

Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy

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Received 20 December 2002; accepted for publication 3 February 2003

Summary. Monoclonal immunoglobulin light chains are deposited as amyloid fibrils in systemic AL (primary) amyloidosis, but the underlying plasma cell dyscrasias are often difficult to detect or unquantifiable. The relationships between circulating monoclonal light chains, amyloid load and clinical outcome, and the relative efficacies of chemotherapy regimens aimed at suppressing monoclonal immunoglobulin production, have not been determined. Circulating free immunoglobulin light chain (FLC) concentration was measured with a sensitive nephelometric immunoassay in 262 patients with AL amyloidosis, and followed serially in 137 patients who received either high-dose chemotherapy or one of two intermediate-dose cytotoxic regimens. Amyloid load was quantified by serum amyloid P component scintigraphy. A monoclonal excess of FLC was identified at diagnosis in 98% of patients. Among 86 patients whose abnormal FLC concentration fell by more

than 50% following chemotherapy, 5-year survival was 88% compared with only 39% among those whose FLC did not fall by half ($P < 0.0001$). Amyloid deposits regressed in 58 patients. The magnitude and duration of the FLC responses to intermediate- and high-dose chemotherapy regimens were similar. The FLC assay enabled the circulating fibril precursor protein in AL amyloidosis to be quantified and monitored in most patients. Reduction of the amyloidogenic FLC by more than 50% was associated with substantial survival benefit, regardless of the type of chemotherapy used. Clinical improvement following chemotherapy in AL amyloidosis is delayed, but treatment strategies can be guided by their early effect on serum FLC concentration.

Keywords: amyloidosis, amyloid protein AL, assay, chemotherapy, diagnosis.

Primary AL amyloid is the most common and severe form of systemic amyloidosis, but its diagnosis and treatment remain difficult and unsatisfactory. Although AL fibrils are derived from circulating monoclonal immunoglobulin light chains, the underlying clonal plasma cell dyscrasias are often remarkably subtle and hard to detect, and are frequently impossible to monitor quantitatively (Buxbaum, 1986; Kyle & Gertz, 1995). Patients may benefit from cytotoxic chemotherapy that suppresses the underlying monoclonal gammopathy, although symptomatic

improvement and functional recovery of amyloidotic organs is usually slow (Gertz *et al*, 1991; Hawkins, 1997). Low-dose oral chemotherapy, comprising melphalan and prednisolone (MP), is the only type of treatment that has been evaluated in randomized controlled clinical trials, and its efficacy is modest (Kyle *et al*, 1997). High-dose melphalan coupled with haematopoietic stem cell support (HDM) (Gertz *et al*, 2000; Sanchorawala *et al*, 2001) is now used widely, but has a procedure-related mortality of 15–40% in patients with AL amyloidosis (Moreau *et al*, 1998; Gillmore *et al*, 1999), and its apparently more favourable outcome may be biased by the selection of fitter patients for this hazardous approach (Dispenzieri *et al*, 2001). We have adopted intermediate-dose infusional regimens such as: vincristine, adriamycin and dexamethasone (VAD) (Samson *et al*, 1989); cyclophosphamide, vincristine, adriamycin and

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methylprednisolone (C-VAMP) (Raje *et al.*, 1997); and intermediate-dose melphalan (IDM) (Schey *et al.*, 1998), but the results have not hitherto been analysed systematically.

A circulating whole paraprotein cannot be identified by serum electrophoresis at the time of diagnosis in more than half of all patients with AL amyloidosis (Kyle & Gertz, 1995), and in many others the concentration is so low that quantification is either imprecise or impossible. Immunofixation is more sensitive and can detect free monoclonal immunoglobulin light chains in the urine in up to 80–90% of patients, but the results are not quantitative and are dependent on renal function (Keren, 1999). In contrast to multiple myeloma in which paraproteins are typically 10–100-fold more abundant, conventional immunochemical techniques are, therefore, often insufficiently sensitive to evaluate the response to chemotherapy of the monoclonal gammopathy in AL amyloidosis.

