To the editor:

Does the FLIPI apply to grade 3 follicular lymphoma?

We welcome the publication of a score (Follicular Lymphoma International Prognostic Index [FLIPI]) assisting in the choice of treatment for patients with newly diagnosed follicular lymphoma (FL). As the authors state, treatment options for this disease range from “watch and wait” to allogeneic bone marrow transplantation, and the selection of the best available option will be favored by a realistic estimation of the expected survival for each particular patient.

The 5 independent prognostic factors retained in the FLIPI reflect important characteristics of the patient (age), of the disease extension and clinical aggressiveness (stage and number of nodal sites involved, lactose dehydrogenase [LDH]), and of the tumor-host interaction (hemoglobin level). Surprisingly enough, histology grade, a disease characteristic considered by many clinicians to be of paramount importance for prognosis and choice of treatment, is missing from the index and was not even significant in the univariate analysis.

The absence of this parameter from the index and the omission of any discussion about this fact in the paper could suggest that histologic grade is indeed not relevant to patient survival and should therefore not be taken as an important information.

We believe that this misunderstanding results from a bias that is not sufficiently covered in the article and that should be clarified.

It was long recognized that an important proportion of large cells (centroblasts) at histologic examination (an observation referred to as grade 3 in the current World Health Organization [WHO] classification) does confer to FL a worse prognosis, despite apparently favorable clinical prognostic features. Nevertheless, the outcome of these cases can be similar to the forms with less centroblasts when they are treated with an anthracycline-containing regimen. This information was known in 1985 (the year of the start of data collection for the FLIPI) and, although details on treatment are not given in the paper, it is probable that the majority of grade 3 FLs of this data set were treated with anthracyclines, thus influencing the prognosis.

Even though the article states that “none of the treatments given during the period of inclusion has significantly changed the natural history of the disease,” this only applies to cases with histologic grades 1 and 2, while for WHO grade 3 we have reason to think that treatment did indeed influence survival, and therefore interacted with other prognostic factors.

The FLIPI could have been a more reliable index if grade 3 FLs were not included in the analysis. We believe that for grades 1 and 2 FL, treatment should be selected based on a number of factors, including the FLIPI score, but an anthracycline-containing regimen should still be favored for patients with grade 3 FL, independent of their FLIPI index.

Michele Ghielmini and Oreste Mora

References

Response:

FLIPI in grade 3 follicular lymphoma

We agree with Prof Ghielmini that grade 3 follicular lymphoma is in many cases a more aggressive disease than other follicular lymphomas, although this is an area of ongoing controversy. Grade 3 follicular lymphoma especially shares the prognosis of and requires treatment approaches similar to that of diffuse large B-cell lymphoma. We welcome the opportunity to comment further on the Follicular Lymphoma International Prognostic Index (FLIPI) observations about grade 3 follicular lymphoma.

First, a central pathology review was not realistic for the 5000 FLIPI cases. Since there are known interobserver variations in the assignment of histologic grade, we felt we would introduce a bias if we excluded these cases arbitrarily. Moreover, they represented only 9% of the entire FLIPI population.

Second, the IPI appears to have one of the same shortcomings for grade 3 follicular lymphoma as it does for all follicular lymphomas, namely the identification of only a small (15%-20%) fraction of cases with high risk. Thus the inclusion of grade 3 cases in the FLIPI, and the applicability of the FLIPI to grade 3 follicular lymphoma, represents a potential benefit to patient management.

