**Appendix 1: Splenectomy**

The primary therapeutic goal for splenectomy in transfusion-dependent thalassaemia (TDT) patients with thalassaemia major (TM) is to decrease blood consumption (splenic sequestration) and transfusion requirements, with the ultimate goal of reducing iron overload.[1]

Indications for splenectomy

a. Worsening anaemia with increasing blood transfusion requirements that prevent adequate control of iron overload with iron chelation therapy.
- If the annual transfusion requirement exceeds 200 - 220 mL/kg/year, with donor blood haematocrit values of 70 - 75%.
- Other causes of increased transfusion requirements such as alloimmunization and infection should be excluded.

b. Hypersplenism
c. Symptomatic splenomegaly

Surgical approaches to splenectomy[1,2]

Approaches to splenectomy in TM, include: (*i*) open splenectomy; (*ii*) laparoscopic splenectomy; (*iii*) partial splenectomy; and (*iv*) splenic embolisation.

The favoured approaches are open splenectomy for larger spleens, or a laparoscopic splenectomy, which appears to be gaining favour in view of a significant reduction in mortality, shorter hospital stay and fewer pulmonary, wound and infectious complications.[2]

Complications of splenectomy[1,3]

a. Perioperative / short term: includes infection, wound sepsis, bleeding and atelectasis
b. Hypercoagulability and thromboembolism
c. Pulmonary hypertension
d. Iron overload
e. Medium to long term: sepsis

Methods to minimise and prevent post-splenectomy sepsis include:

i. Immunoprophylaxis

Vaccination against pneumococcus, meningococcus and haemophilus influenzae type B is usually given 2 weeks prior to splenectomy with boosters every 5 years for pneumococcus and meningococcus. Annual influenza vaccination is also recommended.

ii. Chemoprophylaxis

Prophylactic antibiotics may be given in certain situations such as children up to 5 years of age, in patients up to the age of 18 - 21 years in overcrowded environments and for those with recurrent infections, particularly in the first 2 years post splenectomy.

iii. Education

Early recognition of febrile episodes, as well as the need to seek immediate medical attention following a febrile illness are important. Education should include travel-related infections such as malaria and babesiosis with respect to prevention and appropriate management. A medic-alert bracelet or card indicating that the patient has had a splenectomy is also important.

With the availability of both the polysaccharide and conjugated vaccines, an alternative and more recent schedule is suggested in patients undergoing splenectomy (Table 3).[3-5]

Optimal management of TM, including the efficacy of blood transfusion treatment reduces the likelihood of increasing splenomegaly and hypersplenism and the need for splenectomy. Before considering splenectomy, the patient should be placed on an adequate transfusion program for several months and be properly re-evaluated, based on the risk to benefit ratio of the procedure.

**Appendix 2: Fertility and pregnancy**

Current improvements in the management of thalassaemia major patients has led to improved survival and quality of life into adulthood, with the possibility of having a functioning reproductive system and increased chances of achieving a pregnancy. To ensure optimal outcomes, a multidisciplinary team approach is required to assess issues surrounding fertility, pregnancy and delivery. This includes a haematologist, endocrinologist, reproductive medicine specialist, cardiologist, obstetrician and specialist nurse.

Fertility

The most common abnormality in up to 90% of patients is hypogonadotropic hypogonadism.[6] Patients may have primary amenorrhoea, delayed puberty or secondary amenorrhoea with consequent infertility. The majority will have an intact gonadal function, implying that fertility is potentially salvageable by using exogenous gonadotrophin therapy to bypass the hypothalamic pituitary axis and induce ovulation in females and spermatogenesis in males. Optimising the management of other endocrinopathies like diabetes and hypothyroidism is important, as these may have an impact on fertility.

