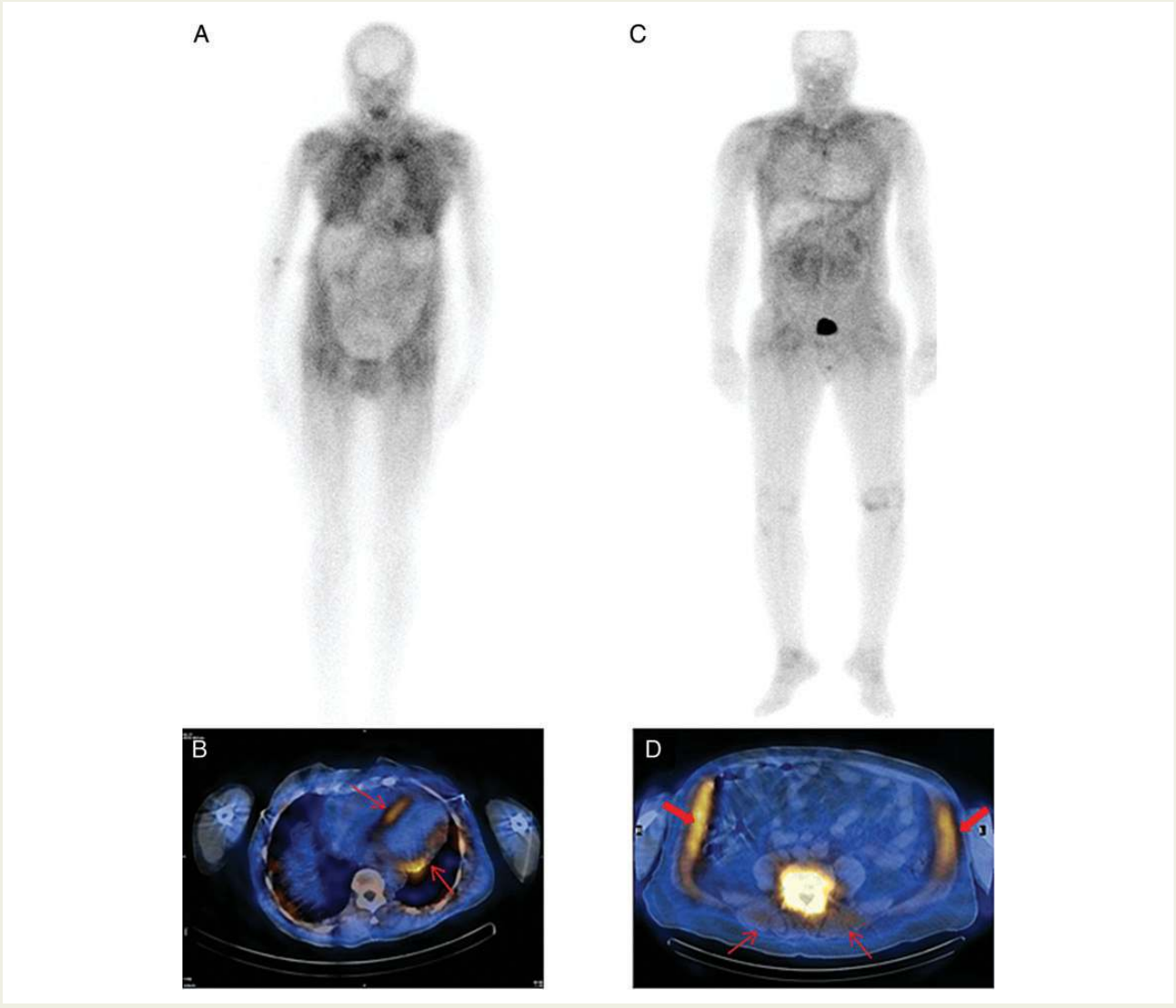

Figure 3 Cardiac and skeletal muscle uptake: (*A*) marked soft-tissue uptake which has obscured cardiac uptake on planar imaging but which is clearly apparent on (*B*) SPECT-CT (arrows). (*C*) A patient with ATTR-V122I amyloidosis following orthotopic heart transplant for cardiac amyloidosis showing no ^{99m}Tc-DPD uptake in transplanted heart but extensive soft-tissue deposition. (*D*) SPECT-CT in a patient with $ATTR_{wt}$ amyloidosis confirming ^{99m}Tc-DPD localization to the anterior abdominal wall (large arrows) muscles as well as subtle uptake in the para-spinal muscles (small arrows).

