The American Journal of Medicine (2006) 119, 355.e15-355.e24

ELSEVIER

THE AMERICAN JOURNAL of MEDICINE®

CLINICAL RESEARCH STUDY

Diagnostic Performance of ^{123}I-Labeled Serum Amyloid P Component Scintigraphy in Patients with Amyloidosis

Bouke P.C. Hazenberg, MD,[a] Martin H. van Rijswijk, MD, PhD,[a] D. Albertus Piers, MD, PhD,[b] Marjolijn N. Lub-de Hooge, Pharm D,[b] Edo Vellenga, MD, PhD,[c] Elizabeth B. Haagsma, MD, PhD,[d] Philip N. Hawkins, FRCP, FMedSci,[e] Pieter L. Jager, MD, PhD[b]

[a]Departments of Rheumatology, [b]Nuclear Medicine, [c]Hematology, and [d]Gastroenterology and Hepatology, University Medical Centre, Groningen, The Netherlands; and [e]National Amyloidosis Centre, Royal Free Hospital, Royal Free and University College Medical School, London, United Kingdom.

ABSTRACT

PURPOSE: To assess the diagnostic accuracy and additional information provided by ^{123}I-labeled serum amyloid P component (SAP) scintigraphy in patients with systemic and localized amyloidosis.

SUBJECTS AND METHODS: ^{123}I-labeled human SAP was injected intravenously into 20 controls and 189 consecutive patients with histologically proven amyloidosis: of AA type in 60 cases, AL type in 80, hereditary ATTR type in 27, and localized amyloidosis in 22 cases. SAP scintigrams were obtained 24 hours after tracer injection and were analyzed for abnormal patterns of uptake. Sensitivity and specificity were determined, and scintigraphic findings were compared with clinical data.

RESULTS: Diagnostic sensitivity of SAP scintigraphy for systemic AA, AL, and ATTR amyloidosis was 90%, 90%, and 48% respectively, and specificity was 93%. The distribution of amyloid was less diverse in AA than in AL type. Myocardial uptake was not visualized in any patient. Splenic amyloid was very frequent (80%) in AA and AL type but rarely detected clinically (14%). Abnormal tracer uptake in the liver and kidneys correlated with disturbed liver function and proteinuria, respectively. Bone marrow uptake was specific for AL (21%) and was more frequent in AL kappa than AL lambda. Localized amyloid deposits were not imaged.

CONCLUSION: SAP scintigraphy is diagnostic of amyloid in most patients with AA and AL type but fewer with hereditary ATTR type, relating to differing distributions and burdens of amyloid in these disorders. It usually reveals more widespread organ involvement than is identified clinically, and certain distributions of amyloid are characteristic of particular fibril types.

KEYWORDS: Systemic amyloidosis; Serum amyloid P component; Scintigraphy

Detection of amyloid in a biopsy specimen warrants further clinical evaluation to determine its type, clinical significance, and extent. It is particularly important to make a distinction between systemic and localized forms of amyloidosis, which can be found in the oropharynx, upper airways, ureters, bladder, skin, and eyelids,[1] and have a vastly better prognosis than systemic forms. The 3 major systemic types are amyloid type A (AA), amyloid associated with light chains (AL), and amyloid associated with transthyretin (ATTR) amyloidosis.[2] AA amyloidosis is associated with longstanding inflammatory disorders, and nephropathy is its predominant clinical feature. AL amyloidosis is associated with free light chains producing monoclonal plasma cell dyscrasias and has remarkably diverse clinical manifestations. Hereditary ATTR amyloidosis is associated with mutations of the transthyretin (TTR) gene and presents mainly with neuropathy and cardiomyopathy.[2]

All amyloid deposits contain the nonfibrillar glycopro-

Requests for reprints should be addressed to Bouke P.C. Hazenberg, MD, Division of Rheumatology, Department of Internal Medicine, Groningen University Medical Centre, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.

E-mail address: b.p.c.hazenberg@int.umcg.nl.

0002-9343/$ -see front matter
doi:10.1016/j.amjmed.2005.08.043

tein amyloid P component, which is derived from and identical to serum amyloid P component (SAP).[1,3] SAP binds in a calcium-dependent manner to amyloid fibrils of all types.[3] SAP labeled with radioactive ^{123}iodine (^{123}I-SAP) has been used as a tracer to detect amyloid and to determine the extent and distribution of amyloid deposits in systemic AL, AA, and ATTR amyloidosis by scintigraphy and turnover studies.[4-9] The majority of experience with SAP scintigraphy has, however, been accrued in just a single center.

The aim of the present study was to independently reproduce and assess the diagnostic accuracy of and additional information provided by ^{123}I-SAP scintigraphy in our Dutch amyloidosis practice. We report here clinical and scintigraphic findings, and their correlation in 189 consecutive patients with biopsy-proven localized and systemic amyloidosis of AA, AL, and hereditary ATTR types who have been assessed over 10 years.

CLINICAL SIGNIFICANCE

- Serum amyloid P component (SAP) scintigraphy is diagnostic in most patients with AA and AL amyloidosis.
- Abnormal tracer uptake in the liver and kidneys correlate with disturbed liver function and proteinuria respectively.
- SAP scintigraphy usually reveals more widespread organ involvement than is identified clinically.
- Certain SAP scintigraphic patterns are characteristic of particular fibril types.

