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Remission status defined by immunofixation vs. electrophoresis after autologous transplantation has a major impact on the outcome of multiple myeloma patients

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Summary. We have retrospectively analysed 344 multiple myeloma (MM) patients (202 *de novo* patients) treated in a non-uniform way in whom high-dose therapy and autologous stem cell transplantation (ASCT) response was simultaneously measured by both electrophoresis (EP) and immunofixation (IF). Patients in complete remission (CR) by EP were further subclassified as CR1 when IF was negative and CR2 when it remained positive. Partial responders (PR) were also subclassified as PR1 (very good PR, $>90\%$ reduction in M-component) or PR2 (50–90% reduction). CR1 patients showed a significantly better event-free survival (EFS) [35% at 5 years, 95% confidence interval (CI) 17–53, median 46 months] and overall survival (OS) (72% at 5 years, CI 57–86, median not reached) compared with any other response group (univariate comparison $P < 0{\cdot}00000$ to $P = 0{\cdot}004$). In contrast, comparison of CR2 with PR1 and with PR2 did not define different prognostic subgroups (median EFS 30, 30 and 26 months respectively, $P = 0{\cdot}6$; median survival 56, 44 and 42 months respectively, $P = 0{\cdot}5$). The non-responding patients had the worst outcome (5-year OS 8%, median 7 months). Multivariate analysis confirmed both the absence of differences among CR2, PR1 and PR2 and the highly discriminatory prognostic capacity of a three-category classification: (i) CR1 (ii) CR2 + PR1 + PR2, and (iii) non-response (EFS $P < 0{\cdot}00000$; OS $P < 0{\cdot}00000$; both Cox models $P < 0{\cdot}00000$). In the logistic regression analysis, the factors significantly associated with failure to achieve CR1 were the use of two or more up-front chemotherapy lines, status of non-response pre-ASCT and inclusion of total body irradiation (TBI) in the preparative regimen. Tandem transplants or the use of multiple agents (busulphan and melphalan) in the preparative regimen resulted in a higher CR1 level; none of the biological factors explored influenced the possibility of achieving CR1. These results confirm that, in MM patients undergoing ASCT, achieving a negative IF identifies the patient subset with the best prognosis; accordingly, therapeutic strategies should be specifically designed to achieve negative IF.

Keywords: multiple myeloma, electrophoresis, immunofixation, response criteria, autotransplant.

The original criteria for assessing response in multiple myeloma patients (MM) (Chronic Leukemia and Myeloma Task Force, 1968; Alexanian *et al*, 1972; Chronic Leukemia and Myeloma Task Force of the National Cancer Institute, 1973) have not been entirely satisfactory (Marmont *et al*, 1991; MacLennan *et al*, 1992) because complete remission is infrequent with conventional chemotherapy (Alexanian *et al*, 1969; Blade *et al*, 1994); this situation has changed with the introduction of high-dose therapy (Cunningham *et al*, 1994; Attal *et al*, 1996; Vesole *et al*, 1996). Complete remission (CR) requires the disappearance of paraprotein in serum and urine, along with less than 5% bone marrow (BM) plasma cells. In the earliest reports (Selby *et al*, 1987; Gore *et al*, 1989), the disappearance of M-component was

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usually evaluated by electrophoresis (EP) and less frequently by immunoelectrophoresis (Duc *et al*, 1988). More sensitive techniques, such as immunoisoelectric focusing (Sinclair *et al*, 1986) and immunofixation (IF) (Whicher, 1983; Duc *et al*, 1988), are available. The latter, a widespread reproducible laboratory technique, is particularly useful in clinical practice (Duc *et al*, 1988; Keren *et al*, 1988, Keren, 1999). The criteria of a negative IF introduced by the Arkansas group in 1989 to assess CR after autologous stem cell transplantation (ASCT) (Vesole *et al*, 1996) defines a better minimal residual disease status that is probably associated with a more favourable prognosis. At present, a negative IF is frequently required to define CR (Anderson *et al*, 1983; Dimopoulos *et al*, 1993; Ballester *et al*, 1997; Joshua *et al*, 1997; Attal *et al*, 1998; Blade *et al*, 1998; Fermand *et al*, 1998; Barlogie *et al*, 1999), nevertheless the coexistence of this method with EP measurement (Cunningham *et al*, 1994; Attal *et al*, 1996; Björkstrand *et al*, 1997, 1999) may be confusing. Moreover, to the best of our knowledge, whether the use of a negative IF as a CR criterion to identify a subset of patients with a better prognosis than those with a negative EP but positive IF has never been adequately assessed.

