has been also used for the $^{32}$P branch of our protocol. Why does Prof Pearson not criticize these data?

On the contrary, I agree with Prof Pearson’s other criticism. If, in fact, some patients with a particular risk are precociously excluded from a therapy protocol, the calculation of this risk for the whole population will be wrong. That was the case in the phlebotomy branch of the P.V.S.G. protocol, where the oldest and those with vascular risk factors or thrombocytosis have been excluded and treated otherwise. So, the true risk of leukemia could be under-estimated. But this is not a criticism of the method of calculation; this a criticism of the design, or of the fulfilment, of the protocol.

Yves Najean
Hôpital Saint-Louis
Paris, France

---

Fifty Years of Studies of the Biology and Therapy of Childhood Leukemia

To the Editor:

The December 1, 1997 issue of Blood included my commentary on 50 years of studies of biology and therapy of childhood leukemia.1 In my acknowledgments, I tried to anticipate the fact that I would be responsible for errors of omission, commission, and also would be unable to properly credit many individuals in the field.

However, there is one glaring omission that I believe requires additional comment. This is the important cloning of the TEL-AML1 fusion gene in two separate laboratories in 1995.2,3 This fusion gene has been particularly important because of its lack of detection by standard cytogenetic methods and because of its independent prognostic significance as reported by several groups as discussed in the Commentary.

I believe that this inclusion helps to clear up one major omission.

John H. Kersey
University of Minnesota Cancer Center
Minneapolis, MN

REFERENCES


---

Factor V Leiden Mutation and Budd-Chiari Syndrome

To the Editor:

Budd-Chiari syndrome is characterized by the hepatic venous outflow obstruction. A multifactorial interaction between the genetic and circumstantial risk factors may be responsible for this kind of disorder. Among these, myeloproliferative syndromes are reported to be the commonest cause of Budd-Chiari syndrome. Other causes include thrombophilia states, oral contraceptives, and cancer.1

Until recently, the major known genetic defects detected for repeated venous thrombosis were deficiencies of protein C, protein S, and antithrombin III, which together accounted for 5% to 10% of these type of cases. The defect in the anticoagulation response to activated protein C has been detected as a new mechanism for thrombophilia, which was subsequently linked to a single point mutation on the factor V gene, resulting in Arg506-Gln substitution in the activated protein C cleavage site.2 Since then, various thrombotic events like deep vein thrombosis, pulmonary embolism, preeclampsia, and pulmonary infarction have been studied for factor V Leiden mutations and reported.3 However, there is not much information on the frequency of these mutations in Budd-Chiari syndrome except for a few isolated case reports.4 This prompted us to report the present findings.

Fig 1. Factor V gene mutations in Budd-Chiari cases.
The RhD⁻ Trait in a White Patient With the RhCCee Phenotype Attributed to a Four-Nucleotide Deletion in the RH D Gene

To the Editor:

The Rh blood group locus comprises two closely linked genes, designated RHCE and RH D, encoding integral membrane proteins that carry the Ce/Ee and D antigens, respectively. The D⁻ phenotype is usually due to the complete deletion of the RH D gene from the Rh locus. The D antigen is extremely immunogenic and is associated with hemolytic disease of the newborn (HDN). HDN occurs when antibodies from D⁻ women, who have been sensitized to the D antigen, cross the placenta and react with antigens on fetal red blood cells. Polymerase chain reaction (PCR) assays to detect the RH D gene have been developed for determining fetal RH D gene type and paternal RH D gene dosage with potential clinical value in the management of pregnancies at risk for HDN. Such assays assume the RH D gene will be completely absent in D⁻ serotypes. This appears valid generally for D⁻ ce haplotypes of white origin (frequency [f] = .39) who account for the vast majority of D⁻ phenotypes. However, exceptions have been reported from whites with the less frequent Ce and eE haplotypes (f = .0098 and .0119, respectively) and amongst nonwhites.

We described two D⁻ CCEE white blood donors where the RH D gene was present in some form. One lacks RH D gene exons between 2 and 9 and would be correctly identified using multiplex PCR assays (unpublished observations, April 1996). We report here that the other donor, designated B1, carries a four-nucleotide deletion at a splice junction along an otherwise normal RH D gene that would prevent expression of the D antigen.

Total RNA extracted from whole blood buffy coat preparations was reverse transcribed into cDNA. This was used as template in PCR reactions to amplify four overlapping products, spanning the entire RH D gene, from the 5' untranslated region (nucleotide 1536) to the 3' untranslated region (nucleotide 1536). Sequencing cDNA-derived PCR products showed a 4-base deletion between nucleotide positions 487 and 492 compared with two previously published RH D gene sequences. The intron 3/exon 4 boundary. Where the sequence ACAGACT was introduced at positions 496-498.

Because Budd-Chiari cases show severe hepatocellular insufficiency, the coagulation tests for factor V Leiden may not have been informative in determining the thrombophilia status. Nevertheless, the detection of factor V Leiden mutation by a simple PCR followed by enzyme digestion is economical and less time consuming, with 100% specificity. With this high frequency of factor V Leiden mutations and comparatively low cost of DNA analysis, it may be suggested that this mutation be examined in all the Budd-Chiari cases.

Dipika Mohanty
Shriram Shetty
Institute of Immunohaematology (ICMR)
KEM Hospital Campus
Parel, Mumbai, India
T.S. Narayanan
Department of Gastroenterology
KEM Hospital
Parel, Mumbai, India

REFERENCES
Factor V Leiden Mutation and Budd-Chiari Syndrome

Dipika Mohanty, Shrimati Shetty, T.S. Narayanan and Philip Abraham