The life of patients with thalassemia major

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Andrea wakes up late, because the night before he went to a party with his girlfriend. He disconnects the needle and puts his desferal pump in the drawer. Then he rushes to work: he's a computer designer and his job is quite safe, because it was granted under the law protecting thalassemia patients and other people with medical problems. Nevertheless he does not want to irritate his boss. Besides, in a few days, he will be absent, and work will pile up, as he needs to go to the hospital for his regular blood transfusion.

Salvatore does not sleep well. He has a cast on his leg, which makes sleeping difficult. Playing football with his friends he broke a tibia, and it is not a first. Last time it was a rib, jumping from a wall in the countryside. He lost his job as a mechanic, recently, just as many people seem to be doing these days. Fortunately he has a pension, given to him because he has this blood disease and also because he was infected with hepatitis C virus as a child. But he's also worried about his health. Iron has been accumulating in his organs over the years and the doctors have warned him that he can no longer skip chelation. He's now faithfully gulping his deferiprone pills and at night he injects desferal with the pump. He's determined to take his life back into his hands again.

Marisa's morning is always difficult, with the two children to send to school (one adopted and the other conceived, unexpectedly, just one year later) and the house to clean, shopping to do and dinner that needs to be ready when her husband comes home. She takes this new chelating pill that dissolves in water, her vitamins and prophylactic antibiotics, her aspirin, and gets ready for the day. Around noon she has an appointment for her yearly check ups: audiometric tests, an ophthalmologic examination, and abdominal ultrasound. Next week it will be the turn of the MRI to check for iron in her heart and liver. Everything seems to be going well, so far. She thinks about her life: she's satisfied with it. Who would ever have thought that she could carry on and look like a normal woman? Certainly not her mother who kept looking at her, as a child, with eyes full of tears. And now, instead … Just a pity about that ugly splenectomy scar on her belly.

Thalassemia major was described for the first time in the early 1920s by Dr Denton Cooley, a Detroit pediatrician who recognized a peculiar clinical picture in seven children of Italian or Greek origin. The signs of the disease included anemia, a large spleen, and characteristic bone deformities. Despite several therapeutic attempts all the children died within a few months of diagnosis.[1] Almost at the same time an Italian pediatrician, Antonio Maccanti, wrote his "*Contribution to the knowledge of the familial splenic anemia of infancy*".[2] Both authors were, in fact, describing the same hemoglobinopathy, now also called Mediterranean anemia or Cooley's anemia. Ten years later the recessive character of the Mendelian transmission of the disease was recognized. It soon appeared clear that the distribution of the disease was not limited to Mediterranean countries, but was also common in South East Asia, in India and in the Middle East. It is now commonly accepted that thalassemia heterozygotes have a selective advantage, being more resistant to malaria.[3] However, until not long ago, the prognosis of homozygotes with thalassemia major was dismal. From the end of the 1950s transfusions were given to symptomatic patients when their hemoglobin reached extremely low levels but, in any case, the patients died after a few years of infection or heart failure. Between 1949 and 1957, in Ferrara, only 9% of the patients reached the age of 6 years and at the end of the 1970s half of Italian patients had died before reaching 12 years of age.[4] The appearance of thalassemic children in those years is well known, as they are still reproduced in most hematology textbooks: large heads, small noses, protruding zygomatic bones, distended abdomens and thin limbs. Today, thalassemia major is a completely different disease. A lot more is known about the pathophysiology of the disease and molecular biology can discern the majority of thalassemic defects. The patients are transfused from early in life, so that bone deformities as well as splenomegaly are prevented. Transfused blood is safer, at least in industrialized countries, where viruses in donors' blood are identified by enzyme-linked immunosorbent assays and nucleic acid amplification technology. An Italian multicenter study that has been going on for the past 26 years and has included almost 1,000 patients born since 1960, has demonstrated that, in 2009, 60% of the patients were older than 30 years (*unpublished data*, Borgna-Pignatti) (Figure 1). Survival was better for patients born in more recent years, and for females, who were less likely to die than males (relative risk= 0.65). An article from Cyprus, which appeared in a recent issue of this journal, confirmed the now excellent survival of thalassemia patients, due especially to a decrease in cardiac disease.[5] Similar results have been reported for UK patients.[6] The first cause of death, in all series, continues to be heart failure, fol-