Pre-pregnancy counselling and fertility assessment of patients, i.e. site of damage to the hypothalamic-pituitary-gonadal-axis and the carrier status of the partner, are important. If both the patient and partner are homozygous for β-thalassaemia, use of donor gametes is preferable, i.e. donor sperm and eggs. If the partner is heterozygous, then pre-implantation genetic gonadotrophin-induced ovulation and/or reproductive technologies can be used. These facilities are unavailable in the majority of South African public sector healthcare facilities, but are potentially viable options in the private healthcare sector.

Pregnancy

Pregnancy does not alter the natural progression of the disease. However, cardiac failure, alloimmunisation, viral infection, thrombosis, osteoporosis and endocrinopathies, e.g. diabetes mellitus, hypothyroidism and hypoparathyroidism, are possible complications to consider in a pregnant TM patient (Table 4). Folate supplementation should continue throughout pregnancy.

Risks associated with pregnancy:[6]

i. Risks of miscarriage and pregnancy-specific complications are the same as the background population. There is no increased risk of fetal malformations.
ii. There appears to be a two-fold increase in fetal growth restriction and an increased risk for pre-term labour.
iii. As chelation therapy is contra-indicated during pregnancy and with the need for ongoing blood transfusions, iron continues to accumulate, particularly in the liver. Chelation should resume promptly after delivery.
iv. There is a risk of thrombosis, especially in patients who have been previously splenectomised. The use of low molecular heparin is therefore recommended during pregnancy.

**Appendix 3: Haematopoietic stem cell transplantation**

Allogeneic haematopoietic stem cell transplantation (HSCT) is currently the only available curative strategy. The outcome of HSCT, however, is strongly influenced by factors such as age at transplantation, irregular iron chelation history, histocompatibility and source of stem cells.

Since the 1970s, the main therapeutic approaches for TDT remain blood transfusion in combination with iron chelation therapy. This approach can only succeed where there is adequate blood provision and good compliance to chelation therapy.[7] However, both regular blood transfusions and chelation therapy are expensive, and in one study, it was estimated that only 12% of children received adequate blood transfusions and less than half had effective iron chelation therapy. Additionally, compliance was often suboptimal.[8] Therefore, curative treatments such as haematopoietic stem cell transplantation (HSCT) would seem a cost-effective option if there was a compatible stem cell donor.

Allogeneic HSCT with an HLA matched (unaffected or heterozygous) sibling started in 1980s, with some of the largest experiences published in Italy.[9] While the approach proved to be effective, the outcome seemed to be influenced by various factors that gave a risk probability for survival. These included quality of the pre-transplant chelation therapy, presence of fibrosis in the liver and hepatomegaly (greater than 2 cm). Age younger than 7 years at transplantation also led to a significantly more favourable outcome.[10] Additionally, there have been improvements in the conditioning regimens and the source of the stem cells, which has impacted on the outcome of HSCT.[9,10] In a study, patients <14 years old, with adequate patient selection and with the recent improvements in transplantation technology, overall survival and event free survival of 91% and 86% were described.[9,10] Graft rejections from expanded haemopoiesis as well as sensitisation of the immune system by lifelong transfusions and severe forms of graft v. host disease (GvHD) remain significant challenges. As HLA-identical donors are available to less than 20% of patients, registries with non-remunerated donors typed at high resolution have become available. Currently more than 39 million donors are registered, substantially expanding the donor pool. Intensifying the conditioning regimens with anti-thymocyte globulin and post-transplant immunosuppression with cyclosporin and methotrexate have reduced non-engraftment significantly, and also the more severe forms of GvHD. Nevertheless, while mortality rates remain low, transplantation with unrelated donor leads to lower thalassaemia free survival at around 63%.[11]

Experience with unrelated cord blood transplantation for patients lacking an HLA-matched donor has showed high rates of graft failure and delayed haematopoietic recovery. It is recommended that only units with at least 3.5 × 10^7 nucleated cells/kg body weight before cryopreservation be used, with a single HLA mismatch allowed.[12]

