

Different types of amyloid concomitantly present in the same patients

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Abstract

Different types of amyloid concomitantly present in the same patient is believed to be improbable. We reported four cases of patients with plasma cell disorders who were found to have biopsy proven concomitant different types of amyloid fibrils deposition. We characterized amyloid fibrils using immunogold electron microscopy. There is lack of experience in the treatment of these frail and elderly patients, who are on the threshold between necessity of chemotherapy for AL amyloidosis and necessity to avoid harmful treatment related toxicity. All patients with a systemic amyloid syndrome require therapy to prevent deposition of amyloid in other organs and prevent progressive organ failure. Stem cell transplant (SCT) is preferred, but only 20% of patients are eligible. Requirements for safe SCT include systolic blood pressure >90 mm Hg, troponin T <0.06 ng/mL, age <70 years, and serum creatinine ≤1.7 mg/dL. Nontransplant candidates can be offered melphalan-dexamethasone or cyclophosphamide-bortezomib-dexamethasone.

Introduction

Amyloidosis covers a group of systemic disorders characterized by the deposition of misfolded protein within the extracellular space, with subsequent deposition in tissues and organ damage. The diagnostic approach is based on two fundamental steps: i) The identification of amyloid in bioptic samples (although testing for amyloid deposits by Congo red staining under polarized light microscopy is generally considered as the diagnostic gold standard, this method lacks in sensitivity and specificity; ii) The typing of amyloid fibrils in order to identify the chemical nature of the amyloidogenic protein.¹⁻⁶ At our Centre the characterization

of amyloid fibrils is achieved by immunogold electron microscopy. Immunogold labeling is a classic high-resolution method that enables the selective localization of macromolecules in biological samples observed at the electron microscope by the use of specific antibodies coupled with colloidal gold particles. Clinically, the presence of amyloidosis in patients with plasma cell dyscrasia is usually assumed to be AL amyloidosis. We reported four cases of patients with plasma cell disorders who were found to have biopsy proven concomitant distinct type of amyloid fibrils deposition. Typing of amyloid deposits has significant implications in patient's management and prognosis.

Materials and Methods

We describe four patients, affected by monoclonal gammopathy, who underwent fat pad biopsy, because of clinically suspected amyloidosis.

Tissue samples obtained by periumbilical fat biopsies were fixed with 2.5% (vol./vol.) glutaraldehyde in 0.1 mmol/L cacodylate buffer, pH 7.4 for 1 h at 4°C, and then post fixed in 1% (vol./vol.) cacodylate-buffered osmium tetroxide for 2 h at room temperature. Samples were dehydrated in a graded series of ethanol, transferred to propylene oxide and embedded in Epon-Araldite. Ultrathin sections (60-80 nm thick) were cut with a diamond knife, placed on formvar/carbon-coated copper grids (200 mesh), stained with uranyl acetate and lead citrate and observed under a Zeiss 902 transmission electron microscope. Immunogold labeling for immunoglobulin light chains κ and λ, seroalbumin A, and trans-thyretin was performed according to Bendayan [Bendayan M. Double immunocytochemical labeling applying the protein A-gold technique. *J Histochem Cytochem* 1982; 30: 81-85] by the use of guinea pig specific primary antibodies (Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, Milano, Italy), together with protein A conjugated gold particles sized 15 nm (Agar Scientific, Stansted, UK). When deposits of TTR amyloid were found, we screened for genetic mutations to discriminate between hereditary (hATTR) and wild type forms of the disease (wtATTR).

Case Report #1

A 73-year-old Caucasian male presented with longstanding chronic kidney disease

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(CKD) G2 related to arterial hypertension stage II. Past medical history: prostate cancer and monoclonal gammopathy of undetermined significance (MGUS) IgG k, rosacea dermatitis, right hand arthritis.