current study, all 159 patients with cardiac ATTR amyloidosis, fulfilling criteria for cardiac involvement by ICC/CMR, had cardiac uptake of ^{99m}Tc-DPD. Furthermore, 12 patients who had no clinical or other imaging evidence of cardiac amyloidosis showed ^{99m}Tc-DPD uptake. These results suggest that ^{99m}Tc-DPD is highly sensitive for detecting cardiac ATTR amyloid deposits, even at the early/asymptomatic stage, and may therefore be useful as a screening tool for cardiac ATTR amyloid deposition. The high incidence of cardiac involvement in this study reflects selection of patients suspected to have cardiac amyloidosis for ^{99m}Tc-DPD scintigraphy. With promising novel drugs in clinical trials for treatment of ATTR, early diagnosis utilizing ^{99m}Tc-DPD scintigraphy may enable therapeutic intervention prior to development of significant ATTR cardiac amyloidosis, and a prospective study screening the elderly is in progress at our centre.

In contrast to ATTR amyloidosis, only half of patients with AL amyloidosis with cardiac involvement showed uptake of ^{99m}Tc-DPD which was almost always low grade. However, some patients with early asymptomatic ATTR cardiac amyloid deposition also had Grade 1 uptake, and therefore the grade of uptake in isolation cannot distinguish between amyloidosis of AL or ATTR type.

^{99m}Tc-DPD scintigraphy shows a clinically useful phenomenon of reciprocal changes in cardiac uptake and the bone uptake; increasing cardiac uptake associated with an apparent parallel reduction in bone uptake on planar imaging which forms the basis of a visual scoring system for cardiac uptake. The basis for this phenomenon is not known but it has hitherto been assumed that the tracer is avidly and competitively taken up by the heart with reciprocal reduction in localization to bone. The soft-tissue/muscle uptake reported

