Glutathione S-transferase P1 Genotype and Prognosis in Hodgkin’s Lymphoma

Stefan Hohaus, Annalisa Di Ruscio, Annalaura Di Febo, Giuseppina Massini, Francesco D’Alo’, Francesco Guidi, Giovanna Mansueto, Maria Teresa Voso, and Giuseppe Leone
Istituto di Ematologia, Universita’ Cattolica S. Cuore, Rome, Italy

ABSTRACT

Purpose: Glutathione S-transferase P1 (GSTP1) is a member of the GST enzyme superfamily that is important for the detoxification of several cytotoxic drugs and their by-products. A single nucleotide polymorphism results in the substitution of isoleucine (Ile) to valine (Val) at codon 105, causing a metabolically less active variant of the enzyme. We assessed the impact of the GSTP1 codon 105 genotype on outcome in patients with Hodgkin’s lymphoma.

Experimental Design: The Ile^{105}Val polymorphism in the GSTP1 gene was analyzed using a PCR-RFLP technique. Ninety-seven patients with Hodgkin’s lymphoma were included and associations with patient characteristics and treatment outcome were analyzed.

Results: The GSTP1 Ile^{105}Val polymorphism was associated in a dose-dependent fashion with an improved failure-free survival in patients with Hodgkin’s lymphoma (P = 0.02). The probability of 5-year survival for patients homozygous for the 105 Val/105 Val GSTP1 genotype was 100%, for heterozygous patients 74% (95% confidence interval, 56-85), and for patients homozygous for the 105 Ile/105 Ile genotype 43% (95% confidence interval, 23-61). The Cox multivariate analysis showed that GSTP1 codon 105 genotype was an independent prognostic factor.

Conclusions: The GSTP1 genotype predicts clinical outcome in patients with Hodgkin’s lymphoma.

INTRODUCTION

Hodgkin’s lymphoma is one of the most chemosensitive malignant diseases. Risk-adapted treatment protocols including intensified chemotherapeutic regimens for patients with advanced disease have certainly led to further improvement of disease control (1, 2). Short-term and long-term side effects of intensified therapies are of concern, whereas less intensive initial therapy cures fewer patients. Treatment decisions are based on prognostic factors. Most prognostic factors reflect the tumor burden and the biological activity of the disease (3–5). One area that received little attention is that of pharmacogenetics, wherein studies inherited differences in drug metabolism caused by genetic polymorphism resulting in interindividual differences in the capability to metabolize drugs and subsequent by-products (6). An altered metabolism of chemotherapeutic drugs or derivative reactive oxygen species may prevent their cytotoxic activity toward tumor cells.

Cytotoxic drugs and their by-products are metabolized in vivo by enzymatic reactions that are classically divided into two categories: the phase I enzymes, mediating oxidation and activation, which are almost exclusively cytochrome P450 enzymes, and the phase II enzymes, mediating glucorinidation, acetylation, or conjugation with glutathione producing more water-soluble conjugates that are less toxic and readily excretable (7). Glutathione S-transferases (GST) are involved in the conjugation of several anticancer drugs, including alkylating agents, anthracyclines, and cyclophosphamide (8). We have recently shown that the presence of at least one deletion in two of these enzymes, GSTT1 and/or GSTM1, is associated with an improved disease-free survival for patients with Hodgkin’s lymphoma (9).

GSTP1 is another member of the GST supergene family. A single nucleotide polymorphism in the GSTP1 gene causes the substitution of isoleucine to valine at amino acid codon 105 (Ile^{105}Val; ref. 10). The valine allele occurs at a frequency of ∼30% in Caucasian populations and is associated with a decreased activity of the enzyme compared with isoleucine allele (11). Recently, the GSTP1 Ile^{105}Val genotype has been associated with favorable prognosis following chemotherapy with drugs known to be GSTP1 substrates in a variety of malignancies, such as pediatric acute lymphoblastic leukemia, myeloma, breast, and colon cancer (12–15). In the present report, we studied the prognostic impact of genetic polymorphism of the GSTP1 gene in patients with Hodgkin’s lymphoma.

PATIENTS AND METHODS

Patient Characteristics. Our retrospective analysis included 97 patients (median age 33 years, range 14-71 years; 39 females and 58 males), diagnosed with Hodgkin’s lymphoma between October 1984 and March 2003. Further patient characteristics are detailed in Table 1. Peripheral blood samples were obtained at the time of initial diagnosis or during follow-up. All but one patient were treated with standard chemotherapy regimens: 53 patients received doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD); 28 patients a modified Stanford V regimen (substituting 6 mg/m^2 mechlorethamine with 650 mg/m^2 cyclophosphamide); 5 patients mechlorethamine, vincristine, procarbazine, and prednisone (MOPP); 9 patients bleomycin, etoposide, doxorubicin,
cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP); and 1 patient etoposide, epirubicin, bleomycin, cyclophosphamide, and prednisolone (VEBEP; refs. 1, 16–19). Radiotherapy was included for consolidation in 50 patients. Complete response was achieved after first-line treatment in 77 patients. Informed consent was obtained from all patients according to institutional guidelines.

