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Original Research Article

Diffuse large B-cell lymphoma with concordant bone marrow involvement has peculiar genomic profile and poor clinical outcome

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Abstract

Bone marrow (BM) involvement in diffuse large B-cell lymphoma (DLBCL) can be morphologically discordant from the primary tumor. Concordant BM infiltration has been shown associated with a poorer outcome in patients treated with CHOP. In order to evaluate tumor-related factors leading to BM involvement in DLBCL, we performed an integrated analysis of i) genomic profiles obtained with a high-density genome wide SNP-based arrays ii) immunomorphological and iii) clinical data from 133 patients uniformly treated with R-CHOP. BM infiltration was found in 27 of 133 (20%) cases; and it was concordant in 18/27 (67%) cases. Concordant infiltration, but not discordant, influenced negatively OS, PFS and DFS and was associated with higher serum LDH, lower CR and higher PD rates. No association with cell of origin was found between BM+ and BM- DLBCL. As compared with BM- cases, BM+ DLBCL showed absence of 7q gain.

Keywords: **bone marrow; lymphoma; CGH; R-CHOP; prognosis; chromosome 7; microarray**

Introduction

Bone marrow (BM) involvement occurs in 10–30% of diffuse large B-cell lymphoma (DLBCL) [1,2]. In up to 70% of the cases, BM infiltration can be morphologically and immunomorphologically discordant from the primary tumour, mainly reflecting features of a low grade B-cell lymphoma. In clonally related cases, transformation from a low-grade B-NHL can be assumed, whereas the presence of a second coexisting B-cell neoplasm should be considered in clonally unrelated cases [2–4]. An important impact of concordant BM involvement on overall survival (OS), independent of the IPI, has been previously observed in patients treated with CHOP [1,2]. In concordant cases, the percentage of neoplastic B-cells as well as the proportion of hematopoietic tissue involved have also a prognostic significance [5]. The molecular mechanisms leading to BM involvement by DLBCL are largely unknown and may include tumour- and host-related factors. In order to partially elucidate the role of tumour-related parameters we analyzed clinical data and genomic profiles obtained with high-density genome-wide microarrays in DLBCL patients treated with R-CHOP with and without BM involvement.

Methods

Cases with the information on BM involvement were identified within a series of 166 consecutive cases of DLBCL [6], selected based upon the availability of frozen material, for having a fraction of malignant cells in the pathological specimen representing >70% of overall cellularity as determined by morphological and immunophenotypic studies. The study was approved by the Bellinzona ethical committee.

In patients with BM infiltration, BM samples and flow cytometry images obtained at time of initial diagnosis were reviewed and compared with the primary DLBCL biopsies. In doubtful cases, immunohistochemistry (IHC) was repeated and benign lymphoid infiltrates were excluded, according to earlier published criteria [7]. BM Infiltration by large CD20+ cells was defined as concordant, while by small or mixed-small and large CD20+ cells as discordant. No information regarding possible clonal relationships of the discordant BM-component with the primary DLBCL tumours was available. The cell of origin (COO), germinal centre B-cell-like (GCB) or non-GCB types was defined in 84 cases, using IHC in 45 according to the algorithm of Hans *et al*. [8] and with gene expression profiles (GEPs) in 39 samples [9].

Genomic profiles were obtained with the GeneChip Human Mapping 250K NspI (Affymetrix, Santa Clara, CA, USA), as previously reported [6,10]. The modified Bayesian Piecewise Regression (mBPCR) method [11] was used to estimate the copy number (CN) starting from raw CN values obtained with Affymetrix CNAT 4.01. For minimal common regions (MCRs) [12] occurring in at least 15% of cases, differences in frequencies between subgroups were evaluated using Fisher's exact test: no adjustments for multiple test correction were made due to the exploratory nature of the study. OS, progression free survival (PFS), disease free survival (DFS) and response criteria were defined according to Cheson *et al*. [13]. The actuarial duration of PFS, DFS and OS were plotted according to the Kaplan–Meier method. Univariate analysis was performed with the Log-Rank test. Chi-square test was used to compare differences in clinical parameters. A p-value <0.05 was considered as statistically significant. Statistical analyses were performed with SPSS 17.0.1 (SPSS, Chicago, IL, USA) and R statistical package.