We report here the application of a new highly sensitive and quantitative nephelometric immunoassay (Bradwell *et al.*, 2001) with which we were able to identify free monoclonal immunoglobulin light chains (FLC) in the serum of virtually all patients with AL amyloidosis. Use of the assay in conjunction with serial serum amyloid P component (SAP) scintigraphy, a technique for quantitatively imaging amyloid deposits (Hawkins *et al.*, 1990), enabled us to define the relationships between the abundance of the light chain precursors of the AL amyloid fibril protein, whole body amyloid load and clinical outcome in a series of patients with AL amyloidosis. The study demonstrated that AL amyloid deposits frequently regressed following treatment and suggested that the efficacy of intermediate-dose infusional chemotherapy is comparable to that of autologous stem cell transplantation.

MATERIALS AND METHODS

Protocol. A protocol for the diagnosis, investigation and monitoring of patients with AL amyloidosis was implemented in 1992 in a single UK centre. Recommendations for cytotoxic chemotherapy, favouring infusional approaches, were offered on an individual basis that reflected clinical characteristics, potential for adverse effects and patients' preferences. Clinical evaluations included assessment of monoclonal immunoglobulin production and quantitative whole-body ^{125}I -labelled SAP scintigraphy to monitor amyloid load, in order to investigate the relationship between the circulating monoclonal immunoglobulin concentration and amyloid fibril deposition. A bone marrow examination, including immunophenotyping, was performed at baseline. Serum samples obtained at each visit were stored at -30°C .

Patients and diagnosis. Two hundred and sixty-two patients with systemic AL amyloidosis were studied, comprising all such patients referred to our centre between 1992 and 2002 who had not received previous chemotherapy, in whom concurrent multiple myeloma and other malignant B-cell dyscrasias had been excluded, and in whom amyloid had been confirmed histologically. The

diagnosis of AL-type amyloidosis was confirmed immunohistochemically (Tan & Pepys, 1994) and, when staining for AL amyloid was not definitive, the genes encoding transthyretin, fibrinogen A α -chain, apolipoprotein AI and lysozyme were sequenced to exclude all known amyloidogenic mutations (Lachmann *et al.*, 2002). One hundred and sixty-four of these patients underwent infusional chemotherapy in the form of VAD/C-VAMP, IDM or HDM, of whom 137 survived for more than 6 months and were followed-up on at least one occasion.

Clinical assessment. Patients underwent comprehensive baseline clinical assessment and biannual review at the UK National Amyloidosis Centre. Monoclonal immunoglobulin was sought by electrophoresis and immunofixation of serum and urine, and quantitative assessment of the distribution and extent of visceral amyloid deposits was determined by whole-body ^{125}I -labelled SAP scintigraphy (Hawkins *et al.*, 1990). Blinded comparisons were made between the scans obtained at baseline and 1 year after undergoing chemotherapy. Accumulation of amyloid was defined as an increase in tracer uptake in at least one of the amyloid-infiltrated organs compared with the initial scan, or involvement of a previously unaffected organ, and regression was defined as a decrease in tracer uptake in a least one affected organ. The amyloid load was defined as steady when the relative amount of tracer in target organs and in the blood-pool background was unchanged between scans (Rydh *et al.*, 1998). Echocardiography was used to evaluate cardiac amyloid.

Serum free immunoglobulin light chain assay. Serum kappa and lambda FLC were measured in stored serum samples using a latex-enhanced immunoassay (The Binding Site, Birmingham, UK) on a Behring BN II nephelometric analyser (Dade Behring, Deerfield, IL, USA) (Bradwell *et al.*, 2001; Drayson *et al.*, 2001). The assay utilizes antibodies directed against FLC epitopes that are hidden in whole immunoglobulin molecules, and has a sensitivity of < 5 mg/l. This compares with typical detection limits of 150–500 mg/l by immunofixation and 500–2000 mg/l by electrophoresis. The reference range was established using 282 healthy volunteers' sera in which the 95% confidence interval (CI) for polyclonal free kappa and free lambda light chains was 3.3–19.4 mg/l and 5.7–26.3 mg/l, respectively, with a mean kappa to lambda ratio of 0.59 (95% CI 0.3–1.2) (Katzmann *et al.*, 2002). Monoclonal FLC were identified as values for kappa or lambda that exceeded the respective reference ranges and produced an abnormal kappa to lambda ratio. In each patient, the serum concentration of the monoclonal class of FLC 6 months after undergoing chemotherapy was expressed as a percentage of the pretreatment value.

