LETTER TO THE EDITOR

Through translational prospective study, the GSTP1 Ile105Val polymorphism emerges as prognostic marker in de novo large B-cell lymphoma patients

Blood Cancer Journal (2017) 7, e560; doi:10.1038/bcj.2017.38; published online 28 April 2017

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of non-Hodgkin lymphoma (NHL), and its current standard of care is chemoimmunotherapy with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). Clinical-pathological markers such as the International Prognostic Index in rituximab era (R-IPI) and β2-microglobulin serum level (B2M) have been used as prognostic factors in DLBCL, but do not predict patients’ outcomes in several cases.

Glutathione S-transferase (GST) M1, T1 and P1 detoxify chemotherapeutic agents, such as cyclophosphamide (CP), doxorubicin and/or their metabolites, by conjugating them to glutathione. GSTP1 also regulates cellular chemical stress and death through interaction with the c-Jun N-terminal kinase (JNK1) protein. GSTM1, GSTT1 and GSTP1 are encoded by polymorphic genes. Homozygous deletion of GSTM1 and GSTT1 genes results in loss of functional enzyme activity. The GSTP1 isoleucine (Ile) 105 valine (Val) polymorphism influences enzyme activity; protein encoded by the Ile allele is more efficient in detoxification than that produced by the Val allele.

Studies conducted in patients with follicular lymphoma and DLBCL treated with CP-based regimens have shown that deletion of GSTM1 and/or GSTT1 confers worse event-free survival (EFS) and more adverse effects when compared with undeleted genes. On the other hand, breast cancer patients with the Val allele genotype of the GSTP1 Ile105Val polymorphism had longer overall survival (OS), better therapy response and reduced risk of myelosuppression than those with the Ile allele when treated with CP-based chemotherapy.

The GSTM1, GSTT1 and GSTP1 Ile105Val polymorphisms have been used as prognostic factors in DLBCL, but do not predict patients’ outcomes in several cases. Analyses were conducted according to their assumptions. Logistic regression model assessed possible associations between genotypes and clinic-pathological features. Kaplan–Meier method was applied to EFS and OS, where date of diagnosis was the baseline to calculate the time, EFS until first event date (relapse, progression or death) or last seen date, whereas, OS until death or last seen date. Hence, it was applied the Cox regression for EFS and OS. To ensure the stability of model was used the bootstrapping (n = 1000) based on repeatedly random sampling, applying the bias-corrected and accelerated method. P-values were two-sided, considering significantly significant when \( p \leq 0.05 \) using the SPSS 21.0 software (IBM Corp, Armonk, NY, USA).

Table 1 presents toxicity, response rate and final status by GST genotypes distributions of 144 patients. On December 2015, 97 patients were alive (just one with disease) and 47 died (21 due to toxicity, 25 of disease progression and 1 of unrelated cause). Until here, these data were similar to those previously published. No association of clinic-pathological features and GSTM1 and GSTT1 genotypes were observed. Regarding GSTP1 Ile105Val polymorphism, the Ilelele genotype was more frequent in patients who presented grade III or IV toxicity (most myelosuppression), in patients who did not obtain complete response to R-CHOP and in patients who advanced to death than in those with the remaining genotypes of the gene; carriers of the Ilelele genotype were under a 2.94-, 2.18- and 2.80-fold increased risks of toxicity, not achieving complete response to chemoimmunotherapy and evolving to death (logistic regression analysis). All associations were confirmed by bootstrapping method (Table 1). Only Korean DLBCL patients with GSTT1 null genotype and GSTM1/GSTT1 null combined genotype displayed more frequent grade III–IV R-CHOP-related myelosuppression than those with undeleted genes, but excesses of patients with ECOC > 2 and IPI 3–5 scores were identified in the GSTT1 null genotype group. Whereas, the treatment response rate in these patients did not differ according to GSTM1, GSTT1 and GSTP1 polymorphisms.

