Histological regression of amyloid in AL amyloidosis is exclusively seen after normalization of serum free light chain

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ABSTRACT

Background
Histological regression of amyloid has not been studied systematically but is assessed by clinical parameters. We analyzed the change of amyloid deposition in fat tissue in patients with AL amyloidosis following chemotherapy and studied the relation with type of hematologic response.

Design and Methods
Between January 1994 and July 2007 all consecutive patients with AL amyloidosis were evaluated in whom fat tissue aspirate was obtained before and following chemotherapy. Patients were divided into three groups depending on response of serum free light chain: complete, partial or non-responders. Fat tissue was assessed using a validated semi-quantitative test (grading 0-4). A change of 2 grades of amyloid deposition in fat tissue was considered significant and used as event to construct Kaplan-Meier curves of the patients who were able to reflect such a change.

Results
One hundred and twenty consecutive patients were studied. Fifty-one patients fulfilled inclusion criteria. Thirty patients had a complete response of the amyloidogenic free light chain a median 0.5 year (range 0.3-2.9 years) following chemotherapy. Reduction of 2 grades of amyloid deposition in fat tissue was seen in 50% of these patients after 2.4 years and in 80% after 3.2 years. In contrast to complete responders, none of the patients with partial (n=9) and non-response (n=12) showed reduction of 2 grades (p=0.02) with median follow-up of fat tissue analysis of 1.3 and 0.8 years, respectively.

Conclusions
This study in a selected group of patients with AL amyloidosis shows significant histological regression of amyloid deposition in fat tissue exclusively after normalization of serum free light chain.

Key words: AL amyloidosis, free light chain response, regression of amyloid.

Citation: van Gameren II, van Rijswijk MH, Bijzet J, Vellenga E, and Hazenberg BP. Histological regression of amyloid in AL amyloidosis is exclusively seen after normalization of serum free light chain. Haematologica 2009;94:1094-1100. doi:10.3324/haematol.2008.004119

Funding: this study is part of the EURAMY project 037525 that is supported by funding of the Sixth Research Framework Programme of the European Union.

Manuscript received on November 26, 2008. Revised version arrived on March 8, 2009. Manuscript accepted on March 10, 2009.

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Introduction

AL amyloidosis is one of the major types of systemic amyloidosis. It is associated with high morbidity and mortality, due to deposition of amyloid fibrils in major organs such as the heart, the kidney, the liver and the nervous system, leading to loss of organ function and eventually to death.[1] The diagnosis of amyloidosis is based on histological analysis, demonstrating positive staining with Congo-red and the characteristic apple-green birefringence when viewed in polarized light. Subcutaneous abdominal fat tissue is the preferential biopsy site, because of the convenient and non-invasive character and excellent diagnostic accuracy.[2] The validated semi-quantitative assessment of amyloid in fat tissue[3] provides the opportunity to monitor severity of amyloid deposition in tissue during follow-up.

In AL amyloidosis the precursor protein of amyloid fibrils in organs is an immunoglobulin free light chain (FLC) produced by an underlying clonal plasma cell dyscrasia. Often the plasma cell dyscrasia is of low grade character, and cannot be found with conventional immuno-electrophoresis,[1] but can be demonstrated with the recently developed FLC assay. The FLC assay provides the opportunity to monitor disease activity more accurately since this is a quantitative and highly sensitive method for assessing free κ and λ light chains in serum.[4-6]

Amyloidosis is thought to be a dynamic process of deposition and resolution. This implies that after elimination of the precursor protein, amyloid deposits in organs might resolve in the course of time resulting in improvement of both organ function and clinical performance.[7] Therapy in all types of systemic amyloidosis is focused on eliminating the precursor protein thereby preventing further amyloid deposition in organs and eventually reaching resolution of amyloid. Because in AL amyloidosis the precursor protein is an immunoglobulin free light chain produced by a plasma cell dyscrasia, chemotherapy schemes used in multiple myeloma are also applied in AL amyloidosis. In general, due to major organ involvement in AL amyloidosis, these chemotherapy schemes are less well tolerated in AL amyloidosis compared to multiple myeloma.

Although histological regression of amyloid in tissue after successful treatment has been documented sporadically,[8,9] this regression has not been studied systematically in patients with AL amyloidosis.

