Clinical Value of Absolute Lymphocyte Counts before Bortezomib-Dexamethasone Therapy in Relapsed Multiple Myeloma Patients

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Key Words
Bortezomib · Lymphocyte · Multiple myeloma

Abstract
A high absolute lymphocyte count (ALC) at diagnosis is known as a surrogate marker of favorable prognosis in newly diagnosed multiple myeloma (MM). Recent studies showed tumor sensitization and enhanced cytotoxicity of bortezomib. We hypothesized that a high ALC before bortezomib treatment would contribute to tumor sensitization and activated cytotoxicity of bortezomib in relapsed MM. Ninety-seven relapsed MM patients who underwent bortezomib-dexamethasone (Vel-Dex) therapy were analyzed. Median follow-up duration was 21 months and median age was 61 years. Complete response (CR) and very good partial response (VGPR) after 2 cycles of Vel-Dex therapy were higher in the high-ALC group (≥1.1 × 10^9/l) (CR + VGPR 50.0% in the high-ALC group vs. 10.4% in the low-ALC group, p = 0.001), and stable disease (SD) rate was lower in the high-ALC group (SD 11.8% in the high-ALC group vs. 44.8% in the low-ALC group, p < 0.001). In the univariate analysis, the low-ALC group before therapy was associated with shorter progression-free survival (PFS) [hazard ratio (HR), 2.780; 95% confidence interval (95% CI) 1.703–4.536, p < 0.001]. Multivariate analysis revealed that a low ALC represented an independent predictive factor for PFS (HR 1.937, 95% CI 1.168–3.212, p = 0.010). A low ALC just before Vel-Dex therapy was associated with a poor prognosis in relapsed MM.

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Introduction
Recent studies have identified recovery of absolute lymphocyte count (ALC) after autologous stem cell transplantation as an independent prognostic factor for survival in several hematologic malignancies, including multiple myeloma (MM) [1–3]. ALC at diagnosis in newly diagnosed MM patients was associated with outcome [4]. Furthermore, ALC has been shown to predict survival in relapsed diffuse large B cell lymphoma, suggesting that host immunity is an important factor in the prediction of survival [5]. Considering the above data on ALCs in hematologic malignancies, ALC appears to be important as a surrogate marker of outcome.
MM is a neoplastic proliferation of plasma cells, which normally serve as engines for large-scale synthesis of immunoglobulins. One of the most successful therapies of the disease disrupts normal protein homeostasis by targeting the proteasome. Targeting of intracellular protein turnover by inhibition of the ubiquitin-proteasome pathway as a strategy for cancer therapy is a new addition to our chemotherapeutic armamentarium, and appears to be one of the greatest successes against MM.

Bortezomib is a proteasome inhibitor recently approved for the treatment of MM; it is currently being investigated in other types of cancer either as a single agent or in combination with chemotherapy. Several studies have recently shown that bortezomib could sensitize tumor cells by several cellular pathways [6–10].

The present study was launched to investigate whether ALC before therapy in relapsed MM is associated with outcome.

**Patients and Methods**

**Patients**

Of a total of 134 patients who received bortezomib-dexamethasone (Vel-Dex) therapy, 97 patients who did not progress after 2 initial cycles of the therapy in a relapsed status were retrospectively analyzed between July 2005 and August 2008. Another 37 patients were excluded due to progression after 2 initial cycles and stopping the therapy. The median follow-up after Vel-Dex therapy was 21 months. The median age was 61 years (range 43–80 years) and the male-to-female ratio was 1.25:1. Patients were excluded if they had previously received bortezomib or had refractory disease after the 2nd, 4th, or 6th cycle of Vel-Dex therapy (defined as progressive disease, PD), or stopped Vel-Dex therapy due to severe side effects. In conventional cytogenetic analysis, 35 of 97 (36.1%) patients had abnormal findings, and 17 of 97 (17.5%) patients had a deletion of chromosome 13.

**Treatment Schedule**

Eligible patients had measurable PD after 1–3 previous treatments. Bortezomib 1.3 mg/m² was administrated as an intravenous bolus on days 1, 4, 8, and 11 every 3 weeks for up to 6 cycles. Oral dexamethasone (40 mg) was administered on days 1, 2, 4, 5, 8, 9, 11 and 12 every 3 weeks. Platelet and red cell transfusions and administration of granulocyte growth factor were allowed. All patients received intravenous bisphosphonates every 4 weeks, unless such treatment was clinically contraindicated.

**Assessment**

The response was assessed after 2, 4 and 6 cycles, according to International Myeloma Working Group criteria. Briefly, complete response (CR) was defined by negative immunofixation on serum and urine, <5% plasma cells in bone marrow and disappearance of any soft-tissue plasmacytoma if present at baseline; a very good partial response (VGPR) was defined as a reduction of 90% or greater in serum M-protein plus urine M-protein level <100 mg/24 h; partial response (PR) was defined as ≥50% reduction in serum M-protein level and a reduction in 24-hour M-protein ≥90% or <200 mg, and a ≥50% reduction in the size of soft-tissue plasmacytoma. Patients not meeting the criteria for CR, VGPR, PR or PD were defined as having stable disease (SD). PD was diagnosed as the presence of at least one of the following conditions: increase of ≥25% from baseline in serum or urine M-protein (with an absolute increase of at least 0.5 g/dl and 200 mg/24 h, respectively).