But we completely agree with Prof Ghielmini’s observation that an anthracycline-containing regimen is favored for patients with grade 3 follicular lymphoma. It was beyond the scope of the initial FLIPI project to analyze in any detail the various treatment modalities used. Moreover, such a retrospective analysis would be open to valid criticism. But we agree that the literature supporting the inclusion of anthracyclines in grade 3 follicular lymphoma is
The achievement of partial or complete cytogenetic remission (CCR) in response to imatinib mesylate in chronic myeloid leukemia (CML) is an indicator of favorable response to the drug.\(^1\) The current assays for prediction of response rely on immunoblot analyses of Bcr-Abl or its substrates Crkl\(^2\) or Stat5.\(^3\) However, these require large numbers of primary cells and are technically demanding. We previously showed that the inhibition of total phosphotyrosine was a reliable indicator of the effect of imatinib mesylate on BCR-ABL\(^+\) cell lines.\(^4\) We have now further developed this flow cytometric test to assess the response of primary cells to in vitro imatinib treatment.

We analyzed cryopreserved mononuclear cells from 23 patients with CML in chronic phase, 16 of whom had responded to imatinib mesylate, reaching at least a partial cytogenetic response within the first year of therapy. There was no significant difference in the interval between diagnosis and the initiation of imatinib mesylate treatment between the 2 groups. After thawing, CD34\(^+\) cells were isolated by immunomagnetic separation, kept overnight in culture, and subsequently exposed for 2 hours to doses of imatinib mesylate ranging from 0.1 to 50 \(\mu\)M, and intracellularly stained as described in Figure 1. The overall tyrosine phosphorylation was determined by flow cytometry and expressed as a percentage of the level of phosphorylation of the nonexposed control cells.

The results showed a significant difference between patients who achieved a complete or partial cytogenetic remission and those who did not (Figure 1). For the most discriminatory concentration of 20 \(\mu\)M imatinib mesylate, the median level of tyrosine phosphorylation inhibition in cells from the responders was 46\%, as opposed to only 32\% in the nonresponders (\(P = 0.34\), Mann-Whitney test). None of the nonresponders had more than 45\% inhibition of their total cellular phosphotyrosine content, whereas this was achieved in half of the responding patients. The molecular response to imatinib mesylate also correlated with its capacity to inhibit tyrosine phosphorylation in vitro: in none of the patients who failed to exhibit a greater than 2-log reduction in the number of BCR-ABL transcripts or a BCR-ABL/ABL ratio less than 2\% was imatinib able to reduce the cellular phosphotyrosine content below 55\% of the pretreatment levels, whereas 75\% (6/8) of those with at least a 50\% reduction in its level.

**References**


2. Bosga-Bouwer AG, van Imhoff GW, Boonstra R, et al. Follicular lymphoma grade 3b includes 3 cytogenetically defined subgroups with primary t(14;18) or other translocations: t(14;18) and t(9;22) are mutually exclusive. Blood. 2003;101:1145-1154.


To the editor:

The interleukin-10 gene promoter polymorphism (−1082) does not correlate with clinical outcome in diffuse large B-cell lymphoma

Interleukin-10 (IL-10) is a cytokine important in suppressing the immune response by inhibiting proinflammatory cells. However, this cytokine is multifunctional and can also stimulate proliferation in general, are older (P = .014), had B symptoms more often (P = .034), and had elevated serum lactate dehydrogenase (S-LDH; P = .012) levels, no difference in survival was shown when considering only patients younger than 60 years of age or history) from Uppsala and Umeå University Hospitals to establish their IL-10−1082 genotype and compare it with overall survival. We also assessed the genotype in 195 healthy controls. In the present analysis, the frequency of the IL-10−1082 allele was not significantly different in our patients with DLBCL versus the control group (0.43 vs 0.46; P = .38). Thus, the increased IL-10−1082 allele frequency could not be verified in our analysis of DLBCL (Table 1). Furthermore, we did not find any difference in overall survival between the 174 patients with IL-10−1082AG/GG genotype and the 70 patients with IL-10−1082AA (median survival, 39 vs 37 months; log-rank test, P = .50; Figure 1). No significant difference in the clinical presentation was indicated between the 2 cohorts considering sex, clinical stage, complete remission rate, and international prognostic index. Although patients in our analysis, in general, were older (χ²; P = .014), had B symptoms more often (χ²; P = .034), and had elevated serum lactate dehydrogenase (S-LDH; P = .012) levels, no difference in survival was shown between patients with IL-10−1082AG/GG and IL-10−1082AA genotype when considering only patients younger than 60 years of age or

