Impact of chromosome 13 deletion and plasma cell load on long-term survival of patients with multiple myeloma undergoing autologous transplantation

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Abstract. High-dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) is the most common treatment for patients under 65 years of age with multiple myeloma (MM). In this study, we present a retrospective analysis of the prognostic impact of different factors in patients who have received this treatment as first line therapy in our centre. Abnormalities in chromosome 13 were identified by fluorescence *in situ* hybridization at the time of diagnosis. The median overall survival (OS) and progression-free survival (PFS) from transplantation time in the whole group of 193 patients were 90 and 48 months respectively. The median follow-up was 65 months (range: 6-186 months). The complete remission (CR) rate in patients with and without del(13) was 31 and 40% respectively whereas the median OS in patients with del(13) was 58 months but not reached in patients without del(13) (p=0.006). The PFS was 26 months in patients with del(13) and 84 months in those without del(13) (p=0.001). The transplantation related mortality was 2.5% both in the absence and presence of del(13). Patients who achieved CR following ASCT had longer OS and PFS when compared to those who only achieved partial remission. Thus, this study confirms the role of del(13) as a marker of poor prognosis. Multivariate analysis showed that the existence of del(13) was the only single independent factor effecting survival (p=0.001). In patients without del(13), the prognostic impact was even stronger when combined with the plasma cell load in the bone marrow (p=0.020), whereas the plasma cell load had no effect on survival of patients with del(13). Overall, the absence of del(13) in combination with low plasma cell infiltration at diagnosis predicts the best survival.

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Introduction

Multiple myeloma (MM) accounts for approximately 2% of all cancer deaths and 20% of deaths caused by haematological malignancies (1). Although novel drugs like thalidomide, lenalidomide and bortezomib have improved response rates, their impact on long-term survival is still unclear (2-4). Allogeneic stem cell transplantation (5-8) might be curative for a small group of eligible patients, but the current gold standard for the treatment of patients under 65 years of age is high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) (9). For these patients, the overall survival has been improved from about 3 years to more than 4 years, but, in spite of this progress, almost all patients treated with ASCT relapse due to residual disease (10,11). Yet, a fraction of patients have become long-term survivors and better assessment for applying current treatment modalities is required in order to understand the nature of different responses to the therapy.

Factors that predict survival in MM such as ß-2-microglobulin (B2M), creatinine, haemoglobin levels and others have been well characterized previously (12-14). Another widely studied prognostic factor in MM is the occurrence of chromosomal abnormalities among the malignant cells. The IgH locus at 14q32 has strong transcriptional activity in B cells and the translocation of an oncogene to this region may result in dysregulation of its expression (15). Expectedly, many B-cell tumours, including MM have chromosomal translocations mediated by recombination errors which position an oncogene under the influence of a strong immunoglobulin enhancer in this region. Several chromosomal abnormalities have been shown to have prognostic importance, the most common being del(13), t(4;14), t(11;14), del(17p), t(14;16), t(14:20), and 1q gains (16).

Del(13) can be demonstrated in 39-54% of newly diagnosed MM patients (17) and it is observed in all subtypes of MM, as well as monoclonal gammopathy of undetermined significance (MGUS) (18-20). Conventional cytogenetics fail to recognize del(13) in 20-40% of the cases compared to FISH. About 80-85% of multiple myeloma cases with del(13)

Table I. Patient characteristics at diagnosis.

	All	Unknown del(13)	del(13)	No del(13)
Number of patients	206	101	56	49
Age (years)	55 (31-72)	54 (31-70)	56 (37-70)	58 (34-72)
B2M (mg/l)	2.8 (0.9-67)	2.6 (1.3-67)	2.9 (0.9-18)	2.8 (0.9-18)
Creatinine (μmol/l)	126 (30-980)	110 (30-366)	108 (38-344)	176 (47-980)
Hb	115 (75-151)	115 (75-151)	111 (78-141)	120 (99-137)
Plasma cells in BM (%)	24 (5-99)	24 (5-99)	28 (10-66)	20 (10-65)
Time to HDT (months)	6.2 (1-115)	7.2 (1-115)	5.9 (1-19)	6.2 (1.4-47)
IgA-subtype	17%	17%	17%	16%
IgG-subtype	55%	55%	54%	56%
Light chain disease	26%	25%	26%	26%
Durie-Salmon I	9%	13%	4%	6%
Durie-Salmon II	17%	18%	19%	13%
Durie-Salmon III	74%	69%	77%	81%
Tandem HDT	36%	33%	39%	39%

Values are presented as median (range).

consist of monosomy 13, the remaining are deletions located at 13q14 (21).