**Statistical Analysis**

Progression-free survival (PFS) was calculated as the time between the first dose of bortezomib and the date of disease progression or death, or the last follow-up for censored cases. The Mann-Whitney U test was used for the comparison of the high-ALC group with the low-ALC group. PFS was estimated using the method of Kaplan-Meier and compared with these two groups or with the response of each group after the 2nd cycle using the log-rank test. Cox proportional hazard models were used for univariate and multivariate analysis to evaluate the value of ALC for predicting PFS compared to known relevant predictive factors. Estimated hazard ratios (HRs), along with 95% confidence intervals (CIs) and p values were reported based on Cox proportional regression. All statistical analyses were performed using SPSS 15.0 software for Windows (SPSS Inc., Chicago, Ill., USA).

**Results**

**Patient Characteristics**

Thirty-seven (38.1%) of 97 patients achieved CR (n = 19) and VGPR (n = 18) after 2 cycles of Vel-Dex therapy, whereas 39 patients achieved PR (40.2%), and 21 patients achieved SD (21.7%).

A LC was measured just before the 1st cycle of Vel-Dex therapy. Analysis of different cutoff levels between the 25 and 75% quartile (1.001–2.365 × 10⁹/l) using the log-rank test determined that an ALC ≥1.1 × 10⁹/l as the cutoff point yielded the highest difference in PFS, which was used as cutoff level in statistical analysis. Sixty-eight patients were included in the high-ALC group, while the other 29 patients were allotted to the low-ALC group.

Baseline demographics and other characteristics of the two groups were balanced. The rate of CR and VGPR after 2 cycles of Vel-Dex therapy was higher in the high-ALC group than in the low-ALC group (CR + VGPR 50.0% in the high-ALC group vs. 40.4% in the low-ALC group, p = 0.001), whereas SD was lower in the high-ALC group than in the low-ALC group (SD 11.8% in the high-ALC group vs. 44.8% in the low-ALC group, p < 0.001, table 1).

**Clinical Outcome according to Response after 2 Cycles or Pretreatment ALC Level**

Patients achieving CR and VGPR after 2 cycles of Vel-Dex therapy had a significantly longer median PFS (CR,
19 months; VGPR, 15.5 months) than patients achieving PR or SD (PR, 11 months; SD, 7 months; p < 0.001, p < 0.001, respectively), and patients achieving PR also had a longer PFS than those achieving SD (p = 0.009), whereas median PFS in patients achieving CR was similar compared to those achieving VGPR (p = 0.194, fig. 1a). Comparison of PFS between the two ALC groups before therapy showed that the median PFS of the high-ALC group was significantly longer than that of the low-ALC group (high-ALC group, 15 months; low-ALC group, 7 months, p < 0.001, fig. 1b).

**Correlation between Pretreatment ALC Level and Response after 2 Cycles of Vel-Dex Therapy**

Comparison of pretreatment ALC levels according to response after 2 cycles of Vel-Dex therapy showed that the median ALC level in patients achieving CR and VGPR was significantly higher than in those achieving PR (median ALC level of CR and VGPR, 1.89 × 10^9/l vs. PR, 1.39 × 10^9/l, p = 0.021). Similarly, the ALC level in patients achieving PR was also higher than in those achieving SD (median ALC level of PR, 1.39 × 10^9/l vs. 1.00 × 10^9/l in SD, p = 0.022, fig. 2).
Prognostic Impact on PFS of Pretreatment ALC Level Compared to Other Prognostic Factors

A low pretreatment ALC was associated with a shorter PFS (HR 2.780; 95% CI 1.703–4.536, p < 0.001) in the present study, whereas only one previous treatment before Vel-Dex therapy and achievement of a good response (CR + VGPR) after 2 cycles of therapy were associated with longer PFS (HR 0.341, 95% CI 0.189–0.616, p < 0.001; HR 0.226, 95% CI 0.129–0.396, p < 0.001, respectively, table 2). Other factors (age, deletion of chromosome 13, stage I at diagnosis, pretreatment β2-microglobulin, albumin, and lactate dehydrogenase levels) were not predictive of PFS after therapy. Multivariate analysis using a Cox proportional hazard model confirmed that a low ALC before therapy represented an independent predictive factor for PFS (HR 1.937, 95% CI 1.168–3.212, p = 0.010, table 3).

Discussion

The present study showed that relapsed MM patients with a high ALC before Vel-Dex therapy experienced higher overall response rates and PFS, and that documented ALC before Vel-Dex therapy could be one of the predictive factors for PFS in relapsed MM.

