Original Article

Significance of Abnormal Protein Bands in Patients with Multiple Myeloma following Autologous Stem Cell Transplantation

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Abstract

Aim: We studied the characteristics of small abnormal protein bands (APB) (including oligoclonal bands and new apparent monoclonal bands) that are frequently detected by serum protein electrophoresis (SPEP) and isoelectric focusing (IEF) in the post-autologous stem cell transplant setting.

Methods: In a retrospective analysis of patients with multiple myeloma undergoing transplantation, paraprotein identity and quantification were performed using standard immunofixation electrophoresis. The nature of any new bands was determined by IEF which distinguished between oligoclonal bands and apparent monoclonal bands.

Results: Of 49 myeloma cases, the median follow-up was 33.7 months (range, 5.6-97.5 months) and 24 patients had relapsed. Thirty six (73%) developed APB. 22 patients had more than one episode of APB and 6 patients had more than 2 episodes resulting in a total of 69 episodes of APB observed post-transplant. IEF demonstrated 54 of these APB were oligoclonal bands and 15 appeared to be monoclonal. Of the 15 episodes of apparent monoclonal bands, 10 had differing heavy or light chain restriction compared to the original myeloma paraprotein and 5 had the same heavy and light chain restriction but different band location in the SPEP lane. Ten of these apparent monoclonal bands resolved, 5 persisted, and only one represented true disease progression. The presence of APB impacted favourably on event-free survival (p=0.05).

Conclusion: Small APB are very frequent post-transplant for myeloma, and IEF can identify these APB as oligoclonal or monoclonal. Apparent monoclonal bands may represent relapsed disease, but in the vast majority of cases it does not, and most likely represents a transient phenomenon representing regeneration of a limited immune response.

Introduction

Multiple myeloma is characterised by the clonal expansion of malignant bone marrow cells engaged in the production of a unique monoclonal immunoglobulin. While bi- and tri-clonal paraproteins are occasionally present at diagnosis[1] and switching of paraprotein types can occur during disease relapse,[2] the myeloma associated paraprotein usually has a constant isotype, light chain restriction and electrophoretic mobility which are maintained throughout the course of the disease. This characteristic underpins the pivotal role of serum and urine electrophoresis in the monitoring of patients with multiple myeloma.

APB are not infrequently seen on serum electrophoresis following myeloablative therapies.[3-5] This phenomenon has been reported across the spectrum of haematologic disorders treated with high dose chemotherapy and stem cell transplantation (both autologous and allogeneic). Often immunofixation reveals oligoclonal bands but small discrete bands with the appearance of a monoclonal paraprotein are also seen. These APB are likely to be due to transient dysregulation of the regenerating B cell compartment during recovery post transplant.[3,5] Specifically for myeloma patients, this may potentially represent either a change in the antibody

production of the original plasma cell clone or the emergence of a new malignant clone. The appearance of these APB can pose significant problems to the laboratory as they may be mistakenly reported to suggest relapse.

IEF is a technique whereby proteins are "focused" on a gel incorporating a pH gradient. While traditional SPEP and/or immunofixation electrophoresis (IFE) may not be able to determine whether APB are oligoclonal or monoclonal, IEF can be helpful in distinguishing between these possibilities. Oligoclonal bands show a random pattern on IEF whereas monoclonal bands show a distinct equidistant laddering pattern which is due to varying degrees of deamidation of the paraprotein.[6] IEF combined with immunofixation is also a more sensitive technique than SPEP and IFE and thus may be able to provide greater insight into the nature of the small APB during the post transplant period (see article by Findley Cornell).

We conducted a retrospective audit of patients with myeloma undergoing autologous stem cell transplantation to determine the characteristics and significance of small APB on SPEP during the post-transplant period and to provide a framework for appropriate reporting of APB by the pathology laboratory.