A less discussed parameter for prognosis of MM is plasma cell infiltration in the bone marrow at the time of diagnosis. Plasma cells in the BM microenvironment produce several osteolytic factors including RANK ligand (RANKL), immunosuppressive factors such as IL-10 and large amounts of MIP-1α which stimulate osteoclast (OCL) precursors to differentiate into bone resorbing OCL (22,23). Hence, hypothetically the plasma cell load in the BM should directly correlate with bone destruction rate. Although there seems to be a reasonable correlation between the infiltration of plasma cells in BM and the aggressiveness of the disease (24), this parameter is rarely included in analysis of large studies of prognostic factors in multiple myeloma.

As a reflection of our own experience in the field of MM treatment, in this study, we have analyzed data from 210 newly diagnosed MM patients who have received HDT followed by single or tandem ASCT at our centre since 1990, in an attempt to identify factors that have a role in determining the prognosis of patients receiving this treatment.

Materials and methods

Patients and treatment. Out of 484 patients with multiple myeloma admitted to our department during the period between January 1990 and April 2008, 336 were under 72 years of age. One hundred and sixty-three were under the age of 60 and 142 (87%) of those received an autologous transplant. One hundred and seventy-three patients were between 60 and 72 of which 68 (39%) received an autologous transplant. In total, 210 patients were treated with HDT followed by ASCT. Out of these 210 patients included in the study, 27 had a subsequent allogeneic SCT and were therefore censored at the time of allogeneic SCT during statistical analysis. Patient characteristic are given in Table I. All patients were conditioned with Melphalan 200 mg/m^2 followed by an autograft 24 h later, the median time from diagnosis to HDT was 10 months. Depending on age, (below 60 years) after the first transplant, 74 patients received a second course of HDT with Melphalan 140 mg/m^2 and ASCT. All patients had a standard debulking treatment with VAD (vincristine, doxorubicin, betametason) induction with a median of 3 (range 2-5) cycles prior to HDT. VAD-resistant patients received a 2nd line therapy and were not treated with HDT unless they reached a stable disease. Stem cells were mobilized with cyclophosphamide and G-CSF after the debulking therapy and harvested from the peripheral blood by leukapheresis.

Response and progression criteria. EBMT response criteria were used with the important modification of using agarose gel electrophoresis instead of immunofixation for determining response. Therefore some patients might have been only in near CR (nCR) according to EBMT response criteria. Patients in complete remission with agarose gel electrophoresis are therefore called patients in CR + nCR. Patients were considered to be in PR if there was at least a 50% reduction of the initial paraprotein levels. Progression was defined in accordance with the same criteria.

FISH (fluorescence in situ hybridization). FISH was performed on unstimulated bone marrow cells cultured for 12 h without any cell separation steps. Prepared cell slides were pre-treated in 0.1 mg/ml pepsin /0.01 M HCl in order to digest covering proteins. Cell preparations and probes were simultaneously denatured in a HYbrite (Abbott-Vysis Inc., IL, USA) at high temperature (73˚C) and hybridized for 18 h at 37˚C. Post-hybridization washes were the same for all slides and performed at high stringency to avoid unspecific bindings. The cells were counterstained with DAPI in mounting medium Vectashield for easier detection (Vector Laboratories, Inc., Burlingame, CA, USA). Slides were analyzed using a fluorescence microscope equipped with an appropriate filter set (Nikon E800, Nikon Corp. Tokyo, Japan) and documented in