METHODS

Patients

All 219 consecutive patients with histologically proven amyloidosis who were evaluated at Groningen University Hospital, a tertiary referral center, from February 1990 until December 2003 were prospectively screened for the study. Patients were classified to have systemic amyloidosis of the AA, AL, or hereditary ATTR type, or localized amyloidosis.[10]

Amyloid was diagnosed in all patients by the presence of typical apple-green birefringence in polarized light in a tissue specimen stained with Congo red dye. Localized amyloidosis was defined by its typical clinical presentation of only one tissue affected with amyloid, despite a rigorous search of amyloid in other sites, such as rectum, bone marrow, and subcutaneous fat. Systemic amyloidosis was defined either by the detection of amyloid in a biopsy of kidney, liver, nerve, spleen or subcutaneous fat, or by positive biopsies derived from at least 2 different organs or tissues. AA amyloid was distinguished immunohistochemically using monoclonal murine anti-human AA antibodies (Reu.86.2, Euro-Diagnostica, Arnhem, The Netherlands). AL amyloid was defined by the detection of a clonal plasma cell dyscrasia in patients whose amyloid deposits were negative immunohistochemically for AA type. A clonal plasma cell dyscrasia was diagnosed when a free kappa or lambda light chain was detected in serum or urine by immunofixation electrophoresis or when a relative excess of cells producing 1 of the 2 light chains was detected in bone marrow. In patients with only cardiomyopathy or neuropathy, a mutation in the TTR gene was excluded before the diagnosis of AL amyloid was accepted. ATTR amyloid was defined by the detection of a TTR mutation in patients whose amyloid deposits stained specifically with anti-TTR antibodies (Dako, Copenhagen, Denmark).[10] Twenty control subjects were studied, comprising patients who had diseases that can underlie amyloidosis, but in whom biopsies for amyloid had been negative and no features suggesting amyloidosis had developed during follow-up of 2 to 8 years. The local Ethics Committee approved the study, and all patients and controls who gave informed consent were included. Thirty amyloid patients who did not participate (12 AL, 8 AA, 2 ATTR, 3 localized, and 5 unclassified) were not included for a variety of reasons, including inability to classify their fibril type in 5, logistical problems in 10, individual preference in 6, or through severe illness or death before scintigraphy could be scheduled in 9.

Clinical Evaluation of Organ Involvement

All patients were evaluated in a standardized way, and organ involvement was assessed according to established criteria[11] with some small modifications. The heart was considered to be involved when clinical heart failure (NYHA grade 3 or 4), low voltage electrocardiogram, or mean left ventricular wall thickness $>$ 12 mm was present. Liver involvement was defined as liver span measuring $>$ 16 cm or an elevated serum alkaline phosphatase ($>$180 IU/l, ie, 150% of upper reference limit) was present. Pulmonary amyloid was defined as pulmonary infiltrates on standard chest radiography that were not related to heart failure. Splenic amyloid was defined when it was $>$ 13 cm on ultrasound scanning or when Howell Jolly bodies were present in the blood film. Bone marrow involvement was defined as amyloid present in a bone marrow biopsy, although these were performed systematically only in patients with AL amyloidosis. Renal amyloid was defined as proteinuria ($>$0.5 g per day) or endogenous creatinine clearance (ECC) $<$ 60 mL/min. Clinically overt adrenocortical insufficiency was sought in all patients. Joint involvement was defined by the 'shoulder pad' sign or other unexplained stiffness, deformity, or restriction of hand joints. Carpal tunnel syndrome (CTS) was present when both typical symptoms and a positive Tinel sign were present. In all cases, other causes that might explain the increased size or disturbed function of the organ were excluded.

Table 1 Guidelines for Visual Assessment of SAP Scintigraphy

Sequence	View	Organ	Abnormal Uptake Indicative of Amyloid Deposition
1	Anterior	Liver	More than over the heart
2	Posterior/ LAO	Spleen	Obviously more than liver. If liver is positive, similar uptake is abnormal. LAO view is valuable to distinguish spleen from radioactivity in the stomach
3	Posterior	Bone marrow	Obviously visible sacral bone and pelvis, long bones, or individual vertebrae
4	Posterior	Kidneys	Obviously more than the interrenal region. If bone marrow is strongly positive, similar uptake is abnormal
5	Posterior	Adrenals	One or both visible separate from kidneys, liver, and spleen
6	Both sides	Joints	More than surrounding tissues. NB. False positive in patients with arthritis
7	Both sides	Body	At least one of the criteria above positive without any doubt (+ or more)

LAO = left anterior oblique position. Semi-quantitative score of uptake: overwhelming (3+); intense (2+); positive without any doubt (1+); weak or doubtful (±); normal (−); inadequate image, no judgment possible (X).

Radiolabeling and Quality Control

From 1990 to 1999, highly purified human SAP was obtained from London (provided by PNH).[4] Since 1999 SAP was independently purified and prepared from Dutch blood donors according to Dutch pharmaceutical standards in our center in Groningen. Radiolabeling with radioactive iodine (^{123}I, t½ = 13.2 hours, obtained from Amersham Cygne, Eindhoven, The Netherlands) and quality control were performed as described elsewhere.[12] TCA-precipitable fraction of the injected product was > 95% (median 99%).

Scintigraphy

Two hundred MBq ^{123}I-SAP containing 100 μg protein was given as an intravenous bolus.[12] Thyroid uptake of free iodide was prevented by oral administration of potassium iodide. A history of adverse reactions to intravenous radiological contrast media was excluded before administration.

Scintigraphy was performed 24 hours after injection. Total body anterior and posterior views as well as abdominal anterior, left anterolateral (LAO), and posterior spot views were acquired on large-field-of-view rectangular gamma cameras (Siemens DIACAM or MULTISPECT 2, Hoffman Estates, Illinois), equipped with a medium energy all-purpose collimator.

Visual Assessment

The guidelines used by two blinded investigators (PLJ and BPCH) to assess increased organ uptake are shown in Table 1. Provisional criteria were added to be used for myocardium and lungs: more uptake than background outside and "blood pool" inside the heart was indicative of myocardial uptake, while more uptake than in liver or inside the heart was indicative of pulmonary uptake. Patients and controls were scored in random order in a semi-quantitative way and compared with normal blood-pool distribution established previously by PNH: overwhelming (3+); intense (2+); positive without any doubt (1+); weak or doubtful (±); normal (−); or inadequate image, no judgment possible (X). Differences were resolved by consensus, in which usually the most conservative score was chosen. For final analysis uptake scores − and ± were considered negative and uptake scores 1+, 2+, and 3+ were considered positive for all organs investigated. The highest organ score present in a patient determined the body uptake score. A Receiver Operating Characteristic (ROC) curve was made showing the tradeoff between sensitivity and specificity for the different possible cutoff points of body uptake scores of all patients with systemic amyloidosis, versus the pooled group of controls and patients with localized amyloidosis.