**DNA Extraction and Amplification.** DNA was extracted from peripheral blood leukocytes using DNAzol (Invitrogen, Carlsbad, CA) as previously described (9, 20, 21). The Ile<sup>105</sup> Val polymorphism of the GSTP1 gene was analyzed using the PCR-RFLP technique as described by Harries et al. (22). Four hundred nanograms DNA was amplified by PCR in a mixture containing 200 ng primer P105F (5'-ACC CCA GGG CTC TAT GGG AA-3') and P105R (5'-TGA GGG CAC AAG CCC CT-3'), 1.5 mmol/L MgCl<sub>2</sub>, and 1 unit Taq DNA polymerase in a total volume of 25 μL. Following initial denaturation at 95°C for 5 minutes, 30 PCR cycles were done. Amplification conditions were strand separation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, polymerization at 72°C for 30 seconds, and a final elongation step of 72°C for 5 minutes. Amplification leads to a 176 bp band. The A to G polymorphism introduces a restriction site recognized by the BsmA1 restriction enzyme (New England Biolabs, Beverly, MA). Digestion of the PCR product with 5 units BsmA1 in a 25 μL volume results either in retention of the 176 bp product or complete digestion to 91 and 85 bp fragments corresponding to individuals homozygous for the Ile or Val alleles, respectively. The presence of all three fragments corresponded to individuals heterozygous at codon 105. The products were separated on a 3.0% agarose gel and visualized by staining with ethidium bromide.

**Statistical Analysis.** Fisher’s exact test was used to examine for differences in patient characteristics according to GSTP1 genotypes. Survival curves were estimated using the Kaplan-Meier product limit method. Differences in the survival curves were evaluated with the log-rank test. The primary end point was freedom from treatment failure, with progression during treatment, lack of complete remission at the end of first-line treatment, relapse, and death from any cause counted as adverse events. Cox regression was used for multivariate analysis including patient characteristics that had prognostic significance in the univariate analysis. All computations were done using the Stata 6.0 software (Stata Corp., College Station, TX).

### Table 1: Patient characteristics and GSTP1 genotype (Fisher’s exact test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Val/Val, n (%)</th>
<th>Val/Ile, n (%)</th>
<th>Ile/Ile, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>65</td>
<td>8 (12)</td>
<td>24 (37)</td>
<td>33 (51)</td>
<td>0.95</td>
</tr>
<tr>
<td>≥45</td>
<td>32</td>
<td>3 (9)</td>
<td>12 (38)</td>
<td>17 (53)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
<td>3 (8)</td>
<td>12 (31)</td>
<td>24 (61)</td>
<td>0.3</td>
</tr>
<tr>
<td>Male</td>
<td>58</td>
<td>8 (14)</td>
<td>24 (41)</td>
<td>26 (45)</td>
<td></td>
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<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td>73</td>
<td>8 (11)</td>
<td>26 (36)</td>
<td>39 (53)</td>
<td>0.7</td>
</tr>
<tr>
<td>Other histotype</td>
<td>23</td>
<td>3 (13)</td>
<td>10 (44)</td>
<td>10 (44)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-IIB</td>
<td>34</td>
<td>3 (9)</td>
<td>17 (50)</td>
<td>14 (41)</td>
<td>0.18</td>
</tr>
<tr>
<td>IIB-IV</td>
<td>61</td>
<td>8 (13)</td>
<td>18 (30)</td>
<td>25 (57)</td>
<td></td>
</tr>
<tr>
<td>B symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>3 (7)</td>
<td>20 (47)</td>
<td>20 (47)</td>
<td>0.10</td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>8 (16)</td>
<td>13 (27)</td>
<td>28 (57)</td>
<td></td>
</tr>
<tr>
<td>Bulky disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>3 (7)</td>
<td>17 (40)</td>
<td>23 (53)</td>
<td>0.42</td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>7 (16)</td>
<td>14 (31)</td>
<td>24 (53)</td>
<td></td>
</tr>
<tr>
<td>CR after first-line treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>0</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>11</td>
<td>30</td>
<td>36</td>
<td>0.12</td>
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<tr>
<td>Relapse from CR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>66</td>
<td>11</td>
<td>27</td>
<td>28</td>
<td>0.15</td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CR, complete remission.