Recent studies have shown that ALC at diagnosis in MM patients was an independent predictive factor for overall survival [4]. Other studies analyzing lymphocyte...
subsets in MM at diagnosis have suggested that the quantitative numbers of CD19+ or the development clone were associated with survival, and T cells were shown to have a suppressive effect on polyclonal immunoglobulin production in MM patients [11–14]. Peripheral blood lymphocytes from MM patients were shown to have direct antimyeloma activity by proliferative and cytotoxic responses to autologous and allogeneic myeloma cells, which suggests a contribution of the host immune system to tumor control in MM patients [15].

Previous studies of relapsed MM using a regimen that included bortezomib have shown that stage I, a few previous courses of treatment and achievement of a good response could be predictive factors of the outcome of therapy [16, 17]. The present study also showed that only one previous course of treatment and achievement of a good response as well as a high ALC were favorable predictive factors. To the best of our knowledge, this study is the first report linking pretreatment ALC to prognosis in relapsed MM.

Bortezomib is a first-class proteasome inhibitor for the treatment of MM. Recent evidence indicates that bortezomib may also be involved with natural killer (NK) cell-mediated antitumor immunity. Several in vitro and clinical studies evaluating adoptive NK cell infusions in patients with cancer have hypothesized therapeutic roles of adoptive NK cell infusions in the treatment of malignant diseases [18–22]. NK cells have multiple potential mechanisms for lysis of tumor target cells. The major cytotoxic pathway is thought to result from the release of perforin/granzymes. The ability of NK cells to kill target cells is also associated with target cell expression of major histocompatibility complex (MHC) class I expression. Thus, NK cells kill target cells with low-level or lacking MHC class I expression and cannot kill target cells with high levels of class I expression. NK cells have surface-inhibiting killer immunoglobulin-like receptors that bind to classic MHC class I molecules in a polymorphic way and inhibit NK cell killing of human leukocyte antigen-positive cells. Sayers et al. [23] recently demonstrated

| Table 2. Univariate analysis of prognostic factors |
|--------------------------------------------------|
| **Univariate analysis**                          |
| **HR**                                           |
| **p value**                                      |
| Age ≥65                                          | 1.101 (0.673–1.802) | 0.701 |
| Deletion of chromosome 13                        | 0.877 (0.480–1.604) | 0.670 |
| ISS stage I at diagnosis                         | 0.702 (0.393–1.254) | 0.232 |
| Serum albumin <3.5 before Vel-Dex therapy        | 1.179 (0.738–1.884) | 0.492 |
| Serum β2MG ≥3.5 before Vel-Dex therapy           | 1.453 (0.900–2.345) | 0.126 |
| Serum LDH above UNL before Vel-Dex therapy       | 1.666 (0.795–3.492) | 0.177 |
| Low ALC before Vel-Dex therapy                   | 2.780 (1.703–4.536) | <0.001 |
| Only one treatment before Vel-Dex therapy        | 0.341 (0.189–0.616) | <0.001 |
| Good response after 2nd cycle of Vel-Dex therapy (CR + VGPR) | 0.226 (0.129–0.396) | <0.001 |

Figures in parentheses are 95% CI. LDH = Lactate dehydrogenase; UNL = upper normal limit.

| Table 3. Multivariate analysis of prognostic factors |
|-----------------------------------------------------|
| **Multivariate analysis**                           |
| **HR**                                              |
| **p value**                                         |
| Low ALC before Vel-Dex therapy                      | 1.937 (1.168–3.212) | 0.010 |
| Only one treatment before Vel-Dex therapy           | 0.525 (0.284–0.972) | 0.040 |
| Good response after 2nd cycle of Vel-Dex therapy (CR + VGPR) | 0.329 (0.284–0.972) | <0.001 |

Figures in parentheses are 95% CI.
that bortezomib can sensitize neoplastic cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis. Lundqvist et al. [6] also demonstrated that bortezomib could sensitize murine tumor cells to perforin/granzyme as well as tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis. Another recent study demonstrated that bortezomib can down-regulate MHC class I molecules and enhance the sensitivity of myeloma to NK cell-mediated lysis [8], and that bortezomib can also promote upregulation of NKG2D, thereby enhancing NK cell cytotoxicity [24, 25].

Therefore, the results of the present study appear to reflect the importance of tumor cell sensitization and enhancement of NK cell cytotoxicity by bortezomib. These findings support the concept that a high ALC containing a high fraction of NK cells before bortezomib-containing therapy could be an important predictive factor for PFS.

Unfortunately, this retrospective study excluded patients who showed disease progression after 2 initial cycles because the patients could not receive further bortezomib-dexamethasone therapy. Therefore, a well-designed prospective study that includes the T/NK cell subset would be needed to provide further information with regard to this observation.

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Pretreatment ALCs in Vel-Dex Therapy in MM Patients