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Table 1. Allele and genotype frequency of the IL-10−1082 polymorphism in 244 DLBCLs and 195 control subjects

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>DLBCL cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10−1082G</td>
<td>0.43</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype frequency (%)</th>
<th>DLBCL cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10−1082 AG/GG</td>
<td>38 (16)</td>
<td>46 (24)</td>
<td></td>
</tr>
<tr>
<td>IL-10−1082 AG</td>
<td>136 (56)</td>
<td>89 (46)</td>
<td></td>
</tr>
<tr>
<td>IL-10−1082 AA</td>
<td>70 (29)</td>
<td>60 (31)</td>
<td></td>
</tr>
</tbody>
</table>

P values were calculated using χ² test. The P value for the genotype frequency is calculated for IL-10−1082AG/GG versus IL-10−1082AA.

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References

when patients with or without B symptoms or elevated S-LDH levels were analyzed separately. Moreover, no difference in outcome was evident for patients with or without the IL-10-1082G allele when separately analyzing de novo DLBCL and cases with previous history of low-grade lymphoma. In conclusion, we could not confirm the findings in the report by Lech-Maranda et al2 of increased frequency of the IL-10-1082G allele and its association with superior outcome in our analysis of patients with DLBCL. We therefore believe that the clinical relevance of the IL-10-1082G promoter polymorphism may be limited in DLBCL, although larger studies have to be performed to conclude this properly.

Mattias Berglund, Ulf Thunberg, Göran Roos, Richard Rosenquist, and Gunilla Enblad

Correspondence: Mattias Berglund, Departments of Oncology, Radiology, and Clinical Immunology, Rudbeck Laboratory, Uppsala University, SE-751 85 Uppsala, Sweden; e-mail: Mattias.Berglund@genpat.uu.se.

Response:

Different patients, different results: the need for prospective controlled studies

In this issue, Berglund and colleagues report their analysis of the IL-10 gene polymorphism (IL10) in a series of 244 lymphoma patients. This series included 168 de novo diffuse large B-cell lymphomas whereas other cases had a previous history of low-grade lymphoma and should probably be considered as transformed lymphoma. The authors did not find a different distribution in IL10-1082 alleles between the patient and the control populations. Of note, the IL10-1082G allele disequilibrium frequency that we previously described only applies to diffuse large B-cell lymphoma and not to other lymphoma subtypes (E.W. and G.S., unpublished results, May 2005). In this context, these authors also failed to observe a prognostic significance for the IL10-1082G allele in diffuse large B-cell lymphoma.

The reasons for this discrepancy are unclear and may include several biases such as patient characteristics and treatment options and results. For instance, the median survival reported by Berglund et al is close to 38 months for the whole population but was projected to be 70 months in our study. The fact that this study includes older patients is also significant since the prognostic significance of the IL10-1082G allele was more pronounced in patients younger than 60 years than in older patients in our study. Finally, how inherited immune response may actually influence patient outcome may indeed depend on the initial characteristics of the patient, the disease, and the efficiency of treatment. Altogether, these results emphasize the need for prospective and multicentric genomic DNA analysis linked to a clinical database, such as the one currently built with Groupe d’Etude des Lymphomes de l’Adulte (GELA) clinical trials, which will be analyzed in the coming years.

Gilles Salles and Ewa Maranda

Correspondence: Centre Hospitalier Leon-SUD, 69495 Pierre-Bénite cedex, France; e-mail: gilles.salles@chu-lyon.fr.