Results and discussion

BM involvement was observed in 27/133 (20%) of the patients. The infiltration was concordant in 18/27 (67%) and discordant in 9/27(33%) cases (Figure 1). All

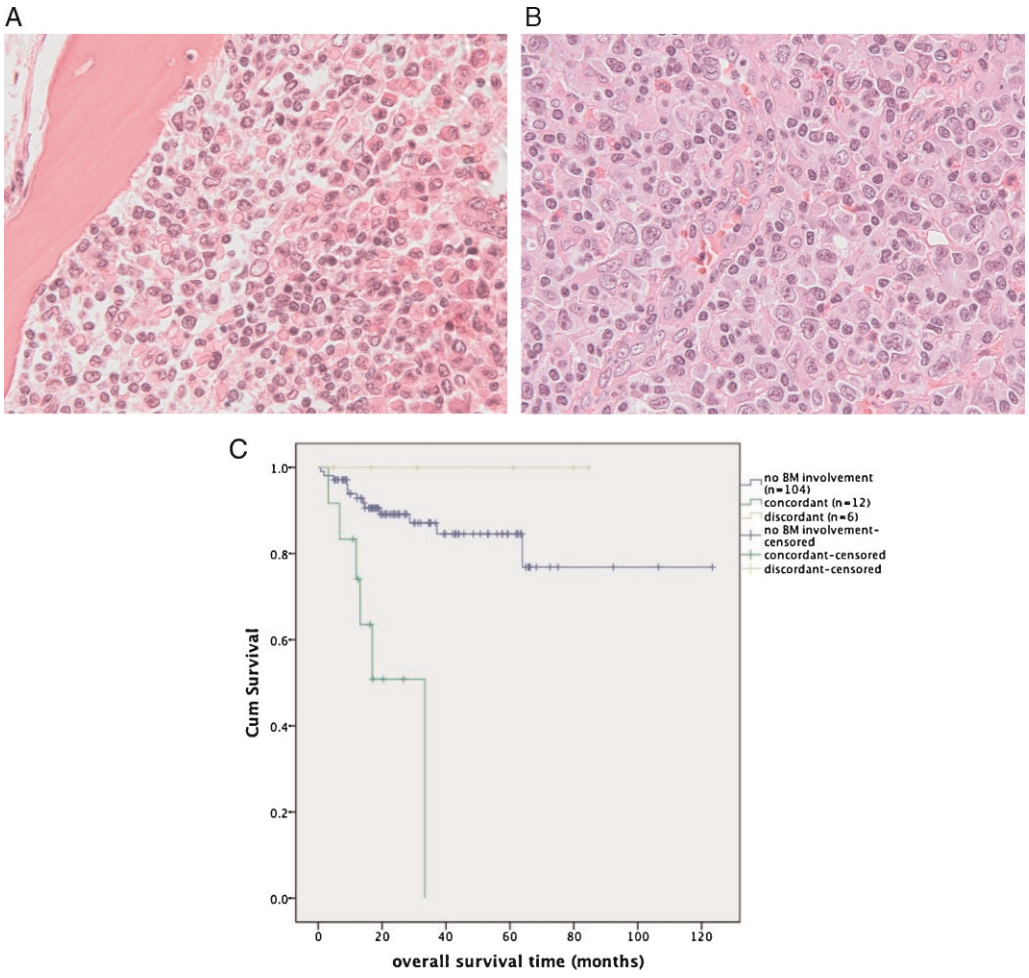

Figure 1. Pattern of BM involvement in DLBCL: morphology and clinical outcome. (A) Example of discordant BM involvement, H&E 40×. (B) Example of concordant BM involvement, H&E 40×. (C) Overall survival according to BM-status in 12 patients with a concordant pattern (green), six with discordant pattern (yellow) and 104 without any involvement (blue) ($p < 0.001$).

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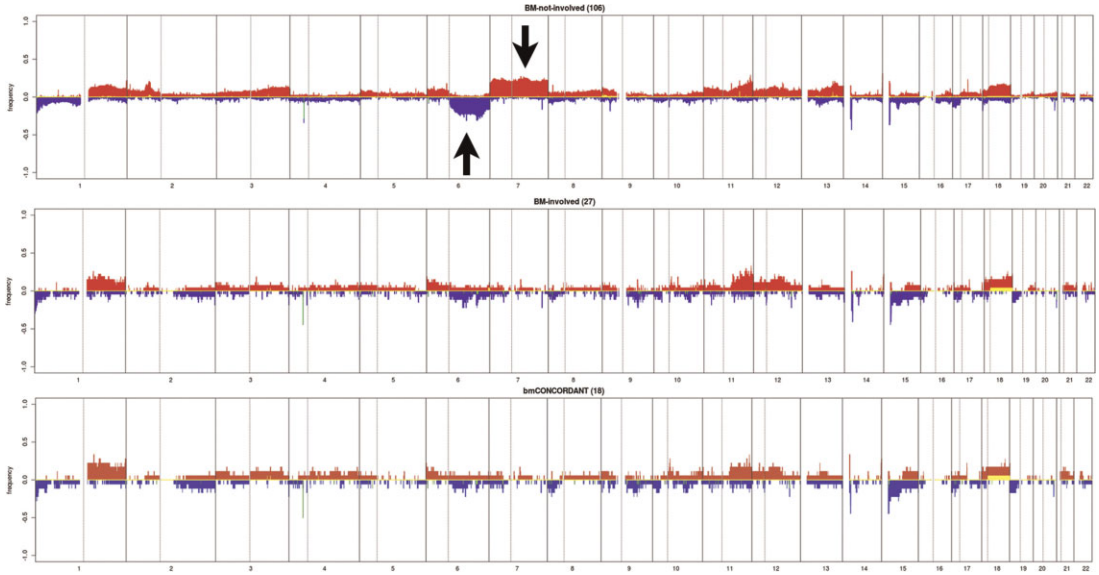