Methods

Patients

Consecutive patients having their first autologous stem cell transplant between June 1996 and June 2004 were identified retrospectively from the transplant database at the Princess Alexandra Hospital. Only patients with multiple myeloma or multiple plasmacytomas were considered. Data abstracted included patient and disease characteristics, and outcome data including response to treatment, relapse and survival. Myeloma responses were defined according to the criteria published by the European Group for Bone Marrow Transplantation.[7]

Chemotherapy and Transplantation

The choice of induction therapy was at the treating physician's discretion. The most commonly utilised regimen was cyclophosphamide, idarubicin and dexamethasone.[8] Peripheral blood progenitor cells were mobilised with a variety of chemotherapy and cytokine protocols with a target minimum CD34+ cell count of $2.0x10^6$/kg. Patients all received high-dose therapy consisting of melphalan 200 mg/m^2 i.v. on day –1. Peripheral blood progenitor cells were infused on day 0. Routine maintenance chemotherapy post-transplant was not utilised in the early study period. After 2002, some patients were managed on an Australian Leukaemia and Lymphoma Group Trial which included prednisolone and thalidomide maintenance therapy.

Serum and Urine Protein Electrophoresis and Isoelectric Focusing

Serum and urine protein electrophoresis and immunofixation were performed using the Paragon System (Beckman Coulter, Brea, CA). Agarose gel IEF (pH 3-10) and immunofixation were performed using Pharmalyte™ ampholytes (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and Dako antisera (DakoCytomation Denmark A/S, Glostrup, Denmark) according to the method of Cornell and McLachlan.[9]

An APB was defined as a single, generally small discrete band seen on SPEP which had different characteristics from the original disease paraprotein. IEF was used to define an oligoclonal band as one that produces a random pattern of bands after IEF and IFE. An apparent monoclonal band was defined as one that produces a distinct ladder pattern of equidistant bands. A monoclonal spectrotype may occur in addition to oligoclonal bands in which case serum samples were noted as having both oligoclonal and monoclonal banding (Figure 1).

Statistical Methods

Study endpoints included: frequency, type and duration of APB detected on SPEP; event-free survival (EFS); and overall survival (OS). The probabilities of event-free and overall survival were calculated from the day of the transplant according to the method of Kaplan and Meier. Variables affecting these endpoints were compared using the log rank test (categorical variables) or Cox proportional hazards (continuous variables). Statistical analysis was performed using a Stata (StataCorp, College Station TX, USA) package. Summary statistics use the median values with ranges in brackets.

Results

Patients

Fifty-one patients with plasma cell malignancies underwent autologous stem cell transplantation during the study period. Two were excluded from analysis due to lack of follow-up data. Of the remaining 49 patients, 45% were female and the median age at the time of transplantation was 57 years. Myeloma was stage I in 8%, stage II in 2% and stage III disease in 90%. Following transplantation, 47% of patients achieved complete remission and 47% achieved partial remission. With a median follow-up of 34 months, 5 year survival is 76% (95%CI, 59-86%) and 24 patients had relapsed at a median of 15 months (range, 3-65). Patient characteristics are detailed in Table 1.