The main objective of this study was to demonstrate regression of amyloid deposition in fat tissue of patients with AL amyloidosis following chemotherapy. Subsequently the relation between type of hematologic response as measured by FLC response and regression of amyloid deposition was studied.

Design and Methods

Patients

All consecutive patients with AL amyloidosis seen in our tertiary referral center between January 1994 and July 2007 were studied. Systemic amyloidosis was diagnosed either by detection of amyloid in a biopsy at a site typically involved in systemic amyloidosis, such as abdominal fat tissue, kidney, liver, spleen or nerve, or by a positive biopsy derived from at least 2 different organs or tissues, including the heart, gastro-intestinal tract (including rectum), and bone marrow. AL amyloidosis was defined by detecting a clonal plasma cell dyscrasia in patients who were immunohistochemically negative for AA type. A clonal plasma cell dyscrasia was diagnosed when a free kappa or lambda light chain was detected by free light chain assay in serum and/or urine, using a commercial FLC reagent and carried out on serum stored at -80°C, or immunofixation electrophoresis, or when a relative excess of plasma cells producing one of the two light chains was detected in the bone marrow. In patients with only cardiomyopathy or neuropathy a mutation of the TTR gene had to be excluded before AL amyloidosis was diagnosed. Organ involvement and response was assessed using established criteria[10] with minor modifications.

Included in the study were all patients in whom at least two fat tissue aspirates were obtained, one before chemotherapy and at least one after start of chemotherapy, all simultaneously with free light chain measurement in serum. The pre-treatment FLC measurement had to be abnormal by showing a single elevated FLC or, in case of renal insufficiency, an abnormal ratio of kappa and lambda. Patients were divided into three groups depending on the type of response of the amyloidogenic serum FLC. Complete response (CR) was defined as complete normalization of pre-treatment elevated amyloidogenic serum FLC concentration or, in case of complete renal failure, normalization of the ratio of both light chains. Partial response (PR) was defined as >50% decrease of pre-treatment elevated amyloidogenic serum FLC in serum but without complete normalization. Non-response (NR) was defined as <50% change or increase in pre-treatment elevated serum FLC in serum. Relapse was defined as unambiguous recurrence or an increased amyloidogenic FLC after complete or partial response, respectively. Reference levels were set using healthy blood donors: κ<20 mg/L and λ<32 mg/L, with a κ /λ reference ratio 0.28–1.20.

Chemotherapy was applied according to local protocol at the time of diagnosis. As of 1995 all patients were screened for intensive treatment with 3 courses of induction therapy with vincristin, doxorubicin and dexamethasone (VAD) followed by high-dose melphalan (HDM, 200 mg/m^2) and autologous stem cell transplantation (ASCT). Patients >65 years and those with renal insufficiency received intermediate dose melphalan (IDM, 140 mg/m^2) followed by ASCT. Eligibility was assessed using the Dutch national study protocol (*www.HOVON.nl*, HOVON 41 study).[11] Patients ineligible for intensive treatment were scheduled to less intensive treatment according to the opinion of the hematologist related to the amyloid center. These schemes consisted of orally administered melphalan with prednisolone (MP), melphalan with dexamethasone (MDex) and as of 2004 the combination with thalidomide. During follow-up, fat tissue was analyzed in the first

year every 3-6 months, thereafter (bi) annually. FLC was measured every three months. Follow-up was until death, until relapse of amyloidogenic FLC, or until end of survey (January 2008). Time to response of FLC was measured as of start of therapy. In the majority of patients therapy was started within a month of diagnosis. Follow-up of response was until last assessed fat tissue aspirate and FLC assay. The study was approved by the local ethics committee and informed consent was obtained in all patients.

Amyloid deposition in fat tissue

Subcutaneous abdominal fat tissue was aspirated and stained with Congo-red dye as previously described.[2] All fat smears were reviewed by two experienced observers who independently graded all smears at 40 x magnification, 3 smears per patient with total screening time being up to five minutes, using an Olympus 100-watt BX 50 microscope. A validated semi-quantitative grading system was used ranging from 0 to 4+: 0 (negative), 1+ (minute, <1% of surface area), 2+ (little, between 1% and 10%), 3+ (moderate, between 10-60%), and 4+ (abundant, >60%).[3] In case of disagreement between the 2 observers, the 3 smears were reviewed and discussed to obtain consensus. All fat smears were assessed in a blinded manner for clinical and laboratory data.