Laboratory tests (Table 1) showed normal complete blood count (CBC), normal serum calcium value, creatinine 1.4 mg/dL, B type natriuretic peptide (BNP) 161 pg/mL, proteinuria 946 mg/24 h, albuminuria 200 mg/dL. His serum protein electrophoresis (SPEP) and immunofixation (IFE) revealed a monoclonal IgG k M-protein at 2 g/dL. Twenty-four-hour urine protein electrophoresis (UPEP) demonstrated positive k light chain proteinuria, Bence Jones Proteinuria (BJP). Kappa serum free light chain (FLC) was 126 mg/dL (normal range 0,330-1,940 mg/dL) and lambda serum FLC was 0.858 mg/dL (normal range 0.571-2.630 mg/dL) with kappa/lambda ratio at 146 (normal range 0.26-1.65).

Bone skeletal survey didn't detect osteolytic bone lesion.

Bone marrow biopsy revealed

plasmocytosis with scattered CD138+ plasma cells at 30% (Congo red staining not evaluated). We assessed amyloidosis as a concomitant CKD's cause: Congo red staining test on fat pad biopsy was positive. Immunogold on fat pad sample revealed transthyretin (ATTR) and serum amyloid A (SAA) amyloidosis. We screened for genetic mutations: the patient was affected by wild type form of the disease (wtATTR) (Figure 1).

The patient didn't have systemic AL amyloidosis. The value of FLC ratio above 100 was confirmed in serial testing. This is an IMWG criteria for treatment (FLC ratio >100 with concomitant FLC k >100 mg/L), but in this case, treatment was delayed and FLC ratio monitored.

In clinical practice it is a common error to assume AL primary amyloidosis in patients presenting with monoclonal gammopathy. Rheumatologic assessment in order to identify underlying cause of SAA amyloid will be needed, considering patient history of rosacea dermatitis and hand arthritis. As a result of the correct diagnosis being made, the prognosis was much more favorable. It is known the high prevalence of coexistent MGUS with ATTR amyloidosis in the elderly population. The incidence of MGUS in wild type TTR cardiac amyloidosis is 23%;^{7,8} moreover, it could be not uncommon to discover a plasma cell disorder in patients with SAA amyloid, given the increasing incidence of both conditions with chronic inflammatory disease.

Case Report #2

A 70-year-old Caucasian male presented with CKD G3 related to arterial hypertension stage II. Past medical history: cardiac diastolic dysfunction New York Heart Association (NYHA) class II, mitral insufficiency and pulmonary hypertension; hypercholesterolemia and coronary artery atherosclerosis without hemodynamically significant stenosis.

The patient complained about dysgeusia, hyporexia and severe weight loss (10 kg in 6 months); a chronic antral gastritis had been diagnosed by gastroscopy.

Laboratory tests (Table 1) showed normocytic anaemia (11.9 g/dL), normal serum calcium value, creatinine 1.36 mg/dL,

N-terminal prohormone of brain natriuretic peptide (NT pro BNP) 7400 pg/mL, proteinuria 975 mg/24 h, albuminuria 70 mg/dL. His serum protein electrophoresis (SPEP) and immunofixation (IFE) revealed a monoclonal IgG lambda M-protein at 0.6 g/dL. Twenty-four-hour urine protein electrophoresis (UPEP) demonstrated positive lambda light chain proteinuria, Bence Jones Proteinuria (BJP). Kappa serum FLC was 1.740 mg/dL (normal range 0.330-1.940 mg/dL) and lambda serum FLC was 8.240 (normal range 0.571-2.630 mg/dL) with kappa/lambda ratio at 0.211 (normal range 0.26-1.65).