Figure 2. Frequency of gains (up, red) and losses (down, blue) in DLBCL patients according to bone marrow involvement. *X*-axis, chromosome localization and physical mapping; *Y*-axis, percentage of cases bearing the aberration. Arrows: The two regions showing significant differences between cases without BM involvement and cases with concordant BM involvement. Upper panel: Frequency of DNA gains and losses observed in 106 cases without BM-involvement. Intermediate panel: Frequency of DNA gains and losses observed in all 27 cases with BM-involvement. Lower panel: Frequency of DNA gains and losses observed in 18 cases with concordant BM-involvement.

discordant cases were CD5 negative. Only one patient resulted in having a history of a pre-existing low-grade B-cell lymphoma (follicular lymphoma, FL) transformed to DLBCL, and indeed morphological features of FL (grade I-II) in BM. Clinical characteristics at time of diagnosis, reflecting the general population of DLBCL patients [14], are listed in Supplementary Table I. No statistically significant differences in terms of COO was found between BM+ and BM− DLBCL.

Patients with concordant BM infiltration were younger than all the remaining cases, presented poorer parameters such as LDH serum values, lower complete response rate and higher rate of progression or relapse. Patients with discordant BM involvement presented characteristics similar to patients without any BM infiltration.

Univariate analysis according to OS (Figure 1), PFS and DFS showed that, also for patients treated with R-CHOP, concordant BM involvement influenced survival negatively ($p < 0.001$), whereas no events were observed among patients with discordant involvement. These data further support the importance of precisely reporting the pattern of BM involvement in the clinical practice, since the presence of a small cell component does not seem to affect the outcome.

The genomic aberrations detected in the whole group of 133 cases reflected what has already been reported in DLBCL [6,12,15,16]. The analysis of genomic aberrations further highlighted that cases with concordant BM infiltration are biologically different (Figure 2). When compared to the patients without BM involvement, patients with concordant BM infiltration completely lacked the otherwise common gains of the whole chromosome 7 or of its long arm (p-value = 0.04), and also presented a much lower frequency of losses affecting the 6q and in particular the 6q13-q27 region, containing *PRDM1* and *TNFAIP3* (p-value = 0.04). On the converse, other common aberrations, such as deletions at 1p or 17p or gains of chromosome 12 or occurring in the long arms of chromosomes 1, 11 and 18 were observed in both groups of patients. No differences were observed in terms LOH. Due to their small number, we did not perform statistical analyses on the group of patients with discordant BM infiltration.

Gain of 7q is a common genetic lesion among DLBCL, possibly more common in GCB than in non-GCB type, whereas 6q deletion is more frequent in non-GCB DLBCL [12,17,18]. Up to now, none of the two genomic aberrations have been described in relationship with BM localization. Based upon the literature, DLBCL cases with double translocations affecting *BCL2* and *CMYC* have a higher tendency for BM involvement [19]. Interestingly, cases with single translocations of *CMYC* do not seem to have a high rate of BM involvement despite a poor outcome [20], and, indeed, often present gains of chromosome 7 [21]. Gains of 7q gain seem to occur at similar percentage among DLBCL arising from both immune-privileged and immune-competent anatomical sites [22]. The presence of gains at 7q have been associated with a reduced T-cell infiltrate [23].

In conclusion, DLBCL with concordant BM infiltration showed distinct clinical and biological features. Only concordant BM infiltration had a negative impact on survival in patients with DLBCL, also when treated with R-CHOP. We provided evidence that 7q gain were never observed in primary tumour samples from these patients.

Conflict of interest

The authors have no conflict of interest.

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