Statistical Analysis

Statistical analysis was performed by using the statistical package GraphPad Prism, version 4.02 (GraphPad Software Inc., San Diego, California). The one-way ANOVA test was used in combination with Dunnett's multiple comparison test to detect differences in variables of patient groups versus the control group. Fisher's exact test was used to calculate differences in 2 × 2 tables where appropriate. In all tests, 2-tailed P values <.05 were considered significant.

RESULTS

Diagnostic Performance

All Patients. A total of 189 patients were included: 60 with AA, 80 with AL (21 kappa and 59 lambda), and 27 with hereditary ATTR types of systemic amyloidosis, whereas 22 had localized disease. Nineteen patients without amyloid and one healthy person served as controls. Patient characteristics are shown in Table 2.

Positive uptake was seen in 54 AA, 72 AL, and 13 ATTR patients, as shown in Table 3; abnormal images were also obtained in 3 patients with localized disease but in none of the controls. Thus, diagnostic sensitivity for AA was 90%, with a 95% confidence interval (95% CI) between 80% and 96%. Sensitivity for AL was 90% (95% CI, 81% to 96%). Sensitivity for ATTR was much lower, 48% (95% CI, 29%

Table 2 Characteristics of 20 Controls and 189 Patients with Systemic and Localized Amyloidosis Studied from 1990 until 2003

Type	Controls	AA	AL	ATTR	Localized
Number	20	60	80	27	22
Duration* (median, range)	NA	3 (0-467)	2 (0-167)	3 (0-140)	7 (1-111)
Male : Female	14 : 6	19 : 41	46 : 34	13 : 14	12 : 10
Age (mean, SD)	53.2 (16.6)	56.5 (14.7)	61.8 (10.2)†	51.8 (10.5)	50.5 (12.1)
Association	Associated disease:	Associated disease:	Associated disease:	Mutation:	Tissue site:
	RA 7	RA 37	MGUS 59	Cys114: 9	Larynx 6
	TTR mutant 6	JIA 6	MM 16	Met30: 8	Eyelid 4
	MGUS or MM 3	Spond ank 3	NHL 3	Glu47: 5	Skin 2
	Polyneuropathy 1	Rec pulm infect 2	Plasmocytoma 1	Ala71: 3	Joint 3
	FMF 1	FMF 2	M. Waldenström 1	Ala94: 1	Bowel 2
	JIA 1	M. Crohn 1		Unknown: 1‡	Amyloidoma 2§
	Healthy 1	Infected burns 1			Bronchus 2
		Psoriatic arthritis 1			Oropharynx 1
		Paraplegia 1			
		M. Wegener 1			
		Tuberculosis 1			
		SAPHO 1			
		TRAPS 1			
		M. Waldenström 1			
		Idiopathic 1			

NA = not applicable; RA = rheumatoid arthritis; JIA = juvenile idiopathic arthritis; FMF = familial Mediterranean fever; MGUS = monoclonal gammopathy of undetermined significance; MM = multiple myeloma; Spond ank = spondylitis ankylopoietica; Rec pulm infect = recurrent pulmonary infections; SAPHO = acronym for synovitis, acne, pustulosis, hyperostosis, and osteitis; TRAPS = TNF-receptor-associated periodic syndrome; NHL = non-Hodgkin's lymphoma.

*Duration of amyloidosis in months between histological proof and scintigraphy.

†$P < .05$ vs controls.

‡Patient with variant TTR detected in plasma and without any sign of monoclonal plasma cell dyscrasia, who died before DNA analysis was performed.

§Amyloidoma in one patient localized in mediastinum and retroperitoneum and in the second patient in the cervicobrachial plexus.

to 68%). Pooling controls and patients with localized amyloidosis into one nonsystemic amyloidosis control group yielded a diagnostic specificity for systemic amyloidosis of 93% (39 of 42; 95% CI, 81% to 99%). The ROC curve showed that the cutoff point ≥1+ was the best discriminator (Figure 1). No adverse reactions to the tracer were observed.

Systemic AA Amyloidosis. Abnormal organ uptake in AA amyloidosis was seen in spleen in 87%, in kidney in 67%, in adrenal glands in 20%, and in liver in 8% of the patients. No uptake in bone marrow, myocardium, or lung was detected in any AA patient. Nonspecific uptake in joints was present in 13%, all known with arthritis. No differences in specific organ uptake were detected between patients with and without rheumatoid arthritis.

Systemic AL Amyloidosis. Organ uptake in AL amyloidosis was seen in spleen in 75%, in liver in 43%, in kidney in 31%, in bone marrow in 21%, and in joints in 16% of the

Table 3 Organ and Body Uptake of ^{123}I-SAP; Percentages of Patients with AA, AL, and ATTR Amyloidosis for Each Uptake Score

	AA (n = 60)				AL (n = 80)				ATTR (n = 27)			
Tissue	-	1+	2+	3+	-	1+	2+	3+	-	1+	2+	3+
Myocardium	100	0	0	0	100	0	0	0	100	0	0	0
Liver	92	3	2	3	58	6	8	29	100	0	0	0
Lungs	100	0	0	0	100	0	0	0	100	0	0	0
Spleen	13	20	20	47	25	20	16	39	63	30	7	0
Bone marrow	100	0	0	0	79	18	4	0	100	0	0	0
Kidneys	33	52	15	0	69	18	14	0	89	11	0	0
Adrenals	80	18	2	0	100	0	0	0	100	0	0	0
Joints	87	13*	0	0	84	15	1	0	100	0	0	0
Total body	10	20	23	47	10	28	20	43	52	41	7	0

*Denotes nonspecific uptake in joints of AA patients with arthritis.