The present study uses M-component values measured simultaneously by EP and IF to evaluate the response in a series of MM patients who underwent ASCT. The patients' outcome according to the different degree of response, including CR assessed by IF and/or EP, as well as the biological or therapeutic factors that influenced the achievement of CR have been analysed.

PATIENTS AND METHODS

Patients and therapeutic procedures. A total of 344 patients with MM from 17 different institutions transplanted between 1989 and 1998 have been retrospectively analysed in the study. The numbers of de novo patients (transplant after 0 or 1 chemotherapy line and duration of previous chemotherapy < 6 months) and those who had received more extensive prior treatment were 202 and 142 respectively. At the time of analysis (June 1999), the median follow-up times (range) of the entire series was 76 months (8–266) from diagnosis and 54 months (4–86) from ASCT; 56% of the 344 patients had relapsed and 39% have died. The medians for overall survival (OS) and event-free survival (EFS) from the time of ASCT are, respectively, 56 and 31 months. The stem cell source was peripheral blood stem cells (PBSCs) in 335 cases; BM + PBSCs in six cases and selected PBSCs in three cases. The main conditioning high-dose regimens were: 200 mg/m^2 melphalan (MEL200) in 143 cases; 140 mg/m^2 melphalan plus total body irradiation 8/12 Gy (MEL140 + TBI) in 75 cases; 12 mg/m^2 busulphan plus 140 mg/m^2 melphalan (BUMEL) in 55 cases; and double ASCT with 200 mg/m^2 melphalan followed by 300 mg/m^2 BCNU with 750/m^2 etoposide and 6 g/m^2 cyclophosphamide (TandemTx) in 46 cases. The remaining 25 patients received other high-dose regimens that did not individually have enough patients to be statistically analysed. Of the 318 cases that had achieved some type of response, 179 (56%) received alpha interferon (IFN) after ASCT therapy of between 3 and 5×10^6 UI three times a week. IFN administration was begun with stable peripheral blood values and was abandoned if toxicity or relapse appeared.

This study included $> 90\%$ of the total MM ASCT cases, responders and non-responders, in each institution. All data entered in the data base were reconfirmed by different investigators from the clinical and data management teams.

Paraprotein determinations. Information about remission status, evaluated simultaneously by EP and IF for serum and urinary M-protein, was available for all these patients. All patients were also monitored by both EP and IF in serum and urine throughout follow-up. Paraprotein was determined at each institution by homogeneous methods (Keren, 1999). Briefly, for EP, protein was subjected to an electrical field in agarose support, stained with protein binding dyes and scanned by densitometry. In IF, serum and urine protein were separated in agarose gel media by electrophoresis in an alkaline pH. Antisera against specific Ig classes and light-chain types were applied, and identification was made after staining of immunoprecipitated antigen–antibody complexes. Urine monoclonal free light-chain detection was taken from 24-h samples, and the aliquot was concentrated at least 100-fold before EP and IF.

Response criteria. Table I summarizes the types of response and the abbreviations used in the text, tables and figures. Post-ASCT CR required the absence of detectable paraprotein in serum and urine by EP on at least two measurements over a period of at least 6 weeks, together with $< 5\%$ plasma cells in BM and correction of anaemia, hypercalcaemia, renal insufficiency, etc. Cases already classified as CR were subclassified as CR1 when in addition to the disappearance of the monoclonal band on the electrophoretic pattern IF study also showed the absence of the original M-protein. If the EP was negative but the IF was positive, showing oligoclonal bands with the original isotype, the response was classified as CR2. Partial remission (PR) was defined by a $\geq 50\%$ reduction in paraprotein levels for at least 1 month. Patients in PR were also subclassified as PR1 when there was a decrease of at least 90% in the paraprotein level, whereas decreases between 50% and 90% were considered as PR2. The remaining patients were considered as non-responders and a distinction was made between cases with progressive disease (PD) and patients with stable disease (StD). In patients with PR or StD, an increase $> 50\%$ with respect to the lowest paraprotein level previously achieved was required to consider progression. For patients in CR, relapse was established at the time of reappearance of serum or urinary paraprotein confirmed by at least one further test and excluding oligoclonal immune reconstitution; in CR1 patients, relapse was defined by recurrence of detectable paraprotein on IF, even if EP remained negative, whereas in CR2 patients a positive EP was required to constitute relapse. In all cases, the reference paraprotein level was the one immediately before ASCT. The best serological status achieved was used to evaluate the response.

