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ORIGINAL ARTICLE

Amyloid in bone marrow smears of patients affected by multiple myeloma

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Abstract Systemic AL amyloidosis is associated with nearly 15% of cases of multiple myeloma, but data on the frequency and significance of amyloid deposits in the bone marrow of patients affected by multiple myeloma without clinical signs of systemic amyloidosis are scanty. Bone marrow smears of 166 unselected patients affected by multiple myeloma (126 at diagnosis and 40 after treatment) were stained with Congo red and studied by transmission and birefringence microscopy. Both focal and diffuse storages were considered positive. Overall, 67 patients were positive and 99 were negative to Congo red and apple-green birefringence. In particular, 51 of the 126 patients studied at diagnosis and 16 of the 40 patients with advanced disease were positive. Seventeen patients were

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reassessed after a mean follow-up of 32 months (range: 6–91): disappearance of amyloid deposits was verified in three cases, all responsive to bortezomib-based regimens. The preliminary data available suggest that amyloid deposition in the marrow of myeloma patients is frequent, as it can be traced in nearly 40% of cases. We failed to find correlations between bone marrow amyloid deposits and immunoglobulin type, disease stage, plasma cells percentage, hemoglobin, calcium, creatinine, albumin, or $\beta_2$microglobulin. Significantly higher incidence of moderate/severe peripheral neuropathy was found in patients with marrow amyloid exposed to potentially neurotoxic antineoplastic agents. Further studies and prolonged follow-up are needed to validate our findings and to define possible prognostic aspects.

Keywords Amyloidosis · Multiple myeloma · Congo red staining · Bone marrow amyloid

Introduction

AL amyloidosis is the most frequent type of systemic amyloidosis in Western countries [1]. It is caused by tissue-infiltrating proteins derived from monoclonal immunoglobulin light chains, frequently observed in patients affected by multiple myeloma (MM) or, less frequently, by Waldenström's macroglobulinemia or other lymphoproliferative disorders [2]. Monoclonal protein, either in the form of intact immunoglobulins or as light chains only, are often detectable in serum and/or in urine; in most cases, only protein fragments rather than intact molecules are detectable in tissues [2, 3].

AL amyloid is determined by proteins arranged in cross-β-pleated sheet structures. These fibrils are usually formed by N-terminal fragments of variable region of an immunoglobulin light chain, although they may occasionally include part of a constant region or are formed by whole light chain [1, 4]. Only a small portion of light chain is able to form amyloid fibrils: this property is likely correlated with distinctive structural features of amyloidogenic light chains, as almost 70% of them are lambda isotype and the genes Vλ6a and Vλ3r encode 42% of these chains [5, 6]. The light chains of λVI family are quite steadily linked to amyloidosis [5, 6]. Structural features may also be responsible for the organ tropism of amyloid deposits: in fact, it has been shown that λ6a light chains are associated with renal involvement [7]. Amyloid deposits differ from those observed in light chain disease because, in the latter, the protein fragments do not form β fibrils, have no P component, nor are they stained by Congo red [2, 8, 9].

Amyloid can be detected in the bone marrow by Congo red staining as amorphous pink material of variable amount or as intracellular reddish, sometimes spindle-shaped, inclusions that recall Auer rods.

Systemic AL amyloidosis may be associated with MM in approximately one-tenth of affected patients [10]. However, the significance of amyloid deposits in the marrow of MM patients is not yet established. Our study aimed to verify the frequency of amyloid deposits in the bone marrow of MM patients in the absence of signs or symptoms of systemic amyloidosis, and the possible clinical implications.

Patients, materials, and methods

We performed a retrospective study on 166 patients affected by MM, 126 at diagnosis, and 40 after treatment. MM was diagnosed and staged according to Durie and Salmon criteria. Amyloid was retrospectively investigated by cytological techniques: bone marrow smears, necessary for both diagnosis and therapeutic decision-making, were obtained by aspiration with 15G biopsy needle from the posterior superior iliac spine. Smears were air-dried and stained with May-Grünwald-Giemsa for cytological evaluation and with Congo red for amyloid detection. All Congo red positive cases were evaluated by transmission birefringence microscopy. Archive material was considered suitable for the analysis, as Congo red positive deposits were also found in old, unfixed, air-dried smears.