Statistical analysis

Statistical analysis was performed by using the statistical package GraphPad Prism, version 4.02 (GraphPad Software Inc., San Diego, CA, USA). Frequencies were compared with the use of χ^2 or Fisher's exact test. Logarithmic transformation was used if indicated to obtain normally distributed values. More than two groups were compared with ANOVA analysis followed by Bonferroni's multiple comparison test. Pearson's r was used for correlation.

A change of one grade of amyloid was expected to occur frequently by chance because of variability of both fat samples and observer assessment in this visual grading method. Therefore, only a change of 2 grades of amyloid was considered to reflect a significant change.[12] The change of at least 2 grades of amyloid in fat tissue was used as event to construct Kaplan-Meier curves of the patients who had the potential to reflect such a change. The log-rank test was used to detect differences between the curves. In all tests, two-tailed p values of less than 0.05 were considered significant.

Results

Patients and free light chain response

Between January 1994 and July 2007, 120 consecutive patients with AL amyloidosis were seen in our tertiary referral center. Fourteen patients were lost during follow-up because they were only admitted to our hospital for diagnosis and therapeutic advice and treated in centers in their own neighbourhood. In 48 patients only one fat tissue specimen was available because the majority of these patients died before a second biopsy could be performed. Two patients had pre-existing multiple myeloma and received previous chemotherapy and therefore were excluded. In 5 patients free light chain assessment was not determined: in 3 patients a pre-treatment free light chain was not available, 2 other patients had renal insufficiency with normal ratio of the light chains.

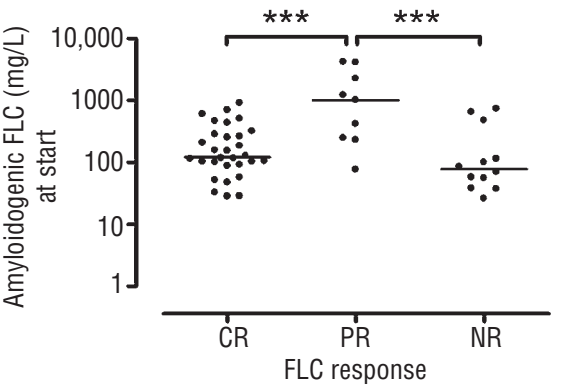

Figure 1. Amyloidogenic free light chain (FLC) (mg/L) at start of therapy. Patients were divided into 3 groups: complete responders (CR, n=30), partial responders (PR, n=9), and non-responders (NR, n=11). Horizontal bars denote median values; ***p<0.001.

The remaining selected 51 patients had more than one fat aspirate, at least one before and one after (chemo)therapy and simultaneous measurement of the FLC in serum. They all showed pre-treatment FLC elevation, and were included in the study. Of these 51 patients, 30 were identified with a complete response of the amyloidogenic FLC median 0.5 year (range 0.3-2.9 years) following therapy. Nine patients only reached a partial response of the amyloidogenic FLC after a median 0.5 year with median follow-up being 1.6 years (0.3-8.7 years) following therapy. In 12 patients no response of the amyloidogenic FLC was noticed after a median follow-up of 1.1 years (0.3-7.4 years). FLC values at start were significantly higher in partial responders (median 1010 mg/L) than in complete responders (median 122 mg/L) and non-responders (median 78 mg/l) (p<0.001) (Figure 1). In contrast with non-responders, complete responders and partial responders showed significant decrease of FLC following therapy (Figure 2).

Patients' characteristics are listed in Table 1. There was no significant difference in age (p=0.4) or number of organs involved (p=0.3), but significantly less frequently heart and nerve involvement (p<0.05) was seen in complete responders compared to partial responders and non-responders. Initial treatment differed therefore in these groups: almost all complete responders (80%) were treated with HDM/IDM plus ASCT, whereas only a minority of partial responders and non-responders (19%) were eligible to receive this intensive treatment (p<0.0001).

In this landmark analysis a significant difference in survival was seen between complete responders and the group of partial responders and non-responders (p<0.004). In 76% of the partial responders and non-

responders death was related to amyloidosis compared to 3% in complete responders.