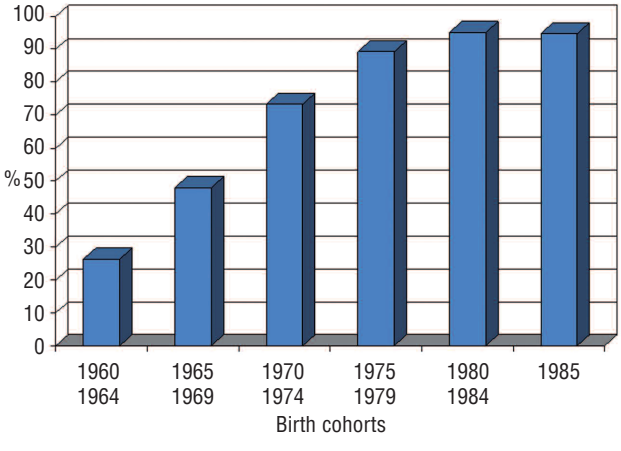

Figure 1. Survival in 2009 of Italian patients included in the Seven Centers Study, according to birth cohort.

lowed by infection. Splenectomy, in the past performed in the first decade of life, is now delayed for as long as possible, in order to prevent previously unrecognized infectious and thrombotic complications.

The major step forward in improving survival and reducing complications was the introduction, in the 1960s, of the chelating agent deferoxamine, first as an intramuscular injection and later as a subcutaneous infusion. Two oral chelators, deferiprone and deferasirox, have more recently become available, making therapy easier and more efficacious. Compliance, although improved by the switch to oral therapy, still represents a problem and is the major obstacle to effective prevention, or limitation, of iron overload. Methods to quantify the iron content of the heart and liver are precious in monitoring the course of iron accumulation and the effect of chelation therapy. The so-called magnetic resonance T2* technique is now widely used for the evaluation of myocardial and liver iron.[7] Myocardial T2* values less than 20 ms indicate iron overload, which is considered severe when T2* is less than 10 ms and is associated with systolic and diastolic ventricular dysfunction. A recent paper reporting results in 652 thalassemia major patients found that the relative risk for heart failure with cardiac T2* values less than 10 ms (compared with more than 10 ms) was 160. Heart failure occurred in 47% of patients within 1 year of cardiac magnetic resonance imaging when the T2* was less than 6 ms.[8] Despite these excellent results, complications are not infrequent. In the above mentioned Italian study,[9] at least one complication was present in 22% of the patients. Hypogonadism was the most frequent complication, affecting 54% of the patients, followed by hypothyroidism (11%), and diabetes (6%). In addition, with increasing age of the patients, new life-threatening complications are being recognized, including severe thromboembolic events,[10] pulmonary hypertension,[11] hepatocellular carcinoma,[12] and pseudoxanthoma elasticum.[13]

Hypogonadotropic hypogonadism is probably attributable to the free-radical oxidative damage caused by non-transferrin bound iron to the anterior pituitary gland and to the hypothalamus. Hormone replacement therapy is given in order to induce secondary sexual characters, enhance height growth velocity, and to help to prevent osteoporosis. Oligospermia and asthenospermia affect more than one half of men with thalassemia.[14] Treatment with human chorionic gonadotropin plus human follicle-stimulating hormone or gonadotropin-releasing hormone stimulates spermatogenesis and induces fertility in males. In females, fertility is usually normal in the presence of normal menstrual function, or it can be induced with human menopausal gonadotropin or follicle-stimulating hormone.[15] Overall, hundreds of pregnancies have taken place in women with thalassemia, but pre-existing cardiomyopathy, expanded plasma volume, increased cardiac output, and reduced glucose tolerance all represent menaces to maternal health. The study by Origa *et al.*, published in this issue of the journal,[16] is an important testimony on how improvements in therapy have completely changed the long-term prognosis and quality of life of thalassemic patients. The authors report on the course of 75 pregnancies in 46 women with thalassemia major and in 11 women with thalassemia intermedia followed in four Italian centers participating in a cooperative study. Ninety-one percent of pregnancies resulted in successful delivery and the proportion of neonates with intrauterine growth retardation did not differ from that reported in the general Italian population. Five sets of twins and one set of triplets were born as a consequence of gonadotropin-induced ovulation. Origa *et al.* concluded that pregnancy is possible and usually safe, although the need for ovarian stimulation remains a concern for thalassemic women who wish to become pregnant. All the problems that one could expect to complicate pregnancy were kept in check, underlying the importance of surveillance and treatment by a multidisciplinary team. No secondary complications of iron overload developed, in particular no irreversible cardiac problems, which is particularly encouraging because in the past a few cases of cardiac failure, occasionally fatal, had been reported. Unfortunately, T2* findings, which could have provided interesting information on the amount of iron accumulated during the months when chelation was not given, were available only for two patients. The most threatening problem was severe alloimmune anemia complicating the course of pregnancy in a previously minimally transfused woman. The same problem had been previously reported by other authors,[17,18] and should be kept in mind when counseling a woman with thalassemia, especially thalassemia intermedia, who wants to become pregnamt. In fact, this complication is most often observed in patients starting transfusion therapy after the first few years of life, and should be a major concern when previously untransfused patients require transfusion during pregnancy. No thrombotic events were observed, probably as a consequence of careful monitoring of coagulation and anticoagulant prophylaxis. Interestingly, in one case of spontaneous pregnancy, both parents had thalassemia and the newborn was consequently affected. No better proof could be offered that the lives of patients with thalassemia are becoming more and more normal. Not only did two patients get married and reproduce, but they were satisfied enough with their lives that the birth of a thalassemic child was happily accepted.