Table I. Definition of response by M-component in multiple myeloma.

Response and progression criteria	Abbreviations*	Definition†
Response		> 50% reduction
Complete remission	CR	Unmeasurable by EP, sustained 6 weeks
New definition		
Type I complete remission	CR1	No detection by EP and IF
Type II complete remission	CR2	No detection by EP but positive IF
Partial response	PR	≥ 50% reduction but positive EP, stable 1 month
New definition		
Very good partial response	PR1	90–99% reduction
Partial response	PR2	50–90% reduction
No response		< 50% reduction
Stable disease	StD	0–50% decline
Progressive disease	PD	Increase

* For use in figures, tables and text.

† The value of the M-component is for serum and/or urine and was determined with respect to pretreatment values.

EP, electrophoresis; IF, immunofixation.

Statistical analysis. OS and EFS were analysed from the day of transplantation. The impact of IFN maintenance on the groups with the most significant responses was also evaluated. For univariate analysis, survival curves were calculated according to the Kaplan–Meier method and differences between curves were evaluated with the log-rank test (two samples) or with the life table and χ^2 test (multiple samples). The multivariate study was performed using a Cox proportional regression hazard model. For inclusion in the Cox model, response classes or their groupings with statistical significance from the univariable analysis were summarized into individual variables. For a single response type, the code was 'yes/no', and for various response types the variable was coded into categories with a presumably better to worse prognosis. To avoid bias produced by the incidence of the prognostic factors on each of the response groups, the model was adjusted with other previously identified prognostic factors.

A Cox stepwise proportional hazard model was fitted to identify the factors that might have a significant independent influence on OS and EFS. Variables included in the maximum model were: sex; age (≤/> 60 years); M-protein class (IgA, other Ig); Durie–Salmon stage (I, II, III); general condition on diagnosis and on ASCT (ECOG); bone lesions (normal, osteoporosis, lysis); β_2-microglobulin on diagnosis and at ASCT (mg/l, continuous variable); lactate dehydrogenase (LDH) levels at diagnosis and ASCT (≤/> normal value); serum creatinine at diagnosis and at ASCT (μmol/l, continuous variable); haemoglobin at diagnosis and at ASCT (g/dl, continuous variable); serum albumin at diagnosis and at ASCT (g/l, continuous variable); time between diagnosis and ASCT (months, continuous variable); pre-ASCT response status (CR, PR, StD, PD); and number of chemotherapy lines before ASCT (number 1, 2, 3, 4).

A two-step logistic regression procedure was used to identify the factors that might have a significant independent influence on the failure to achieve each of the different types of response. Initially, an independent logistic regression analysis was carried out for each of the variables. In the second stage, the variables with a significant odds ratio that had either a favourable or an unfavourable effect on the possibility of achieving the type of response under analysis were included in a multivariate logistic regression model. Variables included the main conditioning regimens (MEL200, yes/no; MEL140 + TBI, yes/no; BUMEL, yes/no; and TandemTx, yes/no) together with all the factors already mentioned in the survival study (age: reference level, ≤ 60 years; ECOG: reference level, 0 + 1; Durie–Salmon stage: reference level, II; chemotherapy lines before ASCT: reference level, 0 + 1; time from diagnosis to ASCT: reference level, ≤ 6 months; status at ASCT: reference level, response; for haemoglobin, creatinine, albumin, β_2-microglobulin and LDH, the reference levels were 10 g/dl, 177 μmol/l, 35 g/l, 2·5 mg/l and normal value respectively).

RESULTS

Patient characteristics

Tables II and III show the patients' characteristics at diagnosis and at transplant according to response group and the main significant differences between the response groups. Overall characteristics were similar except for myeloma isotype distribution and the number of cases with high LDH in the PR2 group. The non-responding patients showed the worst prognostic profile before ASCT: more conventional chemotherapy lines, more time between diagnosis and ASCT, worse performance status and more had high β_2-microglobulin and low haemoglobin levels. In contrast, the CR1 group was characterized by more cases treated with a single up-front chemotherapy line and more cases in CR with low β_2-microglobulin values before ASCT than the remaining response groups.

Prognostic factor selection

After stepwise Cox analysis, the following variables were selected for OS (model, $P < 0{\cdot}00000$): Durie–Salmon stage

Table II. Main patient characteristics at diagnosis according to response group.

