Incidence of amyloidosis over 3 years: the AMYPRO study

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Abstract

Objectives

There is a lack of epidemiological information concerning amyloidosis, particularly in France. We started a 3-year prospective study (AMPYRO) to analyze the epidemiological features of amyloidosis in the eastern part of France.

Methods

From 2003 to 2005, all patients with a tissue sample showing amyloid deposits, were included in this study. Immunohistochemistry using anti-P component, anti-SAA, anti-light chains immunoglobulins and anti-transthyretin was applied for each tissue sample. For each patient, past and present medical histories along with biological features were recorded.

Results

Seventy-six patients with amyloid were identified over 3 years. The age-standardized incidence rate of amyloidosis was estimated at 14 cases per million person-years. The final entire population included in the AMYPRO study was composed of 66 patients with a mean age of 71.7±11.5 years old. The amyloid typing after clinical, biological and immunohistochemistry revealed senile amyloid in 40 cases (60.6%), AL amyloid in 13 (19.7%) and AA amyloid in 9 (13.6%). Neither clinical nor biological features differed significantly between the transthyretin-positive and transthyretin-negative populations.

Conclusion

Regarding only tissue samples, senile amyloid was the most prominent amyloid type identified. Therefore, the clinician needs to be aware that in most of the amyloid cases identified on the pathologic examination there is no need for additional examination unless there are clinical or biological signs of a primary or secondary amyloidosis.

Key words
Amyloid, epidemiology, biopsy.

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Introduction

The diagnosis of amyloidosis is first suspected after clinical and biological examination, but is only confirmed with the pathologic examination of a tissue sample stained with Congo Red showing a green apple birefringency under polarized light. After identifying the amyloid nature of the deposit, the physician needs to type the amyloid to decide whether the patient needs a particular treatment or not.

As for many rare diseases, there is a lack of epidemiological information concerning amyloidosis. Most of the data concerns the systemic primary amyloid and the secondary amyloid and among them most are derived from autopsy studies. The questions of how many, what type, and from where remained unsolved.

We started a prospective study (AMYloidosis PROspective: AMYPRO) in the eastern part of France (Franche-Comté) to record each tissue sample with amyloid deposits. The aim of AMYPRO was to determine the incidence of amyloidosis in 3 French geographical regions of Franche-Comté, whether the amyloid deposit was associated with a real disease or not, and what type of amyloid was identified among primary, secondary, senile and hereditary amyloidoses.

Material and methods

Patients

The records of all patients with a histopathological diagnosis of amyloid (*i.e.*, Congo Red-positive tissue) were obtained from the Besançon University Hospital and the Belfort-Montbeliard Community Hospital and their affiliated Departments of Pathology for the period January 1, 2003 to December 31, 2005. Both Hospitals were required to care for the population of 3 French geographical regions: Doubs, Haute-Saône, Territoire de Belfort belonging to the Franche-Comté Region (eastern part of France). A residency criterion was applied so that only those patients who had lived in the 3 French geographical regions for at least 1 year before the diagnosis of amyloidosis were considered to be residents. Population by age and French geographical region was estimated by the French National Census Bureau (Insee) (1). The 2004 values (883,726 persons) were used in the statistical analyses. The only inclusion criterion was the demonstration of amyloid in paraffin blocks from biopsy on the basis of apple-green birefringence when stained with Congo Red (2) and viewed under polarized light. Exclusion criteria included amyloid diagnosis made before the inclusion period, patients living in another French geographical region than those mentioned above, and patients deceased just after the diagnosis implying no possibility of a complete clinical examination. For each patient, location of the initial biopsy, the extension of the amyloid process (multiple positive biopsies), and a complete clinical examination and history were recorded and standard biological tests were performed including C reactive protein, creatinine level (creatinine clearance), ASAT, ALAT, gammaGT, and, if necessary, immunoelectrophoresis and Bence-Jones proteinuria. Because of abnormalities at the initial clinical examination, some patients had echocardiography and/or electromyography.