Tables 1 and 2 show the characteristics of the patients at diagnosis or with advanced disease, respectively. Briefly, of the126 patients studied at diagnosis, most had the IgGk isotype, nine were micromoleculars (4 k and 5 λ, respectively), six were non-secretory, and two had solitary plasmacytoma. The IgGk isotype was also prevalent among the 40 patients with advanced disease: among them, four micromolecular λ, five non-secretory, and two solitary plasmacytomata were also present. Seventeen patients (11 at diagnosis and six advanced) were reassessed after a mean follow-up of 32 months (range: 6–91). Patients for whom there was clinical or instrumental suspicion (i.e., by heart or liver ultrasound scan) of systemic amyloidosis, either at diagnosis or during the follow-up (mean: 21 months, range: 2–91) were excluded from the analysis.

A statistical analysis was performed to evaluate possible associations between amyloid deposits and the following parameters: clinical stage (I–II vs. III), type of chain (IgG vs. IgA; k vs. λ), percentage of bone marrow plasma cells (>median vs. <median), amount of monoclonal component (>median vs. <median), levels of hemoglobin (>median vs. <median), creatinine (normal vs. abnormal), calcium (normal vs. abnormal), albumin (normal vs. abnormal), and β_2-microglobulin (normal vs. abnormal), World Health Organization (WHO) grade of peripheral neuropathy (<2

Table 1 126 patients at diagnosis of multiple myeloma

	Ig					Stage				% BM	MC	Hb	Creatinine	Albumin	β2 mic.	Ca
	G	A	Micro	k/λ	n.s	I	II	III	A/B	pl. cells	g/dI	g/dI	mg/dI	g/dI	ng/mI	mg/dI
Congo red positive: 51 patients																
n	35	12	2	28/20	2	13	14	23	43/8	49	41	46	45	44	38	46
Mean										40.7	3.1	11.1	1.2	3.8	4,475	9.8
Range										1–100	0.2–7.4	6.9–15.5	0.5–10.4	1.9–4.8	1,583–51,000	8.2–15.1
Congo red negative: 75 patients																
n	45	19	7	35/28	4	23	18	33	60/15	75	63	71	75	68	63	71
Mean										37	3	11.6	1.2	3.8	2,963	9.8
Range										2–100	0.2–5.8	6.3–17.7	0.5–4.8	2.2–5	1,185–53,000	8–17.3

n.s. non secretory, *MC* monoclonal component

Table 2 Forty patients with advanced multiple myeloma

	Ig					Stage				% BM	MC	Hb	Creatinine	Albumin	β2 mic.	Ca
	G	A	micro	k/λ	n.s	I	II	III	A B	pl. cells	g/dI	g/dI	mg/dI	g/dI	ng/mI	mg/dI
Congo red positive: 16 patients																
n	10	1	1	11/1	4	n.a.				16	10	14	12	14	13	11
Mean										31	2.7	11.2	1	3.6	6,460	9.4
Range										0–100	0.6–4.4	7–17.6	0.7–2.3	2.3–4.5	1,845–25,468	7.1–15.3
Congo red negative: 24 patients																
n	12	8	3	12/7	1	n.a.				23	17	22	21	20	18	21
Mean										28	2.8	11	0.9	3.6	3,449	9.1
Range										1–88	0.5–6.4	7.4–14.8	0.3–7.8	2.1–4.6	1,930–5,867	8–10.2