	CR1	CR2	PR1	PR2	No Response
n	84	66	54	114	26
Sex, male	60	62	52	67	69
Age, years	53 ± 8	55 ± 7	54 ± 8	53 ± 10	52 ± 7
M-protein, IgG/IgA/IgD/IgM/light chain	45/27/4/1/23	45/27/0/2/26	69/21/2/0/8	69/18/1/0/12	62/19/7/0/12
PS (ECOG), ≥ 2	56	54	49	54	54
Durie–Salmon stage, I/II/III	1/27/72	7/14/79	6/33/61	8/20/72	8/38/54
Serum creatinine (μmol/l)	141 ± 106	132 ± 79	141 ± 106	106 ± 62	106 ± 88
Serum albumin (g/l)	38 ± 6	37 ± 6	39 ± 7	38 ± 6	34 ± 7
Serum calcium (nmol/l)	2·5 ± 0·4	2·4 ± 0·3	2·3 ± 0·3	2·4 ± 0·3	2·4 ± 0·4
Haemoglobin (g/dl)	10·7 ± 2·5	10·7 ± 2·6	10 ± 2·4	11·1 ± 2	10·5 ± 2·3
β_2-Microglobulin, ≥ 2·5 mg/l	73	82	70	65	66
LDH > normal limit	18	13	9	4	15

PS, performance status.

Mean values (± SD) are given to continuous variables or percentage of cases with that characteristic.

Main statistically significant differences $P < 0{\cdot}05$ (Anova test or Fisher's exact two-sided test): CR1 and CR2, < cases with IgG than in PR1, PR2 and no response groups; CR1 and CR2, > cases with IgA than in PR1 and no response groups; CR1 and CR2, > cases with light chains than in PR2 group; PR2, > cases with normal LDH than the other groups.

($P = 0{\cdot}04$), number of chemotherapy lines before ASCT ($P = 0{\cdot}00001$), pre-ASCT status ($P = 0{\cdot}04$), β_2-microglobulin ($P = 0{\cdot}02$) and pre-ASCT LDH ($P = 0{\cdot}01$). The following variables were selected as significant for EFS (model, $P = 0{\cdot}0003$): LDH on diagnosis ($P = 0{\cdot}00002$), number of chemotherapy lines before ASCT ($P = 0{\cdot}006$) and pre-ASCT status ($P = 0{\cdot}009$).

Survival analysis

Table IV summarizes the EFS and OS values at 5 years and 95% confidence interval for different response categories. In the univariate analysis, comparing EFS in CR1 with CR2, the outcome was significantly better in the former group (35% vs. 21% of event-free patients at 5 years; median EFS 46 vs. 30 months; $P = 0{\cdot}004$) (Fig 1A). A similar difference was observed for OS: at 5 years, 72% of patients were alive in CR1 compared with 48% alive in CR2, with median OS not reached compared with 56 months ($P = 0{\cdot}0006$) (Fig 1B). In contrast, as also shown in Fig 1, no significant differences in either EFS or OS were detected upon comparing CR2 with PR1 and with PR2 (median EFS 30, 30 and 26 months, respectively, $P = 0{\cdot}6$; median survival 56, 44 and 42 months, respectively, $P = 0{\cdot}5$). Nevertheless, when we pooled CR1 + CR2 (CR, 150 cases) and compared the resulting values with PR1 + PR2 (PR, 168 cases), the differences were favourable for CR patients in both EFS (30% vs. 18% at 5 years, median 43 and 28 months, respectively, $P = 0{\cdot}0003$) and OS (62% vs. 41% at 5 years, median 70 vs. 42 months, respectively, $P = 0{\cdot}001$). Concerning non-responding patients, those with PD did worse than those with StD, although, probably as a result of the small number of cases, these differences did not reach statistical

Table III. Patient characteristics at transplant.

	CR1	CR2	PR1	PR2	No response
Haemoglobin (g/dl)	11·8 ± 1·5	11·6 ± 1·7	11·3 ± 1·7	11·2 ± 1·7	10·4 ± 0·6
Serum creatinine (μmol/l)	106 ± 132	88 ± 26	80 ± 26	95 ± 61	80 ± 26
Serum calcium (nmol/l)	2·3 ± 0·2	2·3 ± 0·2	2·2 ± 0 1	2·2 ± 0·2	2·2 ± 0·1
Serum albumin (g/l)	30 ± 16	29 ± 15	30 ± 15	31 ± 13	30 ± 14
β_2-Microglobulin, ≥ 2·5 mg/L	29	52	42	43	58
LDH, > normal limit	9	11	6	13	26
PS (ECOG), ≥ 2	9	21	22	14	47
Pre-ASCT chemotherapy lines, ≥ 2	24	36	42	50	70
Time diagnosis to ASCT, months	15 ± 22	16 ± 19	16 ± 16	24 ± 31	31 ± 31
Pre-ASCT response status, CR/PR/StD/PD	23/61/2/14	18/58/6/18	2/72/6/20	0/73/6/21	0/35/30/35

PS, performance status; ASCT, autologous stem cells transplantation.