Changes of amyloid grade in fat tissue

Regression of amyloid at last observation was seen almost exclusively in complete responders and was significantly higher in the group of complete responders than in the non-responders ($p<0.05$, Figure 3A). Only complete responders showed a significant correlation (r=-0.54; $p<0.002$) between the decrease of amyloid in fat tissue and duration of follow-up (Figure 3B).

Of the 25 patients able to reflect significant regression, 8 patients (32%) demonstrated this regression following chemotherapy, after a median follow-up of 1.9 years (0.9-6.4 years). In the following fat tissue aspirates this response was sustained in 6 patients, one patient showed a FLC relapse at the consecutive biopsy and one patient developed acute leukemia that was rapidly fatal. Amyloid regression was followed by stabilization or improvement of organ response: one patient with cardiac disease remained stable and all 4 patients with proteinuria, the 2 patients with elevated alkaline phosphatase and one of 3 patients with neuropathy showed normalization. ^{123}I-serum amyloid P component scintigraphy[13] showed improvement in 7 patients and stabilization in one patient.

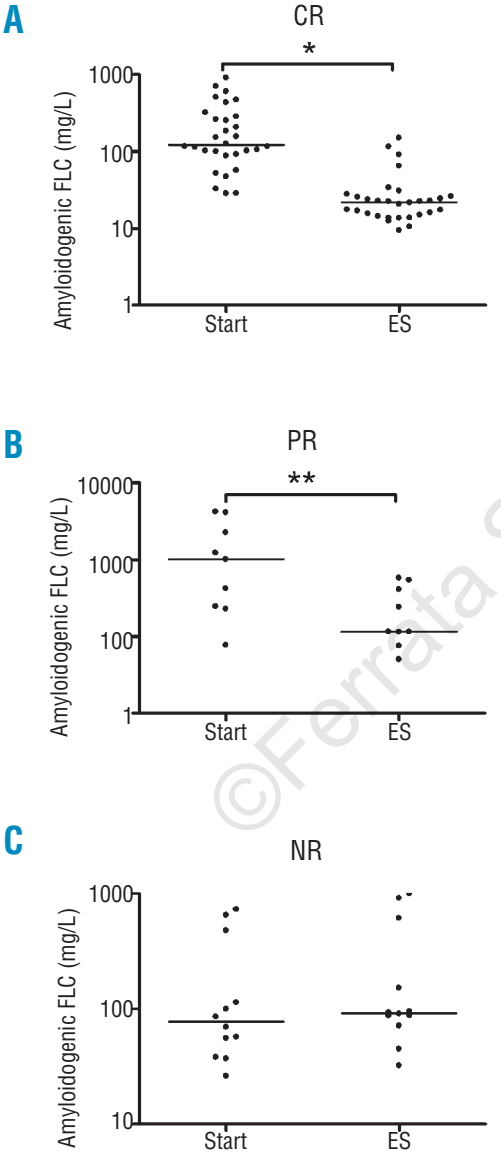

Figure 2. Response of amyloidogenic FLC (mg/L) following chemotherapy. (A) Complete responders (n=30). (B) Partial responders (n=9). (C) Non-responders (n=12). ES denotes end of study, horizontal bars denote median values; *$p<0.05$, **$p<0.005$.

Table 1. Patients' characteristics based on free light chain response.