Chemotherapy. Among the 164 patients who underwent infusional chemotherapy, 92 received monthly courses of VAD (Samson *et al.*, 1989) or C-VAMP (Raje *et al.*, 1997) on three to six occasions; 24 patients had two to six courses of intravenous IDM at 25 mg/m²/month (Schey *et al.*, 1998) and 48 patients had HDM (Gertz *et al.*, 2000). Twenty-seven of these patients died within 6 months of commencing treatment (eight VAD/C-VAMP, four IDM, 15 HDM) and,

therefore, 137 patients were followed-up. Most patients were treated in their local haematology centres.

Statistical analysis. Patient survival from baseline visit was estimated by Kaplan–Meier analysis and groups were compared by log-rank tests. Cox regression analysis was used to assess the impact on survival of age, initial serum concentration of the monoclonal class of FLC, cardiac involvement, class of aberrant FLC and renal function at presentation. The responses to IDM, VAD/C-VAMP and HDM were compared by the Kruskal–Wallis and Fisher’s exact tests. Patients were classified according to whether their amyloid load had accumulated, remained unchanged or diminished between the initial SAP scan and the follow-up scan 1 year after undergoing chemotherapy. Relationships between changes in amyloid load and the effect of chemotherapy on the serum concentration of the monoclonal class of FLC were sought by the Kruskal–Wallis test.

RESULTS

Monoclonal immunoglobulin could not be detected at presentation, by electrophoresis or immunofixation in either serum or urine in 55 (21%) of the 262 patients. In a further 67 patients (26%), monoclonal light chains could be detected only qualitatively by immunofixation of serum or urine. A quantifiable circulating whole monoclonal immunoglobulin was identified in 140 patients (53%), in more than three-quarters of these at a concentration of less than 10 g per litre (median less than 7 g per litre). In contrast, circulating monoclonal FLC were identified by the nephelometric assay in 257 patients (98%) at presentation (Fig 1A). The kappa or lambda class of monoclonal immunoglobulin demonstrated by the FLC assay in 79 and 178 patients, respectively, was corroborated by immunofixation or bone marrow immunophenotyping studies in each of 207 patients in whom these other investigations gave positive results. In most patients, the concentration of the monoclonal class of FLC was within the range of 30–500 mg/l, and these values did not correlate with the concentration of whole monoclonal immunoglobulin when this was present.

The characteristics of 164 patients who were assessed prior to their chemotherapy are shown in Table I. Of these, 137 patients survived for more than 6 months and could, therefore, be included in the analysis. Each of these patients had an excess of one or other class of FLC by nephelometric assay at presentation. The serum concentration of the clonal FLC fell by more than 50% in 86 (63%) of these patients. There was no significant difference in FLC response or survival beyond 6 months among the patients who had been treated with IDM, VAD/C-VAMP and HDM (Fig 1B), and the probability of the amyloidogenic class of serum FLC value remaining below 50% of pretreatment values after 5 years was also the same in the three chemotherapy groups (Fig 1C). Survival at 5 years was 88% among patients whose FLC concentration fell by more than half, and 39% among patients whose FLC concentration remained above this value ($P < 0.001$) (Fig 1D). Median survival was 15 months in the 27 patients whose FLC

showed no response at all ($P < 0.0001$). Suppression of the amyloidogenic FLC concentration by greater than 75% or 90% was not associated with significantly better survival than a fall of more than just 50%. Using Cox regression analysis, the relative risk of death was 10-fold higher in patients whose FLC values were suppressed by less than 50% ($P < 0.0001$, 95% CI 4.15–24.12). Among the 73 patients in whom a whole serum paraprotein could be quantified serially, survival was also better in those whose concentration fell by more than 50% compared with those in whom it fell by less than this extent, but at a lower level of significance ($P < 0.05$). In this post-treatment study period, survival was not influenced by age, renal failure, or by class or initial concentration of serum FLC, although there was a trend towards increased mortality in patients with echocardiographic evidence of amyloid. Patient characteristics at diagnosis that have been identified in other studies to be associated with a better prognosis, comprising age < 70 years, preserved renal function, absence of hyperbilirubinaemia and cardiac involvement (Dispenzieri *et al*, 2001), did not differ significantly among the patients who achieved more than 50% suppression of their amyloidogenic serum FLC concentration compared with those who did not.

