Infections are a major cause of morbidity and mortality in sickle cell disease. Patients present with pulmonary, meningeal, bone, or bloodstream infections usually caused by bacteria, although there is increasing awareness of the role of mycoplasmas and viruses in sickle lung disease. Fungal infections in sickle cell disease are very rare. This case constitutes the first description of fungal abscess in a sickle cell patient with no other identifiable immunocompromising factors. Serology for human immunodeficiency virus (HIV)-1 and -2 was negative. Lymphocyte counts, T-cell subsets, serum Ig, and complement levels were unremarkable. Neutrophil superoxide production and integrins were normal and no defect in opsonization was observed. These results notwithstanding, the occurrence of candida infection, salmonella osteomyelitis, and recurrent pneumonias in a single individual reflects profound and wide-ranging immune deficiency. Further investigation is required, particularly in light of the use of myelosuppressive agents such as hydroxyurea in sickle cell patients, which can potentially heighten susceptibility to infection.

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An Even Easier Method for One-Step Detection of Both FV Leiden and FII G20210A Transition

To the Editor:

Activated protein C resistance (APCR) has emerged as the most common inherited risk factor for venous thrombosis. Heterozygosity for the underlying DNA mutation Factor V Leiden (Arg 506 Gln) is associated with a 5- to 10-fold increased risk of developing a venous thrombotic episode, whereas homozygosity is associated with a 50- to 100-fold increased risk. Interaction of APCR with other co-inherited risk factors such as the recently described prothrombin gene variant (G20210A transition at position 20210) is expected to synergistically increase the thrombotic risk. Therefore, a one-step detection of both genetic variants is very attractive for the diagnosis and management of deep vein thrombosis. We read with interest the recent report by Gomez about the rapid screening of Factor V Leiden and the G20210A prothrombin variant. The investigators described a multiplex polymerase chain reac-

Fig 1. Electrophoretic patterns for multiplex FV and FII PCR. The PCR mixture in a final volume of 50 \( \mu \)L consisted of 1 \( \mu \)g genomic DNA, PCR buffer (16.6 mmol/L ammonium sulphate, 67 mmol/L Tris-HCl, pH 8.8, 6.7 mmol/L magnesium chloride, 67 \( \mu \)mol/L Na2EDTA, 170 \( \mu \)g bovine serum albumin per mL, 10 mmol/L (\(-\)-mercaptoethanol), 400 \( \mu \)mol/L of each desoxynucleotide triphosphate, 30 pmol of each FV primer, 10 pmol of each FII primer, and 2 U Taq polymerase. Thermocycling conditions are 94°C (1 minute), 58°C (1 minute), 72°C (2 minutes) for 40 cycles. Fifteen microliters of PCR product is digested with 15 U HindIII restriction enzyme. The restricted products are separated by electrophoresis through a 2% agarose gel stained with ethidium bromide and directly visualized under UV light. The smallest restricted fragments (32 and 23 bp) are not visible on the gel. Lane 1, size marker (1-kb ladder); lane 2, undigested PCR products; lanes 3 through 8, digested PCR products; UD, undigested PCR products; N, normal allele; m, mutated allele.
tion followed by a combined restriction digest of both the factor V gene and the prothrombin gene PCR products. They simultaneously use two different endonucleases, leading to a complex restriction pattern with several cleavage sites for wild-type and mutated amplified fragments.

In our laboratory, we have developed a very simple method based on multiplex polymerase chain reaction (PCR)-mediated site-directed mutagenesis using primers with mismatched 3'-ends. This duplex PCR is followed by restriction using a single and inexpensive endonuclease, HindIII. The electrophoretic patterns can be easily identified whatever the genotypic combination. This method is used as a routine diagnosis strategy for both mutations.

We use either genomic DNA prepared by a standard salting out procedure or a rapid extraction procedure using Chelex resin (20 µL of extraction product from whole blood).6

Primers for the factor V gene G1691A determination are those described by Gandrille et al6 and primers for the prothrombin gene 20210A determination have been described by Poort et al.7 For each pair of primers, the 3' antisense has been modified to create a single HindIII cleavage site in the presence of both mutated PCR products.

After HindIII restriction, the digested products can be easily separated on a 2% agarose gel. Figure 1 shows the different migration patterns observed. For both factor V and factor II alleles, the normal genotypes produce undigested PCR products (241 and 345 bp, respectively), whereas mutated homozygous lead to restricted fragments (209 + 32 and 322 + 23 bp, respectively). The heterozygous patterns are characterized by the presence of undigested and digested amplified fragments.

To check the accuracy of the results, we systematically include in each assay two positive controls corresponding to homozygous patients for FV or FII mutations, respectively. Furthermore, a single reaction mix for the digestion, including the restriction endonuclease, is prepared for each sample set. This allows both samples and controls to be assayed with the same conditions.

Our method has at least two main advantages. First, the same inexpensive restriction endonuclease is used for the detection of the mutated alleles in both FV and FII, allowing the size of the PCR digests allowing identical for both mutations. This method thus offers a reliable tool for routine diagnosis.

| Table 1. Correlation Between Glucksberg Grade and IBMTR Index |
|-------------------|---|---|---|---|---|
| Glucksberg Grade | 0 | A | B | C | D |
| 0     | 142 |   |   |   |   |
| I     | 16  | 34 |   |   |   |
| II    | 141 | 211|   |   |   |
| III   | 25  | 179| 41 |   |   |
| IV    | 49  |   |   |   | 49 |
| Total | 142 | 16 | 200| 390| 90 |
|       |     |   |   |   | 838 |

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Methods for Assessment of Graft-Versus-Host Disease

To the Editor:

The International Bone Marrow Transplant Registry (IBMTR) has recently adopted a new severity index for grading acute graft-versus-host disease (GVHD) after allogeneic marrow transplantation. This index is based on the results of an analysis in which distinct patterns in the peak severity of GVHD involvement in the skin, liver, and gut were correlated with the risks of transplant-related mortality (TRM) and treatment failure (defined as relapse or death) among 2,129 adults who received an unmodified marrow graft from an HLA-identical sibling with the use of methotrexate and cyclosporine for GVHD prophylaxis.1

In this analysis, acute GVHD was entered into Cox proportional hazards regression models as a time-dependent variable so that all patients were categorized as not having GVHD until they developed the disease. As a further refinement, the analysis was stratified across two age categories and three pretransplant risk categories. Results showed that patients with Glucksberg grade I GVHD did not have a significantly increased risk of TRM compared to those with grade 0 GVHD (relative risk = 1.12), while the relative risks for patients with Glucksberg grades II and III-IV GVHD were 2.19 and 5.33, respectively. Patients with IBMTR Index of A did not have an increased risk of TRM (relative risk = 0.84), while the relative risks for patients with IBMTR Indices of B, C, and D were 1.9, 4.34, and 11.9, respectively. The pooled relative risk for patients with IBMTR Indices of C or D was not reported.

The IBMTR Index was designed to avoid the need for subjective assessment of performance status which has been included as an element in the Glucksberg scale. In practice, performance status is used in the Glucksberg grading system only to distinguish between grades III and IV GVHD. Use of the term “extreme” to describe the reduction in...
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