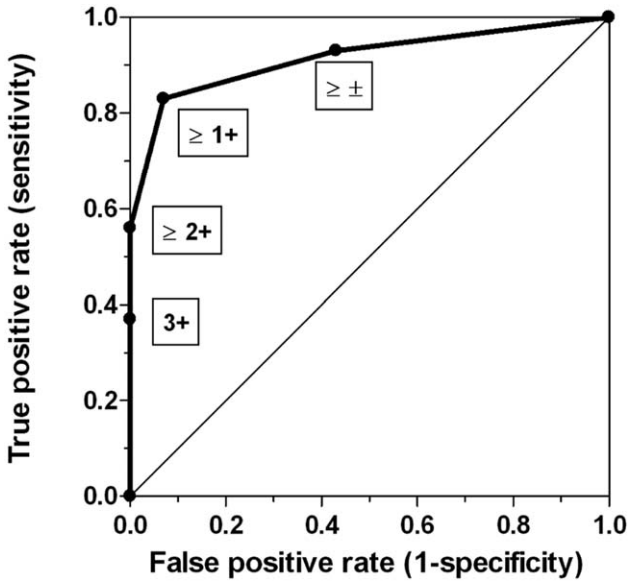

Figure 1 Receiver Operating Characteristic (ROC) curve showing the tradeoff between sensitivity and specificity for different possible cutoff points of positive body uptake score of ^{123}I-SAP scans: ie, the cutoff points ≥± (doubtful), ≥1+ (positive without any doubt), ≥2+ (intense), and 3+ (overwhelming).

patients. No uptake in adrenal glands, myocardium, or lung was detected in any AL patient. No differences in organ uptake were detected among 16 patients with multiple myeloma (MM) and 59 patients with monoclonal gammopathy of undetermined significance (MGUS). Bone marrow and joint uptake was more frequent in patients with kappa, as compared with lambda light chain involvement (43% vs 14% and 33% vs 10%, respectively; $P < .05$ for both).

Hereditary Systemic ATTR Amyloidosis. Organ uptake in hereditary ATTR amyloidosis was seen only in spleen in 37% and in kidney in 11% of the patients. Uptake was seen less frequently (1 of 8) in patients with a TTRMet30 mutation, than in patients (12 of 19) with a non-Met30 TTR mutation (13% vs 63%; $P < .05$).

Localized Amyloidosis and Controls. Three (14%) of 22 patients who had been deemed to have localized amyloidosis showed abnormal uptake (all 1+): in spleen (2 cases) and kidney (1 case); it was not deemed ethical to obtain biopsies to confirm or refute the positive scan findings. No accumulation of tracer in visceral organs was observed in any of the 20 nonamyloid controls. However, some nonspecific signal in joints was evident in 2 of 8 controls with arthritis, consistent with the expected ingress of tracer into the expanded synovial fluid space.

Patterns of Tracer Localization

Controls. Scans of control subjects showed a normal bloodpool distribution of the tracer (ie, within major blood vessels and heart and within the major viscera reflecting their blood content). Variable minor nonspecific signal was evident in sites in which it is known that free radioiodine or tracer degradation products can accumulate or be excreted, including the blocked thyroid, salivary glands, nasopharynx, stomach, and the bladder. An example of a normal scan is shown in Figure 2, panel A.

AA Amyloidosis. The most common abnormality was uptake in the spleen, occurring in 87% of cases. Five different patterns of organ uptake were present in the 54 AA patients with abnormal scans: increased signal in the spleen and kidneys in 35%; spleen only in 23%; spleen, kidneys and adrenal glands in 20%; spleen, kidneys and liver in 8%; and kidneys alone in 3%, as shown in Figure 2, panels B-F.

AL Amyloidosis. The most common abnormality in AL amyloidosis also was increased signal in the spleen, in 75% of cases. Organ uptake otherwise was extremely diverse, including 38% of patients whose abnormal scan findings were encompassed by the patterns described above that occurred in patients with AA amyloidosis. Figure 2, panels G-I shows 3 frequent patterns that differed from AA amyloidosis.

Hereditary ATTR Amyloidosis. The spleen or kidneys were the only organs that showed abnormalities in hereditary ATTR amyloidosis.

Correlation Between Clinical Evidence of Organ Involvement and SAP Scan Findings

All Patients. Cardiac involvement was identified by echocardiography in 31% of patients with systemic amyloidosis and in none of those with localized amyloidosis, but was not evident on SAP scintigraphy in any case. Pulmonary abnormalities were not identified on SAP scans or radiographs in any patient.

AA Amyloidosis. The findings are summarized in Table 4. Liver involvement, defined by size and alkaline phosphatase, was present in 5 patients (5%), of whom 3 showed abnormal increased liver uptake. Liver scans were also positive in a further 5% whose liver size was not increased and whose alkaline phosphatase was not elevated above the threshold to be categorized as involved by amyloid clinically. There were clinical indications of splenic involvement by amyloid in 8%, all of whom had positive spleen images; a further 78% of patients showed abnormal uptake in the spleen without clinical evidence of involvement. More patients (71%) with splenic uptake than patients (25%) without had proteinuria ($P < .05$). A bone marrow biopsy was performed in only 17% of patients. Bone marrow uptake was not seen on any of the patients' scans.

Presence or absence of renal involvement accorded with scan findings in 73% of cases. Two percent had normal kidney function and positive scans, but 25% had impaired renal function and showed normal tracer uptake. Creatinine clearance did not differ between patients with abnormal and