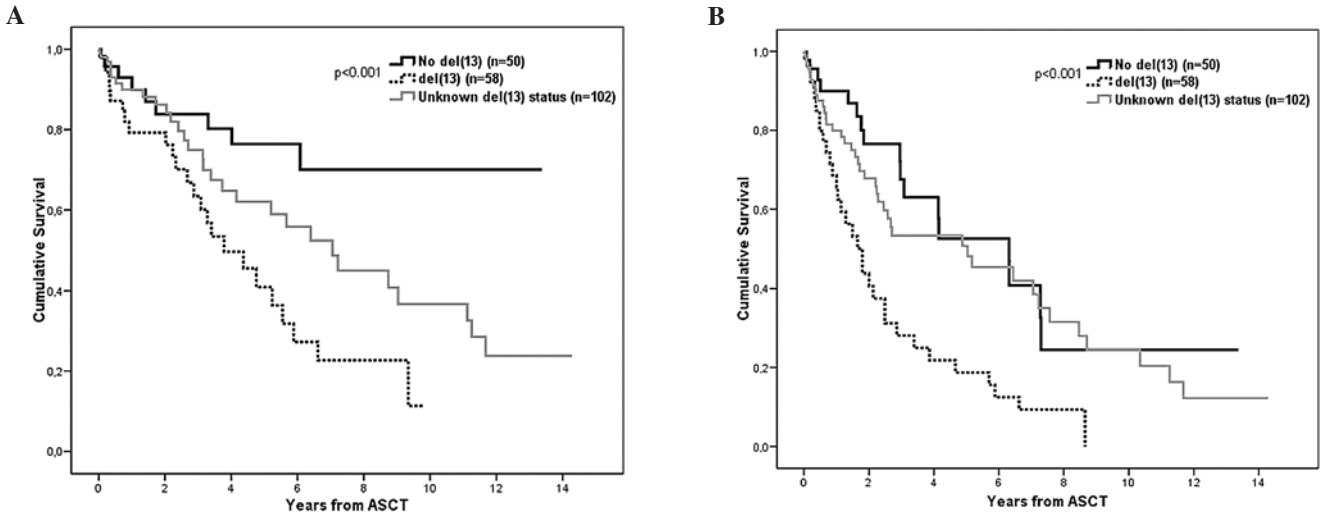

Figure 1. The impact of del(13) on the survival of MM patients. Both the OS (a) and PFS (b) curves show that patients with del(13) have significantly lower survival rates. The group of patients, that were not evaluated for del(13), were plotted on the same graphs in order to show that there was no selection bias.

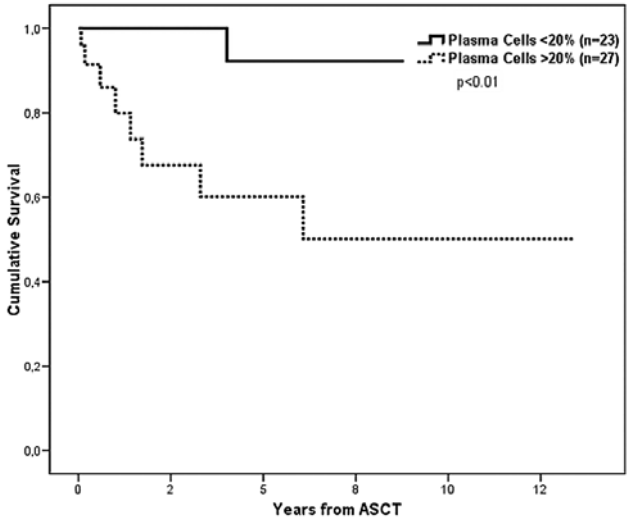

Figure 2. The impact of plasma cell load in MM patients without del(13). An increased plasma cell load at the time of diagnosis significantly decreases the survival of patients without del(13). No such effect was seen in patients with del(13).

CytoVision image system (Applied Imaging, Newcastle upon Tyne, UK). A minimum of 400 interphase nuclei were assessed during each hybridization procedure. As control normal donor cells (PBMCs) were used. Del(13) was defined as the presence of more than 5% mono allelic cells for the specific probe among the plasma cells. Probes used for FISH analysis were from the beginning, TelVysion 13q Spectrum Orange (33-260013), LSI 13/Rb-1 Spectrum Orange (32-190001), LSI D13S25 Spectrum Orange (32-190029) all from Vysis Inc (Downers Grove, IL, USA). Also three overlapping cosmids, c1a, c30a, c32a were used from the region D13S319 (25). These cosmids were labeled with spectrumGreen-dUTP by Nick translation (kit from Vysis Inc.). All probes were substituted with a commercial probe mix, covering loci D13S25 and D13S319 also including sub-telomere specific probe, when this was available (Cytocell Technologies Ltd., Cambridge, UK).

Statistical analysis. All statistical analysis was carried on SPSS 15.0.0 software (SPSS Inc. Chicago, IL, USA). Two-tailed non-parametric correlation test was utilized for analysis of correlation between survival and the following variables: Age, sex, del(13) by FISH, BM plasma cell content, disease stage, response quality, single vs. double transplantation, time to progression, Hb, Albumin, LD, M-component size and type, B2M. The variables that exhibited significant correlation with survival were analyzed by plotting Kaplan-Meier plots to identify their impact on PFS and OS and the curves were compared using log-rank test (26). Each variable was also compared using del(13) as a stratum to detect any possible difference between patients with and without del(13). Prognostic factors for survival were also subjected to multivariate analysis using the Cox proportional hazards regression model with forward stepwise method. Progression-free survival (PFS) and overall survival (OS) were defined as the time from the day of ASCT to progression or death and censored at last contact.