n.s. non secretory, *MC* monoclonal component

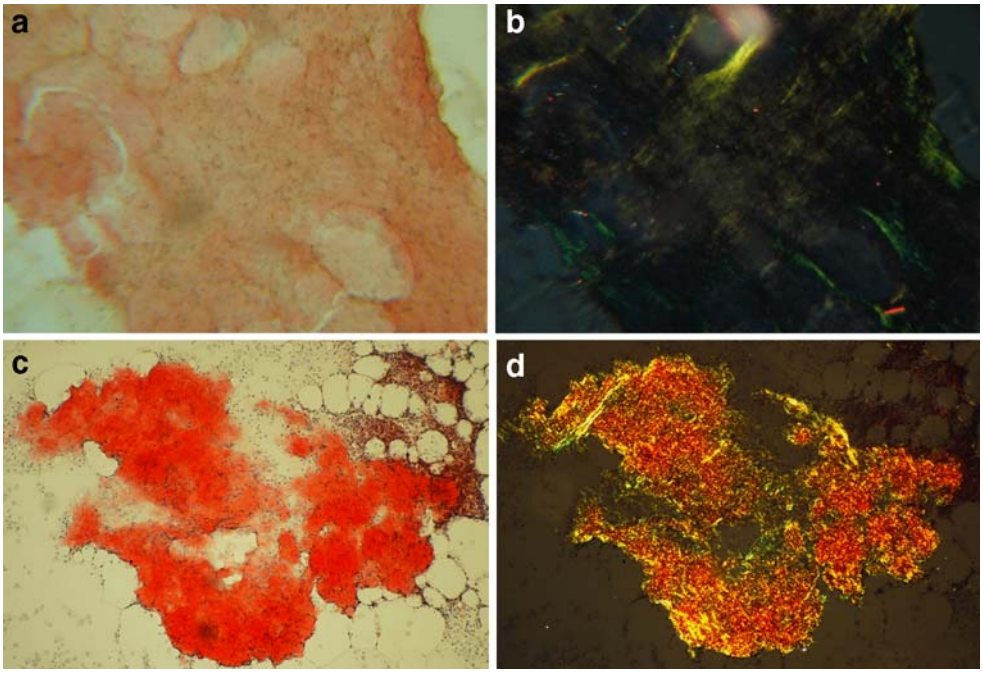

Fig. 1 Two representative examples of diffuse amyloid deposits in bone marrow smears stained by Congo red: conventional microscopy shows typical cherry red color (**a**, **c**); polarized light microscopy on the same samples shows apple-green birefringence (**b**, **d**); ×100

vs. >1) during therapy with thalidomide and/or bortezomib. For each variable, a contingency table with the presence of amyloid deposits was constructed and a χ^2 test for independence was carried out. All p values were two-sided.

Results

Tables 1 and 2 show the results of Congo red staining in patients studied at diagnosis or after treatment, respectively.

Overall, 67 patients were positive and 99 were negative to Congo red and apple-green birefringence.

At Congo red, amyloid deposits have the typical optical features, i.e., cherry red if observed with non-polarized light source, and apple-green birefringent if observed with polarized light source (Fig. 1). Quantitative differences were common: some smears showed diffuse, dense, blob-like amorphous pink material with sharp outlines, while focal strips of pink, stroma-like material with bone marrow cells tightly attached were evident in other cases (Figs. 1, 2). In

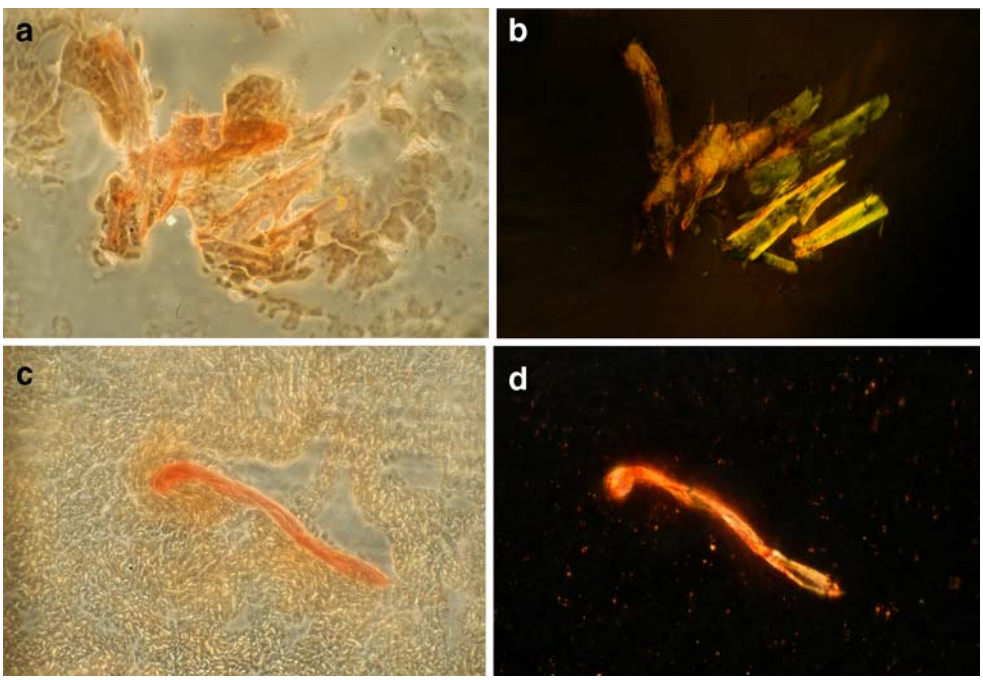

Fig. 2 Two representative e xamples of focal amyloid deposits in bone marrow smears stained by Congo red: conventional microscopy shows typical cherry red color (**a**, **c**); polarized light microscopy on the same samples shows apple-green birefringence (**b**, **d**); ×100