To the editor:

The bone marrow microenvironment influences the differential chemokine receptor expression of normal and neoplastic plasma cells

A number of mechanisms may explain the clinical and biologic differences in behavior between monoclonal gammopathy of undetermined significance (MGUS) and myeloma plasma cells (PCs). These include inherent differences in PC programming, immortalization of PCs at different stages of differentiation, different cells of origin, and potentially the influence of the normal/neoplastic bone marrow (BM) microenvironment. Chemokines and their receptors are known to be paramount to normal cell differentiation and homing. As B cells differentiate into PCs they undergo a coordinated change in chemokine receptor expression and chemokine responsive. We therefore hypothesized that the expression of chemokine receptors on myeloma PCs would be altered compared with normal PCs and may explain some of the differences in behavior between normal and malignant populations. As our previous studies have demonstrated that 2 populations of PCs (normal and neoplastic) can be identified in myeloma and MGUS cases based on expression of CD19 expression1-3 and that MGUS cases with a predominantly neoplastic phenotype have a higher chance of progressing to myeloma than those with a mixed normal and malignant phenotype,3 we also hypothesized that by examining both normal and neoplastic PCs from the same BM in MGUS patients we could determine whether any variation in chemokine receptor profile was due to the marrow microenvironment or to neoplastic transformation.

Normal (CD38+CD138+CD19+CD56−) and neoplastic PCs (CD38+CD138+CD19−CD56+) were distinguished from other leukocytes using 4-color flow cytometry with CELLQuest version 3.1 on a FACSort (BD Biosciences, Oxford, United Kingdom) as
Figure 1. Flow-cytometric analysis of chemokine receptor expression. (A) Chemokine receptor expression on normal and myeloma PCs. The mean expression level and range is shown. (B) CCR2 expression is significantly lower on CD19+ PCs from MGUS patients than that on CD19+ PCs from the same bone marrow. The figure shows 5th, 25th, 50th, 75th, and 95th percentiles for expression, measured as mean fluorescence intensity.

To the editor:

Patients with thalassemia in the United States

Thalassemia is one of the most common monogenic diseases of man, prevalent in tropical and subtropical regions of the world. Population migration during the past decades has led to increasing numbers of these patients being encountered in all parts of the world, including in the United States. A clinical research network, supported by National Institutes of Health (NIH), was established to study β-thalassemia major/intermedia. A report on disease complications was published in July 2004, based on 342 patients (222 in the United States and 120 in Toronto)\(^1\).

Our laboratory was established in 2003 to provide molecular diagnosis of hemoglobinopathies and thalassemias. In the past 18 months, we have genotyped 40 β-thalassemia major/intermedia patients of all ethnic backgrounds (Table 1). Only 4 cases are of Mediterranean ancestry, hitherto the source of most β-thalassemia patients in our country. Seventy percent of the cases are 15 years of age or younger. They reside in Massachusetts (18), Rhode Island (3), West Virginia (4), New York (1), Maryland (3), and Georgia (11). Surprisingly, only one patient in this recently diagnosed cohort has the β-thalassemia mutation genotype [IVS1-6 (T>C)/IVS1-110 (G>A)] that corresponds to 1 of the 5 genotypes reported to be most commonly found in the United States\(^1\).

These findings highlight the fact that β-thalassemia is more...
Additionally, our laboratory has diagnosed 11 cases of clinically significant hemoglobin (Hb) H disease from Massachusetts, New York, and Georgia: 1 (—MED/—α<sup>3.7</sup>); 5 (—SEA/—α<sup>3.7</sup>), including 1 Hb E heterozygote; and 5 (—SEA/Constant Spring α), including 2 Hb E heterozygotes. Efforts devoted to community public education appropriately adapted to different cultures and languages, continuous education for health care providers, and research in disease pathophysiology and treatment for α- and β-thalassemias are needed.

Hong-yuan Luo, Jeanne Boudreaux, Martin H. Steinberg, and David H. K. Chui

Correspondence: David H. K. Chui, Hemoglobin Diagnostic Reference Laboratory, Boston Medical Center, 88 East Newton Street, Boston, MA 02118; e-mail: david.chui@bmc.org.