	CR n=30	PR n=9	NR n=12
M:F	11:19	5:4	6:6
Median age (range) in years	57 (33-74)	59 (51-82)	62 (51-82)
κ:λ	7:23	6:3	2:10
BM PC < 10%:PC > 10%	27:3	6:1*	10:2
Median number (range) of fat aspirates	5 (2-11)	3 (2-6)	2 (2-10)
Median FU (range) of fat tissue in months	19.5 (3-104)	15 (3-68)	10 (4-75)
Amyloid grade at diagnosis			
0	0	0	0
1	5 (17%)	2 (22%)	1 (8%)
2	5 (17%)	1 (11%)	1 (8%)
3	11 (37%)	3 (33%)	5 (42%)
4	9 (30%)	3 (33%)	5 (42%)
Major organ involvement†			
heart	10 (33%)	6 (67%)	8 (67%)
liver	11 (37%)	3 (33%)	3 (25%)
kidney	23 (77%)	5 (56%)	10 (83%)
nervous system	10 (33%)	6 (67%)	7 (58%)
N. of major organs involved			
0	2 (7%)	1 (11%)	0
1	10 (33%)	1 (11%)	3 (25%)
2	12 (40%)	2 (22%)	4 (33%)
3	4 (13%)	4 (44%)	3 (25%)
4	2 (7%)	1 (11%)	2 (17%)
Major therapy			
HDM/IDM +ASCT	24 (80%)	2 (22%)	2 (17%)
MP or MDex	2 (7%)	6 (67%)	10(83%)
MP/MDex + thalidomide	4 (13%)	‡	0
Median FU (range) in months	19.5 (3-104)	15 (3-68)	10 (4-75)
End of FU due to			
Death	7 (23%)	7 (78%)	9 (75%)
caused by amyloidosis	1 (14%)	7 (100%)	9 (100%)
Relapse of FLC	10 (33%)§		
End of survey	13 (43%)	2 (22%)	3 (25%)
Survival			
Median (years)	Not reached	2.0	2.9
Range (years)	0.3 - 12.3	0.5 - 8.2	0.5 - 8.9

*CR: complete response of FLC; PR: partial response of FLC; NR: no-response of FLC; BM: bone marrow; PC: plasmocytosis; FU: follow-up; MP: melphalan/prednisolone; MDex: melphalan/dexamethasone; HDM: high-dose melphalan: 200 mg/m^2; IDM: intermediate dose melphalan 140 mg/m^2; ASCT: autologous stem cell transplantation, *one patient had a plasmocytoma and one patient had a Non-hodgkin's lymphoma. †Major organ involvement[10] ‡one patient had radiotherapy for plasmocytoma;§Relapse of FLC after a CR that had been reached a median 1.7 years (0.7-7.0) after start of therapy.*

Kaplan-Meier analysis (Figure 4A) showed a significant regression of 2 amyloid grades in 50% of the patients after 2.4 years and in 80% after 3.2 years. In 6 patients amyloid disappeared completely from fat tissue (4 out of 5 with pre-treatment score of 1+, one with pre-treatment score of 2+ and one with pre-treatment score of 3+). None of the partial responders or non-responders who had the potential to demonstrate a reduction of 2 grades of amyloid deposition showed such a reduction after a median follow-up of fat tissue analysis of 1.3 years (0.3-5.7 years) and 0.8 years (0.3-6.3 years), respectively. The 2 partial responders at the end of survey who survived did not show a complete response of FLC or amyloid regression at extended follow-up.

Clinical condition of the remaining 17 patients with a complete response of FLC but without 2 grades regression of amyloid improved in 4, stabilized in 10 and deteriorated eventually after a period of stabilization in 3 patients. Of the 5 patients with a complete response of FLC and a Congo red score of 1+ at start, clinical condition improved in 3 patients, stabilized in one patient and deteriorated in one patient. Of the 9 partial responders, 2 had stable clinical disease whereas 7 deteriorated clinically and all 12 non-responders showed clinical deterioration.