Haploidentical transplant from family donors with intense T-cell depletion (CD34+ donations, TcRa/b, CD19+ depletion) avoided severe forms of GvHD, but have been associated with slow immune reconstitution and increased mortality from infections.[13]

Allogeneic HSCT with an HLA-compatible sibling or high-resolution-matched unrelated donor remains an effective alternative for young transfusion-dependent patients, with good chelation and who have not developed complications from iron overload. Better patient and donor selection with improvements in immunosuppression have led to high rates of disease-free survival. Indeed, work from Sardinia suggests that the cost of transplantation compares favourably after 8.7 years of conservative management,[14] showing that HSCT is a cost-effective option if an adequate stem cell donor is available.

**Appendix 4: Gene therapy**

While transplantation from HLA-identical siblings leads to 85% disease-free survival, the outcome of transplants from matched unrelated donors results in lower cure rates and increased mortality, and haploidentical transplantation still carries unacceptably high risks. Thus, allogeneic stem cell transplantation is a viable and effective option in <20% of patients. Gene therapy is theoretically available to all patients due to the autologous origin of the transplanted cells. The goal of gene therapy is to achieve stable introduction of functional globin genes into the patient's own haematopoietic stem cells, thus obviating the need for transfusions.[15-17]

Gene therapy using the lentiglobin vector can lead to cure without any mortality, graft rejection or clonal dominance issues.[16,17] Genome editing is a novel approach that makes use of targeted nucleases to correct the mutations in specific DNA sequences. Genome editing mediated by CRISPR/Cas9 has the ability to restore the normal β-globin function. Similarly, using CRISPR/Cas9, expression of BCL11A (responsible for modulating HbF expression) can be downregulated, leading to increased production of γ-globin and of HbF, thereby minimising the clinical severity of β-thalassaemia.[18] However, these genome-editing tools are still under *in vitro* trials.

The first successful gene therapy trial for thalassaemia was performed using lentiviral vectors by transducing autologous CD34+ HSCs which encode functional β-globin and the patient did not require transfusions for the following 2 years.[17] Development of lentiviral vectors with self-inactivating capacity without any pathogenic elements will be a significant milestone in the search and development for the cure for thalassaemia. Two subsequent clinical trials addressing transfusion-dependent β-thalassaemia started in 2013 based on the use of the BB305 vector. At a median follow-up of 26 months, all but one of the 13 patients with a $\beta^0/\beta^0$ thalassaemia genotype had discontinued red cell transfusions and remained transfusion-independent with levels of total Hb of 8.2 - 13.7 g/dL, of which the therapeutic HbAT87Q accounted for 3.4 - 10 g/dL.[19] Another clinical trial was carried out in Italy in transfusion-dependent β-thalassaemia. The study included three cohorts of adult (*n*= 3), adolescent (*n*= 3) and paediatric (*n*= 4) subjects with various phenotypes. After myeloablative conditioning with treosulfan and thiotepa, mobilised CD34+ cells were transduced with the GLOBE vector and administered by intra-osseous injection. The procedure was well tolerated, with no treatment-related adverse events. All tested patients showed multilineage cell engraftment and no evidence of clonal abnormality. With a follow-up of >12 months, the transfusion requirement in the adult patients was significantly reduced, while three of the four paediatric participants remained transfusion free.[20]

Several hurdles remain in the implementation of gene therapy for patients with TM, such as adequate harvest of CD34+ cells and transduction with the optimal vector.[21] Use of an insufficient quantity of cells may result in graft rejection. Lentiviral vectors are required in large concentrations for effective integration; however, their use is limited by their high cost and complex nature of the production. Additionally, integration of viral vectors at regions other than the target region may result in the activation of proto-oncogenes resulting in different types of cancer.[22,23] Clearly the future appears bright, but the optimal strategy will only be determined by comparing competing approaches in the context of properly designed clinical trials with an extended follow-up.

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