^{99m}Tc-Aprotinin Scintigraphy in Amyloidosis

Bente K. Schaadt, MD, PhD[1,2]; Helle W. Hendel, MD, PhD[1]; Peter Gimsing, MD, MSc[3]; Viggo Jønsson, MD, DMSc[3]; Heidi Pedersen, Bpharm[4]; and Birger Hesse, MD, DMSc[1]

[1]Department of Clinical Physiology and Nuclear Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [2]Department of Clinical Physiology and Nuclear Medicine, Hillerød Sygehus, Hillerød, Denmark; [3]Department of Haematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; and [4]Section for Radiopharmacy, Danish Medicines Agency, Copenhagen, Denmark

Changes in the amount and distribution of amyloid lesions have been difficult to monitor because they can usually be demonstrated only by evident symptoms or from a biopsy. The recent progress in the treatment of amyloidosis stresses the need for an early diagnosis and the need for noninvasive monitoring during the course of treatment. To validate ^{99m}Tc-aprotinin scintigraphy, we studied 23 consecutive patients with known or suspected amyloidosis. **Methods:** ^{99m}Tc-Aprotinin (500–700 MBq) was injected intravenously and whole-body scans, regional images, and SPECT tomograms were obtained 90 min after tracer injection. **Results:** Focal accumulations of ^{99m}Tc-aprotinin were seen in different organs of 22 patients with a total of 90 lesions, of which 20 were confirmed by biopsy or autopsy. Scintigraphy revealed "silent" amyloid deposits in at least 5 patients who later developed clinical symptoms. Physiologic uptake or excretion in liver and kidneys could not be differentiated from pathologic lesions in those organs. **Conclusion:** ^{99m}Tc-Aprotinin scintigraphy appears to be a fairly sensitive and specific diagnostic modality in patients with suspected amyloidosis. The technique is noninvasive, and it entails a minimal stress to the patient and is useful for detection of a wide range of lesions.

Key Words: amyloidosis; ^{99m}Tc-aprotinin; scintigraphy

J Nucl Med 2003; 44:177–183

Amyloidosis is a disorder of protein metabolism in which autologous proteins are deposited extracellularly in a characteristic fibrillar fashion (*1*). This deposition leads to organ impairment, ranging from an initially asymptomatic state to later progressive organ failure and death (*2*). Amyloidosis can be focal, localized, or systemic, and the disease may be hereditary or acquired (*3*). The rate of progression varies greatly among patients and among different organs of the same patient.

Received Feb. 1, 2002; revision accepted Sep. 25, 2002.

For correspondence or reprints contact: Bente K. Schaadt, MD, PhD, Department of Clinical Physiology and Nuclear Medicine, Hillerød Sygehus, Helsevej 2, 3500 Hillerød, Denmark.

E-mail: besch@gentoftehosp.kbhamt.dk

Amyloidosis is classified according to the nature of precursor plasma proteins that form the fibril deposits. These plasma proteins are diverse and unrelated—for example, monoclonal immunoglobulin light chains in AL (primary [immunocyte derived]) amyloidosis. They occur mainly in malignant B-lymphoproliferative disorders, amyloid A protein, or amyloid derived from β_2-microglobulin in AA (secondary [reactive systemic]) amyloidosis, seen in patients with chronic inflammation, and transthyretin in familial amyloidosis transthyretin (ATTR) (*2*). Other familial types are related to deposition of fibrinogen, oligoprotein, and prion proteins (*2*). The diagnosis and staging of amyloidosis are often difficult because the clinical symptoms may be weak and a specific imaging technique has been lacking so far. However, the need for such an imaging technique has been emphasized on the basis of the recent observation that early intensive chemotherapy with supportive stem-cell transplantation may induce disease remission and clinical improvement in some selected patients. In this process, monitoring of the disease is mandatory to evaluate the effect of the treatment. Thus, a specific, noninvasive method for the estimation of amyloid deposition is highly warranted, both as a diagnostic tool and to evaluate the outcome of treatment.