**RESULTS**

GSTP1 genotype was assessed in 97 patients with Hodgkin’s lymphoma, of which 11 (11%) were homozygous for the Ile<sup>105</sup> Val<sup>105</sup> genotype, 36 (37%) were heterozygous (Ile<sup>105</sup> Val<sup>105</sup>), and 50 (52%) were homozygous for Ile<sup>105</sup> Val<sup>105</sup> GSTP1 genotype. The genotype distribution was in Hardy-Weinberg equilibrium (χ<sup>2</sup> = 1.25, P = 0.26). Our observed allele frequency for the GSTP1 Ile allele was 0.30 (58 of 194) and was similar to previous reports on allele frequencies for healthy Caucasians (22).

We did not observe associations between demographic characteristics of the patients (age and sex) and GSTP1 genotype. Clinical and pathologic characteristics, including histotype, stage of disease, presence of B symptoms, bulky disease, and abnormal laboratory parameters, were not associated with GSTP1 genotypes (Table 1).

We next asked whether the GSTP1 genotype was predictive for response to therapy and prognosis. For patients with increasing number of Val alleles, there was a trend for a better remission rate to first-line therapy (P = 0.12) and a lower risk for relapse (P = 0.15) without reaching statistical significance. The difference in treatment outcome became evident when analyzing treatment failure as end point (P = 0.008). Compared to patients with the Ile<sup>105</sup> Val<sup>105</sup> GSTP1 genotype, failure-free survival was better with increasing number of GSTP1 Val alleles (P = 0.02; Fig. 1A). The probability of 5-year survival for patients homozygous for the Ile<sup>105</sup> Val<sup>105</sup> GSTP1 genotype was 100%, for heterozygous patients 74% (95% confidence interval, 56–85), and for patients homozygous for the Ile<sup>105</sup> Ile<sup>105</sup> genotype 43% (95% confidence interval, 23–61). Overall survival was not influenced by the GSTP1 genotype (P = 0.33).

As patients received different chemotherapy regimens containing anticancer drugs, not all of which are effected by GSTP1, we evaluated subjects according to the different drugs. Ninety patients received doxorubicin and bleomycin as part of ABVD, Stanford V, and BEACOPP. The combination of cyclophosphamide, vincristine, and etoposide was included in the treatment of 37 patients. We also analyzed for GSTP1 effects in patients receiving vinblastine (81 patients) or dacarbazine (53 patients). These analyses showed a significant impact of GSTP1 for patients receiving the known GSTP1 substrate doxorubicin (P = 0.023), whereas failure-free survival was not different according to GSTP1 in patients receiving the combination of cyclophosphamide, vincristine, and etoposide (P = 0.36). When the analysis was restricted to 53 patients treated with ABVD chemotherapy, essentially the same differences in failure-free survival were observed (P = 0.02). We conclude that the effect of GSTP1 on failure-free survival is most likely to the metabolism of doxorubicin; however, the contribution of other drugs in the combination regimen, in particular...
bleomycin, vinblastine, and dacarbazine is not clear, as they are not known GSTP1 targets.

We next analyzed whether the GSTP1 genotype was an independent prognostic factor. We first analyzed the prognostic value of established prognostic factors, as age, sex, stage of the disease, presence of bulky disease or B symptoms, and abnormal laboratory parameters in a univariate analysis. Stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA versus advanced disease in stage IIB-IV, \( P = 0.03 \); Fig. 1B). We therefore included stage in a multivariate analysis using the Cox regression model (Table 2). GSTP1 genotype and stage had an independent prognostic value (\( P = 0.02 \) and \( P = 0.07 \), respectively). We also analyzed how the GSTP1 genotype influenced the outcome in patients grouped according to stage (Fig. 2). The probability of failure-free survival was high in patients with stage I-IIA disease without apparent differences for GSTP1 genotypes (Fig. 2A), whereas the probability of failure-free survival in patients with advanced stage (IIB-IV) disease significantly differed according the GSTP1 genotype (Fig. 2B).

**DISCUSSION**

To our knowledge, this is the first report on the association between GSTP1 genotype and clinical outcome following chemotherapy in Hodgkin’s lymphoma. Our results are in line with reports on an association between \(^{105}\)Val GSTP1 allele and a favorable prognosis in other malignancies (12–15). The impact of the GSTP1 genotype is, therefore, probably not specific for a tumor type but may be related to the metabolism of anticancer drugs. Patients with the \(^{105}\)Val GSTP1 variant have a reduced

**Table 2** Prognostic significance of the GSTP1 genotype for failure-free survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariate analysis (Cox regression analysis)</th>
<th>( P )</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1 V/V vs V/I vs I/I</td>
<td></td>
<td>0.02</td>
<td>0.42</td>
<td>0.21–0.85</td>
</tr>
<tr>
<td>Stage* IIB-IV vs I-IIA</td>
<td></td>
<td>0.07</td>
<td>2.4</td>
<td>0.92–6.45</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval.