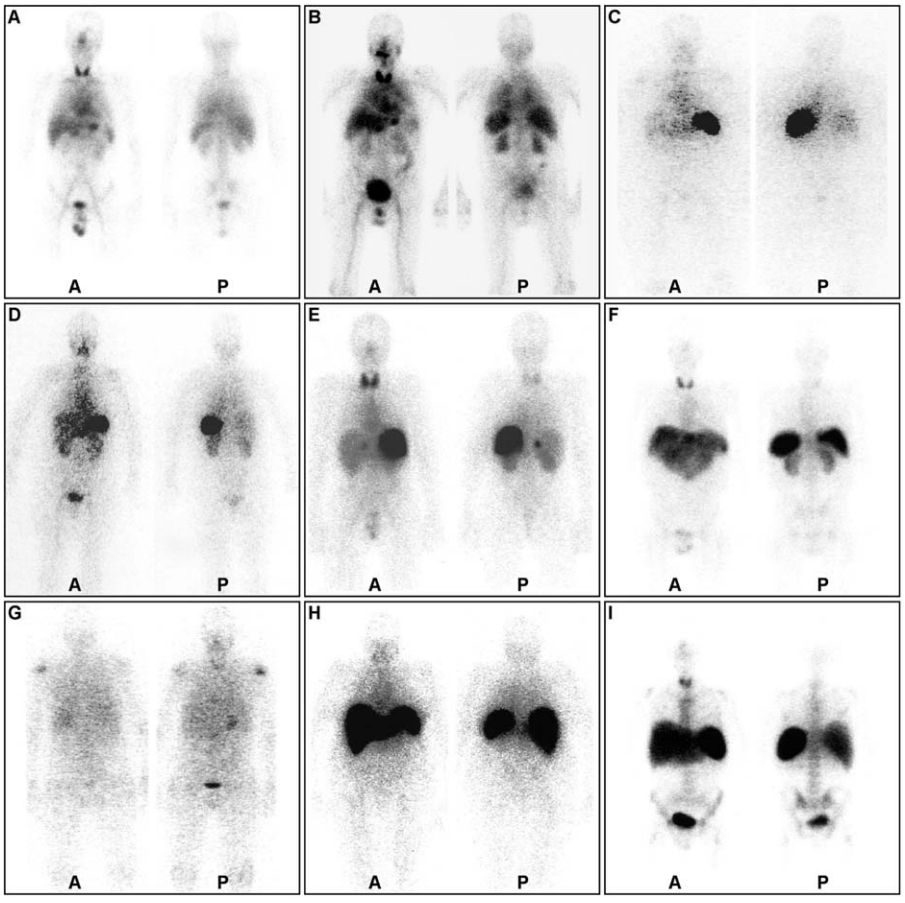

Figure 2 Total body ^{123}I-SAP scan, anterior (A) and posterior (P) view, 24 hours after injection. **(A)** Male control. Blood pool activity as well as minor nonspecific uptake can be seen in the (blocked) thyroid, nasopharynx, stomach, urine in bladder, and testicles. **(B-F)** Five patterns of organ uptake in AA amyloidosis. **(B)** Kidney uptake only (and prostatism with urine retention in bladder). **(C)** Intense splenic uptake only. **(D)** Spleen and kidney uptake. **(E)** Spleen, kidney, and adrenal gland. **(F)** Spleen, kidney and liver uptake (and nonspecific uptake in the stomach adjacent to the left liver lobe, providing the illusion of liver enlargement). **(G-I)** Three examples of organ uptake in AL amyloidosis. **(G)** Joints only (8%). **(H)** Spleen and liver (20%). **(I)** Spleen, liver, and bone marrow (10%).

normal kidney images, but proteinuria increased with renal uptake score (Figure 3A). Median proteinuria values were 0.4, 2.4, and 4.8 g/24 h for uptake scores 0, 1+, and 2+, respectively.

Adrenal uptake was seen in 20%, compared with adrenal failure in just 3%. However, clinical investigation was hampered because many AA patients had been treated with low-dose prednisolone for years. Joint uptake was not seen

Table 4 Percentages of AA and AL Patients with (C+) or Without (C−) Clinical Organ Involvement Who Had (S+) or Had Not (S−) Increased Uptake of ^{123}I-SAP

	AA (n = 60)				AL (n = 80)			
Tissue	C+/S+	C−/S+	C+/S−	C−/S−	C+/S+	C−/S+	C+/S−	C−/S−
Myocardium	0	0	18	82	0	0	55	45
Liver	3	5	2	90	28	16	3	54
Lungs	0	0	0	100	0	0	0	100
Spleen	8	78	0	13	18	58	1	24
Bone marrow	0	0	20*	80*	10	11	36	43
Kidneys	65	2	25	8	30	1	53	16
Adrenals	2	18	2	78	0	0	1	99
Joints	NA	NA	NA	NA	11	5	4	80

NA denotes not applicable.

*Denotes that only 10 of 60 AA patients underwent a bone marrow biopsy.

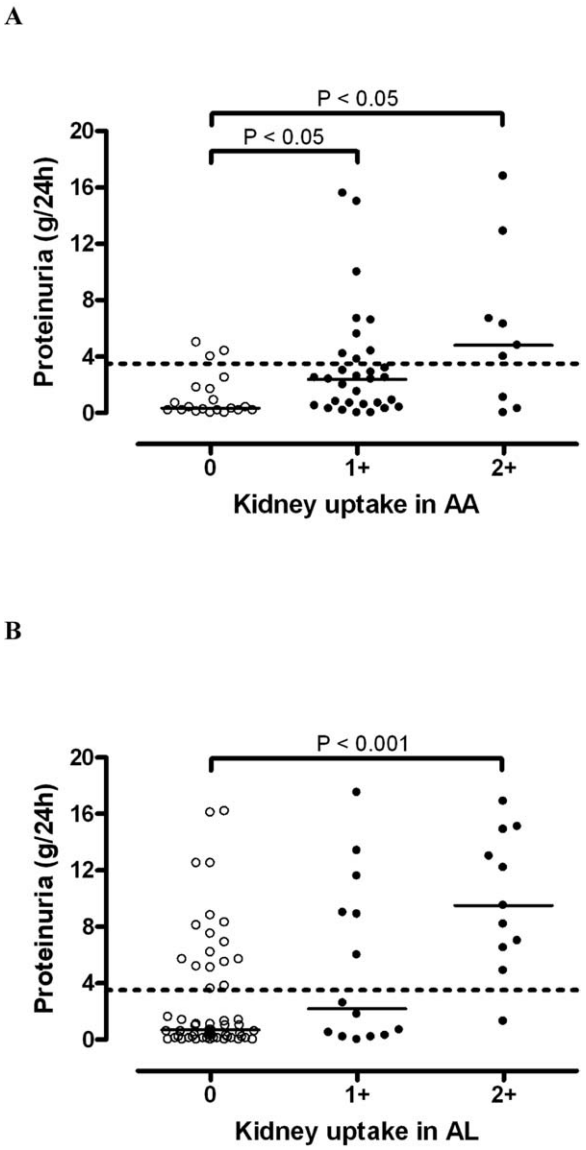

Figure 3 Proteinuria (g/24 h) and ^{123}I-SAP kidney uptake (negative uptake 0; positive uptake 1+ and 2+). The dashed line indicates heavy proteinuria (>3.5 g/24 h). Horizontal lines show median values. **(A)** AA patients. **(B)** AL patients.