Methods

Immunohistochemistry

Each amyloid sample was submitted to immunohistochemistry using different antibodies to confirm Congo Red staining (anti-P component) and to confirm the typing of amyloid (anti-light chains, anti-TTR, anti-SAA). The immunohistochemistry step was performed in the Pathology Department of Besançon University Hospital. Four antibodies were used according to the manufacturer's instructions: anti-P component (A302, dilution 1/400, Dako, Denmark), anti-transthyretine (A002, dilution 1/10, Dako, Denmark), anti-SAA (M759, dilution1/400, Dako, Denmark), anti-kappa light chains (A191, dilution 1/30000, Dako, Denmark) and anti-lambda light chains (A193, dilution 1/5000, Dako, Denmark). At the end of the immunohistochemistry staining, an amyloid typing was proposed including the clinical, biological, echocardiography and/or electromyography and immunohistochemistry findings. Four amyloid types were distinguished: secondary amyloid

(AA amyloid), primary amyloid (AL amyloid), senile amyloid (SA), hereditary amyloid (AF) and if the type was not determined the patient was included in a group called "undetermined".

Statistical analysis

Statistical analyses were conducted using EPI Info 6.04cfr (CDC, Atlanta, USA). The baseline characteristics of the studied patients were expressed as numbers and percentages for categorical variables and as means±standard deviations (SD) for continuous variables. For univariate analysis, the chi-square and Fisher exact tests were used for categorical variables, and the Student's *t*-test was used for continuous variables. For all statistical analyses, $p<0.05$ was considered as significant. Age-standardized rates were calculated using the World global population as a reference (3).

Results

Clinical and biological characteristics (Table I)

During the study period (January 2003 to December 2005), 97 patients were diagnosed as having amyloid deposits on at least one tissue sample. Three patients were deceased before any examination, 17 patients were residents of another geographical region than those mentioned above, 7 patients had an incomplete clinical file and 4 patients had a diagnosis made before 2003. The calculation of amyloid incidence rates used 76 patients because it included the 3 patients that were deceased and the 7 patients with an incomplete file. Actually, for those 10 patients the previous medical history and the results of the immunohistochemistry were used to type presumptively the amyloid deposit (8 were suspected as having senile amyloid, 1 AL amyloid, 1 hereditary amyloid and 1 undetermined amyloid) (Fig. 1). The age-standardized incidence of amyloidosis was estimated at 14 per million person-years with a 95% confidence interval (CI) between 10.5 and 17.5 (Table II). Forty-four patients (57.9%) were living in the Doubs, 22 (28.9%) in the Haute-Saône and 10 (13.2%) in the Territoire de Belfort. Thirty-one diagnoses (40.8%) were made in 2003, 27 (35.5%) in 2004 and 18 (23.7%) in 2005. The population was composed with 42 men (55.2%) and 34 women (44.7%) with a mean age of 71.7 years±11.5 (41-96 yrs). Only 66 patients (called *whole population*) were enrolled completely in the AMYPRO study and were used for recording clinical and biological criteria. Among them, 18 (28.3%) had a previous history of atrial fibrillation and 18 patients had a previous history of renal failure. Only one patient had a familial history of amyloid. The mean level of CRP was 24.5 mg/L±47, the mean creatinine clearance was 56.1 ml/min ± 31, the level of ASAT or ALAT was slightly elevated (1.5-2N) in 2 patients, and no modification of gammaGT level was observed. An abnormal immunofixation was identified in 13 patients (19.7%) and a positive Bence-Jones proteinuria in 5 patients (7.6%). Forty-six patients (69.7%) had echocardiography, in which the mean of left ventricular ejection fraction was 31.6%±30.7; none of the 46 patients showed typical features of amyloid cardiomyopathy. Seven patients (10.6%) had an electromyography and 6 of them had abnormalities, in which 3 showed a typical amyloid neuropathy.

Table I. Clinical and biological findings of the amyloid population.