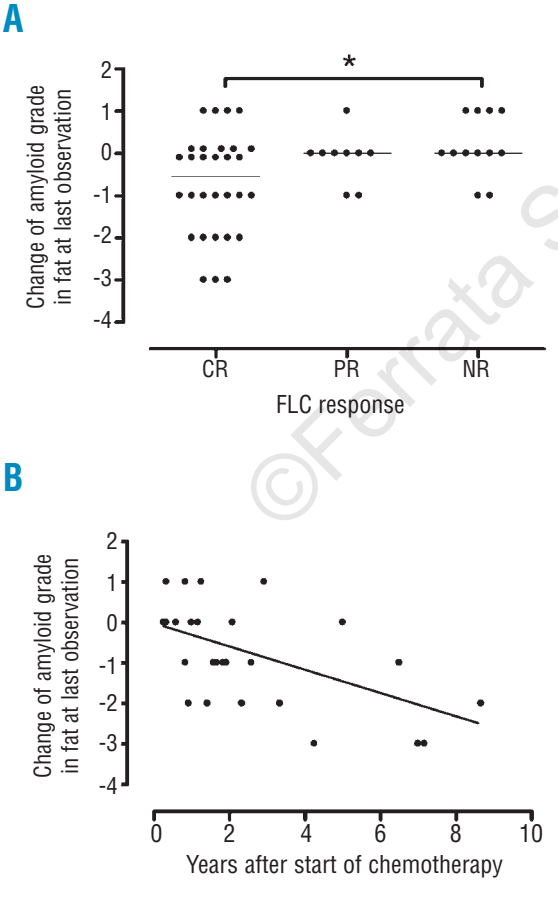

Figure 3. (A) Change of amyloid grade in fat tissue at last observation in complete responders (CR), partial responders (PR), and non-responders (NR) of FLC; * $p<0.05$. (B) Linear regression of the change of amyloid grade in fat tissue at last observation for complete responders ($r=-0.54$, $p<0.002$).

Finally the end-point of 2 grades reduction of amyloid deposition in fat tissue differed significantly ($p=0.01$) between patients with complete response and the combined group of patients with partial response and no response (Figure 4B).

Initial therapy and free light chain response

In retrospect, 28 of the 51 studied patients in this landmark analysis had received intensive therapy including high or intermediate dose melphalan and autologous stem cell reinfusion. In 83% a complete response of serum FLC had been reached, 7% had a partial response and 10% had shown no response. Twenty-two patients had not been eligible for intensive chemotherapy and had been treated with MP or MDex. A patient with a solitary plasmacytoma had been treated with radiotherapy. Of these 23 patients, 29% had shown a complete response, 29% had shown a partial response and 43% had no response. Only 4 patients had been treated primarily with MP or MDex combined with thalidomide and all 4 patients showed a compete response.

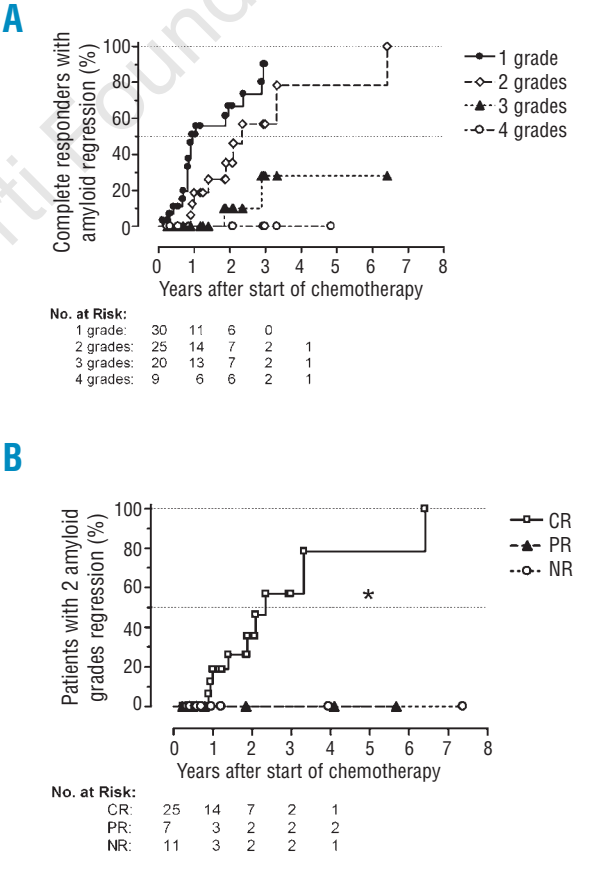

Figure 4. (A) Kaplan-Meier curves of patients with complete response (CR) of serum free light chain. Endpoints are decreases of 1, 2, 3 and 4 grades of amyloid in fat tissue, respectively. The numbers of CR patients at start and later who had the potential to reflect 1, 2, 3, and 4 grades are shown at the bottom. (B) Kaplan-Meier curves of patients with complete response (CR), partial response (PR), and no response (NR) of serum free light chain. Endpoint is a significant decrease of 2 grades of amyloid in fat tissue. The numbers of patients at start and later who had the potential to reflect such a decrease are shown at the bottom; *$p<0.05$.