in any of 12 patients without arthritis, whereas some joint uptake was seen in 8 of 48 arthritic patients. The 8 arthritic patients with joint uptake had higher serum C-reactive protein (CRP) levels (median value 47 vs 14.5 mg/L) than the 40 arthritic patients without joint uptake ($P < .005$), consistent with joint images representing the expected nonspecific extension of the blood-pool in patients with larger synovial effusions.

AL Amyloidosis. The findings also are summarized in Table 4. Presence or absence of liver involvement accorded with scan findings in 82% of cases. Positive liver scans were obtained in 16% of the patients in whom hepatic amyloid was not suspected clinically, whereas the opposite occurred in 3%. Serum alkaline phosphatase values among patients with 3+ hepatic uptake (median 244 kU/l) were higher ($P < .001$) than those without uptake (median 80 kU/L). Hepatic uptake was not associated with signs of backward failure of the heart.

Clinical involvement of the spleen was present in 19% of all AL patients, whereas abnormal splenic images were seen in 75%. Howell Jolly bodies were present in 26% of patients with 3+ splenic uptake and absent in all patients with lower uptake or no uptake ($P < .001$).

Bone marrow uptake was seen in 21% of AL patients. Small amyloid deposits were present in periosteal tissue or blood vessels in 43%, but this was not associated with bone marrow uptake. However, diffuse deposition of amyloid was present in bone marrow biopsies in 4% of patients, and all of these had intensive bone marrow uptake (2+).

Positive or negative renal images accorded with clinical evidence of involvement in 46% of the patients. Abnormal tracer uptake was not seen in 53% of cases; intense adjacent liver or spleen signal in 18 (43%) of these 42 cases obscured visualization of the kidneys. Creatinine clearance did not differ between patients with abnormal and normal kidney images, but proteinuria increased with renal uptake score (Figure 3B). Median proteinuria values were 0.7, 2.2, and 9.5 g/24 h for uptake scores 0, 1+, and 2+, respectively.

Adrenal uptake was not seen and adrenal failure was present in only 1 patient. Presence or absence of clinical joint involvement accorded with scan findings in 91% of AL patients. Thirty-eight percent of patients with joint uptake had CTS, compared with 15% of those without (n.s.).

ATTR Amyloidosis. Clinical evidence of splenic involvement was absent in all patients, whereas splenic amyloid was evident on SAP scintigraphy in 37%. Renal dysfunction was present in 15% of cases, which did not overlap at all with 11% of patients who had abnormal renal uptake on SAP scintigraphy. CTS was present in 22%, whereas joint uptake was absent in all patients. Neither clinical involvement nor abnormalities on SAP scintigraphy were present in liver, adrenal glands, joints, and bone marrow.

Localized Amyloidosis. No definite abnormal uptake of tracer was seen at the sites of the amyloid lesions. The significance of apparent minor and variable uptake in the spleen and kidneys in 3 patients is unclear. None of these latter patients had developed clinically overt systemic amyloidosis during follow-up of 3, 10, and 10 years, respectively.

DISCUSSION

This study confirms that ^{123}I-SAP scintigraphy is an effective noninvasive tool for diagnosis of systemic AA and AL amyloidosis, which provides additional information on the distribution and amount of amyloid in various visceral sites. Characteristic patterns of organ involvement can give a strong indication of amyloid fibril type, although substantial overlap between types does occur. The sensitivity of SAP scintigraphy is greatest for larger solid viscera, including the

spleen, liver and kidneys, more modest for bones and joints, but it is inadequate for evaluating the heart muscle. The chief value of SAP scintigraphy in localized amyloidosis is to provide further corroboration of the local nature of the disease by excluding systemic deposits.

The diagnostic sensitivity of SAP scintigraphy for AA and AL amyloidosis was 90% for both types but was substantially lower among patients with hereditary TTR amyloidosis at 48%. The frequency of positive images was especially low among patients with the most common TTRMet30 variant, contrasting with results of Rydh et al.[8] Although factors including the density and absolute amount of amyloid in various organs are major determinants in producing diagnostic images in SAP scintigraphy, the scoring method we chose to use here may also have been more stringent than those used in other studies. The overall diagnostic specificity of SAP scintigraphy for systemic amyloidosis was 93%, based on data derived from the pooled group of controls and the patients who had localized amyloidosis, although it was generally not possible to definitely confirm the presence or absence of amyloid in individual organs histologically.

The utility of SAP scintigraphy in systemic amyloidosis has not been clearly assessed yet. SAP scintigraphy is very useful to detect amyloidosis in patients with strong suspicion on amyloidosis in whom a biopsy is negative or equivocal. Besides the advantage of high diagnostic accuracy in AL and AA amyloidosis, the method lacks potential risks (eg, bleeding, infection, perforation) of invasive procedures such as biopsy of kidney, liver, or heart. On the other hand, however, amyloidosis is, by definition, a histological diagnosis, SAP is available in only a few centers, and ^{123}iodine is not generally available and rather expensive. Not only should the diagnostic value of body uptake be studied, but also the prognostic value of specific organ uptake and the possible influence of organ uptake on the application of specific treatment ultimately leading to a different clinical outcome for the patient.

