old extending this approach well into the next decade of life. The acute GVHD rate in this group was predictably higher (grade 2 and 3, 7 of 15 = 47%) than their pediatric experience (20%)\(^2\); however, the GVHD appeared easily treated by single agent prednisone. With the extensive transplant experience that this group and others have reported,\(^3,7\) there is certainly a place for full ablative HSCT in pediatric patients with SCD, and this new experience now suggests that the same is true for young adults eligible for this approach. These accumulating reports continue to confirm a very favorable benefit to risk ratio making this a truly exciting time for patients and physicians contemplating HSCT for SCD.

Myeloablative conditioning and the ensuing risk of GVHD, however, require robust organ function. We now have transplanted 23 patients with severe disease at our center with nonmyeloablative conditioning, their ages ranging from 17 to 65 years. All patients are alive, and engraftment was achieved in 20 (87%). Importantly, 5 of the first 10 patients reported\(^8\) are now off immunosuppression with continued stable mixed chimerism. Equally important, none of the engrafted patients has experienced any GVHD. In addition, 3 patients have produced offspring naturally (1 male and 2 female). This larger experience suggests that with this nonmyeloablative approach, the risk of rejection is similar, the risk of GVHD is lower, long-term immunosuppression is not absolutely required, and stable mixed chimerism is achievable. It is important to note that nearly half (10 of 23) of our patients would be ineligible for myeloablative transplantation because of comorbidities including cirrhosis and poor lung function.

Thus for patients in their second and third decade of life, options include both full and nonmyeloablative transplant conditioning, with the choice depending on organ involvement, potential transplant-related complications, and the desire for future fertility. It is our opinion that patients in this age group should be transplanted as a part of an ongoing clinical trial. HSCT for SCD remains under-utilized and the time to seriously consider this therapeutic option is now.

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To the editor:

Extensive admixture in Brazilian sickle cell patients: implications for the mapping of genetic modifiers

Genome-wide association studies (GWASs) of sickle cell disease (SCD) patients are a promising tool for identifying genetic modifiers of clinically relevant traits.\(^1,2\) In such a study, Solovieff et al\(^2\) have uncovered a set of SNPs associated with fetal hemoglobin (HbF) concentration, the major modulator of the clinical course of this disease.

After the introduction of the SCD mutations into the Americas by African slaves, African descendants mixed with individuals of Native American and European origin to various extents across the continent. In Latin America, individuals tend to be more admixed than in African-American populations.\(^3,4\) Studying different phenotypes, Solovieff et al\(^2\) did not find an association between fetal hemoglobin concentration and European ancestry, while Creary et al\(^5\) have reported an association between European ancestry and the proportion of erythrocytes containing HbF.

The level of admixture of SCD patients has implications for the design of association studies aimed at identifying new genetic modifiers of SCD clinical manifestations, particularly if these outcomes are associated with ancestry. If both genetic variants and clinical manifestations are associated with ancestry, there are 2 important issues to address. First, an observed association may be spurious if ancestry is not controlled for.\(^6\) Second, the admixture mapping strategy\(^7\) may be used: Thousands of ancestry-informative markers may be genotyped across the genome, and the local ancestry along chromosomes can be inferred. The genetic modifier

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References

To the editor:
alleles are expected to be located in genomic regions with an excess of ancestry of the population associated with the more common clinical outcome.

The risk of spurious association and the power of admixture mapping increase both with the level of admixture. However, estimates of ancestry are rare in SCD patients. Excluding GWASs, association studies between SNPs and SCD subphenotypes seldom control for ancestry.