*Complete data for 95 patients.
ability to detoxify chemotherapeutic agents, which determines an increase in the effective dose of the drug within the cell. Our data are compatible with a gene dosage effect. Watson et al. (11) showed that individuals with two GSTP1 105 Val alleles have a lower catalytic activity when compared with individuals with two GSTP1 105 Ile alleles, whereas an intermediate activity was reported for heterozygotes.

The precise mechanism whereby GSTP1 is responsible for resistance to anticancer agents is still matter of debate. In addition to glutathione conjugation to anticancer drugs and cytosolic sequestration of drugs by direct binding to the enzyme, chemical reduction of hydroxyl radicals formed by anthracyclins has also been described. The detoxification of reactive oxygen species, which may act as intermediaries in the cytotoxicity of many chemotherapeutic agents, may modulate the response to a specific drug even when the chemotherapeutic agent itself is not a substrate.

The patients of our study received different chemotherapy regimens, including drugs not all of which are affected by GSTP1. Gene transfection and pharmacokinetic studies have identified the following anticancer drugs to be targets of GSTP1: doxorubicin, cisplatin, cyclophosphamide, melphalan, busulfan, chlorambucil, thiotapec, vincristine, and etoposide (23–29). The modulating effect of GSTP1 on cytotoxicity of doxorubicin and cisplatin is well documented (23, 27–29). Cisplatin, as well as melphalan, busulfan, chlorambucil, and thiotapec were not part of the regimens used in our study. The evaluation of different drug combinations suggested that the effect of GSTP1 on failure-free survival is most likely to the metabolism of doxorubicin; however, the contribution of other drugs in the combination regimens, in particular bleomycin, vinblastine, and dacarbazine, is not clear as they are not known GSTP1 targets.

Women with breast cancer carrying the GSTP1 105 Val allele had a better overall survival independent of the specific drug or drug combination (14). Dasgupta et al. (13) observed a better survival only in patients with multiple myeloma treated with conventional chemotherapy, and not in those treated with high-dose therapy.

In a cohort of 77 patients with Hodgkin's lymphoma, Sarmanova et al. (30) found a slightly, but significantly higher prevalence of the GSTP1 105 Val allele in the patient versus control population (0.357 versus 0.305, respectively). In our patient cohort, the prevalence was 0.3, which seems not to be different from the prevalence reported in control subjects (22, 30). We cannot exclude that GSTP1 polymorphism could play a role in the etiology of the disease and could contribute to differences in biological characteristics, which could influence prognosis.

On the other hand, individuals with at least one GSTP1 105 Val allele may be at a higher risk for therapy-related acute myeloid leukemia (t-AML; ref. 31). In our series, we did not observe cases of t-AML. This may be because most patients in our series were treated with ABVD, a regimen associated with a minimal leukemogenic risk and only nine patients with a shorter follow-up had received BEACOPP. Larger studies are needed to address the possible association of GSTP1 polymorphism and t-AML in patients with Hodgkin’s lymphoma.

Methylation of CpG islands in the promoter region of the GSTP1 gene is another mechanism of gene inactivation. GSTP1 hypermethylation has been reported in solid tumors, including prostate cancer, as well as in B-cell non-Hodgkin's lymphomas (32, 33). Data on Hodgkin's lymphoma are missing. It will be of interest to analyze for additive effects of combinations of polymorphism and GSTP1 gene hypermethylation, as well as of polymorphisms of different detoxification genes when combined. These studies will require larger patient groups.

The power of our study to detect differences in failure-free survival was 78% when comparing homozygous patients excluding heterozygous, whereas it was only 28% for the difference between heterozygous and 105-Ile homozygous patients. A cohort of 308 patients will be needed to confirm the prognostic difference between these groups at a 80% power level. GSTP1 genotype may be a useful prognostic marker in patients with Hodgkin’s lymphoma in advanced-stage disease once our data have been confirmed on a larger group of patients. The GSTP1 status may help to identify, on one hand, patients for whom standard treatment as ABVD is sufficient and, on the other hand, patients who may benefit from dose-intensified therapy. Moreover, it is tempting to speculate whether adding drugs that may modify or inhibit the enzymatic activity of GSTP1 might improve treatment results in patients with the GSTP1 105 Ile allele (34).

REFERENCES


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