Serum amyloid P component scintigraphy in familial amyloid polyneuropathy: regression of visceral amyloid following liver transplantation

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Abstract. Familial amyloid polyneuropathy (FAP) associated with transthyretin (TTR) mutations is the commonest type of hereditary amyloidosis. Plasma TTR is produced almost exclusively in the liver and orthotopic liver transplantation is the only available treatment, although the clinical outcome varies. Serum amyloid P component (SAP) scintigraphy is a method for identifying and quantitatively monitoring amyloid deposits in vivo, but it has not previously been used to study the outcome of visceral amyloid deposits in FAP following liver transplantation. Whole body scintigraphy following injection of iodine-123 labelled SAP was performed in 17 patients with FAP associated with TTR Met30 and in five asymptomatic gene carriers. Follow-up studies were performed in ten patients, eight of whom had undergone orthotopic liver transplantation 1–5 years beforehand. There was abnormal uptake of ^{123}I-SAP in all FAP patients, including the kidneys in each case, the spleen in five cases and the adrenal glands in three cases. Renal amyloid deposits were also present in three of the asymptomatic carriers. Follow-up studies 1–5 years after liver transplantation showed that there had been substantial regression of the visceral amyloid deposits in two patients and modest improvement in three cases. The amyloid deposits were unchanged in two patients. In conclusion, ^{123}I-SAP scintigraphy identified unsuspected visceral amyloid in each patient with FAP due to TTR Met30. The universal presence of renal amyloid probably underlies the high frequency of renal failure that occurs in FAP following liver transplantation. The variable capacity of patients to mobilise amyloid deposits following liver transplantation may contribute to their long-term clinical outcome.

Key words: Amyloidosis – Polyneuropathy – Serum amyloid P component – Scintigraphy – Liver transplantation

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Introduction

Amyloid deposition occurs in a diverse range of disorders that includes Alzheimer's disease, maturity onset diabetes and the transmissible spongiform encephalopathies as well as the more well-known systemic amyloidoses [1]. Although acquired systemic AA and AL amyloidosis are uncommon, they are significant because the diagnosis is often difficult, and effective treatments have increasingly become available [2]. Hereditary amyloidosis, which usually presents as familial amyloid polyneuropathy (FAP), is extremely rare, but is important as a model for studying the pathogenesis of amyloidosis generally [3]. FAP is an autosomal dominant inherited syndrome associated with more than 60 different transthyretin (TTR) gene mutations and characterised by systemic deposition of amyloid composed of variant TTR [4]. The disease presents at any time from the second decade, typically with peripheral and autonomic neuropathy and varying degrees of visceral amyloid involvement. It is almost always fatal within 5–15 years. Major foci of FAP associated with the commonest TTR Met30 variant occur in Portugal, Japan and Sweden although kindreds have been reported throughout the world.

Treatment of FAP has lately been revolutionised by orthotopic liver transplantation (OLT). This was pioneered in Sweden in 1990 based on evidence that the liver was the main source of circulating TTR [5]. Following surgery, variant TTR in the plasma is rapidly replaced by normal wild-type protein and, following favourable early reports [6], OLT has been performed in hundreds of patients with FAP in many centres throughout the world [7]. Available follow-up data suggest that the mortality of OLT is 20% in patients with FAP (FAP World Transplant Registry), and that, although the autonomic disease can sometimes improve substantially, the

peripheral neuropathy usually only stabilises. Rehabilitation may be very slow and postoperative renal failure has been reported in up to 20% of patients. The long-term outcome of OLT remains to be determined.

All amyloid deposits contain a minor non-fibrillar constituent, amyloid P component, a glycoprotein that is derived from and identical to the normal plasma protein serum amyloid P component (SAP) [1]. SAP binds to amyloid fibrils via a specific calcium-dependent mechanism which is the basis for our development of radiolabelled SAP as a diagnostic nuclear medicine tracer in patients with amyloidosis [8–10]. We have previously shown that AA and AL amyloid deposits can be monitored quantitatively using SAP scintigraphy [11], and here we report radiolabelled SAP imaging studies in 22 Swedish subjects with TTR Met30, eight of whom were followed up after OLT. The results provide the first objective evidence that TTR amyloid deposits in FAP can regress following transplantation.

Materials and methods

Patients. Twenty-two individuals with TTR Met30 were studied. Twelve were male and ten were female and they were aged 25–63 (mean 45) years. Seventeen of the patients had had typical clinical features of FAP for 1–7.5 years, and five individuals were asymptomatic. Follow-up studies were performed 1–5 years after OLT in eight cases, and after 12 and 15 months respectively in two other patients who were not transplanted.