some May-Grünwald-Giemsa stained smears, intense, and diffuse amyloid infiltration was heralded by the presence of amorphous pink, garnet red, or navy blue material, with waxen, vanished, or nebulous aspect (Fig. 3). On the other hand, focal Congo red positive cases were not associated with atypical features of marrow spicules in May-Grünwald-Giemsa stained smears. Both diffuse and focal aspects had typical apple-green birefringence (Figs. 1, 2). Intracellular reddish spindle-shaped inclusions (Auer rod-like) were detected in plasma cells of two positive cases. Both diffuse and focal presentations were considered positive when found within the spicules. In five unselected patients, bone marrow trephine biopsies were available together with the smears obtained by aspiration: cytology and histology were concordant in the two positive and in the three negative cases.

No correlations were found between bone marrow amyloid deposits and clinical staging, heavy or light chain types, percentage of bone marrow plasma cells, serum or urine concentration of monoclonal component, hemoglobin, creatinine, calcium, albumin, or β2microglobulin levels.

A significantly higher incidence of moderate-severe peripheral neuropathy (WHO 0, 1 vs. 2–4), mainly sensorial, was found in Congo red-positive patients treated with potentially neurotoxic drugs, such as thalidomide or bortezomib (50 patients with peripheral neuropathy: p: 0.0001; 28 patients at diagnosis, p: 0.004; see Tables 3 and 4).

Seventeen patients (11 at diagnosis and six in advanced disease) were reassessed after a mean follow up of 32 months (range: 6–91): disappearance of amyloid deposits occurred in three cases (two at diagnosis and one in advanced disease), all responsive to chemotherapy, while five negative patients (two at diagnosis and three advanced) became positive.

After a mean follow-up of nearly 2 years (mean: 21 months, range: 2–99), none of the patients has developed overt signs attributable to systemic amyloidosis.

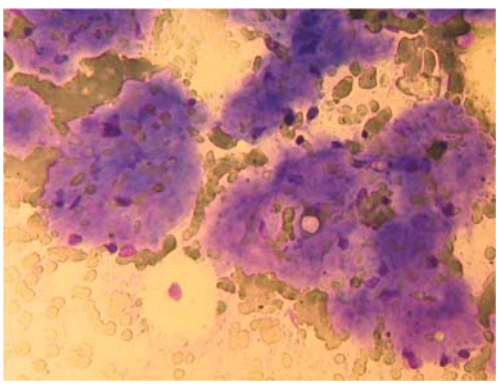

Fig. 3 Diffuse deposits of amyloid in bone marrow smears stained by May-Grünwald-Giemsa appear as amorphous, nebulous navy blue material (samples derived from the same patient of Fig. 1a,b); ×100

Table 3 Incidence of moderate-severe peripheral neuropathy

	+	−
WHO 0–1	11	35
WHO 2–4	23	5

χ^2 test for peripheral neuropathy: moderate/severe (WHO 2–4) vs. no/mild (WHO 0–1), in patients affected by multiple myeloma, at diagnosis or in advanced disease, with Congo red-positive (+) or Congo red-negative (−) bone marrow smears. p=0.0001

No survival difference was found between Congo red positive vs. negative patients (mean survival: 26 months, range: 2–91 vs. 30 months, range: 12–99, respectively).

Discussion

Bone marrow involvement due to amyloid in patients affected by systemic AL amyloidosis is not infrequent [11] However, the frequency and significance of amyloid deposits in the bone marrow of patients affected by MM without signs or symptoms of systemic amyloidosis is not well known.

Our observations show that nearly 40% of the patients affected by MM may have focal or diffuse amyloid deposits in the bone marrow. Also, out of 11 patients affected by non-secretory MM (six at diagnosis and five advanced), six had amyloid deposits in the marrow, and out of four patients with solitary plasmacytoma, three (one at diagnosis and two in advanced disease but with normal marrow plasma cell percentage) showed amyloid in the marrow.

Although bone marrow involvement by amyloid seems frequent in multiple myeloma, it is reported that less than 10% of the patients affected by MM may have or develop systemic AL amyloidosis [1]: this would suggest a lack of prognostic significance, but the influence of chemotherapy cannot be precisely weighed.