Hematopoietic stem cell transplantation is available for patients who have a related or unrelated HLA-identical donor. The probability of cure ranges from 90-95% for recipients of grafts from relatives[1] to 80-85% for those receiving grafts from an unrelated donor, although the probability even in these cases approaches 90% when donor matching is as strict as that between HLA-identical siblings.[19]

Gene therapy offers a potential cure for β-thalassemia and would represent an ideal alternative to both conventional therapy and bone marrow transplantation. Several reports of successful transfer of a human β globin gene into the hematopoietic stem cells of mice, most often using lentiviral vectors encoding β-like genes have been published. One particularly interesting report described conversion to transfusion independence, lasting at least 16 months, of a patient with βE/β°thalassemia whose bone marrow $CD34^+$ cells were transduced *ex vivo* with a lentiviral vector expressing a marked βA-T87Q-globin gene.[20] Other groups of scientists are working at similar projects and phase one clinical trials are being planned to assess the therapeutic value of globin gene transfer.[21]

However, there are still concerns about safety of this approach. Reactivation of γ globin chain production has the potential benefit of improving the imbalance in the α/non-α ratio, reducing the severity of the anemia. The small-scale studies performed on the induction of fetal hemoglobin in thalassemia major have, so far, been unsuccessful. Identification of the three major loci – the *Xmn1-HBG2* single nucleotide polymorphism, the *HBS1L-MYB* intergenic region on chromosome 6q, and *BCL11A* – contributing 20-50% of the individual variation in fetal hemoglobin expression in healthy European Caucasians, has not yet been translated into new therapeutic approaches.[22]

Thalassemia used to be a pediatric disease, but the median age of the patients has now increased in European Mediterranean countries, because of increased survival and birth rate reduction. Population screening, genetic counseling, and the availability of prenatal diagnosis have been extremely effective. In Sardinia the reduction of the birth rate of patients with thalassemia major declined, in the last 20 years, from 1:250 live births to 1:4000.[23] The advent of DNA amplification has made it possible to perform pre-implantation genetic diagnosis on a single cell biopsied from cleaving embryos obtained by *in vitro* fertilization. Only unaffected embryos are transferred into the uterus. Successful experiences with this approach have been reported.[24] Pre-conception diagnosis analyzes the polar body obtained during the maturation of the oocyte.[25] These procedures avoid the need to terminate affected pregnancies and, although they are expensive and require intensive effort, there has been a steady increase in the number of cycles, pregnancies and infants reported annually. Recent advances include HLA typing for embryos undergoing genetic diagnosis, when an elder sibling is affected and could be cured by stem cell transplantation.[26]