Echocardiogram estimated the 2D global left ventricular ejection fraction at 55%; severe biventricular hypertrophy; diastolic

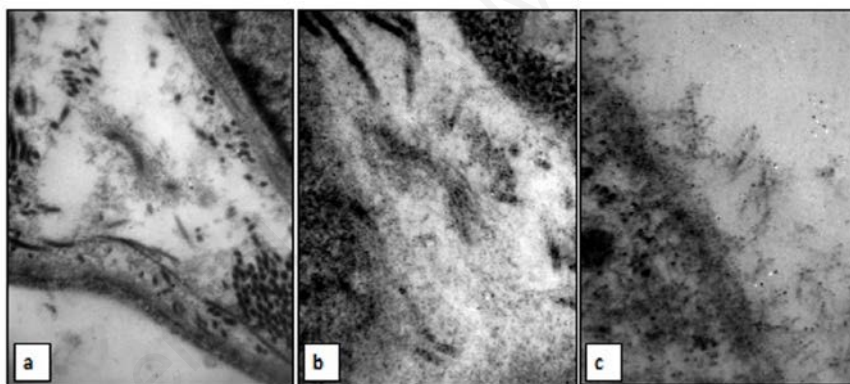


Figure 1. Case 1 (a) electron microscopy assay of fat pad biopsy: amyloid fibrils; (b) immunogold (5 nm) transthyretin (ATTR) and (c) serum amyloid A (SAA) amyloidogenic deposition.

Table 1. Patients' characteristics.

	Case 1	Case 2	Case 3	Case 4
Age (years)	73	70	76	80
Gender	Male	Male	Male	Male
Clinical presentation	CKD G2 arterial hypertension stage II	CKD G3 arterial hypertension stage II; cardiac diastolic dysfunction NYHA II; dysgeusia, hyporexia, weight loss	CKD G3 arterial hypertension stage II	CKD G2 arterial hypertension stage III; peripheral neuropathy; congestive heart failure NYHA II; smoldering myeloma
M protein (g/dL)	IgG k (2)	IgG L (0.6)	IgM k (1.25)	IgG k (1.5)
Kappa/lambda ratio	146	0.211	3.71	1.83
Value of the involved light chain, mg/dL	126	8.240	9.020	4.5 *
BM biopsy	30% plasma cells	10% plasma cells	Not done	10-12% plasma cells
Organ biopsy	Not done	Endomyocardial biopsy	Not done	Not done
Fat pad biopsy	ATTR and SAA amyloid	ATTR and AL (lambda light chain) amyloid	AL (k light chain) and SAA amyloid	ATTR and AL (lambda light chain) amyloid
Diagnosis	ATTR and SAA amyloidosis and multiple myeloma	ATTR and AL amyloidosis	AL and SAA amyloidosis	ATTR and AL (lambda light chain) amyloidosis
Management	Follow-up	Mel-Dex + doxycycline	Follow-up	Follow-up

*Referring to k FLC k.

dysfunction grade 2 (restrictive filling pattern). Cardiac MRI (magnetic resonance imaging) revealed features suggesting cardiac amyloidosis (severe biventricular hypertrophy). Tc 99m pyrophosphate scan was diagnostic for cardiac ATTR amyloidosis.⁹

Bone marrow biopsy revealed plasmocytosis with scattered CD138+ plasma cells at 10%. Congo red staining on bone marrow, endomyocardial and fat pad biopsy were positive. Immunohistochemistry (ICH) staining on fat pad and on endomyocardial biopsy samples revealed ATTR and AL amyloidosis (lambda light chain). Immunogold on fat pad and on endomyocardial biopsy samples revealed only AL amyloidosis. We screened for genetic mutations: the patient was affected by wild type form of the disease (wtATTR) (Figure 2).

Unfortunately, gastric tissue had not been biopsied, so we couldn't confirm suspected soft tissue involvement, resulting in dysgeusia.

It was a challenging diagnostic case: after an in-depth discussion with pathologists patient was diagnosed with wtATTR and AL amyloidosis, supported by Tc 99m pyrophosphate scan and ICH (even if we know that immunohistochemistry is not a reliable typing method unless done by very expert amyloid pathologist with very specific antibody panels); he was classified as intermedium risk according to "Guidelines 2011-2012 for Amyloidosis University of Pavia" and as stage III according to Mayo Clinic staging system. The presence of concomitant ATTR cardiac amyloidosis could represent an interfering factor for the use Mayo clinic staging system.