Diagnosis and clinical evaluation. All subjects in the study were confirmed to be TTR Met30 heterozygotes by PCR or Southern blot analysis [12]. Amyloid deposits were confirmed histologically in intestinal or subcutaneous fat biopsies in each of the 17 patients with clinical features of FAP, all of whom had evidence of an axonal polyneuropathy on electrophysiological testing. Amyloid was not identified in subcutaneous fat biopsies in any of the five asymptomatic individuals, and nerve conduction studies in them were also normal.

The patients' neurological status was graded according to our previously described polyneuropathy disability index [13], scored as follows: grade 0, asymptomatic; grade I, sensory disturbance with normal motor function; grade II, impaired walking ability but not requiring a stick or crutch; grade IIIA, ability to walk but with one stick or crutch; grade IIIB, walking with two sticks or crutches; grade IV, confinement to bed or a wheelchair.

SAP scintigraphy. Radiolabelled SAP scintigraphy was performed as previously described [8, 9]. Purified SAP was labelled with ^{123}I using the *N*-bromosuccinimide method [14]. Each patient received approximately 100 μg SAP bearing 200 MBq of ^{123}I, corresponding to an effective dose equivalent of ~ 3 mSv. Thyroid uptake was blocked by oral ingestion of potassium iodide for 2 days before and following radionuclide injection. Anterior and posterior whole body scintigraphy and static abdominal imaging were performed 24 h after radionuclide administration using a GE-STAR-3000 gamma camera. The images were interpreted by two physicians (A.R. and P.N.H.) with combined experience of more than 1500 labelled SAP studies, including 500 patients with non-amyloid diseases. Abnormal localisation of tracer in various organs was graded + when the signal was discernibly above normal blood pool levels, ++ when the intensity of abnormal uptake was substantially greater than the blood pool signal but could still be visualised within the usual blood pool grey scale, and +++ when the abnormal signal was so intense that definition of blood pool was partially lost when the grey scale was adjusted to encompass the target organ uptake. (Localisation scoring +++ represents substantially greater amyloid infiltration than a score of ++ [9].)

Ethics. The study was carried out according to the Declaration of Helsinki and approved by the Umeå University Committee of Ethics and by the Isotope Committee. The Swedish Medical Products Agency approved the administration of the SAP protein to the patients in this study.

Results

Normal blood pool images were obtained in two of the five asymptomatic TTR Met30 carriers whereas amyloid was identified in the kidneys of the three other cases (Table 1). There was abnormal visceral localisation of labelled SAP in all 17 FAP patients, including the kidneys in each case, the spleen in five cases and the adrenal glands in three cases (Table 1). Liver scintigraphy was normal in all patients, and the absence of hepatic amyloid was confirmed histologically in each of the eight patients who underwent OLT. Accumulation of tracer could not be detected in the heart, gastrointestinal tract or nerves in any subject. The quantity of tracer that localised to the spleen, kidneys and adrenal glands and its relative distribution between these organs varied considerably among the FAP patients (Table 2) and did not correlate with neurological impairment as estimated by the polyneuropathy disability index.

Table 1. Results of ^{123}I SAP scintigraphy prior to liver transplantation

Localisation of tracer		Liver	Spleen	Kidneys	Adrenals
Asymptomatic individuals with TTR Met30 (n=5)					
Minor uptake	+	0	0	3	0
Moderate uptake	++	0	0	0	0
Heavy uptake	+++	0	0	0	0
Patients with FAP associated with TTR Met30 (n=17)					
Minor uptake	+	0	2	15	1
Moderate uptake	++	0	1	2	1
Heavy uptake	+++	0	2	0	1

Table 2. Clinical outcome and follow-up SAP scintigraphy of patients with FAP following liver transplantation

Pt	Before liver transplantation (OLT)					Following liver transplantation				
	Months before OLT	Uptake of tracer at ^{123}I–SAP scintigraphy in			*PND	Months after OLT	Uptake of tracer at ^{123}I–SAP scintigraphy in			*PND
		Spleen	Kidneys	Adrenals			Spleen	Kidneys	Adrenals	
1	3	+	+	0	IIIB	12	+	+	0	IIIA
						55	+	+	0	IIIA
2	3	+++	++	++	IIIB	15	++	+	0	IIIB
						36	+	+	0	IIIB
3	3	0	+	0	II	14	0	+	0	II
						29	0	+	0	II
4	12	0	+	0	I	15	0	+	0	I
5	7	0	++	0	I	12	0	+	0	II
6	11	++	++	+++	II	15	+	+	+	II
7	11	0	+	0	II	18	0	+/0	0	II
8	13	++	+	0	IIIA	12	+	+	0	IIIB