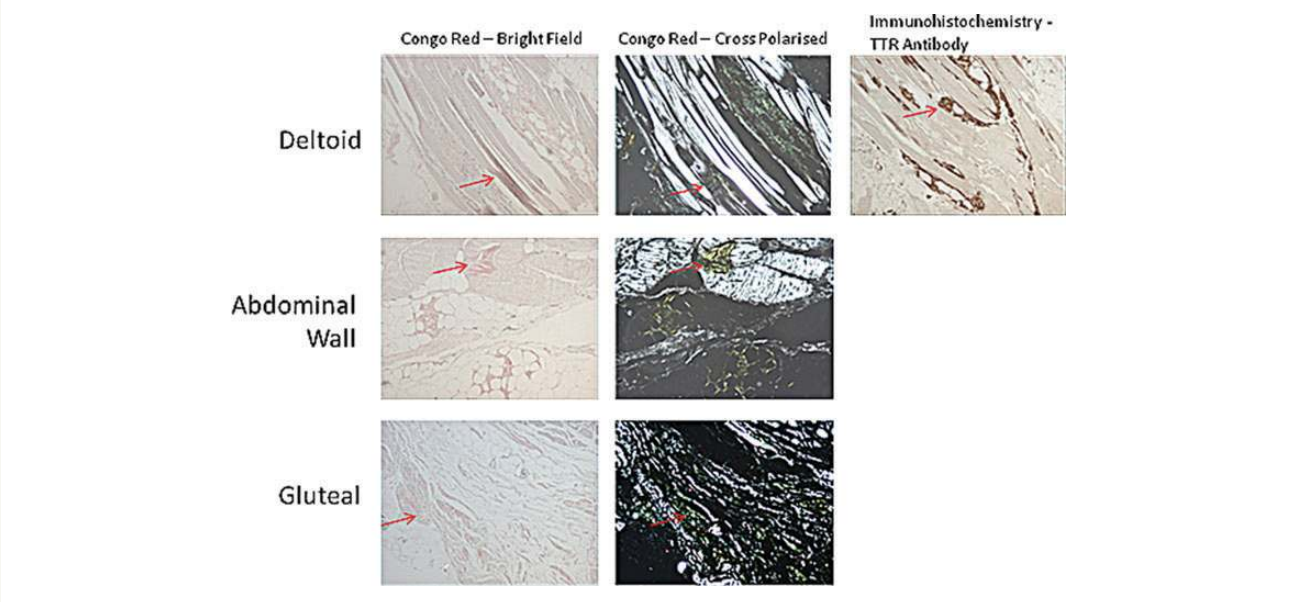

Figure 4 Deltoid, abdominal wall, and gluteal muscle biopsies from a patient who died due to ATTR amyloidosis demonstrating amyloid deposits by Congo Red staining at all sites and confirmed to be of ATTR type by specific staining with antibody to TTR.

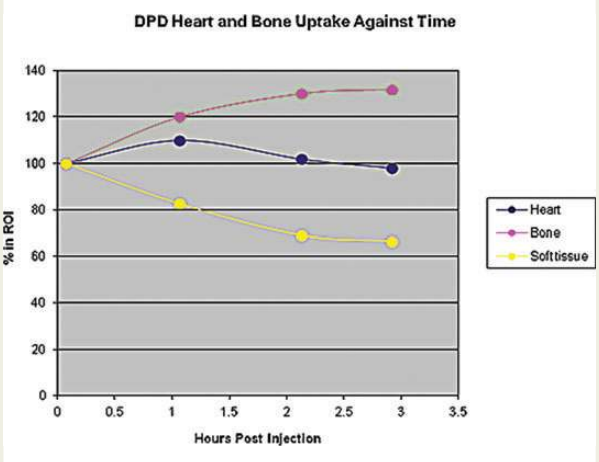

Figure 5 Imaging at multiple time points after ^{99m}Tc-DPD injection showing relative uptake and changing distribution of tracer in a patient with ATTR cardiac amyloidosis. Immediately after injection, tracer is rapidly taken up by heart, skeletal muscle, and bone. Over time, there is a relative gradual decrease in skeletal muscle uptake and progressive increase in bone uptake; cardiac uptake peaks at 1 h post-injection and decreases below baseline at 3 h.

here offers a novel explanation for this finding and a basis of the staging system. Serial imaging confirms that over a 3 h period, there is no significant decrease in bone uptake relative to heart or soft-tissue. Contrarily, the bone uptake progressively increased over the imaging time period and was relatively greater than the uptake in either the heart or soft-tissues—suggesting that the apparent reduction in bone uptake on the whole body images was due to uptake in the overlying soft-tissues. The previously described and quite widely used scoring system comprises three grades of cardiac uptake; this has limitations and does take into account some of the imaging findings noted above. We therefore propose a revised grading system: Grade 0—no visible myocardial uptake in either the planar or cardiac SPECT-CT scan; Grade 1—cardiac uptake seen only by SPECT-CT, or minimal cardiac uptake (less intense than bones) evident on the planar scan and with no apparent reduction in intensity of the normal bone images; Grade 2—moderate cardiac uptake, greater in intensity than the bone uptake with apparent reduction of the latter on planar imaging; Grade 3—intense cardiac uptake with little or no bone uptake visualised on planar imaging; Grade 4—intense soft-tissue uptake partly or completely obscuring cardiac uptake on planar imaging. However, the Grade 2 encompasses a very wide spectrum, with potential for further refinement.