He was treated with melphalan 0,14 mg/kg day 1-4 and dexamethasone 40 mg day 1-4 (Mel-Dex) for six 28 days-cycles. Doxycycline therapy was added (100 mg bis a die-bid-) because doxycycline combined with tauroursodeoxycholic acid improved cardiac outcomes in transthyretin amyloidosis and in a cohort of patients with systemic AL amyloidosis, doxycycline, given as antimicrobial prophylaxis post autologous stem cell transplantation, significantly improved survival compared to controls who were given penicillin. The main impact of doxycycline is reduction in early cardiac mortality.¹⁰ Treatment of AL amyloidosis is not similar to MM in terms of the duration of treatment and these patients should be treated of 2 cycles past best response. We avoided bortezomib because it could be associated with worsening fluid retention and congestive symptoms. Disease assessment after 2 cycles was unsatisfactory,

so the patient received 4 further cycles of chemotherapy. Disease assessment after 6 cycles of chemotherapy revealed a hematological partial response (dFLC reduction >50%), as well as steady heart and

renal functions, however patient still complained dysgeusia and dyspnea. Considering potential chemotherapy toxicity, harmful to senile amyloidosis component, we stopped chemotherapy and the patient

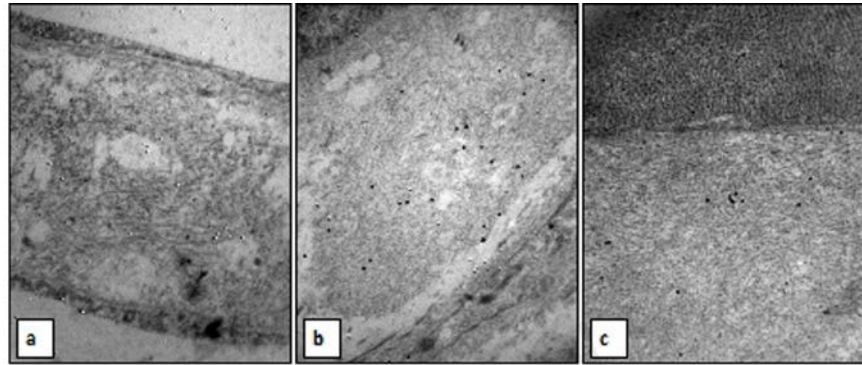


Figure 2. Case 2 (a) electron microscopy assay of fat pad biopsy: amyloid fibrils; (b) immunogold (20 nm) lambda light chains (AL); (c) endomyocardial biopsy immunogold (20nm) lambda light chains amyloidogenic deposition.

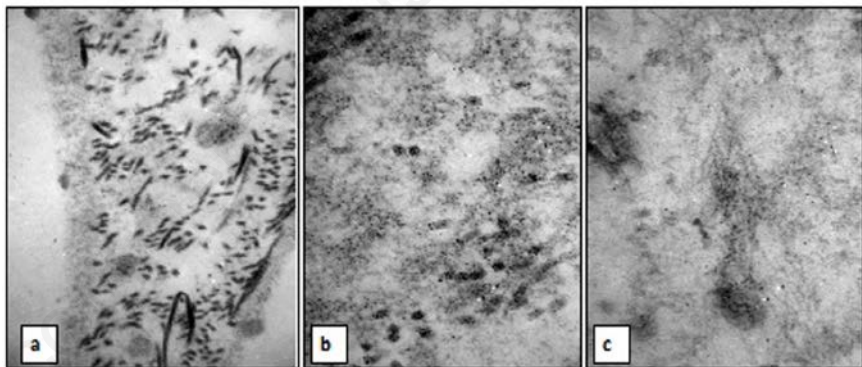


Figure 3. Case 3 (a) electron microscopy assay of fat pad biopsy: amyloid fibrils (b) immunogold (5nm) k light chain (AL) and (c) serum amyloid A (SAA) amyloidogenic deposition.