	Whole Population n=66	TTR-negative amyloid n=25		*TTR*-positive amyloid n=41
		AA Amyloid n=9	AL Amyloid n=13	Senile Amyloid n=40
Age (yr), mean, ± SD	71.6 ± 11.3	70.9 ± 11.1	73 ± 11.3	72 ± 11.3
M/F sex ratio (%M)	37/29 (56)	1/8 (11)	6/7 (46.2)	27/13 (67.5)
Previous history				
Atrial fibrillation	18 (27.3)	1 (11.1)	3 (23.1)	13 (32.5)
Renal failure	18 (27.3)	6 (66.1)	8 (61.5)	3 (7.5)
Initial biopsy site				
Cardiac valvular	28 (42.4)			28 (70)
Liver or GI	14 (21.2)	4 (44.4)	3 (23.1%)	5 (12.5)
Kidney	8 (12.1)	3 (33.3)	5 (38.5%)	
Salivary glands	8 (12.1)	2 (22.2)	5 (38.5%)	1 (2.5)
Yellow ligament	3 (4.5)			3 (7.5)
Carotid artery	2 (3)			2 (5)
Eyelid	1 (1.5)			1 (2.5)
Fat aspiration	1 (1.5)			
Brain	1 (1.5)			
Patients with more than 1 biopsy	10 (15.2)	3 (33.3)	6 (46.2)	1 (2.5)
Biological findings				
C reactive protein (mg/L)	24.5 ± 47	84.3 ± 91	25.1 ± 35.1	11.9 ± 24.2
Creatinine clairance (ml/min)	56.1 ± 31	38.3 ± 29.5	36.7 ± 30.2	65.6 ± 28.7
Abnormal Immunofixation	13 (19.7)	1 (11.1)	10 (76.9)	1 (2.5)
Bence Jones proteinuria	5 (7.6)	0	9 (69.2)	ND

ND: not determined.

Amyloid characteristics of the whole population (Table I)

The initial biopsy site was cardiac valvular in 28 patients (42.4%), liver or GI in 14 cases (21.2%), kidney in 8 cases (12.1%), salivary glands in 8 cases (12.1%), yellow ligament in 3 cases (4.5%), carotid artery in 2 (3%) and eyelidlid, fat aspiration and brain in one case each. Ten patients (15.2%) had more than one positive biopsy. Among the 66 patients, 13 were identified as AL amyloid (19.7%), 9 as AA amyloid (13.6%) and 40 as senile amyloid (60.6%). One patient had a TTR amyloid.

TTR positive population characteristics (n=41, Table I)

The mean age of the senile amyloid population was 72±11.3 years old with 27 men (67.5%) and 13 women (32.5%). The age-standardized incidence rate

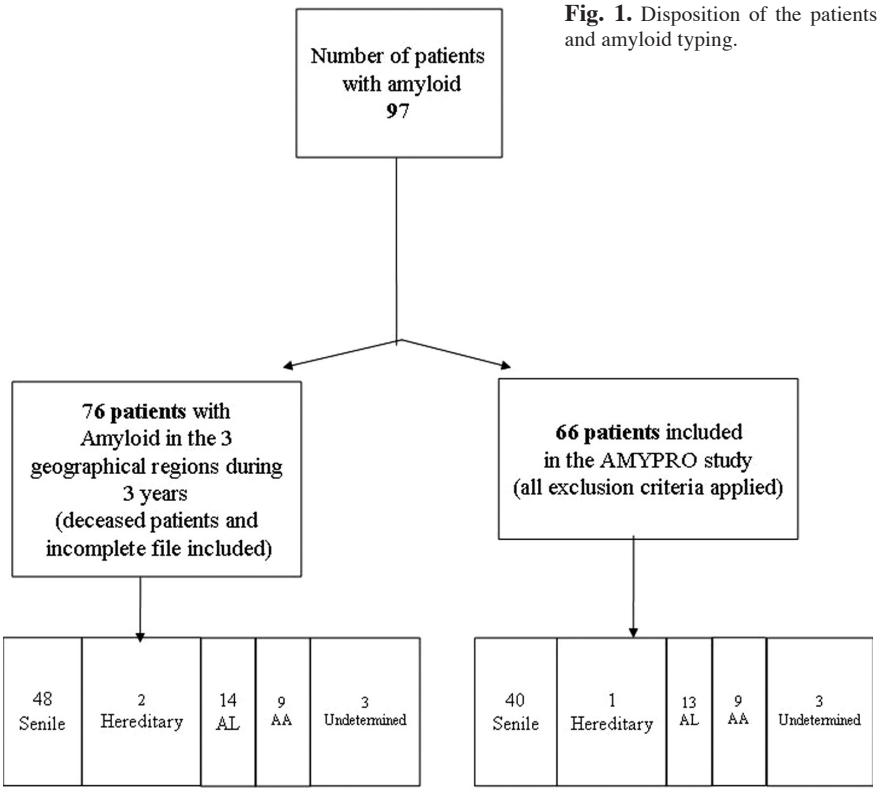

Fig. 1. Disposition of the patients and amyloid typing.