Results

The patient characteristics in detail are given in Table I. Of the analyzed 210 patients, 32% were older than 60 years at diagnosis. There was no statistically significant difference in OS between patients <60 and those ≥60 years. Tandem HDT in itself did not have a significant impact on OS or PFS when compared to the single HDT group. OS differed between the group with B2M ≥2.5 mg/l where median OS was 70 months and the group with B2M <2.5 where median OS was not reached ($p<0.05$).

Occurrence of del(13) predicts shorter OS and PFS. Bone marrow samples from 108 patients were analyzed for del(13). According to FISH results, 58 patients had 5% or more plasma cells with del(13) and 50 patients had <5%. Thus, using the probes described above with a 5% cut-off level, 54% of the patients carried del(13) at the time of diagnosis.

Median OS and PFS in the del(13) group was 45 months and 21 months, and in the non del(13) group, not reached and

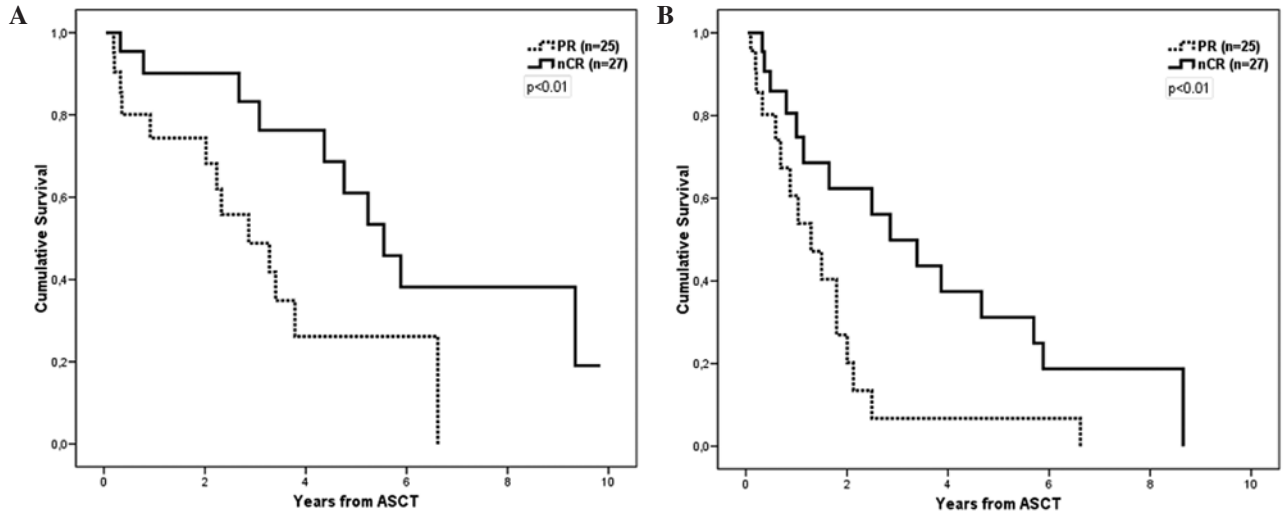

Figure 3. The impact of the quality of response to HDT on the survival of MM patients with del(13). Both the OS (a) and PFS (b) curves show that patients with del(13) who could achieve CR/nCR have significantly higher survival rates. No such effect was seen in patients without del(13).

75 months, respectively (Fig. 1A and B). In order to demonstrate that there was no selection bias between the patients who were cytogenetically analyzed for del(13) and those who were not, the OS and PFS curves for the non-evaluated group are also plotted in the same figures. Median PFS for this group was 60 months and median OS was 85 months.

Plasma cell infiltration and quality of response predict survival in different groups of patients. We have observed plasma cell infiltration (% plasma cells in bone marrow at the time of diagnosis), and quality of response to HDT (nCR/PR) act as secondary indicators of survival depending on del(13) status.

In patients without del(13), a plasma cell content of more than 20% was significantly associated with worse survival (Fig. 2) whereas no such difference was observed in the group carrying del(13). In patients with del(13), those who achieved nCR had a significantly better OS and PFS (Fig. 3). Median OS and PFS in patients with del(13) were 66 and 34 months, respectively, when nCR was achieved, while OS was 34 months and PFS 15 months if only PR was reached. The quality of response had no significant impact on OS in patients without del(13).

Multivariate analysis of prognostic factors. Multivariate analysis using all variables with $p<0.05$ on univariate analysis of effect on survival was done using the Cox proportional hazards regression model. Although our study and previous studies have shown a various number of factors that effect survival, the only factor identified as an independent predictor of survival was del(13) (Table II). Despite the discriminative effect of plasma cell infiltration in patients without del(13), and quality of response in patients with del(13), when data from all patients with or without del(13) were used, multivariate analysis revealed that neither plasma cell load, nor quality of response was an independent prognostic factor by itself.