Based on our data, it seems that the amyloid in the bone marrow of patients affected by MM does not influence the prognosis, as it did not prevent the achievement of a long survival. No correlations were found with the variables commonly used to diagnose and stage MM, and none of the patients has so far developed overt signs attributable to

Table 4 Incidence of moderate-severe peripheral neuropathy

	+	−
WHO 0–1	9	21
WHO 2–4	12	3

χ^2 test for peripheral neuropathy: moderate/severe (WHO 2–4) vs. no/mild (WHO 0–1), in patients affected by multiple myeloma at diagnosis, with Congo red-positive (+) or Congo red-negative (−) bone marrow smears. p=0.0043

systemic amyloidosis (mean follow-up: 21 months, range: 2–99).

However, some points are worth a discussion. Sequential marrow evaluations showed the disappearance of amyloid deposits after effective therapy in three patients (two after diagnosis and one in advanced disease): it is remarkable that in all cases the treatment schedule included bortezomib and dexamethasone. This observation could support therapeutic protocols with bortezomib-based regimens in systemic AL amyloidosis [12]. On the other hand, significantly higher incidence of severe peripheral neuropathy among positive patients exposed to potentially neurotoxic drugs, such as thalidomide or bortezomib, if confirmed on a more numerous population, may indicate the need to modulate treatment in Congo red-positive patients.

Differently from patients affected by systemic AL amyloidosis, who have a clear excess of λ chains, in our patients, the k/λ ratio of Congo red-positive cases at diagnosis did not significantly differ from that of Congo red negative cases (Congo red positive: k/λ=28/20; Congo red negative: 35/28). However, λ chain seems rarer in patients with advanced disease, whether positive or negative to Congo red (k/λ in advanced cases=23/8), thus, suggesting a negative selection. The association between λ chain and poor prognosis brought forward in a recent retrospective analysis of long survivor patients affected by MM [13], could still suggest a link between lambda chain, amyloid, and prognosis. Further studies on a larger population sample with longer follow-up should allow a better biological and clinical definition of marrow amyloid deposits.

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References

1. Müller MS, Geibel A, Neumann HPH, Kühnemund A, Schmitt-Gräff A, B hm J, Engelhardt M (2006) Primary (AL) amyloidosis in plasma cell disorders. The Oncologist 11:824–830
2. Cohen AS (1995) Amyloidosis. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA (eds) Hematology. McGraw-Hill, USA, pp 1137–1146
3. Kyle RA, Gertz MA (1995) Primary sistemic amyloidosis: clinical and laboratory features in 474 cases. Sem Hematol 32:45–59
4. Kyle RA (1995) Amyloidosis. Circulation 91:1269–1271
5. Solomon A, Frangione B, Franklin EC (1982) Bence Jones proteins and light chain of immunoglobulins. Preferential association of the V lambda VI subgroup of human light chain with amyloidosis AL (lambda). J Clin Invest 70:453–460
6. Perfetti V, Casarini S, Palladini G, Vignarelli MC, Klersy C, Diegoli M, Ascari E, Merlini G (2002) Analysis of V(lambda)-J (lambda) expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r (lambdaIII) as a new amyloid-associated germline gene segment. Blood 100:948–953
7. Comenzo RL, Zhang Y, Martinez C, Osman K, Herrera GA (2001) The tropism of organ involvement in primary systemic amyloidosis: contribution of Ig V_L germ line gene use and clonal plasma cell burden. Blood 98:714–720
8. Dobson CM (2003) Protein folding and misfolding. Nature 426:884–890
9. Merlini G, Berlotti V (2003) Molecular mechanisms of amyloidosis. N Engl J Med 349:583–596
10. Invernizzi R, Palladini G, Benatti C, Travaglino E, Nuvolone M, Merlini G (2006) Bone marrow amyloidosis. Haematologica 91 (Suppl.5):23
11. Swan N, Skimmer M, O'Hara CJ (2003) Bone marrow core biopsy specimens in AL (primary) amyloidosis. Am J Clin Pathol 120:610–616
12. Reece DE, Sanchorawala V, Hegenbart U, Merlini G, Palladini G, Fermand JP, Vescio RA, Liu X, Elsayed YA, Cakana A, Comenzo RL (2009) Weekly and twice-weekly bortezomib in patients with systemic AL amyloidosis: results of a phase 1 dose-escalation study. Blood 114:1489–1497
13. van Rhee F, Dhodapkar M, Shaughnessy JD Jr, Anaissie E, Siegel D, Hoering A, Zeldis J, Jenkins B, Singhal S, Mehta J, Crowley J, Jagannath S, Barlogie B (2008) First thalidomide clinical trial in multiple myeloma: a decade. Blood 112:1035–1038