Mean values (± SD) are given to continuous variables or percentage of cases with that characteristic.

Main significant differences $P < 0{\cdot}05$ (Anova test or Fisher's exact two-sided test): no response, > cases with low haemoglobin, > cases with β_2-microglobulin ≥ 2·5 mg/l, > cases with ECOG 2–4, > time diagnosis to ASCT, < cases in pre-ASCT CR and > cases with ≥ 2 pre-ASCT chemotherapy lines; CR1 and CR2, > cases in pre-ASCT CR; CR1, > cases with low β_2-microglobulin, > cases with ECOG 0–1 and > cases treated with a single up-front chemotherapy line.

Table IV. Survival values for different response categories at 5 years.

		Overall survival		Event-free survival	
Post-ASCT response status	Cases (*n*)	%	CI 95%	%	CI 95%
Response	318	51	43–51	25	18–33
CR	150	62	50–75	30	17–43
CR1	84	72	57–86	35	17–53
CR2	66	48	24–72	21	1–41
PR	168	41	31–51	18	*
PR1	54	42	24–59	27	11–43
PR2	114	41	28–53	15	5–25
No response	26	7	0–28	–	–
StD	12	20	0–51	8	0–24
PD	14	0	0–0	–	–

* Asymmetric distribution.
ASCT, autologous stem cells transplantation; CI, confidence interval.

significance (median survival 7 vs. 16 months, $P = 0{\cdot}06$). According to these results, three major subgroups of patients could be established by the response to high-dose therapy: (i) CR1; (ii) CR2 + PR1 + PR2; and (iii) non-responders. This three-category classification showed elevated statistical significance in terms of differences in both EFS ($P < 0{\cdot}00000$) (Fig 2A) and OS ($P < 0{\cdot}00000$) (Fig 2B).

We have analysed whether the above-described benefit of achieving CR1 similarly affects patients receiving and not receiving post-ASCT IFN maintenance. Within the cohort of patients receiving IFN, those that achieved CR1 (54 patients, 64%) showed a significantly better EFS and OS than the CR2 + PR1 + PR2 cohort (125 patients, 53%) (median EFS 46 vs. 30 months, $P = 0{\cdot}0003$; median