It is well plausible that patient with the GSTP1 105Ilelele genotype has worse response and reduced toxicity when exposed to CP, since the Ile allele encodes a more efficient protein in detoxification of chemical agents than that produced by the Val allele, with short exposure of cells to the drug. Indeed, an inverse association of GSTP1 Ile105Val polymorphism and toxicity was found in this study; the Ilelele genotype was associated with higher toxicity when compared with the Val allele, with short exposure of cells to the drug. Nevertheless, a possible alternative mechanism for this unexpected association is through the novel role of GSTP1 in cellular stress response signalling as an inhibitor of c-Jun N terminal kinase (JNK). JNK has been implicated in proapoptotic signalling and may be required for the induced cytotoxicity of a variety of chemotherapeutic agents. Phosphorylation of c-Jun activates JNK resulting in...
subsequent activation of downstream effectors. In non-stressed cells, low JNK1 catalytic activity is orchestrated and maintained through its sequestration within the protein complex that includes GSTP1 and JNK. However, under conditions of oxidative or chemical stress, a dissociation of the GSTP1:JNK complex occurs releasing GSTP1 for oligomerisation, and the activation of released JNK allows for the subsequent induction of apoptosis. It was also speculated that the more active Ile allele results in decreased JNK catalytic activity and reduced expression of downstream cellular stress defense genes, which may predispose cells to chemical cytotoxicity.

With a median follow-up of 42 months (13–83), the 5-year EFS and OS for patients were 63% and 64%, respectively. At this time, EFS and OS were shorter in patients with abnormal B2M (EFS: 55 abnormal B2M vs 88% normal B2M, P = 0.005; OS: 58 abnormal B2M vs 88% normal B2M, P = 0.005), albumin levels < 3.5 mg/dl (EFS: 51 albumin < 3.5 mg/dl vs 74% albumin > 3.5 mg/dl, P = 0.003; OS: 53 albumin < 3.5 mg/dl vs 75% albumin > 3.5 mg/dl, P = 0.001), R-IPI (EFS: 46 poor, R-IPI vs 75% good/very good, R-IPI, P < 0.0001; OS: 47 poor, R-IPI vs 76% good/very good, R-IPI, P < 0.0001), grade III–IV toxicity (EFS: 35 grade III–IV vs 97% grade 0–II, P < 0.0001; OS: 40% grade III–IV vs 98% grade 0–II, P < 0.0001) and GSTP1 Ilele genotype (EFS: 49 Ilele genotype vs 71% IleVal/Val genotype, P = 0.009; OS: 51 Ilele genotype vs 72% IleVal/Val genotype, P = 0.008). Differences between groups remained the same in univariate analysis; abnormal B2M and albumin, poor R-IPI, and Ilele genotype were adverse factors for EFS and OS in multivariate analysis. The Ilele genotype confirmed also having a shorter survival applying the bootstrapping method (Table 2).

Unfavourable outcome for Ilele genotype patients may be attributed to a short exposure of cells to CP and lower antioxidant cellular response, with consequent disease progression and toxicity to therapy, respectively. GSTM1 and GSTT1
double null genotype was associated with shorter EFS in males with DLBCL in Korean DLCBL patients with a median follow-up of 15 months, but OS was not altered by GSTP1 Ile105Val polymorphism.\(^8\)

Differences in associations of GSTM1, GSTP1 and GSTP1 Ile105Val polymorphisms with clinic-pathological aspects and survival found herein and in Korean study\(^8\) may be attributed to distinct sample sizes and median time of follow-up, which were about 1.5 and 2.6 times higher in our study. The imbalance of patients with unfavourable prognosis in groups with GSTT1 null genotype and with undeleted genes in Korean study and distinct frequencies of deleted GSTM1 and GSTT1 genes and GSTP1 Ile105lle genotype in Korean patients and in our patients may also constitute plausible explanations for differences found in both studies.

In summary, despite of some known limitations in this kind of studies, our data present preliminary evidence that GSTP1 Ile105Val polymorphism influences toxicity and response to R-CHOP as well as survival, and it acts as an independent prognostic marker in de novo DLBCL.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
MTD and CSPL performed the study design and data acquisition. MTD, CAS and CSPL performed the data analysis and interpretation. ECMM and GJL performed the statistical analysis. MTD and CSPL drafted the manuscript. All authors approved the final manuscript.

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