Follow-up SAP scintigraphy 1 year after baseline in the 127 survivors showed accumulation of amyloid in 30 patients (24%), no change in 39 patients (31%) and regression in 58 patients (46%). Changes in the amyloid load correlated with changes in serum FLC concentration (Fig 2A and B) ($P < 0.0001$). Among patients whose aberrant FLC concentration was suppressed by more than 50%, regression of amyloid was associated with a substantial survival advantage compared with patients whose deposits merely remained stable, the projected 5-year survival figures by Kaplan–Meier analysis being 95% and 79% respectively ($P < 0.0001$).

DISCUSSION

AL amyloid deposits are derived from circulating monoclonal immunoglobulin light chains, but it has not previously been possible to investigate the relationship between abundance of the AL amyloid precursor protein and its fibril product. The combination of serum FLC quantification and serial SAP scintigraphy indicates that AL amyloid deposits exist in a state of turnover, and that reduction in amyloidogenic FLC concentration by more than 50% is frequently associated with regression of amyloid and prolonged survival. Our demonstration of the clear relationship between the serum concentration of FLC and clinical outcome provides a unique and robust objective criterion for monitoring chemotherapy in AL amyloidosis.

Conventional biochemical techniques were unable to identify monoclonal immunoglobulin in serum or urine in one fifth of patients in this series, and detected it only qualitatively in a further one quarter. In most other patients, quantification was imprecise as a result of the low level of paraprotein, or because a monoclonal immunoglobulin could only be measured in the urine. In contrast,