Table II. Age-standardized incidence rates of amyloidosis per million person-years in the 3 French geographical regions over 3 years.

	Age-standardized incidence rate per million person-years	95% confidence interval (CI)
All patients (n=76)	14.0	[10.5-17.5]
Senile Amyloid (n=48)	8.7	[5.9-11.3]
AA Amyloid (n=9)	2.0	[0.6-3.3]
AL Amyloid (n=14)	2.4	[1.0-3.7]
Hereditary Amyloid (n=2)	0.6	[0.0-1.5]
Undetermined Amyloid (n=3)	0.4	[0.0-1.0]

of senile amyloid was estimated at 8.7 cases per million person-years with a 95% CI (5.9-11.3) (Table II). Thirteen patients (32.5%) had a previous history of atrial fibrillation and 3 had a previous history of renal failure. The mean level of CRP was 11.9 mg/L±24.2; the mean creatinine clearance was 65.6 ml/min±28.7. An abnormal immunofixation was identified in 1 patient and referred to as monoclonal gammopathy of undetermined significance (MGUS). Thirty patients (75%) had echocardiography, in which the mean of the left ventricular ejection fraction was 37.8 %±29.02, while none of the 30 patients showed typical features of amyloid cardiomyopathy. Two patients (10.6%) had electromyography which showed abnormalities unrelated to an amyloid disorder. The initial biopsy site was cardiac valvular in 28 patients (70%), liver or GI in 5 cases (12.5%), salivary glands in 1 cases (2.5%), yellow ligament in 3 cases (7.5%), carotid artery in 2 (5%) and eyelid in 1 patient (2.5%). One patient (2.5%) had more than one positive biopsy. The patient with hereditary TTR amyloidosis had a Tyr78Phe mutation.

TTR negative population characteristics (n=25, Table I)

AL amyloid characteristics (n=13)

The mean age of the AL amyloid population was 73±11.3 years old with 6 men (46.2%) and 7 women (53.8%). The age-standardized incidence rate of AL amyloid was estimated at 2.4 cases per million person-years with a 95% CI (1-3.7) (Table II). Three patients (23.1%) had a previous history of atrial fibrillation and 8 (61.5%) a previous history of renal failure. The mean level of CRP was 25.1mg/L±35.1, and the mean creatinine clearance was 36.7 ml/min±30.2. An abnormal immunofixation was identified in 10 patients (76.9%), showing a monoclonal gammopathy in all. The monoclonal gammopathy was lambda light chain in 5 cases, IgG lambda in 3 cases, IgM lambda in 1 case and IgG kappa in 1 case. Bence-Jones proteinuria was positive in 9 cases. Eleven patients (85%) had echocardiography, in which the mean of the left ventricular ejection fraction was 35.1%±34.5. Three patients (23.1%) had electromyography which showed abnormalities related to amyloid neuropathy. The initial biopsy site was liver or GI in 2 patients (23%), salivary glands in 5 cases (38.5%) and kidney in 5 cases (38.5%). Six patients (46.2%) had more than one positive biopsy.

AA amyloid characteristics (n=9)

The mean age of AA amyloid population was 70.9±11.15 years old with 1 man (11%) and 8 women (89%). The age-standardized incidence rate of AA amyloid was estimated at 2 cases per million person-years with a 95% CI (0.6-3.3) (Table II). One patient (11.1%) had a previous history of atrial fibrillation and 6 (66.1%) a previous history of renal failure. The mean level of CRP was 84.3mg/L±91, and the mean creatinine clearance was 38.3 ml/min±29.5. An abnormal immunofixation was identified in one patient and referred to an MGUS. Three patients (33.3%) had echocardiography, and of which the left ventricular ejection fraction was only determined in one of them and was 60%. One patient (11.1%) had electromyography which showed abnormalities related to alcoholic neuropathy. The initial biopsy site was the liver or GI in 4 patients (44.4%), kidney in 3 patients (33.3%) and salivary glands in 2 patients (22.2%). Three patients (33.3%) had more than one positive biopsy.