When analysing interactions of different variables, and possible secondary prognostic factors that can be combined with del(13), we observed that del(13) and plasma cell load had a strong combined effect on survival (p=0.02). The fact that plasma cell load not on its own but in combination with del(13) shows a statistically significant effect, confirms that it is in fact a strong predictor of survival but dependent on del(13). A similar result is observed with quality of response as well. These results correlate with our observations, suggesting that plasma cell load and quality of response are discriminative for prediction of survival depending on del(13) status.

Table II. Multivariate analysis of factors prognostic for survival.

	p-value
Age	0.176
B2M	0.991
Stage	0.644
Time to ASCT	0.127
Response (CR vs. PR)	0.553
Single vs. tandem ASCT	0.272
% plasma cells	0.849
del(13)	0.001
with B2M	0.052
with stage	0.422
with response	0.006
with single vs. tandem ASCT	0.085
with % plasma cells	0.014

The Cox proportional hazards regression showed that del(13) was the only independent prognostic factor in this group of patients. Combining other factors with del(13) revealed a statistically significant synergy with response to HDT and plasma cell load in the BM at the time of diagnosis.

Discussion

The overall- and progression-free survival of multiple myeloma patients following autologous transplantation in our centre compare favourably to most other similar studies using conventional VAD or VAD like pre-treatment followed by ASCT (27,28). Selection bias cannot be excluded; however, since as many as 87% of all patients less than 60 years and 42% of those between 60 and 72 years had a transplant, selection of patients seems to be similar to that in other ASCT studies. Our results also compare relatively favourably to recent studies using more intensive induction regimens (29) or the new drugs bortezomib and thalidomide for induction (3,4,30-32).

Our study confirms the importance of del(13) as a prognostic factor. Del(13) was the only independent prognostic factor for OS and PFS. The discrimination between poor and good prognosis patients was of the same order as with cytogenetics in contrast to some other studies (17,33). Certain technical differences in evaluating the FISH results may play a role for the better discrimination. Our cut-off for claiming del(13) was at 5% marked cells within the whole sample material. Two similar studies have used separated myeloma cells before FISH analysis and used a 15% cut-off value (16,34). Therefore, the percentage of positive cells is not comparable. It is not clear if the selection procedure may also somehow affect the FISH signal. However, despite such differences in technique, the frequency of del(13) in our cohort of FISH analyzed patients (54%) was similar to that of other centres (18,34) arguing against important impact of technical differences. In order to exclude bias due to incomplete FISH data, the 102 patients who were not investigated were compared to the 108 analyzed ones, and the OS and PFS curves were similar. Thus, the FISH-analyzed patients appear to be representative for the whole material.

As previously reported (35-37) our study shows that the plasma cell infiltration into the BM has a prognostic impact. However, we have observed that this impact is significantly pronounced in patients without del(13) whereas those with del(13) seem to have similar survival rates regardless of the extent of plasma cell infiltration at the time of diagnosis. In the multivariate analysis the plasma cell load did not appear as an independent factor. The reason for the lack of prognostic importance of plasma cell infiltration in patients with del(13) is unclear. The relationship between these two variables remains to be solved, yet it is possible that in some cases the aggressive nature of plasma cells carrying del(13) might be compensating for their low numbers and abandoning the beneficial effects of low plasma cell infiltration.

The importance of obtaining CR has been debated previously, however most previous studies as well as the present study have found that this is an important prognostic parameter for OS and PFS (9). Since del(13) is associated with poor response obtaining CR was not an independent prognostic factor in the multivariate analysis. Nevertheless, we were able to demonstrate that if a CR can be obtained in patients with del(13), a better survival rate can be expected.

Our data presents no better outcome of tandem as compared to single HDT. However, the study was not designed to make this comparison and selection of patients to second transplant may bias interpretation. Some previous larger studies have shown superiority of tandem over single HDT on both PFS and OS but others have not (27,38-40).

In conclusion, our long-term follow-up of MM patients treated with ASCT in our centre compares favorable to many other studies despite conventional induction and conditioning regimens. Although abnormalities of chromosome 13 are associated with shortened survival in MM patients, intensified treatment is nevertheless able to improve the outcome of such patients. Moreover, in the absence of del(13), the fraction of plasma cell content in the bone marrow at the time of diagnosis can be used as a prognostic factor. These prognostic factors could be used together with those previously suggested in order to define patient subgroups with similar characteristics. Thus, the response to HDT can be better predicted and monitoring procedures could be tailored for each subgroup.

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