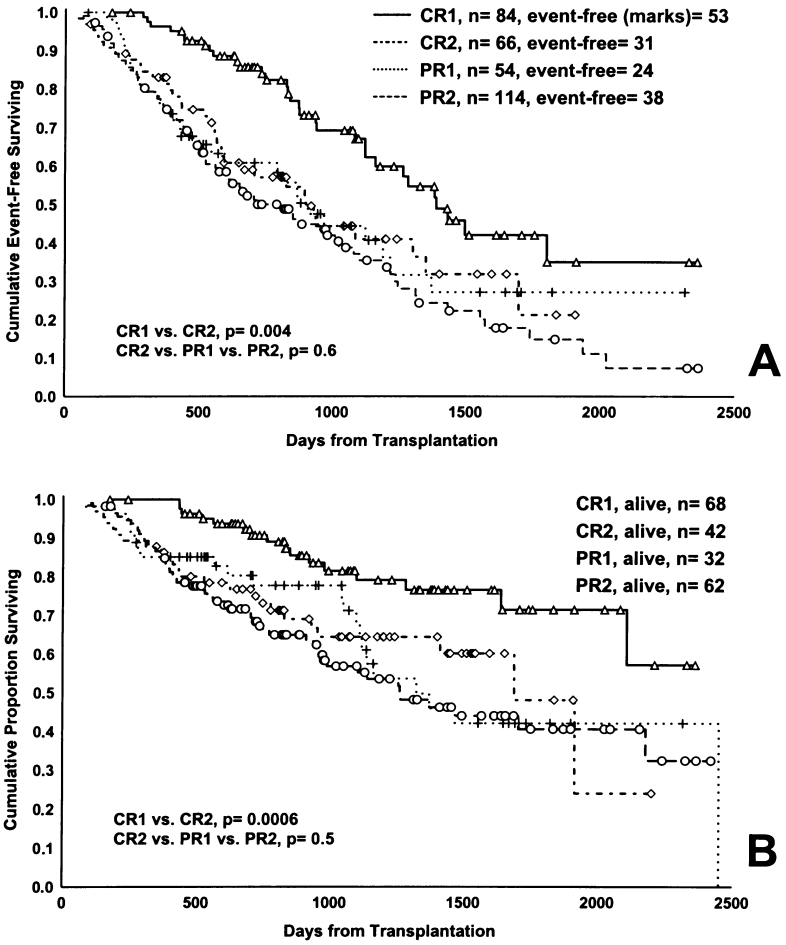

Fig 1. Univariate survival comparison of CR1 (negative inmunofixation) vs. CR2 (negative electrophoresis but positive inmunofixation) vs. PR1 vs. PR2. (A) Event-free survival; (B) overall survival. The line patterns and marks in A are similar to those in B.

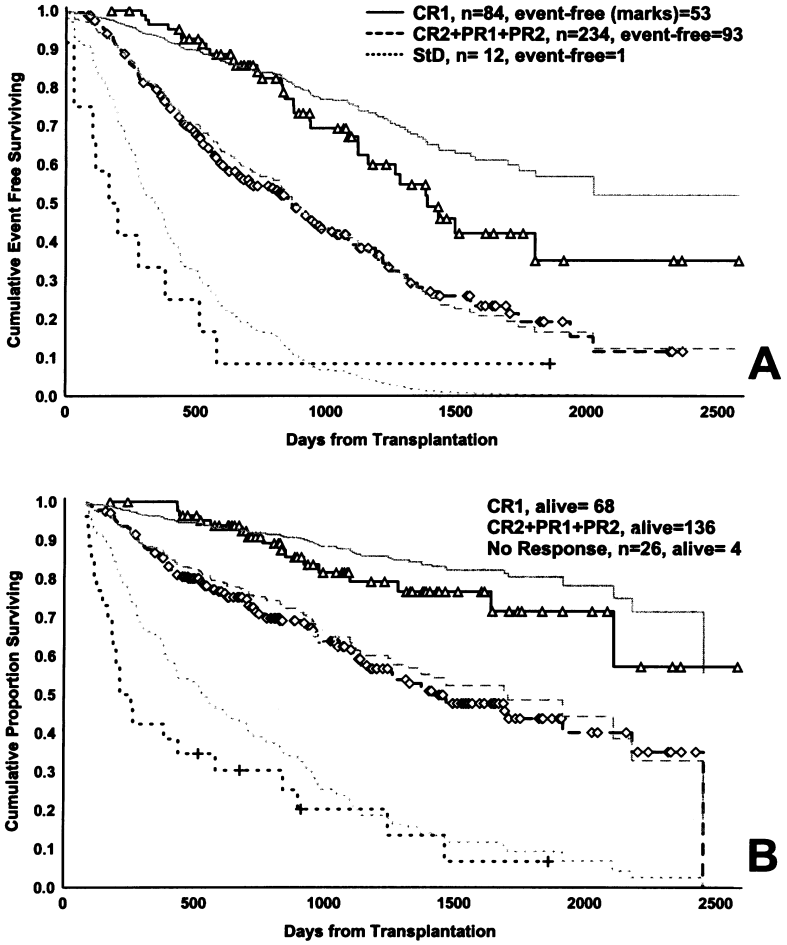

Fig 2. Survival comparison of CR1 vs. (CR2 + PR1 + PR2) vs. no response. Actuarial univariate comparison for event-free survival and overall survival (solid black lines; A and B respectively) and graphs for event-free survival and overall survival generated by Cox proportional hazards models (grey lines; A and B respectively) adjusted by the mean values of the previously identified prognostic factors (both models, $P < 0{\cdot}00000$). In all comparisons, $P < 0{\cdot}00000$.

survival not reached vs. 59 months, respectively, $P = 0{\cdot}009$). Among patients who did not receive IFN, the CR1 group also had a better outcome than the one achieving a lower degree of response (median EFS 37 vs. 17 months, $P = 0{\cdot}01$; OS 54 vs. 32 months, respectively, $P = 0{\cdot}01$).

