analysis, the International Prognostic Scoring System (IPSS),[9] percentage of blasts, M/E ratio, FAB subtype, but not cyclin E IR, were significantly associated with survival (Table 1). Multivariate analysis showed that IPSS (p=0.0068) and RAEB-t subtype (p=0.001) were independent predictors of survival. Interestingly, when the survival analysis was restricted to the low-risk subset of RA/RARS, the variables of age (p=0.0287), IPSS (p=0.0113) and FAB subtype (RA vs. RARS, p=0.0563) were associated with survival. At multivariate analysis, the variable of age (p=0.0284) was the only independent predictor of survival in RA/RARS. Although cyclin E IR was more prevalent in RA/RARS with an adverse clinical course, this trend did not achieve statistical significance, either at univariate (p=0.0759), or multivariate (p=0.0604) analysis, possibly because of the relatively small number of cases.

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References

1. Erlanson M, Portin C, Linderholm B, Lindh J, Roos G, Landberg G. Expression of cyclin E and the cyclin-dependent kinase inhibitor p27 in malignant lymphomas-prognostic implications. Blood 1998;92:770-7.
2. Yokozawa T, Towatari M, Iida H, Takeyama K, Tanimoto M, Kiyoi H, et al. Prognostic significance of the cell cycle inhibitor p27Kip1 in acute myeloid leukemia. Leukemia 2000;14:28-33.
3. Taniguchi T, Endo H, Chikatsu N, Uchimaru K, Asano S, Fujita T, et al. Expression of p21(Cip1/Waf1/Sdi1) and p27(Kip1) cyclin-dependent kinase inhibitors during human hematopoiesis. Blood 1999;93:4167-78.
4. Burger C, Wick M, Muller R. Lineage-specific regulation of cell cycle expression in differentiating myeloid cells. J Cell Sci 1994;107:2047-54.
5. Furukawa Y, Kikuchi J, Nakamura M, Iwase S, Yamada H, Matsuda M. Lineage-specific regulation of cell cycle control gene expression during haematopoietic cell differentiation. Br J Haematol 2000;110:663-73.
6. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol 1999;17:3835-49.
7. Mazumder S, Gong B, Almasan A. Cyclin E induction by genotoxic stress leads to apoptosis of hematopoietic cells. Oncogene 2000;19:2828-35.
8. Shetty V, Hussaini S, Broady-Robinson L, Allampallam K, Mundle S, Borok R, et al. Intramedullary apoptosis of hematopoietic cells in myelodysplastic syndrome patients can be massive: apoptotic cells recovered from high-density fraction of bone marrow aspirates. Blood 2000;96:1388-92.
9. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079-88.

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.[1] Only about 5% of patients show an abnormal karyotype at diagnosis.[2] The most frequent cytogenetic anomalies detected by conventional cytogenetics (CC) are trisomies of chromosomes 8 and 9 and deletions in 13q and 20q.[1]

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[3] 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 the International System for Human Cytogenetic Nomenclature.[4]

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.[5,6] Elis *et al*.[5] reported

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Table 1. Probes and controls used in the study.

Probes	*Controls*	*Studied nuclei*	*Control values* (X±3 S.D.)*
CEN #8	–	500	Trisomy >2.3%
CEN #9	–	500	Trisomy >1.2%
D13S319	tel(13q)	200	Monosomy >5%
D20S108	tel(20p)	200	Monosomy >6%
BCR/ABL	internal	200	Rearrangement <1%

**Control values were established based on peripheral blood of 10 controls. X±3D.S. = mean plus three standard deviations.*

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

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

**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*.,[6] 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*.,[5] Swolin *et al*.[6] and ours. First, in the series examined by Elis *et al*.,[5] patients had a long follow-up (range 1-13 years), while in our study and that by Swolin *et al*.,[6] cytogenetic investigations were performed at diagnosis, in previously untreated patients. Second, the work of Elis *et al*.[5] was carried out using peripheral blood, whereas ours and that by Swolin *et al*.,[6] evaluated bone marrow cells. Additionally 7/10 patients with trisomies 8 and 9 in the series studied by Elis *et al*.[5] were maintained on hydroxyurea at the time of cytogenetic investigation. As the number of cytogenetic abnormalities is greater in treated than in untreated patients,[1] 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*.[6] corroborates the hypothesis that cells with trisomy 8 have a proliferative advantage over disomy 8 cells in culture, since the frequency of trisomy 8 cells is greater in bone marrow metaphases than in interphase nuclei.[7]

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[8] 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[9] 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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References

1. Bench AJ, Nacheva EP, Champion KM, Green AR. Molecular genetics and cytogenetics of myeloproliferative disorders. Baillières Clin Haematol 1998;11:819-48.
2. Anonymous. Report on essential thrombocythemia. Cancer Genet Cytogenet 1981;4:138-42.
3. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. Semin Hematol 1997;34: 29-39.
4. ISCN. Guidelines for Cancer Cytogenetics, Supplement to an International System for Human Cytogenetic Nomenclature. F Mitelman, editor. Basel: S. Karger;1995.
5. Elis A, Amiel A, Manor Y, Tangi I, Fejgin M, Lishner M. The detection of trisomies 8 and 9 in patients with essential thrombocytosis by fluorescence in situ hybridization. Cancer Genet Cytogenet 1996;92:14-7.
6. Swolin B, Safai-Kutti S, Anghem E, Kutti J. No increased frequency of trisomies 8 and 9 by fluorescence in situ hybridization in untreated patients with essential thrombocythemia. Cancer Genet Cytogenet 2001;126:56-9.
7. Bench AJ, Cross NC, Huntly BJ, Nacheva EP, Green AR. Myeloproliferative disorders. Best Pract Res Clin Haematol 2001;14:531-51.
8. Corradini P, Palumbo AP, Battaglio S, Ponzio G, Boccadoro M, Pileri A. Analysis of the breakpoint cluster region in essential thrombocythemia. Haematologica 1990;75:573-5.
9. Solé F, Florensa L, Espinet B, Besses C, Lloveras E, Woessner S. Absence of bcr/abl rearrangement in 41 patients with essential thrombocythemia. Haematologica 2000;85:215-6.

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