Although using muscle and cardiac uptake of ^{99m}Tc-DPD allowed accurate identification of ATTR amyloidosis on blinded reporting by two experienced observers (AQ and AW) in 99% of cases with ATTR cardiac amyloidosis, uptake on ^{99m}Tc-DPD scintigraphy alone is not enough to make a definitive diagnosis as grades may vary. In this series, all patients with endomyocardial biopsy proof of amyloid type and who fulfilled the following criteria: cardiac ^{99m}Tc-DPD uptake of $\geq$Grade 2, typical muscle uptake pattern, no visceral uptake on ^{123}I-labelled SAP scintigraphy, and no detectable monoclonal protein in serum with normal serum free light chains—all had cardiac ATTR amyloidosis. AL amyloidosis is unlikely in any patient satisfying all the above criteria. Elderly patients who satisfy these criteria may, therefore, be able to avoid an invasive cardiac biopsy. An algorithm for using ^{99m}Tc-DPD scintigraphy for the diagnosis of cardiac amyloidosis is suggested in *Figure 6*.

A number of uncertainties still exist for using ^{99m}Tc-DPD scintigraphy for amyloid imaging. The mechanism of DPD uptake is not known nor is the basis for the remarkable sensitivity of DPD for

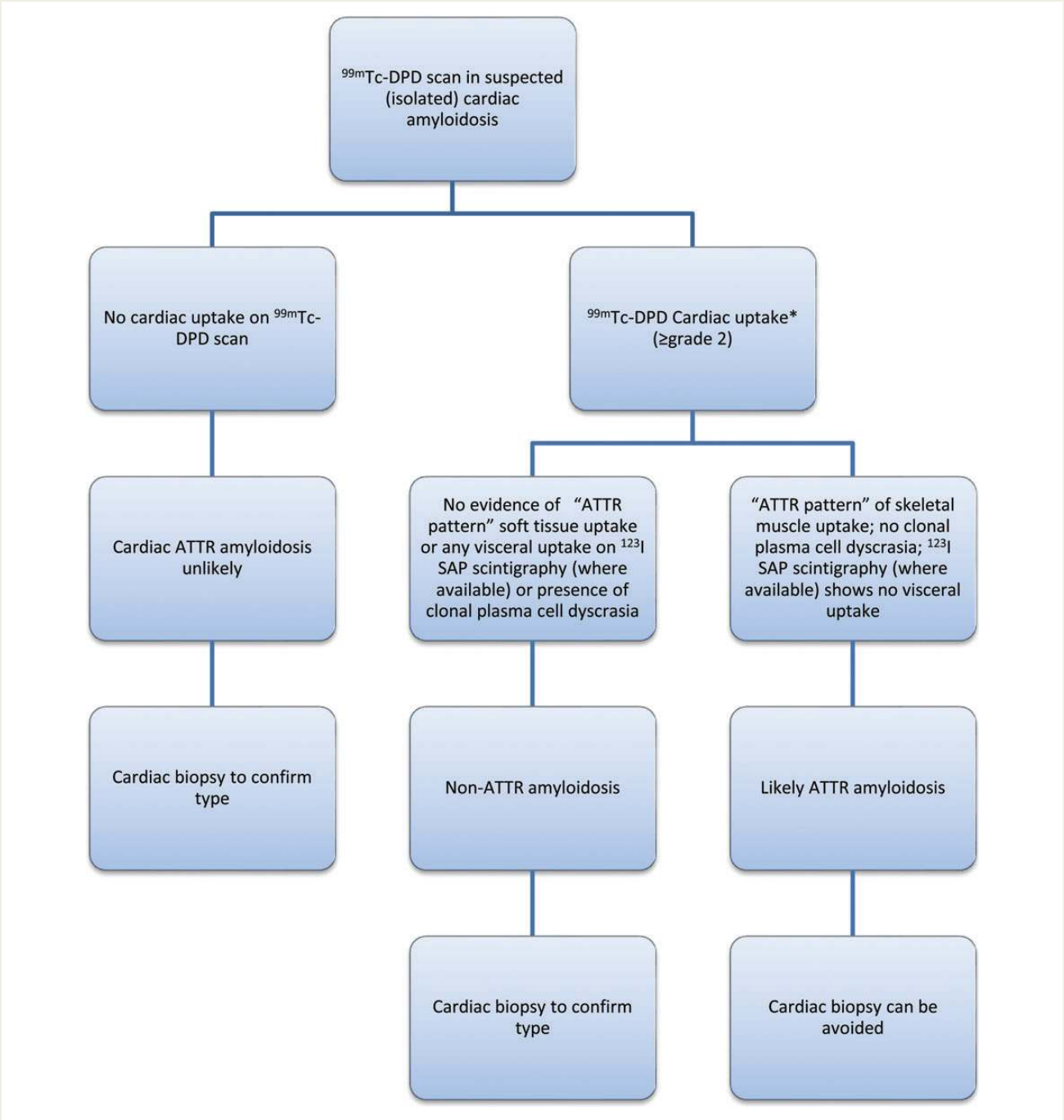

Figure 6 A suggested algorithm for clinical use of ^{99m}Tc-DPD scintigraphy in the diagnosis of cardiac amyloidosis. *Patients with Grade 1 cardiac uptake may have any type of amyloidosis and will need a cardiac biopsy to confirm the diagnosis if clinically appropriate.

ATTR amyloidosis. The lack of ^{99m}Tc-DPD uptake in visceral organs when there is ^{99m}Tc-DPD uptake in the heart in patients with amyloidosis raises more questions—this phenomenon was noted in all types of amyloidosis seen in this series. The uptake and kinetics of ^{99m}Tc-DPD in the heart, muscle, and bone are variable and the potential for interdependence of tracer uptake within these three compartments presents a major challenge to quantifying uptake (and hence amyloid load) in any particular site. The potential for using ^{99m}Tc-DPD scintigraphy to track changes in amyloid load remains to be determined. Another pitfall is the idiosyncratic behaviour of ^{99m}Tc-DPD in some patients with AL amyloidosis, in which the amyloid fibril protein is unique in all cases. Interpretation of cardiac ^{99m}Tc-DPD scintigraphy requires experience, and considerable caution, both in the cases of apparently positive and negative scan results. Use of ^{99m}Tc-DPD scintigraphy in isolation to determine amyloid type is unreliable, and therefore not recommended.