Noninvasive whole-body scanning for the detection of amyloid with a detailed mapping of its anatomic distribution has been attempted using different radiolabeled, specific ligands for amyloid. Tracers include ^{123}I- and ^{131}I-labeled serum amyloid P protein (*3–5*). However, these traces are not commercially available and the image quality is not optimal. ^{67}Ga citrate, ^{99m}Tc-pyrophosphate or diphosphonate, and ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc(V)-DMSA) have also been used but appear fairly nonspecific (*6*). ^{99m}Tc-Aprotinin has been used previously for radionuclide kidney studies (*7,8*). Aprile et al. (*6*) used ^{99m}Tc-aprotinin for amyloid detection and reported promising results in the demonstration of cardiac and pleuropulmonary AL amyloid. The purpose of the present study was to evaluate ^{99m}Tc-aprotinin scintigraphy to determine the extent of amyloid deposits in various organs in patients with known or suspected amyloidosis.

TABLE 1
Characteristics of 23 Consecutive Patients with Known or Suspected Amyloidosis and Findings on ^{99m}Tc-Aprotinin Scintigraphy

Patient no.	Sex	Age (y)	Type of amyloidosis/coexisting malignant disease and clinical conditions	^{99m}Tc-Aprotinin accumulations on scintigraphy	Biopsy or autopsy positive or negative*	Symptoms on scintigraphy†
1	F	86	AL amyloidosis/IgGκ multiple myeloma	Hands		+
				Tongue	+	+
				Submandibular glands	+	+
				Heart, intestines		−
2	M	39	AL amyloidosis renis/nephrotic syndrome, multiple myeloma (κ light-chain disease)	Heart	+	+
				Paraaortic lymph nodes	+	−
				Liver	+	−
				Spleen	+	−
3	M	62	AL amyloidosis/plasmacytoma with amyloidosis, multiple myeloma (λ light-chain disease)	Left eye region		+‡
				Upper mediastinum	+	+
				Left shoulder		+
				Liver		−
4	M	59	AL amyloidosis/IgGκ M component, MGUS	Heart, liver		−
				Spleen		−
				—	+ rectum, + bone marrow, + lip	
				—		
5	M	64	AL amyloidosis renis/(λ light chain in urine)	Heart	+	−
				Right lung/pleura	+	−
				Liver	+	−
				Spleen	+	−
6	F	78	AL amyloidosis/initially plasmacytoma, multiple myeloma (κ light-chain disease)	Both pleura/lungs		−
				Left maxillary sinus	+	+
				Calvarium	+	+
				Right shoulder	+	−
				Left femur		−
7	F	66	AL amyloidosis renis/multiple myeloma IgAλ	Lungs		−
				Liver, intestines		−
8	F	70	Clinically evident amyloidosis/multiple myeloma (λ light-chain disease)	Liver, spleen		−
9	F	56	Localized amyloidosis in plica vocalis/no myeloma	Liver	−	−
				—	+ plica vocalis	+
10	F	66	Clinically evident amyloidosis/IgAλ multiple myeloma	Heart		+§
				Tongue		+
				Liver		−
11	F	75	AL amyloidosis renis	Liver	+	+
				Spleen		−
12	F	45	Localized amyloidosis in biopsy of sural nerve	—	+ of sural nerve	Polyneuropathy

TABLE 1 (*Continued*)

Patient no.	Sex	Age (y)	Type of amyloidosis/coexisting malignant disease and clinical conditions	^{99m}Tc-Aprotinin accumulations on scintigraphy	Biopsy or autopsy positive or negative*	Symptoms on scintigraphy†
13	M	55	AL amyloidosis/IgGκ multiple myeloma	Heart	+	+¶
14	M	50	Hereditary amyloidosis	Heart	+	+
				Pleura, intestines		−
15	M	73	AL amyloidosis renis/IgMκ macroglobulinemia Waldenström	Lungs		−
				Liver, spleen		−
				Intestines		−
16	F	57	Hereditary amyloidosis	Heart		+
				Lungs		+
				Liver, pleura		−
17	F	60	Clinically evident amyloidosis/IgGκ multiple myeloma	Lungs		+
				Liver		−
18	F	58	AL amyloidosis/macroglobulinemia Waldenström, and cold-agglutinin syndrome	Liver		+
				Spleen, intestines, lungs		−
				All major joints		+
				Metacarpophalangeal regions and fingers	+ pathologic fracture of right femur, + bone marrow	+
19	F	58	Localized amyloidosis in bronchial biopsy	Lungs, liver		−
20	M	54	AL amyloidosis/IgMκ	Liver	+	+
				Spleen	+	−
				Intestines		+
					+ heart,‖ + alveolar walls, + vascular walls in lungs, + bone marrow	+
21	F	67	AL amyloidosis/nephrotic syndrome IgAλ	Liver		+
				Spleen, lungs		−
				Shoulders, nasopharynx		−
22	M	53	AL amyloidosis/multiple myeloma, gastrointestinal and bone marrow amyloid deposits	Tongue		+
				Pleura		−
				Liver, spleen		−
23	F	57	AL amyloidosis of lungs	Lungs	+	+
				Liver		−