Abnormal Protein Bands

Thirty-six (73%) patients developed small APB on serum

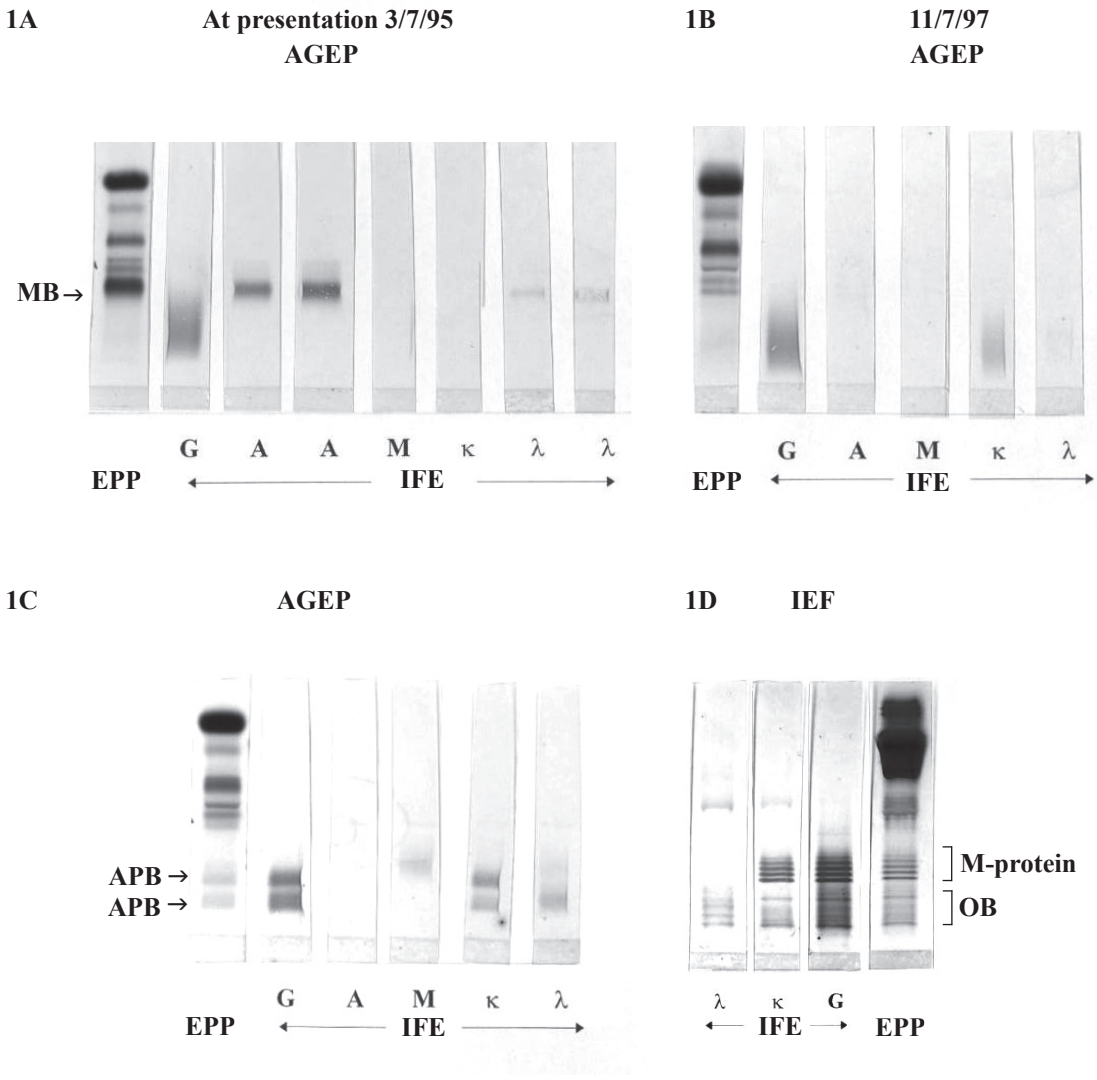

Figure 1. Abnormal protein bands post-autologous stem cell transplantation for myeloma. Panel A shows at diagnosis the presence of an IgA lambda band (MB) in the beta region on agarose gel electrophoresis (AGEP) and immunofixation electrophoresis (IFE); Panel B shows disappearance of the band post-autologous stem cell transplantation in May 1997; Panel C shows the appearance of two small abnormal IgG protein bands (APB) in the gamma region on AGEP 6 months later; Panel D confirms by IEF and IFE the bands to be a monoclonal IgG kappa band (M-protein) and oligoclonal IgG kappa and lambda bands (OB).

protein electrophoresis in the post-transplant period. Twenty-two patients had more than one episode of APB and 6 patients had more than two episodes resulting in a total of 69 episodes of APB observed post-transplant. Maximum number of distinct episodes of APB was six per patient. The median time to development of the first episode of APB was 1.8 months post-transplant (range, 0-29). Similar proportions of patients in complete and partial remission developed APB (85% and 69%, respectively). Isoelectric focusing demonstrated that 54 of these APB were oligoclonal and 15 appeared monoclonal.

Of the 15 episodes of apparent monoclonal bands, 10 had a differing heavy or light chain restriction compared to the original myeloma paraprotein, and five had the same heavy and light chain but different band location on SPEP (Table 2). Ten resolved and five persisted with only one representing true disease progression. In this last case, the band maintained the same isotype and light chain restriction, but with different electrophoretic mobility in the gamma region. The majority of apparent monoclonal bands were quantitatively ≤1 g/L, although bands of up to 5 g/L were observed. They persisted for a median duration of 3 months (range, 1-18 months). Nine apparent monoclonal bands also had concurrent oligoclonal bands, five had oligoclonal bands in close proximity to the episode of apparent monoclonal band, and only one patient with an apparent monoclonal band did not develop any

Table 1. Patient characteristics.