[a] PND (polyneuropathy disability index) and scoring for organ uptake of labelled SAP are described in Materials and methods

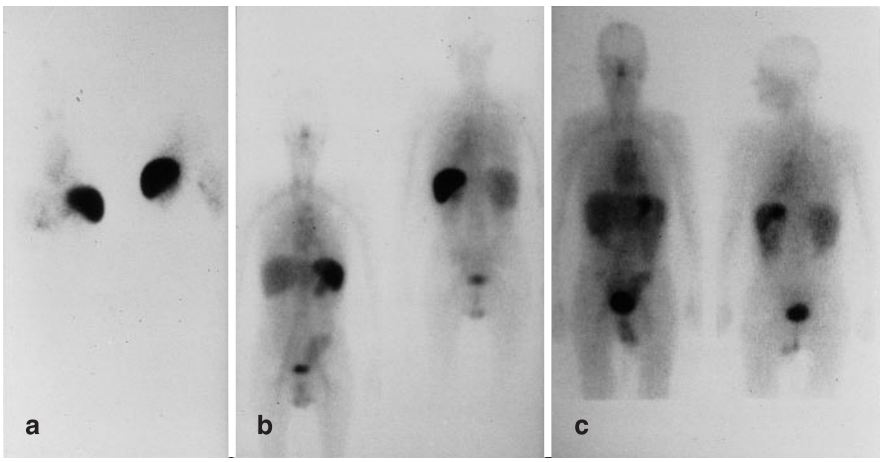

Fig. 1a–c. Serial anterior (*left*) and posterior (*right*) whole body ^{123}I-SAP scintigraphy in a patient with FAP (Table 2 patient 2), showing substantial regression of amyloid deposits in the spleen, kidneys and adrenal glands following OLT. The studies were performed 3 months before surgery (**a**), and 15 and 36 months afterwards (**b** and **c**, respectively)

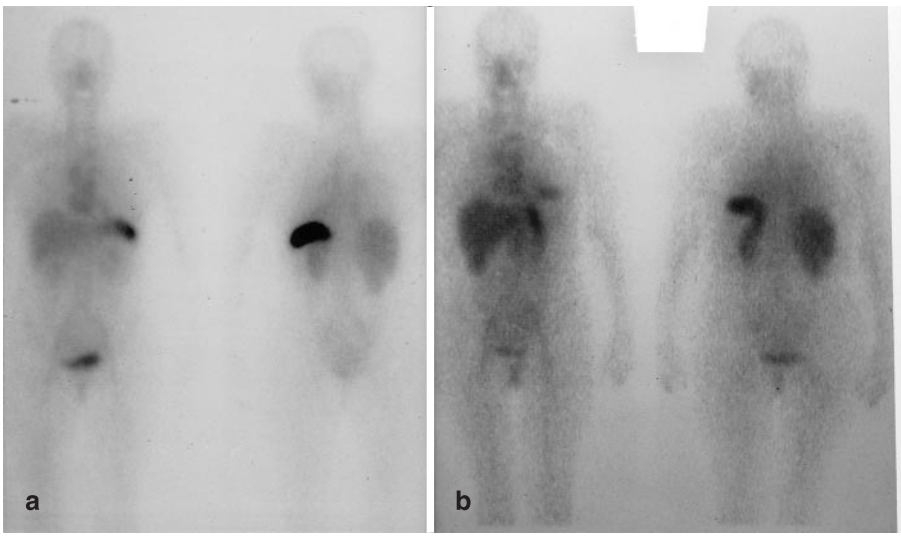

Fig. 2. Serial anterior (*left*) and posterior (*right*) whole body ^{123}I-SAP scintigraphy in a patient with FAP (Table 2, patient 8) before (**a**) and 12 months after (**b**) OLT, showing modest regression of amyloid deposits in the spleen but not the kidneys

Follow-up studies performed after 12 and 15 months in two patients who remained asymptomatic and had not undergone OLT did not show any detectable progression of the amyloid deposits in, respectively, their kidneys, spleen and adrenal glands or kidneys alone. By contrast, among the eight patients who had had OLT, repeat SAP scintigraphy 12–18 months after surgery showed the visceral amyloid deposits had regressed in five cases and had remained stable in the three others (Table 2). Regression of amyloid was substantial in two cases (Fig. 1), signified by reduction of the tracer signal in at least one organ from a score of +++ to ++ or less, and was modest in three patients (Fig. 2). Additional studies performed in three of these patients 29, 36 and 55 months respectively after OLT showed that the changes observed at the first follow-up study were sustained in each case, that is, there had been further regression of amyloid in one patient and the deposits had continued to remain stable in the two others.