*Positive (+) or negative in relation to scintigraphic findings.

†Positive (+) = symptoms on scintigraphy; negative (−) = no symptoms on scintigraphy.

‡Had scintigraphy performed twice within 6 mo after chemotherapy and autologous transplantation of bone marrow and had clinical, paraclinical, and scintigraphic reduction of tumor size in mediastinum.

§After medical treatment with alkaran and prednisolone, patient had regression of symptoms.

¶Had scintigraphy performed twice within 6 mo after chemotherapy and autologous transplantation of bone marrow with remission of multiple myeloma, but no change in cardiac uptake on scintigraphy or cardiac performance.

‖Cardiac region was not sufficiently covered by scintigraphic study.

MGUS = monoclonal gammopathy of unknown significance.

MATERIALS AND METHODS

Patients

Twenty-three consecutive patients with known or suspected amyloidosis underwent ^{99m}Tc-aprotinin scintigraphy diagnosed during a 2-y period (14 females, 9 males; median age, 61 y; age range, 39–86 y). Of the 23 patients with known amyloidosis, 15 had primary AL amyloidosis, 3 had localized amyloidosis (in the bronchial tree, plica vocalis, and sural nerve), 2 had familial amyloidosis, and 3 had multiple myeloma with clinically evident amyloidosis, which had not been proven by biopsy. Nine of the 15 patients with AL amyloidosis had multiple myeloma, whereas 2 had macroglobulinemia Waldenström. Clinical data are shown in Table 1.

Methods

Labeling Procedure. Each patient dose of ^{99m}Tc-aprotinin was achieved by addition of a freshly eluted pertechnetate solution (1,200 MBq in 1.2 mL) to a vial containing a mixture under nitrogen atmosphere consisting of 3,000 kallidenogenase inactivating units of aprotinin (Trasylol; Bayer Corp., Tarrytown, NY) and 0.1 mg of 32 stannous chloride dihydrate in glycine buffer (0.75% glycine and 0.6% sodium chloride), pH 10.5. The mixture was incubated under nitrogen atmosphere at room temperature for 20 min before sterile filtration, dispensing, and quality control. The control of radiochemical purity was performed by thin-layer chromatography (ITLC-SG [Gelman Science, Ann Arbor, MI] with methyl ethyl ketone as solvent) and gel chromatography (Sephadex G25F [Amersham Pharmacia Biotech AB, Uppsala, Sweden] with nitrogen-purged saline as solvent). The amount of impurities was determined to be approximately 1% pertechnetate and 1%–5% reduced unbound technetium.

The estimated radiation dose equivalent has been estimated to be $<$10 mSv in healthy subjects. In patients with amyloidosis the radiation dose equivalent will depend, to some degree, on the varying organ accumulation according to organ involvement of the disease (*6*).

Imaging. ^{99m}Tc-Aprotinin (500–700 MBq) was injected intravenously. The acquisition included anterior and posterior whole-body scans (4 cm/min) and regional static images (acquisition time, 10 min per image) obtained 90 min after tracer injection with a single-head gamma camera (Starcam; General Electric Medical Systems, Milwaukee, WI) or a dual-head gamma camera (Millennium MG; General Electric Medical Systems). Both cameras were equipped with a low-energy, high-resolution, parallel-hole collimator. In some patients, SPECT tomograms of the chest and the abdomen were also obtained.

The images were reviewed in a blinded fashion after termination of the study. Abnormal focal accumulation of the tracer was determined by visual inspection.

