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Sickle cell disease: *primum non nocere* (first do no harm)

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Was Hippocrates aware when he insisted that physicians *primum non nocere* (first, do no harm) that this precept would still prove challenging to his colleagues 2000 years later? In his day, diseases were probably diagnosed only in the presence of overt symptoms, and asymptomatic children were no doubt considered healthy, as there were no means of predicting the subsequent development of diseases that might decrease their chances of living long and happy lives.

Times have changed, and we are now able to diagnose diseases in asymptomatic children, especially through neonatal screening. In sickle cell disease (SCD), for example, neonatal screening can dramatically improve the prognosis;[1] a 2007 report of an English cohort screened at birth and enrolled in a comprehensive follow-up program showed that children with hemoglobin SS had a 99% chance of living at least 16 years.[2] In addition to a substantial improvement in survival, a marked decrease in morbidity, chiefly due to reductions in stroke, recurrent painful crises and recurrent acute chest syndrome, has been achieved in children with SCD.

A characteristic feature of SCD is the wide variation in clinical expression between patients. To date, only a few factors, either at birth or later on, reliably predict the subsequent development of severe complications. Nevertheless, as a rule, children who experience severe complications early in life continue to express a severe disease phenotype throughout their lives whereas those with mild disease continue to do well, although exceptions may occur.

In recent years, various therapeutic approaches have reduced the risk of stroke, pain and acute chest syndrome in SCD. Transcranial Doppler followed by regular transfusions in children with high cerebral blood flow velocities has decreased the risk of a first stroke by 90%.[3] Hydroxyurea (hydroxycarbamide) very significantly decreases the frequency and severity of vaso-occlusive crises in children.[4] However, hydroxyurea and regular transfusion also generate a burden for the patient and family and they fail to completely eliminate the risk of complications such as recurrent stroke or painful events.[5] In addition to the need for daily treatment and fear of uncontrolled complications, SCD usually generates a feeling of being different, misunderstood and inadequate. Families and patients may experience post-traumatic stress disorder, the rate of occurrence of which is not correlated with disease severity.[6] Thus, the disease itself, independently of the number of hospital admissions and complications, adversely affects the quality of life of the patients and their families. This quality-of-life burden is being increasingly recognized by physicians and families as a key component in the risk/benefit ratio of treatments for SCD.[7,8]

A case in point is the risk/benefit ratio of hematopoietic stem-cell transplantation (HSCT) for SCD using marrow from an HLA-identical sibling. Studies from a number of groups of patients transplanted between 1988 and 2004 show an overall survival rate of 93-95% and an event-free survival rate of approximately 85-86% after a median follow-up of 5-6 years.[9,10] The possibility of cure is a major advance for both SCD patients and their families. The

desire to escape once and for all from the complications of SCD and to be released from living in constant fear of these complications provides powerful motivation for many families. A study reported in 1991 found that 37% of parents would accept a 15% HSCT-related short-term mortality risk and that 12% would still view the procedure favorably if the risk were 50% or greater. Importantly, the parents' willingness to accept the risks associated with HSCT was unrelated to the clinical severity of the disease in their children,[11] strongly suggesting that physicians, patients and their families do not consider the same factors when making their decision. Given the short-term mortality rate of HSCT in SCD of approximately 5%, most physicians in SCD centers would consider the procedure in children with cerebral vasculopathy, recurrent acute chest syndrome or recurrent painful crises.

Assessing the appropriateness of treatments such as HSCT in children who have mild symptoms, or no symptoms at all, is a major ethical challenge. Meeting this challenge is a matter of some urgency, as an increasing number of parents now ask for HSCT even when their children have mild disease. It must be acknowledged that the course of SCD is often unpredictable and that an asymptomatic child may suffer complications after several years of good health. Most of the time, however, mild disease remains mild, and most physicians therefore reserve HSCT for patients who have severe SCD. Nevertheless, whether HSCT should be performed in patients with mild disease who request, or more often whose parents request, the procedure is a crucial question.

The answer probably depends on the quality of the information delivered to the families about HSCT and on their ability to comprehend that information. Therefore, meticulous attention should be given to the information delivered, not only about the short-term risks of HSCT including the risk of death, but also about the long-term risks such as the risk of infertility, and the decision should never be rushed. In a recent study of 30 children managed with HSCT, seven of the ten girls had severe ovarian failure and the remaining three recovered some ovarian function with spontaneous pubertal development; one patient had a normal pregnancy. All 20 boys had germinal epithelial failure detected during or after puberty; only two boys had a spermogram, which showed oligo-teratospermia and azoospermia.[12] Similar findings were subsequently reported by Walters *et al.*, who found that only three of the 13 evaluable males had normal testosterone levels, and eight of the 14 evaluable females had findings consistent with primary ovarian failure.[13] These effects on pubertal development and fertility have led several groups to offer routine pre-transplantation sampling of ovarian tissue for cryopreservation, as the ovarian cortex of young girls contains a large number of follicles, which may, in theory, allow them to have children once they reach adulthood.[14] However, so far, there are no known cases of successful procreation after cryopreservation of ovarian tissue harvested before puberty. Neither are any data available on the cryopreservation of testicular tissue. Finally, in considering the place of HSCT in mild SCD, apart from fertility issues, what carries more weight in the minds of these patients and their families: the short-term risk of death with HSCT or the quality-of-life burden generated by the mild disease? There is no single answer to this question since so many personal, social and psychological factors are involved and carefully conducted studies to better identify and understand these factors are urgently needed.