Discussion

Although improvement of AL amyloidosis after successful treatment has been established by means of clinical, laboratory, and scintigraphic parameters, histological regression has been documented only sporadically.[8,9] This is the first prospective study showing actual histological regression of amyloid after successful treatment of AL amyloidosis. Fat tissue biopsy is a convenient and non-invasive technique, being an outpatient bed-side procedure of minutes duration. The recently validated semi-quantitative assessment of amyloid in fat tissue has now been shown to be a useful histological marker to define the effect of therapy in AL amyloidosis.

Although numbers are relatively small, this study in a selected group of patients with AL amyloidosis shows a clear relation between the type of hematologic response and regression of amyloid in fat tissue, in that only patients with complete normalization of FLC in serum show a significant decrease in amyloid grade. This significant decrease started ten months following therapy and, by using Kaplan-Meier analysis, it was seen in 50% of the patients 2.4 years following therapy and in 80% of the patients 3.2 years following therapy. This implies that regression of amyloid in fat tissue is a relatively slow process. Therefore patients should have a rather long life expectancy to show this regression. None of the partial responders or non-responders of FLC in serum showed significant regression of amyloid in fat tissue. In non-responders this is most likely due to insensitivity to the given chemotherapy, since FLC did not change at all. The result of the group of partial responders is intriguing. The amyloidogenic FLC levels of partial responders at start appeared to be almost ten times higher than in the two other groups but showed significant regression without complete normalization following therapy. The lack of significant amyloid regression in partial responders therefore might be due to insufficient time to reach FLC normalization rather than to insensitivity to chemotherapy. One might speculate that the absolute value of remaining elevated FLC levels is responsible for continued amyloid formation or at least for a balance in amyloid formation and regression. At last observation the levels of FLC in partial responders and non-responders were in a similar range and with such a similar supply of amyloidotic FLC to the tissues it is not surprising that no tendency was observed of amyloid regression in fat tissue over time. This might be an argument for starting maintenance therapy in case a partial response has been obtained. Two recent studies in multiple myeloma showed that the complete response group can be enlarged by treating patients following HDM and ASCT with thalidomide maintenance therapy.[14,15] Nearly all partial responders died due to amyloidosis. This suggests that high absolute levels of amyloidogenic FLC at start is a negative prognostic factor for reaching complete response as already described for MM.[16]

In this landmark analysis complete responders showed superior survival when compared to partial responders and non-responders, confirming previous studies.[17] Patient selection plays a role in our study, since a selected group of patients was included in the study and complete responders had less frequently heart and nerve involvement. Complete responders, therefore, were more frequently eligible for intensive therapy than partial responders and non-responders. Dispenzieri *et al.* showed in a case-control study that despite patient selection intensive treatment showed superior results.[18]

Debate is still going on about what is best therapy for AL amyloidosis and randomized controlled trials are scarce. The search is for therapy giving a quick, complete and longstanding response of FLC without side-effects. The results of a recently published randomized French multicenter study did not show higher complete response rate and survival benefit of high-dose melphalan and stem cell transplantation compared to oral melphalan and high-dose dexamethasone (MDex).[19] However, this multi-center study should be interpreted with caution, because the intensively treated group showed remarkable unfavorable results in terms of serum FLC response and especially survival with a nearly doubled percentage of pre-treatment deaths and treatment-related deaths compared to single-center studies.[20,21]

The search for less lethal and non-toxic chemotherapy is imperative. New treatment modalities such as MDex[22] and thalidomide[23] (although frequently not well-tolerated), lenalidomide[24,25] and especially bortezomib,[26] all in combination with dexamethasone show promising results in small studies and might become opportune therapy in the future.

In conclusion, our study is the first to showing actual histological regression of amyloid deposits in tissue after successful treatment of AL amyloidosis and confirms the importance of normalization of the amyloidogenic FLC in serum: only patients with a complete response of FLC showed amyloid regression. FLC is not only an important marker of hematologic response, but possibly also the best surrogate marker of histological response following chemotherapy in patients with AL amyloidosis.

Authorship and Disclosures

IIvG and EV designed the research, analyzed the data and wrote the article; MHvR designed the research, analyzed the data and reviewed the article; JB performed research and reviewed the article; BPCH designed and performed the research, analyzed the data and wrote the article.

The authors reported no potential conflicts of interest.

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