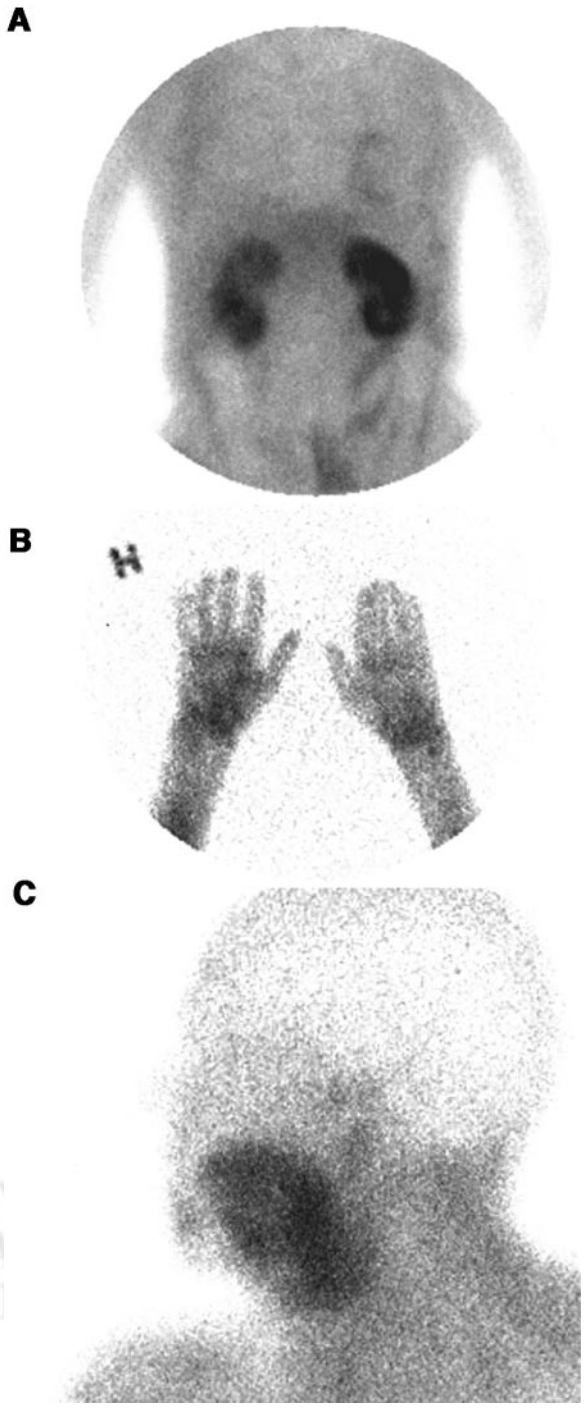

FIGURE 1. An 86-y-old woman (patient 1) with IgGκ multiple myeloma developed carpal tunnel syndrome, progressive submandibular masses, and swelling of tongue. At time of scintigraphy, patient did not have abdominal symptoms but later developed severe constipation due to intestinal amyloidosis. ^{99m}Tc-Aprotinin scintigrams show pathologic uptake in heart and parts of intestine (A, abdominal anterior projection), both hands (B, dorsal view), and tongue and submandibular glands (C, left lateral projection of head).

RESULTS

Scintigraphy

Table 1 shows the scintigraphic findings in the 23 consecutive patients with confirmed or suspected amyloidosis. We observed ^{99m}Tc-aprotinin focal accumulations in various organs. The most frequent sites were the lungs, pleura, liver and spleen, intestines, myocardium, and tongue (Figs. 1–3), organs known to be common sites of amyloid deposits. We