Supported in part by National Heart, Lung, and Blood Institute (NHLBI) Cooperative Agreement 1U54 HL70819.

References

To the editor:

The Marburg I polymorphism of factor VII–activating protease is not associated with venous thrombosis

Table 1. Marburg I polymorphism and the risk of venous thrombosis

<table>
<thead>
<tr>
<th>FSAP nt 1601</th>
<th>Patients, no. (%)</th>
<th>Controls, no. (%)</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>444 (94.3)</td>
<td>441 (93.6)</td>
<td>1* NA</td>
</tr>
<tr>
<td>GA</td>
<td>26 (5.5)</td>
<td>28 (6.0)</td>
<td>0.9 0.5-1.6</td>
</tr>
<tr>
<td>AA</td>
<td>1 (0.2)</td>
<td>2 (0.4)</td>
<td>0.5 0.05-5.5</td>
</tr>
<tr>
<td>GA + AA</td>
<td>27 (5.7)</td>
<td>30 (6.4)</td>
<td>0.9 0.5-1.5</td>
</tr>
</tbody>
</table>

There were 471 patients and 471 controls.
*Reference category.

In a recently published paper in Blood, Hoppe et al showed in 213 patients and 213 controls that carriers of the Marburg I polymorphism of the factor VII–activating protease (FSAP) had an increased risk of venous thrombosis (odds ratio [OR], 3.5; 95% confidence interval [CI], 1.2-10.0).

FSAP is a serine protease that has 2 functions in hemostasis. It activates factor VII, thereby promoting coagulation, but it can also activate single-chain plasminogen activators, thus promoting fibrinolysis. Recently, a single nucleotide polymorphism (1601G>A) was discovered in the gene coding for FSAP, which results in the substitution of glycine 511 by glutamic acid (FSAP Marburg I) and which is present in 2% to 9% of the white population. The Marburg I variant has an impaired prourokinase activating potency, whereas it can still activate factor VII normally.

If true, the finding of Hoppe et al would support the hypothesis that reduced fibrinolysis contributes to the risk of venous thrombosis. However, the authors indicated that their control group might be biased due to the exclusive inclusion of healthy blood donors, or because the control group was relatively young. In thrombosis patients the frequency of the Marburg I allele was similar in the study of Hoppe et al (0.039) and ours (0.030), so the low prevalence in the control group (5/426) seems to be the explanation for the findings by Hoppe et al, either because those were blood donors, in which case the mutant allele would be infrequent in a group selected on health, or because the control group was relatively small. On the other hand we cannot exclude small geographic differences in the Marburg I frequency.

R. van Minkelen, Marieke C. H. de Visser, Hans L. Vos, Rogier M. Bertina, and Frits R. Rosendaal

Correspondence: Rick van Minkelen, Department of Hematology, Haemostasis and Thrombosis Research Center, C2-R, Leiden University Medical Center, PO box 9600, 2300 RC, Leiden, the Netherlands; e-mail: r.van_minkelen@lumc.nl.

Supported by grant 912-02-036 from the Netherlands Organization for Scientific Research (NWO). The LETS study was supported by grant 89-063 from the Netherlands Heart Foundation.

References

Response:

Association of Marburg I polymorphism of factor VII–activating protease with venous thromboembolism is limited to idopathic events

In this issue, van Minkelen et al report on venous thrombosis and the Marburg I variant of factor VII–activating protease (FSAP), commenting on a study published recently by our group in the journal. They essentially describe 2 findings that appear to be in contrast to our study: (1) The frequency of carriers of FSAP Marburg I in the controls of van Minkelen et al was considerably higher (6.4%) than that of our control group (2.3%); and (2) an association of FSAP Marburg I with deep vein thrombosis (DVT) could not be confirmed.