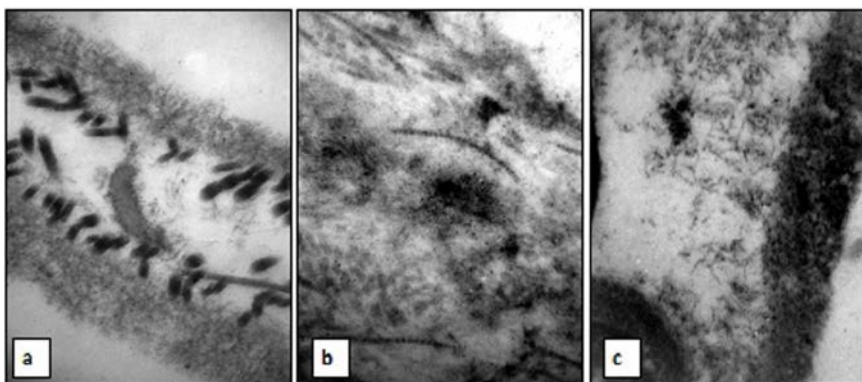


Figure 4. Case 4 (a) electron microscopy assay of fat pad biopsy: amyloid fibrils (b) immunogold (5 nm) lambda light chains (AL) and (c) transthyretin (ATTR) amyloidogenic deposition.

was closely monitored with both clinical and laboratory follow up. After three months from the end of chemotherapy treatment, the patient developed an acute heart failure. He was hospitalized and treated with diuretics, obtaining clinical status improvement. Considering the worsening cardiac function, the increasing in lambda FLC value, and the previous good response obtained with Mel-Dex, the patient was treated with Mel-Dex again (3 cycles) obtaining a mild clinical picture improvement.

In case of disease progression non cardiotoxic immunomodulatory drug, IMiDs, could be administered.

Case Report #3

A 76-year-old Caucasian male presented with CKD G3 related to arterial hypertension stage II. Past medical history: hepatitis C virus (HCV) positivity, MGUS IgM k, hepatic steatosis, hypertensive heart disease, type 2 diabetes mellitus, prostatic hypertrophy. Physical examination revealed lower limb oedema (related to heart dysfunction).

Laboratory tests (Table 1) showed normocytic anaemia (11.0 g/dL), normal serum calcium value, creatinine 3.30 mg/dL, B type natriuretic peptide (BNP) 330 pg/mL, proteinuria 6138 mg/24 h, albuminuria 200 mg/dL. His serum protein electrophoresis (SPEP) and immunofixation (IFE) revealed a monoclonal IgM k M-protein at 1.25 g/dL. Twenty-four-hour urine protein electrophoresis (UPEP) was negative. Kappa serum free light chain (FLC) was 9.020 mg/dL (normal range 0.330-1.940 mg/dL) and lambda serum FLC was 2.430 (normal range 0.571-2.630 mg/dL) with kappa/lambda ratio at 3.710 (normal range 0.26-1.65).

We assessed amyloidosis as a concomitant CKD's and heart disease's cause: Congo red staining test on fat pad biopsy was positive. Immunogold on fat pad sample revealed AL (k light chain) and SAA amyloidosis. The patient refused bone marrow exam (Figure 3).

We decided not to treat the patient, considering the low FLC k value and the concomitant SAA amyloid, that couldn't benefit from chemotherapy. The patient is being followed up every three months to detect promptly any free light chain gain or organ impairment worsening.

Underlying cause of SAA amyloidosis probably was long standing HCV chronic infection. No rheumatic diseases could be reported after rheumatologic investigations.