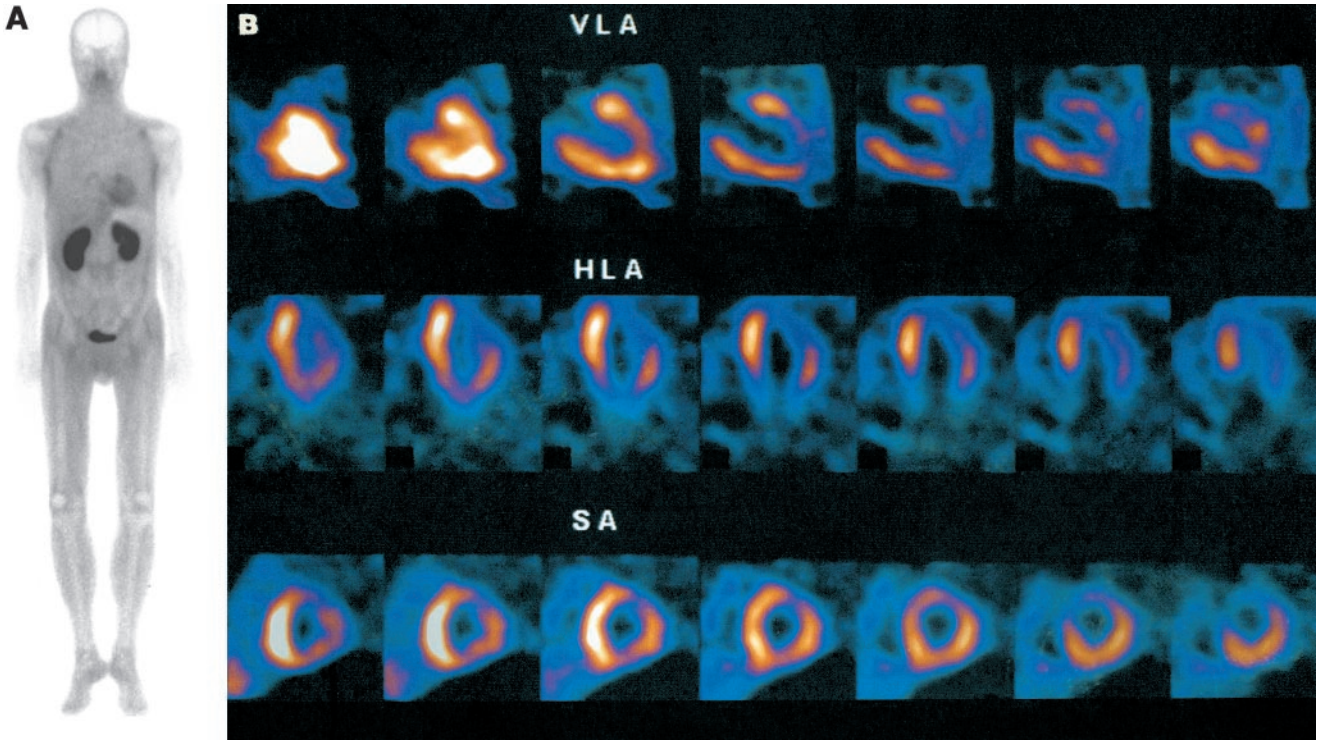

FIGURE 2. A 50-y-old man (patient 14) suffering from severe polyneuropathy during last year, slight functional dyspnea, and coughing. Biopsy of sural nerve showed unspecific axonal atrophy. His mother had died from polyneuropathy and amyloid cardiomyopathy. Scintigram shows pleural and cardiac accumulations of aprotinin (A, anterior view). (B) SPECT tomograms of chest show pronounced aprotinin uptake in interventricular septum and less pronounced uptake in lateral wall and apex of heart. VLA = vertical long axis; HLA = horizontal long axis; SA = short axis.

also found less-expected ^{99m}Tc-aprotinin accumulations in organs such as paraaortic glands, mediastinum and sternum, orbital region, maxillary sinus, calvarium, wrists (carpal tunnel syndrome), joints, and submandibular glands or lymph nodes (Figs. 1, 4, and 5).

Pathology

Autopsy was performed on 3 patients. Scintigraphy on 2 of those patients who had involvement of 9 organs showed no false-positive or false-negative focal accumulations. Autopsy confirmed amyloid deposits in the liver and spleen on the third patient (patient 20) (Table 1) but also revealed amyloid deposits in the heart and lungs (alveolar septa and pulmonary vessel walls). Scintigraphy revealed no pulmonary uptake, and the visualization of the cardiac region was technically not successful.

Of the remaining 20 patients, biopsies were performed on 18 organs from 11 patients. The biopsies confirmed amyloid deposit in all organs with scintigraphic accumulations except the liver in patient 9.