We estimated admixture in 200 patients with SCD and in 291 healthy blood donors; all from the State of Minas Gerais (approximately 20 million inhabitants in South Eastern Brazil). We genotyped 54 SNPs/INDELs validated for admixture studies. The ancestry of the healthy blood donors, who represent the general population reasonably well, was 33.8% African, 57.7% European and 3.5% Amerindian; whereas SCD patients showed 47.3%, 39.7% and 13.0% of African, European and Amerindian ancestry, respectively (estimated following Dupanloup and Bertorelle, see supplemental Table 1, available on the Blood Web site; see the supplemental Materials link at the top of the online article). Considering individual admixture (Figure 1), only 11.05% of SCD patients had > 85% African ancestry. Most of the patients (73.37%) had intermediate levels of admixture (15%-85%), and interestingly, 13.8% had predominant European ancestry (> 85%), and for 13.0%, the lower limit of the 90% credibility interval of European ancestry was > 0.60). Therefore, the prevalence of European ancestry is high, and the individual admixture is very heterogeneous in SCD patients from Brazil. Our results suggest that: (1) despite the association of SCD with African ancestry, the label “ethnic/racial” disease seems inappropriate in this population, (2) in association studies with SCD patients, controlling for ancestry is important to avoid spurious association, and (3) Latin American populations of SCD patients are promising targets for admixture mapping of genetic modifiers of ancestry-associated SCD clinical manifestations.

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Figure 1. Individual admixture in healthy blood donors and Sickle Cell Disease patients from Minas Gerais. Admixture was estimated using the method by Pritchard et al implemented in the software Structure. Each vertical bar represents an individual and his/her admixture proportions based on the parental populations on the left. Additional methodologic information and results are available as supplemental Methods. Structure was run using the following conditions and parameters: K = 3 (number of parental populations), burn-in = 100,000, MCMC cycles after burn-in = 100,000, we used a priori information for the individuals from parental populations to assist the clustering (USEPOPINFO = 1), model = ADMIXTURE for the admixed individuals, α parameter was inferred for each population, GENSBACK = 2, MGRPRIOR = 0.05, allele frequencies was assumed to be correlated.

Response

Genetic admixture in sickle cell disease

Genetic studies require careful thought about ancestry in the study design and analysis to avoid population stratification bias and to maximize the power of finding novel variants. If both the genetic marker and the phenotype vary with respect to ancestry, then a spurious association will occur between the genetic marker and phenotype if one does not adjust properly for ancestry. In this issue of Blood, Silva et al examine the level of admixture in a cohort of Brazilian sickle cell patients and find that patients with sickle cell disease have a wide range of African admixture (15%-85%). They correctly acknowledge that one must appropriately adjust for admixture to avoid false positive findings and that this population may be useful for admixture mapping. However, admixture mapping is only useful if the phenotype of interest also varies with admixture and further research is required to establish this relation in this population. In a cohort of African Americans with sickle cell disease we did not find a significant association between admixture (measured by the first principal component from a principal component analysis) and fetal hemoglobin. However, one should note that the African Americans in this study on average did not have high levels of Caucasian admixture. Admixture mapping has been successfully used to find novel genetic variants and regions for other phenotypes that were related to admixture such as white cell counts and prostate cancer. Furthermore, examining ancestry in genetic studies of sickle cell disease could lead to novel loci that are either more prevalent or specific to certain ethnic groups. For example, sickle cell patients from the Southwestern Province of Saudi Arabia have fetal hemoglobin (HbF) levels twice as high as African Americans despite having similar HBB haplotypes. Furthermore, sickle cell patients from the Eastern Province have even higher levels of HbF. These findings suggest that there are HbF-associated variants that are more prevalent or specific to the Saudi population and that leveraging on ancestry in the genetic analysis can help identify novel variants.

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To the editor:

Prothrombin 20210G>A genotype and C-reactive protein level

Thrombin is central not only in procoagulatory processes like fibrinogen or platelet activation but also in other systems that are related to inflammation control. Recently, Flick et al characterized inflammatory responses of transgenic prothrombin mutant (F2WE) mice in a collagen-induced arthritis (CIA) model. Mice carrying the F2WE transgene that has dramatically reduced procoagulatory effects on fibrinogen and protease-activated receptor 1 exhibited a significantly attenuated inflammatory joint disease in CIA.

Prothrombin 20210G>A (F20210G>A) is a gain-of-function variant resulting in increased prothrombin expression and elevated

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