Patterns of involved organs in patients with AA and AL differed markedly in some respects. The distribution of amyloidotic organs in AA amyloidosis reflected a continuum in which spleen, kidney, and a combination of both are the 3 key findings. Additional involvement of the liver or the adrenal glands was observed in a small proportion of cases. Although renal involvement is the presenting clinical feature in more than 85% of patients with AA amyloidosis,[13] splenic involvement is the most prominent early feature of experimentally induced AA amyloidosis in the widely used mouse model. Amyloid deposition starts in perifollicular regions of the spleen and spreads later to other organs such as liver and kidneys.[14] The high frequency of spleen positivity on scintigrams in our series supports the possibility that it may be possible to identify amyloid in the spleen of patients before clinically significant renal damage has occurred. On this note, the lack of splenic images in 13% of the AA patients in this study, which contrasts with virtually 100% involvement in previous reports, is puzzling and seems most likely to be explained by our inclusion of some patients with minimal clinical disease. For example, creatinine clearance of less than 60 mL/min, used as a criterion for classifying renal amyloid involvement, could have many other etiologies in patients with rheumatoid arthritis. Serial studies in patients with unexpectedly positive and negative findings may provide important clues to the natural history of AA amyloidosis.

The patterns of abnormal tracer uptake in AL were much more heterogeneous, concordant with the clinical differences between AA and AL amyloidosis. Uptake of bone marrow and adrenal glands is particularly dissimilar: bone marrow uptake occurred only in AL in 21% of cases, whereas adrenal gland uptake was seen in this study only in AA in 20% of patients. Adrenal gland uptake, however, is not specific for AA amyloidosis and has been described in AL[4,9] and ATTR patients.[8] Within the group of AL patients, kappa light chain class was more frequently associated with uptake in joints and bone marrow than lambda, in contrast to the greater proportion of patients with AL amyloid of lambda type in general.

The associations between findings on SAP scans and clinical manifestations of disease varied considerably between different organs. For example, splenic uptake is extremely frequent in AA and AL amyloidosis, but only a minority of patients has related clinical signs. On the other hand, all 8 AL patients with manifestations of splenic involvement as evidenced by Howell Jolly bodies had overwhelming (3+) uptake in the spleen on SAP scintigraphy. Liver involvement suggested on scans also was not evident clinically in many cases, possibly reflecting the very nonspecific indicators of size and elevated serum alkaline phosphatase that are widely used to clinically categorize the presence or absence of hepatic amyloid. Enlargement of the liver occurs as a reactive phenomenon in a proportion of patients with chronic inflammatory diseases and in patients with fluid retention, and serum alkaline phosphatase can be increased by more than 50% both as a manifestation of cardiac failure and the acute phase response. In 15 autopsy cases with systemic amyloidosis, performed 8 hours to 5 months after SAP scintigraphy, histological and scintigraphic estimates of the quantity of amyloid in the liver ($r = 0.95$) and spleen ($r = 0.90$) showed a close correlation.[15] Therefore, SAP scintigraphy may have a useful role in detecting splenic and hepatic involvement when it is present without causing detectable clinical abnormalities. Although certain organs can continue to function in an apparently normal manner despite the presence of substantial amyloid deposition, such organs may nevertheless function inadequately or fail altogether under stress, including during chemotherapy, hypovolemia, surgery and infections. More intense bone marrow uptake, equal or greater to 2+ in our scoring scale, accorded with diffuse deposition of amyloid in bone marrow biopsies.

Joint uptake is less useful because, although it occurred in some AL patients with arthropathy, synovial effusions in arthritic joints represent an extension of the blood-pool background in all nuclear medicine procedures. Therefore, nonspecific images of such joints were not unexpected in controls and AA patients who had arthritis.

Our findings here further confirm that SAP scintigraphy is not suitable for evaluating myocardial amyloid, which was present clinically in many patients in this series. Echocardiography and the serum markers NT-proBNP and troponin are currently the best means of evaluating myocardial involvement in routine practice.[16,17] There are many plausible factors that may contribute to the poor performance of labeled SAP studies in imaging amyloidotic heart muscle, including movement artifact, ventricular blood-pool content, and frequent intense uptake of tracer into the adjacent spleen. However, the most important factor is likely to be the lack of a fenestrated endothelium in the myocardium, hindering access of the large 127 kDa tracer to the amyloidotic interstitium within the available timescale of the short half-life ^{123}iodine isotope. Myocardial uptake has been demonstrated over 48-72 hours using ^{131}iodine, which has a half-life of 8 days, but which is generally unsuitable for routine clinical use.[18,19]

Besides diagnosis, SAP scintigraphy can also be used to monitor the amyloid load of the body during treatment. Cure of the underlying disease results in stabilization or regression of visceral uptake on serial SAP scans in systemic amyloidosis of the AA type,[20-22] of the AL type,[23,24] and of the ATTR type.[8] Liver uptake is associated with major amyloid in other organ systems and carries a poor prognosis in AA type.[25]

Other tracers have been described for visualizing amyloidosis, including β2-microglobulin in dialysis-related amyloidosis,[26] aprotinin in AL amyloidosis,[27] pyrophosphates and diphosphonates in AA and AL amyloidosis,[28] and 3,3-diphospho-1,2-propanodicarboxic-acid (DPD) in ATTR amyloidosis.[29] Whereas the mechanism responsible for affinity of the tracer for amyloid may be specific in the case of β2-microglobulin, which is the amyloid fibril precursor protein in dialysis-related amyloidosis, mechanisms responsible for the localization of DPD and other diphosphonates and aprotinin are nonspecific and are probably due to charge interactions.

In summary, SAP scintigraphy permits the noninvasive diagnosis of amyloidosis in the majority of patients with AA and AL types and enables deposits to be imaged in many organs before this is apparent clinically. Although histological examination of tissue remains the diagnostic gold-standard, SAP scintigraphy offers the only means to serially monitor amyloid throughout the body in a quantitative manner. SAP scintigraphy may be of value in addressing the many evident limitations in clinical criteria that are widely used to define involvement by amyloid in organs including the liver, kidneys, adrenals, and spleen in patients who have been shown to have amyloid histologically at other sites.