Transition from pediatric care to adult services has recently become an important issue for the thalassemia community. On the one hand it is difficult for these young adults to leave the cocoon of the pediatric or, more often, pediatric hematology outpatient services where they have been treated for many years and where they know everybody, and everybody knows them. On the other hand they feel the need to cut the cord and finally become emancipated in a world where they are recognized as adults with adult needs and where the physicians are more prepared to face the physical and psychological complications affecting them. In several countries, including Italy, most patients are cared for in thalassemia centers, often sprouted from pediatric day care units, which have developed a large experience in treating the disease, both in children and adults, and where often, in addition to the physicians, specialized nurses are of great clinical and psychological support. The importance of specialized skills in treating patients with thalassemia has been recently reported.[27] Patients and families are well organized in support groups,[28-30] and, in some countries, patients are protected by special laws, favoring their access to work, their leaves of absence for treatment, and even early retirement. In conclusion, both the duration and the quality of life of patients with thalassemia have improved in industrialized countries, while remaining problematic in developing countries where thalassemia and other hemoglobinopathies are highly prevalent. In the future, migratory fluxes of people coming from these areas of the world and settling in affluent countries will bring new challenges both in developing strategies for prevention and in coping with the increasing costs of treatment.

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Pathophysiology and treatment of the myelodysplastic syndrome with isolated 5q deletion

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During the past few years important breakthroughs have been made in the understanding of the 5q- syndrome. Scientists have struggled for decades to unravel the pathophysiology, and several candidate genes located on 5q have been proposed. Now, gene dosage and deletion of micro-RNA due to haploinsufficiency are recognized as key mediators of the disease. Moreover, the clinical management of patients with isolated 5q deletion has changed radically since lenalidomide was shown to have remarkable activity in this particular subgroup of myelodysplastic syndrome (MDS), although safety concerns have been raised recently. These issues will be discussed in detail.

Clinically, the 5q- syndrome is characterized by a macrocytic anemia, normal or slightly elevated platelet counts, hypolobated megakaryocytes, and a long-term risk of evolution into acute myeloid leukemia (AML) of around 10%.[1] The World Health Organization classification of 2008 recognizes MDS associated with isolated del(5q) as a distinct entity, defined as MDS with less then 5% bone marrow blasts in addition to del(5q) as the sole karyotypic abnormality.[2]

Novel insights into the pathogenesis of myelodysplastic syndrome with isolated del(5q)

Haploinsufficiency of the ribosomal gene RPS14 impairs erythropoiesis

MDS with isolated del(5q) is a true stem cell disease, with more than 90% of the hematopoietic stem cells being part of the clone.[3] The del(5q) abnormality has been demonstrated in B cells as well as in natural killer (NK) cells.[3-5] The bone marrow is generally normo- or hypercellular.

It has been an enigma how an expanded clonal progenitor pool could result in anemia, but impaired erythroid development is strongly implied. The commonly deleted region (CDR) at 5q has been shown to contain around 40 genes expressed in hematopoietic progenitor cells.[6] None of these has been found to be mutated, and understanding the role of haploinsufficiency of one or more genes within this region has proven to be a great challenge.

In 2008 Ebert *et al.* performed a series of experiments knocking-down each of the 40 implicated genes located within the CDR at 5q32-33. They elegantly demonstrated that decreased expression of *RPS14* results in poor erythroid development and increased erythroid apoptosis. Moreover, forced expression of *RPS14* in cells from MDS patients with del(5q) reduced the erythroid impairment.[7]

RPS14 is a component of the ribosomal subunit 40S. Intriguingly, germline mutations of other ribosomal protein genes, in particular *RPS19*, have been shown to cause Diamond-Blackfan anemia, which is also characterized by a macrocytic anemia. Furthermore, animal models have demonstrated that defects in ribosomal genes result in ineffective erythropoiesis and an increased risk of cancer.[8] RPS14 deficiency is a plausible explanation of the anemia of 5q- syndrome. However, it does not explain the elevated platelet counts or the stem cell expansion.

Loss of micro-RNA leads to thrombocytosis and contributes to clonal expansion

Starczynowski, Karsan and others recently screened for micro-RNA close to the CDR at 5q and identified *miR-145* and *miR-146a* as being significantly expressed in $CD34^+$ bone marrow cells and significantly less expressed in del(5q) marrow cells compared to normal bone marrow cells. Knock-down of these two micro-RNA in mice resulted in a phenotype mimicking key features of the 5q- syndrome: hypolobated megakaryocytes and peripheral thrombocytosis. Moreover, the innate immune response pathway was shown to be modulated by predicted targets for *miR-145* and *miR-146a*. Two genes in the Toll-like receptor signaling pathway were verified as true targets: *TIRAP* (*miR-145*) and *TRAF6* (*miR-146a*). *TIRAP* interacts