Multivariate analysis adjusted by the most significant prognostic factors confirmed the highly discriminatory prognostic capacity of the three-category classification suggested by the univariate analysis (Fig 2A, EFS; Fig 2C, OS) as well as the lack of differences among CR2, PR1 and PR2 (EFS $P = 0{\cdot}1$; OS $P = 0{\cdot}4$). When IFN was included in the multivariate model, this therapy reached a favourable significance as an independent variable (EFS $P = 0{\cdot}01$; OS $P = 0{\cdot}0002$), but did not modify the discriminatory value of the proposed three-category classification (EFS $P < 0{\cdot}00000$; OS $P = 0{\cdot}00001$).

Logistic regression analysis

In univariate analysis for failure to obtain CR1, none of the diagnostic characteristics were significant. Of the characteristics present at the time of ASCT, ECOG 2–4 [odds ratio (OR) 2·8, $P = 0{\cdot}01$], haemoglobin < 10 g/dl (OR 2·1, $P = 0{\cdot}02$), elevated LDH (OR 1·4, $P = 0{\cdot}02$), β_2-microglobulin $> 2{\cdot}5$ mg/l (OR 2·2, $P = 0{\cdot}008$) and a non-response status (OR 2·1, $P = 0{\cdot}01$) were associated with a lower possibility of achieving CR1. Regarding the conditioning regimens, non-use of BUMEL (OR 2·2, $P = 0{\cdot}01$), non-TandemTx (OR 2·2, $P = 0{\cdot}01$) and the use of MEL140 + TBI (OR 0·3, $P = 0{\cdot}006$) increased the risk of failure to achieve CR1. Finally, the multivariate logistic regression analysis (Table V) did not select any of the above-mentioned biological factors. Multiple treatment lines pre-ASCT (OR 2·2, $P = 0{\cdot}02$), a non-response status before ASCT (OR 2·5, $P = 0{\cdot}04$) and a strategy of a single ASCT rather than TandemTx (OR 3·0, $P = 0{\cdot}02$) are the factors that are unfavourably related to the possibility of achieving a CR1 status after ASCT. This analysis was highly significant (model, $P = 0{\cdot}00001$)

DISCUSSION

Although the response criteria for MM include several parameters (Blade *et al*, 1998), changes in M-protein size are the most important and constitute the basis of disease follow-up. A 50–75% reduction in the paraprotein level was proposed as a response criterion in the earliest treatment reports (Chronic Leukaemia & Myeloma Task Force, 1968; Alexanian *et al*, 1972; Chronic Leukemia and Myeloma Task Force of the National Cancer Institute, 1973), but the

Table V. Factors influencing failure to achieve a CR1 response. Multivariate logistic regression.*

Variable	Reference level	Odds ratio	95% CI	P
LDH at ASCT, > normal value	Normal	0·5	0·2–1·4	0·1
Chemotherapy lines before ASCT, 2 + 3 + 4	0 + 1	2·2	1·1–4·4	0·02
Status at ASCT, no response	Response	2·5	1·0–6·1	0·04
PS at ASCT, ECOG 2 + 3 + 4	0 + 1	2·1	0·7–6·0	0·1
Haemoglobin at ASCT, < 10 g/dl	≥ 10	1·8	0·8–4·0	0·1
β_2-Microglobulin at ASCT, > 2·5 mg/l	≤ 2·5	1·7	0·9–3·2	0·1
Other conditioning regimens	MEL140 + TBI	0·7	0·3–1·6	0·4
Other conditioning regimens	BUMEL	2·0	0·9–4·4	0·07
Other conditioning regimens	TandemTx	3·0	1·2–7·9	0·02

* Model, $P = 0{\cdot}00001$.

PS, performance status; ASCT, autologous stem cells transplantation; MEL140 + TBI, 140 mg/m^2 melphalan plus TBI; BUMEL, 12 mg/m^2 busulphan plus 140 mg/m^2 melphalan; TandemTx, double ASCT with 200 mg/m^2 melphalan followed by 300 mg/m^2 BCNU with 750/m^2 etoposide and 6 g/m^2 cyclophosphamide.

relationship between this degree of response and survival has not been convincingly established (Marmont *et al*, 1991; Blade *et al*, 1994). As CR was rarely achieved in MM patients under conventional chemotherapy, the early trials did not usually include this response category. In the current therapeutic context (San Miguel *et al*, 1999), in which the high-dose regimens achieve a high rate of CR, the original criteria have become inadequate (Blade *et al*, 1998).

