To the editor:

**Subtyping of Fanconi anemia patients: implications for clinical management**

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by progressive bone marrow failure, congenital anomalies, and predisposition to cancer. At least 8 complementation groups for FA are known (A, B, C, D1, D2, E, F, G), and 7 FA genes have been cloned. FA cells are highly sensitive to the DNA crosslinking agents, mitomycin C (MMC) or diepoxybutane (DEB), thus providing the basis for the clinically certified diagnostic MMC/DEB test. FA subtyping is generally considered to be a research tool, and it is not clinically certified. At present, FA subtyping is performed only rarely, in the setting of FA gene therapy, prenatal diagnosis, or preimplantation genetic diagnosis (PGD).

FA patients display a wide range of clinical variability. Recent studies indicate that this variability may be accounted for, at least in part, by their specific FA subtype. FA-A patients, for example, may follow a milder disease course, with later onset of bone marrow failure. FA-G and FA-C patients, in contrast, often have more severe disease, with earlier onset of bone marrow failure and hematologic malignancy. FA-D1 patients, who have biallelic mutations in the FANC/D/BRCA2 gene, may have even earlier onset of malignancies, such as brain tumors (A.S. and A.D.D., unpublished observation, May 2003), and these tumors appear to precede their aplastic anemia. Moreover, FANC/D/BRCA2 (+/−) heterozygote carriers have an increased risk of developing breast, ovarian, or other cancers. Other genetic diseases, such as the Nijmegen Breakage Syndrome (NBS), have partial clinical overlap with FA and some cellular sensitivity to DEB, further confounding the diagnosis of NBS. Compared with FA patients, NBS patients have a predisposition to lymphoma.

Routine subtyping of FA patients will therefore have a significant impact on clinical management. As the relationship between genotype and phenotype is established, identification of patients likely to benefit from earlier institution of curative hematopoietic stem cell transplantation for marrow failure may be feasible. FA-D1 patients may benefit from further reductions in radiation and chemotherapy, due to their enhanced sensitivity to these agents, compared with other FA subtypes (A.S. and A.D.D., unpublished observations, July 2003). Furthermore, BRCA2 carrier status testing of family members of all FA-D1 patients would allow assessment of their own cancer risk. Appropriate counseling and cancer surveillance could be initiated for affected family members. Additional clinical benefits for FA patients and families, derived from FA subtyping, are summarized in Table 1.

Moreover, FA subtyping is relatively straightforward, entailing the systematic use of retroviral complementation, immunoblotting of FA proteins, and direct gene sequencing. For FA patients of particular ethnic backgrounds, more direct tests for specific FANC alleles (ie, the FANCC IVS4+4 A to T allele in Ashkenazi Jews) may be used. Although Fanconi anemia subtyping is not currently clinically certified, once the affected gene is identified from the pool of 8 possible Fanconi genes, mutations can be confirmed via gene sequencing in a Clinical Laboratory Improvement Amendment (CLIA)–certified laboratory. Taken together, the recent advances in FA research now make FA subtyping a straightforward and important element of the clinical management of the FA patient.

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**References**


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To the editor:

**Reduced tumor load in peripheral blood after treatment with G-CSF and chemotherapy in children with tumors of the Ewing sarcoma family but not neuroblastoma**

Peripheral blood (PB) stem cell harvests are reported to contain less tumor burden than bone marrow from the same patient, making them a more attractive source of hematopoietic progenitors for transplantation and improving tolerance to intensive therapy. Although more intensive therapies frequently induce enhanced response rates, this does not necessarily result in an increased overall survival. This may be explained by drug-resistant disease or poor immune response, or tumor cells reinfused during transplantation.
may induce relapse. That reinfused tumor cells contribute to relapse in children with neuroblastoma (NBL) is supported by the presence of gene-marked reinfused tumor cells at the sites of disease relapse.3,5

Using reverse-transcriptase polymerase chain reaction (RT-PCR) for tyrosine hydroxylase (TH) mRNA, tumor cells in PB samples from 6 of 8 children with NBL have been detected following mobilization of stem cells with chemotherapy and granulocyte colony-stimulating factor (G-CSF) (Figure 1A), and EWS-FLI1 fusion transcripts in samples from 7 of 7 children with tumors of the Ewing sarcoma family (ESFT), including 5 of 5 Ewing sarcomas (ES) and 2 of 2 peripheral primitive neuroectodermal tumors (pPNETs) (Figure 1B). No EWS-WT1 fusion transcripts were identified in PB from one child with a desmoplastic small round-cell tumor (DSRCT) (Figure 1B, child 16). All children were entered into multicenter trials using recombinant chemotherapy. On day 5 after the start of chemotherapy, children were treated with recombinant G-CSF (5 µg/kg per day) for 10 days to mobilize stem cells into PB over 4 courses. PB samples taken at intervals (between days 5 and 15 of each course) from the central venous line were analyzed for contaminating tumor cells by RT-PCR: all primary neuroblastomas expressed TH mRNA, all ESFTs expressed EWS-FLI1 fusion transcripts, and the EWS-WT1 fusion product was confirmed in the DSRCT.

In 5 of 8 children with neuroblastoma, TH mRNA was detected in PB throughout treatment (Figure 1A); there was no apparent pattern relating to course number, day in course, hematopoietic cell number, or total RNA isolated from sample. However, in children with ESFT, tumor cells were not detected in PB collected after 2 courses of chemotherapy; this is consistent with a reduced tumor load in courses 2, 3, and 4 compared with that in course 1 (Figure 1B). This restricted pattern may explain the conflicting frequency of tumor contamination reported in PB and PB stem cell harvest from patients with ESFT.5,9 From this study it is not possible to evaluate the clinical significance of reinfused tumor cells; indeed any association between tumor contamination of PB with outcome might purely reflect the disease status of children at the time of PB collection. However, the profiles of tumor contamination in PB after mobilization of stem cells in the group of children with NBL compared with those with ESFT suggest that judicious timing of PB stem cell collection can influence tumor contamination in some cancers. Whether this may reduce the potential risk of secondary disease from reinfused tumor cells in children with ESFT requires further investigation, particularly in view of the recent speculation that reinfused tumor cells might elicit a protective antitumor immune response after autologous transplantation.10

Figure 1. Summary of tumor cells detected in PB from children with high-risk disease following chemotherapy and G-CSF over 4 courses. Results from all 16 children are summarized: 1 to 8 indicate children with neuroblastoma (A); 9 to 15, children with ESFT; and 16, child with DSRCT (B). Patients 9 to 13 were diagnosed with ES; patients 14 and 15, with pPNETs. At diagnosis all patients had metastatic disease detected by conventional imaging, with the exception of patients 13 and 14 who presented with localized disease. Only patients 1 and 5 had bone marrow metastases. Recombinant G-CSF (5 µg/kg per day) was administered for 10 days to mobilize stem cells into PB over 4 courses after chemotherapy, commencing on day 5 after the start of chemotherapy. There were two 2-mL PB samples taken from the central venous line from day 5 after chemotherapy at intervals up to day 15 for analysis by RT-PCR. There was no unexpected toxicity associated with the chemotherapy or G-CSF treatment. A sample per course was analyzed, positive by RT-PCR for contaminating tumor cells. The length of the line for each patient shows the period over which PB samples were collected for analysis.

References

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