Letters to the Editor

Incidence of trisomy 8 and 9, deletion of D13S319 and D20S108 loci and BCR/ABL translocation in non-treated essential thrombocythemia patients: an analysis of bone marrow cells using interphase fluorescence in situ hybridization

We compare conventional cytogenetics (CC) with fluorescence in situ hybridization (FISH) in 53 untreated patients with essential thrombocythemia. CC revealed no abnormalities. When FISH was used, no BCR/ABL rearrangement nor trisomy 8 was found, but one trisomy 9, two del(13)(q14) and five del(20)(q12) were observed. FISH detected chromosome abnormalities in 15% of patients in which no alteration was found by CC.

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Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) with megakaryocytic proliferation in bone marrow resulting in a persistent increase in platelets in peripheral blood. In ET patients chromosome abnormalities detected by G-banding are rare, and no specific abnormality has been identified. Only about 5% of patients show an abnormal karyotype at diagnosis. The most frequent cytogenetic anomalies detected by conventional cytogenetics (CC) are trisomies of chromosomes 8 and 9 and deletions in 13q and 20q.

The finding of an abnormal karyotype would be useful to distinguish ET from secondary thrombocytosis as it gives a clonal hallmark to the disease. For this reason, we re-evaluated genetic findings obtained by CC using fluorescence in situ hybridization (FISH) probes, with the aim of yielding more information about chromosomal abnormalities in ET patients. Herein, we present 53 cases diagnosed as having ET according to the Polycythemia Vera Study Group (PVSG) criteria and who had not previously received cytolytic treatment. Samples from ten healthy volunteers were used as assay validation controls. Chromosome analyses were carried out on hematologic cells from 24-hour bone marrow cultures. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

FISH studies were performed on fixed nuclei from CC following the standard procedures (Table 1). CC results were available in 49/53 patients, all showing a normal karyotype; in the remaining four cases no metaphases were obtained. When FISH was performed, no patient showed BCR/ABL rearrangement nor trisomy 8. One patient showed trisomy 9 in 30% of studied cells, in 2/53 patients a 13q14 deletion was found (frequencies 16% to 26.5%) and 5/53 patients presented a 20q12 deletion (frequencies 10.5% to 13.5%). Both monosomies were hemizygous. There were no cases with more than one abnormality. FISH probes detected chromosomal abnormalities in 8/53 ET patients (15.1%) (Table 2).

FISH studies have been done in ET patients in order to search for chromosome 8 and 9 abnormalities. Eris et al. reported...
Table 1. Probes and controls used in the study.

<table>
<thead>
<tr>
<th>Probes</th>
<th>Controls</th>
<th>Studied nuclei</th>
<th>Control values* (X±3 S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEN #8</td>
<td>–</td>
<td>500</td>
<td>Trisomy &gt;2.3%</td>
</tr>
<tr>
<td>CEN #9</td>
<td>–</td>
<td>500</td>
<td>Trisomy &gt;1.2%</td>
</tr>
<tr>
<td>D13S319 tel(13q)</td>
<td>200</td>
<td></td>
<td>Monosomy &gt;5%</td>
</tr>
<tr>
<td>D20S108 tel(20p)</td>
<td>200</td>
<td></td>
<td>Monosomy &gt;6%</td>
</tr>
<tr>
<td>BCR ABL</td>
<td>internal</td>
<td>200</td>
<td>Rearrangement &lt;1%</td>
</tr>
</tbody>
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* Control values were established based on peripheral blood of 10 controls. X±3 S.D. = mean plus three standard deviations.

Table 2. Summary of chromosome abnormalities detected by conventional cytogenetics and FISH in 53 ET patients.

<table>
<thead>
<tr>
<th></th>
<th>Conventional Cytogenetics* Number of cases (%)</th>
<th>FISH Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>49/49 (100%)</td>
<td>45/53 (84.9%)</td>
</tr>
<tr>
<td>+8</td>
<td>0/49 (0%)</td>
<td>0/53 (0%)</td>
</tr>
<tr>
<td>+9</td>
<td>0/49 (0%)</td>
<td>1/53 (1.9%)</td>
</tr>
<tr>
<td>del(13)(q14)</td>
<td>0/49 (0%)</td>
<td>2/53 (3.8%)</td>
</tr>
<tr>
<td>del(20)(q12)</td>
<td>0/49 (0%)</td>
<td>5/53 (9.4%)</td>
</tr>
<tr>
<td>Total (abnormal)</td>
<td>8/53 (15.1%)</td>
<td></td>
</tr>
</tbody>
</table>

* In four cases no mitoses were observed.

an increased detection of trisomies 8 and 9 by FISH in ET patients with a normal karyotype by CC. They detected 5/18 patients with trisomy 8 and 5/18 with trisomy 9. Swolin et al., however, did not find any new cases of trisomy 8 or 9, not previously found by CC, in a series of 22 patients. Two possible reasons could explain the differences between the results of the studies by Elis et al., Swolin et al., and ours. First, in the series examined by Elis et al., patients had a long follow-up (range 1-13 years), while in our study and that by Swolin et al., cytogenetic investigations were performed at diagnosis, in previously untreated patients. Second, the work of Elis et al. was carried out using peripheral blood, whereas ours and that by Swolin et al. evaluated bone marrow cells. Additionally, 7/10 patients with trisomies 8 and 9 in the series studied by Elis et al. were maintained on hydroxyurea at the time of cytogenetic investigation. As the number of cytogenetic abnormalities is greater in treated than in untreated patients, this may reflect leukemogenic effects of treatment itself or may indicate that patients whose disease requires more aggressive treatment are more likely to develop cytogenetic changes.

The fact that FISH did not detect new cases of trisomy 8 in our series or in that of Swolin et al., corroborates the hypothesis that cells with trisomy 8 have a proliferative advantage over diploid cells in culture, since the frequency of trisomy 8 cells is greater in bone marrow metaphases than in interphase nuclei.

To our knowledge, no other studies have been conducted to test 13q14 and 20q12 probes in ET patients. We identified 2 and 5 patients with a hemizygous deletion in the 13q14 and 20q12 locus, respectively. These results encourage the use not only of centromeric probes from chromosome 8 and 9, but also probes from 13q14 and 20q12 loci, given the relatively high frequency of both deletions.

Although some papers have described ET patients with BCR/ABL rearrangement, we did not find this rearrangement in any patient in our series. Based on our previous experience and present data we can not confirm the presence of BCR/ABL-positive ET cases.

In conclusion, our experience corroborates that interphase FISH allows a higher detection (15%) of cytogenetic abnormalities than CC, and therefore, we suggest that FISH should be incorporated in addition to CC for the detection of specific chromosomal abnormalities in ET.

It will be of interest to follow patients with cytogenetic aberrations in order to establish the prognostic value of these abnormalities.

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Key words: essential thrombocythemia, FISH, conventional cytogenetics.

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References