There was no substantial improvement in the polyneuropathy disability index among the patients who underwent OLT, even those followed up for 3–5 years.

Discussion

The specificity with which labelled SAP localises to amyloid has been confirmed in more than 1500 clinical studies in the Hammersmith unit, involving more than 1000 patients with various types of amyloidosis and 500 disease controls [10, 11]. The proportionate manner in which SAP binds to the fibrils has been demonstrated not only in vitro [15], but also in an animal experiment involving more than 500 mice [16] and in clinical autopsy studies [9, 17], all of which confirm remarkable quantitative concordance between labelled SAP uptake and amyloid histology, and exclude any deiodination or other alteration of the radio-iodinated SAP tracer in vivo. The exceptional capacity for labelled SAP to target amyloid is explicitly illustrated by the abundance of native SAP in amyloid deposits relative to its trace concentration in the plasma: SAP represents only 0.04% of plasma proteins but up to 15% of amyloid deposits [18]. In healthy controls labelled SAP equilibrates very rapidly within the circulation and extravascular space, from which it declines at a constant monoexponential rate that does not vary significantly from subject to subject. In patients with amyloidosis, labelled SAP tracer localises rapidly and quantitatively to the amyloid deposits, in proportion to the amount of normal autologous SAP present [9, 17]. The reversible nature of this interaction, and the ensuing equilibrium of SAP between amyloid and plasma, means that the population of native SAP molecules associated with amyloid deposits is constantly turning over, and that the fibril ligands are uniformly available for radiolabelled SAP irrespective of whether amyloid is being actively deposited or mobilised, or is in a steady state [19].

The present study indicates that SAP scintigraphy has a role in the diagnosis and monitoring of patients with FAP associated with TTR Met30, and provides the first systematic objective evidence that TTR amyloid deposits often regress following OLT. Visceral amyloid was identified in all of the FAP patients and in three out of the five apparently healthy TTR Met30 carriers. Renal deposits were present in all of the patients, and this presumably underlies the high frequency of renal failure that occurs in FAP following OLT. Splenic deposits were present in 30% of patients whereas the adrenal glands were only occasionally involved. Although amyloid deposition in both of these latter sites is rarely evident clinically, its presence could be significant during and after liver transplantation in terms of splenic fragility, susceptibility to certain infections and impaired corticosteroid reserve. Liver deposits were not evident in any patient, and this was confirmed histologically in all eight patients who underwent OLT, supporting the idea that these organs could potentially be re-used in “domino” transplants. Consistent with our extensive experience of SAP scintigraphy in patients with systemic AA and AL amyloidosis, amyloid deposits in the nerves, gastrointestinal tract or heart were not detected because the intensity of tracer signal does not sufficiently exceed blood pool background activity in these sites. Throughout this study, the amount of labelled SAP uptake scored visually, and the subsequent changes in this following OLT, were corroborated by measurements of plasma clearance of the tracer (data not shown).

The factors that determine whether TTR amyloid deposits can regress, and the differing rates at which this occurs after OLT, are unknown. The variable degree of amyloid regression among five out of the eight transplanted patients in this series is similar to findings in patients with AA and AL amyloidosis in whom various treatments had substantially reduced the supply of their respective amyloid fibril precursor proteins [20–22]. The lack of accumulation of amyloid in follow-up studies of the two untreated FAP patients suggests that an equilibrium between the rates of amyloid deposition and mobilisation had been attained in these particular cases.

Liver transplantation is the only available treatment for FAP. The lack of improvement in peripheral nerve function among the eight transplanted patients in this series is consistent with reports from most other centres, and in view of the generally favourable scintigraphic findings may largely be due to the limited capacity of peripheral nerves to regenerate. However, autonomic dysfunction in FAP frequently recovers after OLT [6], leading to substantial improvements in nutritional status, and there have lately been echocardiographic studies that have shown reduction in ventricular wall thickness among patients with TTR Met30 after liver transplantation. These observations provide further evidence that regression of amyloid may be the usual mechanism through which clinical improvements occur following OLT. It remains to be determined in longer term studies

of OLT in FAP to what extent the particular capacity of an individual to mobilise their visceral amyloid deposits might influence their overall outcome.

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