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REFERENCES


Neonatal Screening for the Hemochromatosis Defect

To the Editor:

Hereditary hemochromatosis (HC) is an autosomal recessive disorder of iron metabolism that is characterized by inappropriate iron absorption and storage of excess iron in the parenchymal cells of major organs, primarily the liver, pancreas, heart, pituitary, and joints. High levels of iron stored in these organs can lead to cirrhosis, hepatocellular carcinoma, cardiac dysfunction, diabetes, arthritis, hypogonadism, and premature death. However, patients can have a normal life expectancy if the disorder is diagnosed in the early stages and phlebotomy therapy is undertaken to remove the excess iron.

A novel major histocompatibility complex (MHC) class I-like gene termed HLA-H was recently identified telomeric of the classical MHC complex on the short arm of chromosome 6 and proposed as a candidate for HC. In this initial report, 83% of HC patients were found to be homozygous for a single missense mutation, causing an amino acid substitution of cysteine to tyrosine at residue 282. 30 seconds, 55°C to be between 3 and 5 per 1,000. 8,9

The recent identification of HFE and the presence of a single causative mutation provide an opportunity for the population frequency of the mutant allele to be accurately determined. Comparison of the frequency of HC as determined by biochemical analyses with frequency of homozygotes for this disorder have been lower than this predominantly Caucasian population. Previous estimates of the frequency of homozygotes for the C282Y mutation have been shown to meet clinical diagnostic criteria for HC, further indicating the significance of environmental and possibly other genetic factors in expression.

The Royal Children’s Hospital (Brisbane) and The Queensland Institute of Medical Research.

Three-millimeter squares were cut from dried blood spots using sterile scalpel blades. These squares were then fixed by soaking with absolute methanol and allowed to air dry (for approximately 30 minutes) at room temperature. Samples were then placed in 0.6-mL PCR tubes with 40 μL of sterile ddH2O and incubated at 60°C for 30 minutes, followed by 96°C for 30 minutes in a Perkin Elmer 9600 thermal cycler (Perkin Elmer, Norwalk, CT) to elute the DNA. Twenty microliters of the resulting supernatant was used in subsequent polymerase chain reaction (PCR).

The presence of the C282Y mutation creates a SmaBI restriction site in the amplified product enabling detection of the mutation by enzyme digest. Twelve microliters of the amplified product was digested with 2 U SmaBI (Promega, Madison, WI) for 2 hours at 37°C in a total volume of 20 μL. Samples were then analyzed on a 2% agarose gel. A control sample, known to be homozygous for C282Y, was included to confirm complete digestion of all samples.

Of the 1,660 samples analyzed, 8 (1 in 200 or 0.48%) were homozygous for the C282Y mutation and 186 (1 in 9 or 11.2%) were heterozygous. This gives a frequency for the HC allele of 0.061 in this predominantly Caucasian population. Previous estimates of the frequency of homozygotes for this disorder have been lower than this; however, all previous screening studies have relied on biochemical and/or pathological expression of HC for diagnosis. This indicates that phenotypic expression of HC may be prevented in approximately one third of individuals homozygous for the mutant allele, due to environmental factors such as dietary iron intake and physiological blood loss, as well as sex and age. In addition, a small percentage of patients heterozygous for the C282Y mutation have been shown to meet clinical diagnostic criteria for HC, further indicating the significance of environmental and possibly other genetic factors in expression.
The C282Y mutation can be easily and rapidly detected; thus, population screening is feasible. However, because many of those homozygous for this defect will not develop iron overload requiring treatment, the cost effectiveness of widespread population screening requires further evaluation. However, detection of the mutation is useful in confirming the diagnosis in those with increased iron indices.

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A Prospective Study of Radiation Therapy-Associated Thrombocytopenia

To the Editor:

In the April 1, 1997 issue of Blood, we reported results from a retrospective study of radiation-associated thrombocytopenia. The primary objective of the study was to identify risk factors for unscheduled interruptions in radiotherapy lasting ≥2 days and associated with World Health Organization grade III-IV thrombocytopenia. A group of controls were randomly selected. Potential risk factors for myelosuppression were analyzed using univariate and multivariate analyses. The most important risk factors for treatment interruption with thrombocytopenia based on multivariate analyses were concurrent chemotherapy (odds ratio [OR] 45.5; P < .001), increasing percentage of marrow irradiated (OR 4.1 for each 20%; P < .001), and brain metastases (OR 7.3; P = .01). Other significant (P < .05) factors in univariate analyses were leukemia/lymphoma, bone or bone marrow metastases, and prior chemotherapy.

To validate the criteria identified in the prospective study that were associated with treatment interruptions for thrombocytopenia and to identify new treatment variables that may influence the risk for radiation-induced thrombocytopenia, we performed a prospective study in which we analyzed radiation therapy treatments that were completed between July 6, 1995 and July 29, 1996 at Stanford University Hospital and the Stanford Radiation Oncology facility at Fremont (these dates were selected so that there was no overlap between the retrospective and prospective patient population) and between May 1, 1995 and April 30, 1996 at the Palo Alto Medical Foundation (PAMF). The charts of patients treated at these three facilities were reviewed after completion of the radiotherapy course to identify patients who had unscheduled treatment interruptions of 2 days’ duration or more (excluding weekends and holidays) in which thrombocytopenia was the primary reason for interrupting radiotherapy (cases). Patients with ≥ grade III thrombocytopenia without unscheduled treatment interruptions and those who received platelet transfusions were also considered to be cases. Patients were identified as high risk (HR) if they were scheduled to receive concurrent chemotherapy with myelosuppressive potential (within 1 day of starting radiotherapy or at any time during the course of radiation therapy) or scheduled to have ≥20% of their bone marrow irradiated, including prior irradiation. Complete information was collected on all HR patients treated at the PAMF and on a random sample of approximately 12 HR patients/month at Stanford (from both Stanford University Hospital and the Stanford Radiation Oncology facility at Fremont). Blood count data including differential and platelet counts were recorded. All patients had at least one complete blood count performed during treatment.

Patient courses rather than patients were sampled, increasing the likelihood of selecting those at HR because of multiple courses. Patient charts were reviewed. Detailed information on the extent of any treatment disruption of ≥2 days and possible predisposing factors for myelosuppression, such as previous or concurrent cytotoxic chemotherapy or previous radiation therapy, was extracted and entered into a computer database for statistical analysis as before. In this study, data were not collected for courses of therapy that consisted only of total body irradiation (TBI), electron beam therapy, brachytherapy, intraoperative radiation therapy (IORT), stereotactic radiosurgery, or therapy for benign disease. Otherwise, all adult patients were eligible for inclusion in this study. All cases had at least one blood count during the treatment course that showed at
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