In summary, despite the bias towards patients with cardiac amyloidosis included this cohort, ^{99m}Tc-DPD scintigraphy clearly

remains a highly sensitive technique for cardiac amyloid imaging in ATTR amyloidosis. The role of ^{99m}Tc-DPD scintigraphy in screening for asymptomatic cardiac ATTR amyloid deposition in carriers of ATTR mutations or elderly subjects with heart failure of unknown aetiology requires exploration. Patients with $ATTR_{wt}$ and ATTR-V122I have extensive soft-tissue (skeletal muscle) involvement—an extended disease phenotype that needs further characterization. This extensive extra-cardiac uptake of ^{99m}Tc-DPD also accounts for the picture of apparent change in bone uptake seen on ^{99m}Tc-DPD scintigraphy during cardiac ATTR amyloid imaging. The dynamic nature of tracer uptake and interdependence of tracer kinetics within the bone, cardiac muscle and extra-cardiac soft tissue makes quantification and hence serial scanning with ^{99m}Tc-DPD difficult. *In vitro* studies are in progress to determine the nature of interactions between ^{99m}Tc-DPD and amyloid fibrils. ^{99m}Tc-DPD scintigraphy is becoming a useful tool in the investigation and diagnostic pathways of patients with cardiac amyloidosis, especially in the elderly, but has to be interpreted in context of all investigations for amyloidosis to avoid potentially dangerous diagnostic errors.

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Supplementary data

Supplementary data are available at *European Heart Journal—Cardiovascular Imaging* online.

Conflict of interest: none declared.

Authors' contributions

D.H.: designed study, performed research, analysed data and wrote paper; A.-M.Q.: designed study, performed research and wrote paper; J.P.: performed research, analysed data; M.L.H.: performed research; M.B.: performed research, analysed data and wrote paper; D.G.: performed research; T.L.: performed research and wrote paper; C.J.W.: performed research; H.J.L.: performed research; J.D.G.: performed research; P.N.H.: performed research and wrote paper; A.D.W.: designed study, performed research, analysed data and wrote paper. All authors approved the final version of the manuscript.

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