The 3 other TTR negative patients belonged to the undetermined group. One had a cerebral angiopathy and for the two others, the amyloid typing was unsuccessful.

Comparison of TTR-positive and TTR-negative populations

No clinical or biological data was strong enough to establish a differential diagnosis between TTR-positive and TTR-negative populations. There was a statistical correlation concerning proteinuria and PBJ identified only in systemic amyloids (AA and AL) with a p=0.005 and for creatinine clearance which was statistically lower in systemic amyloids in comparison to TTR-positive patients (p=0.01).

Discussion

In the amyloid field, this is the first time that a prospective study has been realized from the initial to final diagnosis. It is also the first time that an epidemiological study concerning amyloidosis was realized in the eastern part of France. Previous epidemiological studies were published in the USA, in which the study of Kyle *et al*. (4) evaluated the overall sex- and age-adjusted rate of primary systemic amyloidosis (AL amyloid) per million person-years at 6.1 from 1950 to 1969 and at 10.5 from 1970 to 1989, the ratio AL/AA (secondary amyloidosis) was 17:1. In Europe, Hazenberg (5) found an incidence of systemic amyloidoses (AL and AA) of 13.3 million person-years regarding the causes of death in The Netherlands, with a ratio for AL/AA of 1:2. In our study, the age-standardized incidence rate of amyloidosis was 14 per million person-years. This high incidence rate was related to the principal inclusion criteria (amyloid on a tissue sample) and thus to the different types of amyloidoses included in the study (*i.e*., AL, AA, senile and hereditary).

There has been a lack of information concerning the amyloid epidemiological features of France. A retrospective study of a single center in the western part of France (Rennes) revealed 43 amyloid patients included from 1995 to 1999, with a AL/AA ratio of 3:1 (6). Moreover, no information was available concerning senile amyloid. Senile amyloid was the most prominent amyloid type (n=40) in the study, as was the cardiac localization of the amyloid. The typing of senile amyloid was dependent on the immunohistochemistry and the clinical context. No molecular test was proposed to the patients, older than 40 years old, having transthyretin amyloid and no other clinical features of systemic amyloidosis. Because cardiac valvular is a good candidate for senile amyloid (7-9), we estimated that for 28 patients the immunohistochemistry revealed the good amyloid typing. For the twelve remaining patients, there was no clinical or biological findings related to a systemic amyloid other than senile amyloid.

TTR-negative amyloid patients were composed of 13 AL, 9 AA and 3 undetermined patients. The ratio AL/AA was 1.5:1. The observed ratio is different from those observed by Kyle (4), Hazenberg (5) and Casalets (6) but the studied population was not the same. In fact, those epidemiological studies started with the known disease and in our study, the departure point was only the presence of amyloid deposits on a tissue sample without any previous knowledge of a real disease associated with amyloid deposits. In approximately two-thirds of the cases, the amyloid deposit identified on the biopsy was composed of transthyretin and identified as senile amyloid. In most of these cases, no specific or symptomatic treatment was done. In the case of valvular amyloidosis, the treatment was a valvular replacement realized before the amyloid diagnosis was made. In the 12 other cases, the disease was not directly related to the presence of an amyloid deposit. No clinical or biological data was strong enough to make a differential diagnosis between TTR-positive and TTR-negative patients. It was interesting to note that TTR-negative patients had a higher CRP level, a proteinuria and/or PBJ, a lower creatinine clearance and more electromyographic abnormalities. In the TTR negative group, the amyloid diagnosis was made with a renal biopsy in 8 patients presenting with renal failure (61.5%) so there was a selection bias concerning creatinine clearance.

In conclusion, we think that it is important to know that in this population, the finding of an amyloid deposit on a tissue sample is related in two-thirds of the cases to senile amyloid. Therefore, the clinician needs to be aware that in most of the cases there is no need for additional examination in searching for systemic amyloidosis in the absence of other clinical or biological signs of a primary or secondary amyloidosis.

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