ACKNOWLEDGMENTS

We would like to thank C. Th. Smit Sibinga, W. Tuuk Adriani, P.C. Limburg, and J. Bijzet for manufacturing SAP, and A.K. van Zanten, C. Harms, H. Pol, H. Nijnuis, and J.Boorsma for performing labeling studies and laboratory analysis. The "Dutch Arthritis Foundation" and the "Jan Kornelis de Cock Stichting" provided financial support for this study.

References

1. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med*. 2003;349:583-596.
2. Falk RH, Comenzo RL, Skinner M. The systemic amyloidoses. *N Engl J Med*. 1997;337:898-909.
3. Pepys MB, Booth DR, Hutchinson WL, et al. Amyloid P component: a critical review. *Amyloid*. 1997;4:274-295.
4. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with 123I-labeled serum amyloid P component. *N Engl J Med*. 1990;323:508-513.
5. Hawkins PN, Wootton R, Pepys MB. Metabolic studies of radioiodinated serum amyloid P component in normal subjects and patients with systemic amyloidosis. *J Clin Invest*. 1990;86:1862-1869.
6. Hawkins PN, Richardson S, MacSweeney JE, et al. Scintigraphic quantification and serial monitoring of human visceral amyloid deposits provide evidence for turnover and regression. *Q J Med*. 1993;86:365-374.
7. Maulin L, Hachulla E, Deveaux M, et al. 'Localized amyloidosis': ^{123}I-labelled SAP component scintigraphy and labial salivary gland biopsy. *Q J Med*. 1997;90:45-50.
8. Rydh A, Suhr O, Hietala S-O, et al. Serum amyloid P component scintigraphy in familial amyloid polyneuropathy: regression of visceral amyloid following liver transplantation. *Eur J Nucl Med*. 1998;25:709-713.
9. Hachulla E, Maulin L, Deveaux M, et al. Prospective and serial study of primary amyloidosis with serum amyloid P component scintigraphy: from diagnosis to prognosis. *Am J Med*. 1996;101:77-87.
10. Hazenberg BP, van Gameren II, Bijzet J, et al. Diagnostic and therapeutic approach of systemic amyloidosis. *Neth J Med*. 2004;62:121-128.
11. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol*. 1995;32:45-59.
12. Jager PL, Hazenberg BP, Franssen EJF, et al. Kinetic studies with iodine-123-labeled serum amyloid P component in patients with systemic AA and AL amyloidosis and assessment of clinical value. *J Nucl Med*. 1998;39:699-706.
13. Janssen S, van Rijswijk MH, Meijer S, et al. Systemic amyloidosis: a clinical survey of 144 cases. *Neth J Med*. 1986;29:376-385.
14. Snow AD, Kisilevsky R. Temporal relationship between glycosaminoglycan accumulation and amyloid deposition during experimental amyloidosis: a histochemical study. *Lab Invest*. 1985;53:37-44.
15. Vigushin DM, Pepys MB, Hawkins PN. Comparison of histology with SAP scintigraphy for evaluation of amyloidosis. In: Kisilewsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. *Amyloid and Amyloidosis 1993*. New York, New York: Parthenon Publishing; 1994;685-687.
16. Palladini G, Campana C, Klersy C, et al. Serum N-terminal pro-brain natriuretic peptide is a sensitive marker of myocardial dysfunction in AL amyloidosis. *Circulation*. 2003;107:2440-2445.
17. Dispenzieri A, Gertz MA, Kyle RA, et al. Prognostication of survival using cardiac troponins and N-terminal pro-brain natriuretic peptide in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. *Blood*. 2004;104:1881-1887.

18. Hawkins PN, Myers MJ, Lavender JP, Pepys MB. Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet*. 1988;1:1413-1418.
19. Hawkins PN, Aprile C, Capri G, et al. Scintigraphic imaging and turnover studies with iodine-131 labelled serum amyloid P component in systemic amyloidosis. *Eur J Nucl Med*. 1998;25:701-708.
20. Hawkins PN, Richardson S, Vigushin DM, et al. Serum amyloid P component scintigraphy and turnover studies for diagnosis and quantitative monitoring of AA amyloidosis in juvenile rheumatoid arthritis. *Arthritis Rheum*. 1993;36:842-851.
21. Gillmore JD, Lovat LB, Persey MR, et al. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet*. 2001;358:24-29.
22. Lachmann HJ, Gilbertson JA, Gillmore JD, et al. Unicentric Castleman's disease complicated by systemic AA amyloidosis: a curable disease. QJM 2002;95:211-218.
23. van Gameren II, Hazenberg BPC, Jager PL, et al. AL amyloidosis treated with induction chemotherapy with VAD followed by high dose melphalan and autologous stem cell transplantation. *Amyloid*. 2002;9:165-174.
24. Lachmann HJ, Gallimore R, Gillmore JD, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. *Br J Haematol*. 2003;122:78-84.
25. Lovat LB, Persey MR, Madhoo S, et al. The liver in systemic amyloidosis: insights from ^{123}I serum amyloid P component scintigraphy in 484 patients. *Gut*. 1998;42:727-734.
26. Floege J, Burchert W, Brandis A, et al. Imaging of dialysis-related amyloid (AB-amyloid) deposits with 131-I-β2-microglobulin. *Kidney Int*. 1990;38:1169-1176.
27. Aprile C, Marinone G, Saponaro R, et al. Cardiac and pleuropulmonary AL amyloid imaging with technetium-99-m labelled aprotinin. *Eur J Nucl Med*. 1995;22:1393-1401.
28. Janssen S, Piers DA, van Rijswijk MH, et al. Soft-tissue uptake of 99mTc-diphosphonate and 99mTc-pyrophosphate in amyloidosis. *Eur J Nucl Med*. 1990;16:663-670.
29. Puille M, Altland K, Linke RP, et al. 99mTc-DPD scintigraphy in transthyretin-related familial amyloidotic polyneuropathy. *Eur J Nucl Med*. 2002;29:376-379.