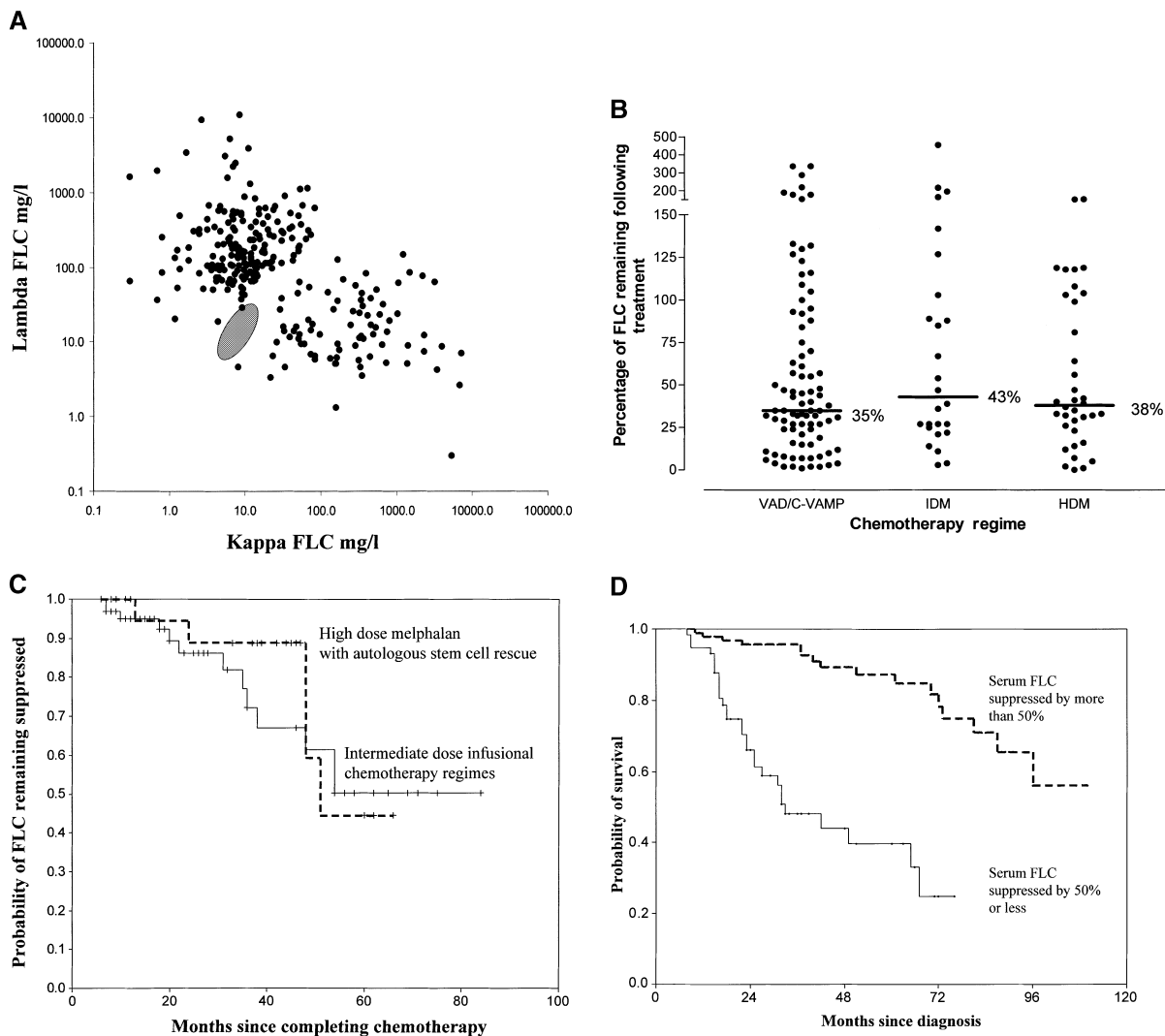


Fig 1. (A) Serum concentration of free kappa and lambda immunoglobulin light chains in 262 patients with AL amyloidosis prior to treatment. The shaded area represents the normal range derived from healthy control subjects. (B) Concentration of the amyloidogenic class of free light chain after 6 months of chemotherapy expressed as a percentage of the value immediately before treatment. The median for each group is marked. There was no significant difference between patients who received VAD/C-VAMP ($n = 84$), IDM ($n = 20$) and those who completed HDM ($n = 33$) (Kruskal–Wallis test, $P = 0.547$). (C) Kaplan–Meier estimate of the probability of the concentration of the amyloidogenic class of free light chain remaining below 50% of the pretreatment value in 86 patients who had responded to intermediate-dose chemotherapy (VAD, C-VAMP or IDM) or HDM. There was no significant difference between the groups. (D) Kaplan–Meier estimate of survival in 137 patients with systemic AL amyloidosis following initial assessment. Eighty-six patients (63%) had a greater than 50% fall in the concentration of the amyloidogenic class of free light chains. Their survival was significantly greater than among the remaining patients whose free light chain concentration fell by less than 50% after completing chemotherapy ($P < 0.0001$).

monoclonal serum FLC were identified in more than 98% of patients at diagnosis and could be monitored quantitatively following chemotherapy. Although the antibodies in the assay do not distinguish monoclonal FLC from low-level polyclonal FLC that exist in the healthy population, a relative excess of kappa or lambda FLC correctly identified the amyloidogenic FLC class in every patient in whom it could be verified. Demonstration of free clonal light chains in virtually all patients with AL amyloidosis supports *in vitro* observations that their free state contributes to their amyloidogenicity (Buxbaum, 1986). In our wider experience, the

results of the FLC assay have been normal in more than half of individuals with uncomplicated low-grade plasma cell dyscrasias (unpublished observations) and, therefore, this test may help to support or argue against a diagnosis of AL amyloidosis in patients with monoclonal gammopathies of undetermined significance.

Rational treatment of systemic amyloidosis centres on the reduction of the supply of amyloid fibril precursor proteins, but in AL amyloidosis only low-dose chemotherapy has been subject to controlled studies. Moreover, in these and all other open chemotherapy studies, the response of the

Table I. Characteristics of patients receiving different chemotherapy regimes.