In reply to these comments we want to remark the following. (1) Indeed, the frequency of carriers of FSAP Marburg I in our controls (5/213; 2.3%) comprising healthy blood donors was lower than that of the Bruneck study cohort (37/810; 4.6%). The controls of van Minkelen et al (30/471; 6.4%), and the Northwick Park Heart Study cohort (176/2066; 8.5%). Different frequencies in the described study populations could be due to low sample size, selection bias, and different geographic distributions. To exclude the possibility that our controls might be biased by negative selection of FSAP Marburg I due to its association with atherosclerosis, we analyzed a larger, independent control group consisting of consecutive patients admitted to our institution for reasons other than venous thromboembolism (non-VTE patients). Main diagnoses of these patients included transient ischemic attack/stroke, and myocardial infarction. Considering the positive association of FSAP Marburg I with progression of atherosclerosis and assuming at least no negative association of this variant with VTE (Hoppe et al and van Minkelen et al), the frequency of FSAP Marburg I carriers in these non-VTE patients (15/327; 4.6%) will presumably overestimate the normal frequency of this variant in our geographic region.

(2) The data presented by van Minkelen et al included patients with DVT, making no distinction between idopathic and secondary events. As we described in the study, the association of FSAP Marburg I with VTE (including DVT and/or pulmonary embolism) was nearly exclusively attributable to idopathic events (Table 1; “Comparison vs controls I”). Secondary VTE due to acquired risk factors (immobilization, surgery, trauma, pregnancy, puerperium, malignancy) was not associated with this variant. (ii) To avoid possible biases by the control group (blood donors), we performed a statistical analysis based on the second, independent control group (non-VTE patients; Table 1; “Comparison vs controls II”). Again, FSAP Marburg I was significantly associated with idopathic but not secondary VTE (odds ratio, 2.7; 95% confidence interval [CI], 1.2-6.1).

The FSAP genotypes have no influence on its capacity to activate factor VII in vitro. Moreover, the physiologic role of FSAP and the site where its action takes place remains unclear. Thus, the absence of a detectable difference in circulating factor VII activities with both FSAP and the site where its action takes place remains unclear. The data presented here (univariate analyses) differ slightly from those of our initial report (logistic regression analyses; independent variables: factor V Leiden, prothrombin 20210G→A, and FSAP Marburg I). The data presented by van Minkelen et al is not surprising.

Based on the additional data described here and as stated in our initial report, FSAP Marburg I is associated with idopathic VTE.

Berthold Hoppe, Guido A. Heymann, Hartmut Radtke, Holger Kiesewetter, Thomas Dörner, and Abdulgabar Salama

Correspondence: Berthold Hoppe, Institute of Transfusion Medicine, Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany; e-mail: berthold.hoppe@charite.de

Table 1. Association of FSAP Marburg I with VTE

<table>
<thead>
<tr>
<th>No.</th>
<th>Carriers of FSAP Marburg I, no. (%)</th>
<th>Comparison vs controls I, odds ratio (95% CI)</th>
<th>Comparison vs controls II, odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic VTE</td>
<td>103 (11.7)</td>
<td>5.5 (1.9,16.0)*</td>
<td>2.7 (1.2,6.1)‡</td>
</tr>
<tr>
<td>Secondary VTE</td>
<td>110 (4.5)</td>
<td>2.0 (0.56,7.0)*</td>
<td>0.99 (0.35,2.8)</td>
</tr>
<tr>
<td>VTE</td>
<td>213 (8.0)</td>
<td>3.6 (1.3,10.0)*</td>
<td>1.8 (0.88,3.7)</td>
</tr>
<tr>
<td>Controls I</td>
<td>213 (5.3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Controls II</td>
<td>327 (4.6)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Controls I indicates healthy blood donors; controls II, non-VTE patients; and NA, not applicable.

*P < .001 (Fisher exact test).
‡P < .05 (Fisher exact test).

References

The bone marrow microenvironment influences the differential chemokine receptor expression of normal and neoplastic plasma cells

Mona H. Al Rayes, Andy C. Rawstron, Gareth J. Morgan and Faith E. Davies