Case Report #4

An 80-year-old Caucasian male presented with CKD G2, arterial hypertension stage III, peripheral neuropathy, congestive heart failure (NYHA-II). Past medical history: heart attack, dyslipidemia, lung cancer, diabetes mellitus caused by recurrent acute pancreatitis of undetermined nature and IgG k smouldering myeloma.

Laboratory tests (Table 1) showed mild normocytic anemia (11.5 g/dL), normal serum calcium value, creatinine 2.21 mg/dL, NT-proBNP 2.337 ng/L, high sensitivity cardiac troponin (HS-cTn) was 76 ng/L, albuminuria 93 mg/dL. His serum protein electrophoresis (SPEP) and immunofixation (IFE) revealed a monoclonal IgG k M-protein at 1.5 g/dL. Twenty-four-hour urine protein electrophoresis (UPEP) was negative (negative BJP). Kappa serum free light chain (FLC) was 4.5 mg/dL (normal range 0.330-1.940 mg/dL) and lambda serum FLC was 2.4 mg/dL (normal range 0.571-2.630 mg/dL) with kappa/lambda ratio at 1.830 (normal range 0.26-1.65).

Echocardiogram estimated the 2D global left ventricular ejection fraction at 58%. There was mild left ventricular hypertrophy with increased septal thickness and myocardial mass index, diastolic dysfunction grade 1, suggesting cardiac amyloidosis. Cardiac magnetic resonance imaging with gadolinium contrast was contraindicated because of moderate kidney disease.¹¹

Congo red staining on fat pad biopsy was positive. Immunogold on fat pad sample revealed ATTR and lambda AL amyloidosis. We screened for genetic mutations: the patient was affected by wild type form of the disease (wtATTR) (Figure 4).

Clinical picture (heart, kidney, peripheral nervous systems damage) was in accord with systemic AL amyloidosis and could benefit from chemotherapy. Concomitant presence of wtATTR, that doesn't benefit from chemotherapy and the recent history of lung cancer advised against chemotherapy, if not really necessary. In order to improve neurological symptoms steroid therapy could be administered, avoiding harmful chemotherapeutic toxicity, but also this therapy could be potentially toxic because of concomitant poorly controlled diabetes mellitus. Therefore, the patient was closely followed up.

Lambda AL is three times more common than kappa AL. It is expected to find the same light chain clonal restriction both in M component and amyloid fibrils. However, this patient presented with an IgG k M-

protein, but immunogold on fat pad sample revealed lambda light chain amyloid fibrils. We could speculate about two distinct concomitant plasmacellular clones with different light chain restriction. There are very few data in medical literature.

Discussion and Conclusions

Accurate amyloid subtyping is extremely important in the management and prognosis of patients. Unfortunately, we couldn't use the gold standard laser capture mass spectroscopy, so the characterization of amyloid fibrils is achieved by immunogold electron microscopy. Concomitant detection of distinct type of amyloid fibrils is believed to be a rare event. Although the mechanism remains unclear, researchers speculate on a synergistic fibrilization of two type of amyloidogenic proteins.¹² The method used does not allow evaluation as to whether there are two separate amyloid or if there are mixed forms. Combination with western blot and/or mass-spectrometry based proteomic characterization would make it possible.

A chemotherapeutic approach is necessary when AL amyloidosis is diagnosed, because of its inexorable progressive course due to uncontrolled organ damage. We described 4 cases characterized by different types of amyloid fibrils deposition concomitantly present in the same patient. We should be able to treat AL amyloidosis patients, avoiding harmful chemotherapeutic toxicity, because other concomitant amyloidosis types, like wtATTR, don't benefit from chemotherapy.^{13,14} Therefore, patients are closely monitored with both clinical and laboratory follow up, as long as we can. If chemotherapy is necessary we choose the less toxic drugs, along with supportive care according to organ damage if present. More clinical experience is needed to further optimize treatment-related toxicity and improve the outcome of these patients.

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