Chemotherapy regime	Number of patients	Mean age, years (range)	Echocardiographic features of amyloid cardiomyopathy, n (%)	Dialysis at time of chemotherapy, n (%)	Median follow-up, months (range)	Deaths, n (%)	Change in amyloid load by SAP scintigraphy at 12-month follow-up, n (%)		
							Regression	Stable	Progression
VAD	92	55.5 (29–77)	34 (37)	9 (10)	27 (0–111)	39 (42)	34 (42)	27 (34)	19 (24)
IDM	24	64 (46–77)	12 (50)	4 (17)	21 (0–79)	9 (38)	9 (50)	4 (22)	5 (28)
HDM	48	53.7 (32–67)	17 (35)	6 (6)	29 (0–68)	19 (40)	15 (52)	8 (28)	6 (21)

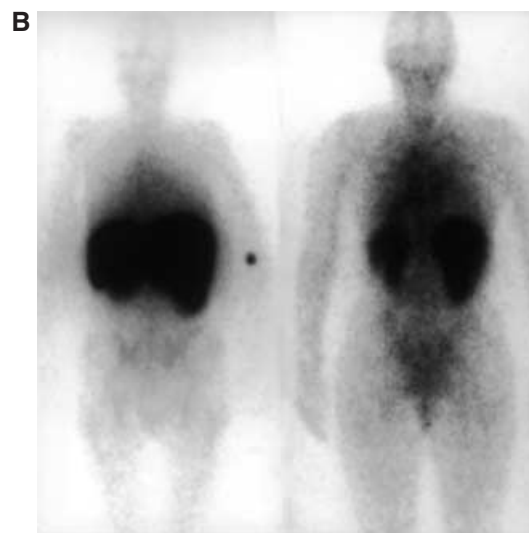
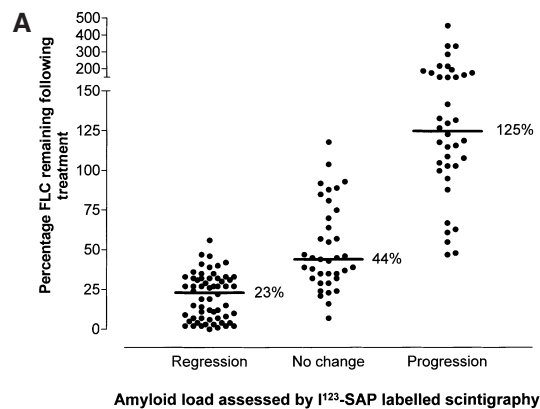


Fig 2. (A) Relationship between the change in amyloid load at the 12-month assessment and the percentage change in serum concentration of the amyloidogenic class of free light chains following chemotherapy, in the 127 surviving patients. The median percentage change in free light chain concentration in each group is indicated (Kruskal–Wallis test, $P < 0.0001$). (B) Radiolabelled SAP scintigraphy: posterior images of a 52-year-old woman with systemic AL kappa amyloidosis, before (left) and 1 year after (right) HDM chemotherapy, demonstrating regression of amyloid deposits in the liver, spleen and bone marrow associated with significant weight regain. The serum concentration of free kappa light chains had fallen from 551 mg/l to 52 mg/l.

underlying clonal disease has been assessed using methods and criteria developed in multiple myeloma. These have insufficient sensitivity and quantitative capacity to effectively monitor the very low levels of monoclonal proteins that typically occur in AL amyloidosis. The high sensitivity and quantitative nature of the nephelometric FLC assay address these shortcomings. In some individuals, the plasma concentration of FLC may increase in association with age or reduced glomerular filtration, but the kappa to lambda ratio that indicates monoclonality is unaffected (Clark *et al*, 2001). Five patients developed renal failure during the study and, therefore, the response to chemotherapy may have been underestimated in these few patients. Conversely,

retention of amyloidogenic FLC in the circulation as a result of renal impairment, which would not be reflected by conventional measurements of whole monoclonal immunoglobulin, might promote amyloid deposition and this possibility merits further investigation.