N	49
Patient and disease characteristics	
Age at transplant (range)	57 years (32-75)
% female	45%
Stage	
I	4
II	1
III	44
Paraprotein type	
IgG kappa	18
IgG lambda	6
IgA kappa	8
IgA lambda	3
Kappa light chains	8
Lambda light chains	2
IgD kappa	1
IgD lambda	1
Non-secretory	2
Median monoclonal band size at diagnosis (range)	25 g/L (0-80)
Median bone marrow plasmacytosis at diagnosis (range)	28.5% (0-85)
Outcomes	
Median follow-up	34 months
Response	
Complete remission	23 (47%)
Partial remission	23 (47%)
No response	3 (6%)
Median time to response (range)	3 months (0.2-40)
Relapse (n)	24
5yr event free survival	34% (95% CI 18-51%)
5yr overall survival	76% (95% CI, 59-86%)

oligoclonal banding. Often the apparent monoclonal band had the same electrophoretic mobility as one of the subsequently developing oligoclonal bands. Concurrent analysis of bone marrow by immunohistochemistry for clonal plasma cells was available for 12 of the 15 episodes. In no case was a definite clonal plasma cell population identified with the same isotype and light chain restriction as the apparent monoclonal protein band.

Clinical Outcomes

The post-transplant response according to the Blade criteria was a strong predictor of outcome with 5yr EFS of 66% vs 6% for those in complete compared to those not in complete remission, respectively ($p<0.001$). Similarly, overall survival was 90% vs 61% for those in complete compared to those not in complete remission, respectively ($p=0.04$).

The clinical outcome of patients with APB was compared to that of patients without APB to determine if the presence of APB had prognostic significance. Compared to those who had a normal SPE, the 36 patients who developed APB post-transplant had superior event-free survival (five year EFS 43% vs 0%, $p=0.049$, Figure 2), but not overall survival (5yr OS 83% vs 57%, $p=0.12$, not shown). A non-significant trend to a higher complete remission rate among patients with APB (53% vs 31%, $p=0.21$) was noted, and when Cox regression was performed to correct for the presence of achievement of complete remission, no effect of APB on outcome was able to demonstrated ($p=0.54$ for EFS, $p=0.43$ for OS).

Discussion

This study confirms the high incidence of APB seen following autologous stem cell transplantation for patients with multiple myeloma. In 73% of patients, APB occurred at a median of 1.8 months post-transplant. This incidence is identical to that reported by Hovenga et al.[4] and higher than the 10% and 43% reported by Zent et al.[3] and Maisnar et al.[10] respectively. The differences in incidence of these small bands may be related to assay sensitivity.[11]

Table 2. Apparent monoclonal band switches post autologous stem cell transplantation.

Patient No.	Monoclonal band change	Quantity (g/L)	Duration (months)
1	IgAλ→ IgAλ	<1	9.0
	IgAλ → IgGκ	1	18.0
2	IgGκ→IgGκ	3 *	21.4+
3	IgGκ→2 x IgGκ	<1	0.9
	IgGκ→IgGκ	<1	0.9
	IgGκ→IgGκ	<1	0.9+
4	IgGκ→IgGλ	1	1.6
5	IgGκ→IgGκ + IgGλ	<1	0.9
6	NS→ IgGκ	<1	4.2+
	NS→IgGκ + IgGλ	<1	1.6+
7	λBJP→2 x IgGκ	<1	5.1+
8	IgAκ→IgGκ	4-5	2.8
9	IgAκ→IgGκ	<1	2.8
10	IgAλ→IgGκ	1	7.8
	IgAλ→IgGκ	1	3.3

* This band progressed to relapse; NS, non-secretory.