All CR criteria require the disappearance of the paraprotein and a normal proportion of BM plasma cells (Blade *et al*, 1998). With respect to paraprotein evaluation, although EP (McElwain & Powles, 1983; Gore *et al*, 1989; Cunningham *et al*, 1994; Attal *et al*, 1996) is gradually being replaced by IF (Fermand *et al*, 1998; Barlogie *et al*, 1999), the two methods still coexist and this complicates the comparison of results based on the different measuring procedures. Given its greater sensitivity (Whicher, 1983; Keren *et al*, 1988), a negative IF should reflect a better residual disease status that would probably be associated with a better prognosis, although this issue has never been appropriately assessed.

This study subdivides patients with classic CR (Gore *et al*, 1989) into two subgroups: cases with negative IF (CR1) and cases with negative EP but positive IF (CR2). Additionally, given the results of the Intergroupe Français du Myelome randomized study (Attal *et al*, 1996), reporting similar survival between patients with CR and those with very good partial response, we also decided to subdivide patients in PR into two subgroups in order to investigate whether or not a reduction > 90% in the M-protein size was associated with a different outcome.

When comparing CR with PR and responders with non-responders, our results are similar to those obtained by other studies using EP paraprotein measurements to evaluate response in myeloablative treatments (Cunningham *et al*, 1994; Attal *et al*, 1996; Björkstrand *et al*, 1997); nevertheless, patients who reach CR with conventional treatments have a significantly longer survival than those who achieve a lower degree of response (Kyle *et al*, 1998). Our study shows the importance of discriminating between the two categories of CR, with a clear advantage in both OS and EFS for CR1 compared with CR2 patients or any of the other response subsets. Additionally, multivariate analysis confirmed the independent value of achieving a negative IF after ASCT. In contrast, when the two types of PR were analysed, neither univariate analysis nor multivariate analysis detected a significant difference. Furthermore, the outcome of PR patients was identical to that of patients in CR2. The similarity in prognosis between CR2 and PR1 is in part concordant with the original observations of Attal *et al* (1996). Probably, both CR2 and PR1 are similar as trace paraprotein may emerge in the electrophoretic spectrum in the presence of severe hypogammaglobulinaemia. Nevertheless, the absence of differences between these two response types and PR2 is a novel finding in this study that needs to be confirmed in other series.

We found CR defined by negative IF to be an independent variable that identifies the subset of patients with the best prognosis. This finding would support the recent criteria proposed by EBMT, IBMTR and ABMTR registries, whose definitions for CR and for relapse from CR (Blade *et al*, 1998) were virtually identical to the definitions for CR1 and relapse from CR1 used in our study. Interestingly, our analysis has shown that the benefit of achieving CR1 applies both to patients that subsequently received maintenance treatment with IFN and to those that did not receive further treatment. Thus, the achievement of a high-quality response should become the main treatment objective in MM patients, as is also the case in acute leukaemia (Brisco *et al*, 1996; San Miguel *et al*, 1997). Discrepancies in the outcome of CR status patients reported by different authors (Attal *et al*, 1996; Barlogie *et al*, 1997) might reflect the use of either EP or IF. In this respect, the OS and EFS reported by the Arkansas team using tandem transplants and the IF criteria (Barlogie *et al*, 1999) were very similar to our results in patients with an equivalent response status. Whether or not this parameter (negative IF) is inferior to other sensitive and more sophisticated methods of minimal residual disease evaluation (Bjorkstrand *et al*, 1995) should be investigated in order to establish optimal, but also practical, methods for assessing CR.

As a whole, our analysis leads to a new model for

response criteria in transplanted MM patients that shows an elevated statistical significance for discrimination of three prognostic subgroups: (i) complete response defined by negative IF; (ii) any other type of non-complete response; and (iii) non-response.

The multivariate logistic regression analysis performed to identify the factors with a real independent influence on the achievement of CR1 after ASCT showed that none of the diagnostic disease characteristics studied here had a major impact. In contrast, the only factors significantly associated with CR1 were those that tend to define the best therapeutic strategy: the pre-ASCT response, particularly if achieved with only one line of chemotherapy, and the conditioning regimen (BUMEL had a positive influence whereas MEL140 + TBI had a negative impact), including the favourable effect of a tandem transplant. These findings are in agreement with observations derived from international registry studies (Björkstrand *et al*, 1999) and emphasize the importance of appropriate up-front and myeloablative chemotherapy regimens in order to induce the best possible response and so prolong survival.

In summary, our study confirms the presumption that, after ASCT, MM patients in a complete response defined by a negative IF have a better prognosis than those in complete response identified by EP. Any response other than CR1 has a worse prognosis and every effort should be made to increase the level of CR1 in MM patients.

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