Clinical Symptoms and Scintigraphy

^{99m}Tc-Aprotinin scintigraphy revealed focal uptake in organs that could easily be related to symptoms. However, at the time of the scintigraphic examination approximately 50% of the focal accumulations detected corresponded to apparently asymptomatic lesions (Fig. 1). The scintigraphic findings preceded the symptoms by several months in 5 patients (patients 1, 2, and 4–6), including 3 cases with myocardial involvement in patients who subsequently suffered from rapid progression of cardiac failure and 1 patient with intestinal accumulation of aprotinin who developed classical intestinal symptoms several months later.

Repeated scintigraphies were performed on 3 patients. One patient showed increasing cardiac uptake, and the patient suffered from progressive heart failure (patient 5). Patient 3 had 2 courses of chemotherapy (vincristin, doxorubicin, dexamethasone) with regression of the tumor size, according to both symptoms and findings on a CT scan. ^{99m}Tc-Aprotinin scintigraphy revealed regression in size and intensity of focal uptake in the left orbital region and mediastinum but no changes in the hepatic and pulmonary uptake. Patient 13 had scintigraphy performed twice within a 6-mo period after chemotherapy and autologous bone marrow transplantation; he obtained complete remission of his multiple myeloma but showed no change in cardiac uptake of aprotinin and his cardiac performance was unchanged clinically.

DISCUSSION

At present, a diagnosis of amyloidosis is based on successful biopsies. Amyloidosis cannot be diagnosed by spe-

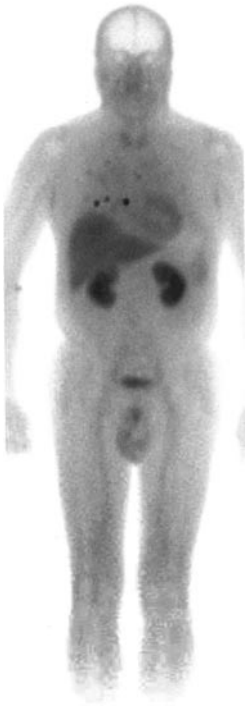

FIGURE 3. A 64-y-old man (patient 5) with renal AL amyloidosis. Whole-body scintigram shows pathologic uptake in heart, liver, and right lung or pleura (anterior view). Patient had no symptoms from heart at time of scintigraphy but died of heart failure 8 mo later.

cific biochemical tests. Imaging modalities such as CT, MRI, and ultrasonography, including echocardiography, show varying sensitivity depending on organ involvement and amount of amyloid present in the lesions. All of these techniques share a limited specificity.

Radionuclide imaging of amyloid deposits has been attempted previously with various tracers such as ^{99m}Tc-pyrophosphate or diphosphonates, ^{67}Ga citrate, or ^{99m}Tc-DMSA (*6*). All of these appear to have relatively low sensitivity and specificity for amyloidosis. Pentavalent DMSA exhibits high background activity in the chest, complicating evaluation of pleuropulmonary and cardiac involvement (*7*)**.** Previous investigations have demonstrated that radionuclide imaging of liver and spleen with ^{99m}Tc-sulfur colloid or ^{99m}Tc-phytate can detect amyloidosis of these organs; however, these techniques are nonspecific (*9,10*).

^{123}I-Labeled serum amyloid P component (SAP) appears to have a relatively high sensitivity and specificity and is useful for monitoring amyloid deposit (*6*). The peptides are currently available only in limited amounts because they are isolated from human sera, and the radiolabeled peptides are not commercially available.

Earlier studies have shown that ^{99m}Tc-aprotinin scintigraphy can be used to detect cardiac and pleuropulmonary amyloid deposits (*6,7*). We performed this study to evaluate the diagnostic value of this technique in the assessment of the different organs of the body in patients suspected of having amyloidosis. ^{99m}Tc-Aprotinin scintigraphy seems to be characterized by a fairly high signal-to-noise ratio of amyloid lesions in the head and neck region, chest, and extremities. The well-known, physiologic urinary excretion complicates the assessment of the kidneys and urinary tract. Hepatic uptake was observed in most patients, and it is not evident whether this is related to disease.