In assessing APB, isoelectric focusing proved a useful tool in that it immediately demonstrated that 54 of 69 APB were oligoclonal and thus were not clinically concerning for relapsed disease. Isoelectric focusing also documented the presence of discrete bands of up to 5g/L which appeared monoclonal, but had different immunoglobulin or electrophoretic properties to the original myeloma paraprotein. In only one case out of fifteen did this discrete band progress to frank disease with a paraprotein showing an isotype switch (i.e. having different isotype, light chain restriction or electrophoretic mobility). Thus, only one out of 69 APB proved to be related to the underlying myeloma and this low frequency reinforces the fact that small APB which have different characteristics to the original myeloma paraprotein are usually benign. This observation supports a small study by Guikema et al,[5] in which reverse transcriptase polymerase chain reaction (RT-PCR) on bone marrow samples did not detect clonal plasma cell populations in those patients with APB.

Similar to Zent et al.[3] and Maisnar et al.[10], in univariate analysis, we found that the presence of APB was a favourable predictor of event-free survival with a trend to predicting

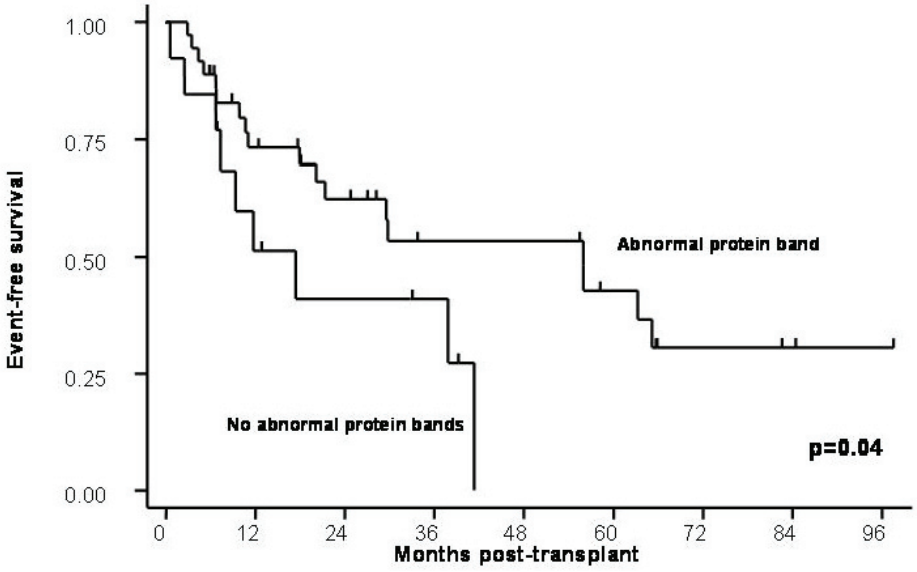

Figure 2. Event free survival post-transplant according to the presence or absence of abnormal protein bands on serum protein electrophoresis.

improved overall survival. When the presence of APB was corrected for the achievement of complete remission post-transplant, however, there was no impact of APB on outcome. Thus, the initial development of APB appears to be associated with marked reduction in the malignant plasma cell clone as evidenced by the achievement of complete remission and may be a surrogate marker for myeloma eradication. Post-transplant regeneration of the B cell compartment may occur more efficiently when the myeloma is in remission. Other studies have demonstrated that early lymphocyte recovery post-transplant predicts superior survival,[12,13] and one could envisage the development of APB in association with this lymphocyte recovery.

The frequent presence of these APB also has relevance for appropriate reporting of SPEP results. In our experience, the majority of reports causing clinician confusion relate to patients with small bands in the post-transplant setting, particularly when the small band was reported as being monoclonal. Rather than characterising these APB as a "monoclonal" protein on the report, we prefer to describe the new band: 'There is a small discrete (type: e.g. IgG kappa) band, approximately (amount: e.g. 2 g/L) on a background of polyclonal and/or oligoclonal gamma globulins. This band is different from the original paraprotein. Its clinical significance is uncertain.' Conversely, the lack of detection of a previous monoclonal M-protein requires validation by the laboratory to confirm its absence. This requires laboratory knowledge of the diagnostic paraprotein in terms of its isotype, light chain and electrophoretic mobility which should be kept in paper or electronic data systems for comparison purposes.

In summary, the development of small APB post-transplant in patients with myeloma is common, appears to have no adverse clinical significance and cannot be considered a sign of disease relapse. Nonetheless, the appearance of such small APB requires careful reporting and monitoring as these bands may occasionally represent true isotype switching leading to disease relapse.

Competing Interests: None declared.

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