The present results showed that reduction of the amyloidogenic serum FLC concentration by greater than just 50% was associated with a favourable clinical outcome in many patients with AL amyloidosis. Reduction of amyloidogenic FLC production by this degree is usually sufficient to prevent further accumulation of amyloid, can lead to regression of existing amyloid deposits and substantially improves survival. In contrast to the situation in multiple myeloma, there were no significant differences between the degree of suppression of the clonal disease or the durability of this response among patients who were treated with intermediate-dose or high-dose chemotherapy regimens. The efficacy of VAD/C-VAMP, IDM and HDM in the non-randomized and selected patients in this study was similar to the efficacy of HDM in other reported series (Santhorawala *et al.*, 2001). Interestingly, substantial and prolonged suppression of FLC production was achieved in some patients who, for various reasons, had received only one or two courses of VAD/C-VAMP or IDM. These intermediate-dose regimens were given monthly in up to six or more cycles and, in contrast with HDM, the cumulative risks were, therefore, reduced in patients who required fewer courses of treatment. These findings may reflect differences in the biology of the plasma cell clones that typically underlie AL amyloidosis compared with those in multiple myeloma, and may have implications regarding the choice of chemotherapy in individuals and in future randomized controlled studies in AL amyloidosis. Although the present study necessarily comprised patients who survived 6 months after baseline evaluation, our wider experience has been that 7%, 13% and 25% of patients treated with VAD/C-VAMP, IDM and HDM, respectively, died within 6 months of commencing therapy. This lends further support to the intermediate-dose chemotherapy approach.

The beneficial effect of a reduction in serum FLC concentration, even by only 50%, in terms of regression of amyloid and improved survival reflects the natural turnover of the deposits, uniquely demonstrated here by SAP scintigraphy. This specific and safe technique overcomes the sampling error inherent in biopsy histology and provides a readily repeatable quantitative survey of whole-body amyloid load. Regression of amyloid has lately been confirmed by SAP scintigraphy in series of patients with amyloidosis of AA (Gillmore *et al.*, 2001a), hereditary (Rydh *et al.*, 1998; Gillmore *et al.*, 2000, 2001b) and B₂-microglobulin types (Tan *et al.*, 1996) following treatments that reduce the circulating concentration of the respective amyloid fibril precursor proteins. Although the process by which amyloid deposits are cleared is not understood, serial SAP scintigraphy has demonstrated that the rate of turnover of amyloid varies greatly between patients, underlying the need to use it in conjunction with the monitoring of circulating amyloid precursor proteins to guide the degree by which the

precursor supply needs to be reduced in individual patients. For example, suppression of the amyloidogenic FLC concentration by 40% was sufficient to lead to regression of amyloid in one patient in this study, but was associated with progression in another. Although as complete as possible suppression of the clonal plasma cell disorder would seem intuitively desirable in AL amyloidosis, and the likelihood of the deposits regressing did correlate with the degree by which the amyloidogenic FLC concentration was suppressed, survival benefit was not shown to be significantly better among patients whose clonal disease was suppressed by greater than 75% or even 90% as compared with those in whom it fell by more than just 50%. This may reflect the small numbers of patients whose clonal disease was suppressed so substantially, and the relatively short follow-up of some of the patients.

In conclusion, our study demonstrates the utility of the nephelometric serum FLC assay in AL amyloidosis and indicates that treatment strategies in AL amyloid may be guided by their effect on reducing the amyloidogenic serum FLC concentration. Although many factors may contribute to outcome in AL amyloidosis, including age, early diagnosis, pattern of organ involvement and supportive measures, including organ transplantation, therapy that lowers the amyloidogenic FLC concentration can stop accumulation of AL amyloid deposits, lead to their regression and greatly improve survival. Unlike whole immunoglobulins, FLC have a circulating half-life of several hours rather than many weeks, and their measurement enables response to chemotherapy to be evaluated rapidly. Frequent FLC analysis may enable exposure to chemotherapy to be minimized and help define patients with refractory disease at an early stage. Finally, quantitative monitoring of serum FLC will contribute importantly to systematic studies comparing different chemotherapy regimens in AL amyloidosis.

ACKNOWLEDGMENTS

We thank our many colleagues for referring and treating the patients, Sheril Madhoo, Dorothea Gopaul and Jayshree Joshi for the care and investigation of patients in the National Amyloidosis Centre, and Beth Jones for expert preparation of the manuscript.

This work was supported in part by grants to M.B.P. and P.N.H. from the Medical Research Council (UK) and The Wellcome Trust, a Wellcome Trust Research Training Fellowship to J.D.G., and by NHS Research and Development Funds.

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