Whether hydroxyurea should be used in asymptomatic SCD children is another crucial question, although in contrast to HSCT, hydroxyurea therapy does not carry a known increased risk of death. Hydroxyurea lowers transcranial flow velocities, whose elevation correlates closely with the risk of stroke,[15] and hydroxyurea in adults with severe SCD seems to decrease mortality.[16] These effects constitute a powerful incentive for using hydroxyurea to prevent brain damage and neurodevelopmental sequelae in asymptomatic children with SCD, particularly as the short-term safety profile is excellent.[17] However, the safety of hydroxyurea in the long term remains unclear. In particular, there may be a risk of infertility in males. The toxicity of hydroxyurea in animal studies was described in a 2008 NIH report.[18] Since then, a study in male transgenic sickle mice revealed 69% and 95% decreases in sperm density and motility, respectively.[19] A comparison of semen before and after cessation of treatment in 44 SCD patients showed that observed alterations of semen due to SCD seemed to be exacerbated by hydroxyurea treatment.[20]

The development of new technologies is lengthening the life of patients with SCD and improving its quality. However, at the same time, these advances generate new ethical dilemmas. HSCT and hydroxyurea are valuable therapeutic options in patients with severe SCD. In patients with mild SCD, however, the ancient precept *primum non nocere* may dictate that we protect patients from the life- and fertility-threatening complications of therapy. Ongoing studies will perhaps help us to better understand the respective burdens generated by the disease and by its treatments. For now, we must focus on fully informing patients of the risks associated with available treatments.

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Hereditary myeloproliferative disorders

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Hereditary forms of myeloproliferative disorders (MPD) can be divided into two broad categories. First, inherited syndromes that affect a single lineage with Mendelian inheritance, high penetrance and polyclonal hematopoiesis, and second, inherited predisposition to true MPD, characterized by low penetrance, clonal hematopoiesis and presence of somatic mutations, e.g. *JAK2*-V617F.[1,2] Relatively little is known about the clinical course and complications of inherited MPD-like syndromes. This short review will focus on recent reports that examined familial thrombocytosis caused by mutations in the genes for thrombopoietin (*THPO*) or its receptor "myeloproliferative leukemia" (*MPL*)

The prototypes for the first category are mutations in the erythropoietin receptor (*EPOR*) which truncate the intracellular domain of the erythropoietin receptor protein and render erythroid progenitors hypersensitive to erythropoietin. Patients with these mutations have an elevated hematocrit and low levels of erythropoietin in the serum,[3,4] but lack additional somatic mutations or the propensity to leukemic transformation, unless inappropriately treated with chemotherapy.[5] Mutations in genes which regulate the response to hypoxia, such as *VHL* and *PHD2* and *HIF-2α* have recently been found in a number of familial polycythemia syndromes with high serum erythropoietin levels.[6,7] Familial thrombocytosis without involvement of other lineages can be caused by mutations in *THPO* or *MPL*. Some families with elevated serum thrombopoietin levels carry activating mutations in the *THPO* gene which cause overproduction of the thrombopoietin protein by a mechanism of increased translational efficiency for the mutant *THPO* mRNA.[8,9] A mutation altering the transmembrane domain of Mpl protein (*MPL*-S505N) was discovered in a family with autosomal dominant thrombocytosis.[10] Interestingly, the identical mutation was also found in a mutational screen using retroviruses in mice,[11] and recently also as a somatic mutation in cases of sporadic essential thrombocythemia.[12]

In contrast, the primary defect that underlies the second category of hereditary MPD, i.e. the inherited predisposition to MPD that frequently comes with a somatic *JAK2*-V617F mutation,[13] remains to be defined. Numerous families with such a predisposing phenotype have recently been described.[14] Mutations in the putative tumor suppressor gene *TET2* occur in patients with sporadic MPD and other hematologic malignancies,[15,16] but *TET2* mutations do not appear to be involved as the primary germline event in familial MPD. Rather they occur as somatic mutations restricted to the hematopoietic system.[17]

In terms of molecular mechanisms, inherited MPD-like syndromes with mutations in erythropoietin and thrombopoietin ligands and receptors are among the best studied hematologic diseases. However, relatively little is known about the clinical course of patients with these disorders, in particular in respect to overall survival and the risk of complications. This is because of the low incidence of most of these familial disorders and the fact that familial MPD-like syndromes have been reported from diverse parts of the world, which makes it difficult to collect data on sufficient numbers of affected family members. Regional clustering has been described for Chuvash polycythemia, which is endemic in the Chuvash Republic, part of the Russian Federation,[18] and for the *MPL*-S505N mutation, which was present in four out of five Italian families with thrombocytosis studied.[19] The fact that all of these families originated from the region around Rome suggests