The relatively small number of patients examined in this study prevents meaningful statistical analysis. Focal aprotinin accumulations corresponding to amyloid deposits were demonstrated in many different organs and tissues in the 23 patients. Biopsy or autopsy of 20 of 73 accumulations confirmed these results. The remaining pathologic lesions were not verified directly but appeared highly probable in most of the cases, either by the presence of or subsequently developed evident symptoms (Table 1).

This finding is in agreement with the observations by Aprile et al. (*7*), who were also able to demonstrate cardiac involvement before the occurrence of symptoms. Very recently, this group reported a serious long-term prognosis of patients with suspected cardiac amyloidosis and myocardial

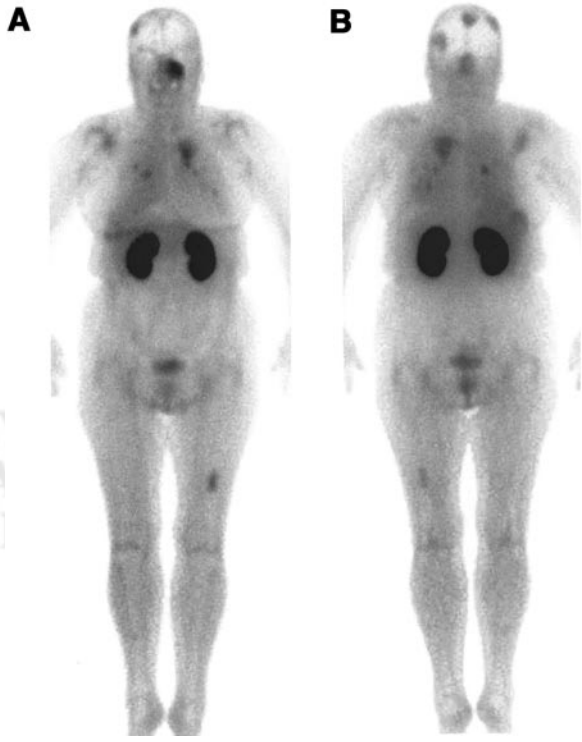

FIGURE 4. Whole-body scintigram shows pathologic uptake in left maxillary sinus in 78-y-old woman (patient 6) with plasmacytoma. Several accumulations in calvarium, lungs or pleura, left femur, and shoulder are also revealed. Patient had no symptoms from these sites, but physical examination of calvarium revealed palpable masses and biopsy confirmed amyloid. (A) Anterior view. (B) Posterior view.

FIGURE 5. A 62-y-old man (patient 3) with plasmacytoma in upper mediastinum with destruction of sternum and extrathoracic component. CT scan also showed tumor in region of left os zygomaticus. ^{99m}Tc-Aprotinin scintigram reveals pathologic uptake in upper mediastinum, left eye region, left shoulder, and liver (anterior view).

visualization by ^{99m}Tc-aprotinin scintigraphy (*11*). We confirmed that observation both in the myocardium and in various other organs: We were able to explain clinical findings, such as palpable masses in the chest and calvarium, symptoms from joints, lymph node enlargement, swelling of the tongue, and symptoms from the lungs. In 1 patient the degree of clinical remission during chemotherapy for AL amyloidosis correlated with changes seen on repeated scintigraphic studies.

CONCLUSION

This study shows that ^{99m}Tc-aprotinin scintigraphy appears to be a fairly sensitive and specific diagnostic modality for patients suspected of amyloidosis. The technique is noninvasive, entails minimal stress to the patient, and is useful for detection of a wide range of extraabdominal lesions. Because of renal excretion, ^{99m}Tc-aprotinin scintigraphy is not suited for detection of amyloid deposits in kidneys or bladder. It failed to reveal lesions of the central or peripheral nervous system. ^{99m}Tc-Aprotinin uptake in the liver is probably less specific. Screening with whole-body scintigraphy may reduce the need for more invasive techniques and be utilized as a guide to accurately locate sites for taking biopsies. Furthermore, pretransplantation screening of patients with amyloidosis secondary to myelomatosis or isolated AL amyloidosis may improve the evaluation of these patients, but further prospective studies are needed.

ACKNOWLEDGMENTS

The authors are grateful to Jørgen Marqvarsen for developing the labeling procedure and to Ole Krogsgaard for implementing it in our laboratory.

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