Thalassemia: a dreadful disease turned to a chronic condition

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Thalassemia is a disease with many challenging aspects. It is caused by a variety of interesting molecular mechanisms, has a complicated pathophysiology, constitutes a multifaceted medical problem with pain and misery for the patients and unhappiness for their families, creates several diagnostic and therapeutic questions for their physicians, and has a huge social and economic impact across the countries where it occurs in high frequencies.

Thalassemia major may have existed in the Mediterranean basin since the antiquity; skeletons with severe *porotic hyperostosis* have been found in several tombs across Sicily and Sardinia and are considered to denote a severe congenital hemolytic condition, which may be thalassemia. In our era, especially at the beginning of the last century, pediatricians have reported cases which resemble thalassemia, but can also be cases of leishmaniasis, leukemia or even iron deficiency. The first formal description of thalassemia is due to Thomas Cooley, a physician from Detroit in the US, who reported a *series of cases of splenomegaly in children with peculiar bone changes* in 1925. The term thalassemia was coined by Whipple and Bradford later. Subsequently, several authors all over the world added various details concerning the clinical and laboratory aspects of the disease, while Caminopetros pointed out conclusively in 1936 that this was a familial condition inherited in an autosomal recessive mode.

The systematic study of thalassemia developed rapidly after the second world war at three levels: (a) understanding the molecular and cellular pathophysiology of the disease including; (b) improving the quality as well as the expectancy of life of the patients, and (c) alleviating the pressure of the disease on the Public Health System of each country harboring high numbers of patients. This lecture will address mainly the clinical part of thalassemia, which is of greater importance for this Conference.

I belong to the generation who have seen in person, examined and taken care of patients with thalassemia major several years ago. The

picture was quite different from what we see to-day; it was a dreadful picture indeed. I remember of several little children with marked maxillary deformities, a tiny face backed by a huge skull, a protuberant abdomen carrying a huge spleen, atrophic, thin arms and legs, and large eyes with a sight of despair. Apparently, the situation did not change appreciably over several years after the initial description of the disease because the second world did not leave space for the care of those unlucky children who died soon after birth because of anemia and malnutrition. The early literature contains some reports of typical patients, the hemolytic jaundice with red cells featuring increased osmotic resistance, *i.e.*, the condition known as Rietti Greppi Micheli syndrome, and the familial incidence of *microcytemia*, along with various therapeutic suggestions such as administration of iron and vitamins, splenectomy, and, whenever possible, support with transfusions of blood. Obviously, this approach failed to provide any significant relief, the only real effect being that it prolonged by a few years the miserable life of the patients and the difficulties and unhappiness of their families. However, as a result of this insufficient gain of survival along with the jump of natality which followed the second world war, the number of thalassemic children, especially in countries like Italy, Greece, and Cyprus, started increasing to numbers which could not allow any studies and progress in the management of thalassemia, except for some improvement of the conditions under which the patients were seen and transfused. In fact, this is the reason why several competent but mostly academic physicians involved in thalassemia focused their scientific interests in deciphering its pathophysiology and reporting interesting variants. The care providing Units were crowded with an ever increasing of patients who were simply undertransfused, splenectomised and given folic acid.

The initial attempts towards improving the care of thalassemia took place in the United Kingdom, through the persistent efforts of Bernadette Modell and her collaborators. Bernadette had the possibility to offer adequate care to a small number of Cypriot and Indian-Pakistani patients which was appropriate for her means. This was really important because it allowed the establishment of the concept of regular transfusion, which in some instances was exaggerated in the sense of *hypertransfusion*, in an attempt to suppress the non-effective thalassemic erythropoiesis, hence preventing the bone deformations and splenomegaly and providing a good activity to the patients. The significance of the enlarged spleen constituted a major problem. Splenectomy was decided either empirically or following elaborate erythrokinetic and chromium 51 studies in order to weigh the potential contribution of splenic erythropoiesis against the splenic hydremia. As a rule, the overall outcome was favourable. Later on, when the contribution of the spleen in protecting the patients against various infectious agents became apparent, the indication for its removal was put with more caution and required preparation with anti-pneumococcal vaccine and long term penicillin. At that time, we witnessed also other interesting alternatives such as regional splenic embolization or partial splenectomy, which are not being practiced anymore.

Meanwhile, through the efforts of several known and unknown people all over the world, blood donation ceased to be a remunerated act

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This lecture is dedicated to the late Professor Antonio Cao.

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Parts of this work were presented at the "3rd Pan-European Conference on Haemoglobinopathies and Rare Anaemias", Limassol (Cyprus), 24-26 October 2012.

and became a voluntary gesture with varying success in several countries. One much debated concept was that of assigning specific donors for specific patients, the so called *nonni*. Although this was coming in contrast with the general philosophy of anonymous blood donation, we must acknowledge that it was a bright idea, which is still practiced in Italy and elsewhere, relieving several patients and physicians from the agony how to secure the necessary blood on time. In parallel, the safety of the transfused blood increased by developing and gradually expanding techniques for the detection of the hepatitis viruses as well by improving the compatibility testing. The frequency of infection with the hepatitis B virus among multitransfused thalassemics was as high as 60% in the early days; it is an exceptional finding to-day. Infection with the hepatitis C virus followed a similar pattern a few years later. Fortunately, the AIDS problem did not involve largely the thalassemic population, the main reason being that the infection started spreading mainly in the United States, where the thalassemic patients were relatively few, thus allowing a margin of time until the development and broad application of virus detection techniques which prevented the spread of the disease across Europe and the Near East.

The relatively rare post-transfusion complication of high fever due to sensitization of the patients against various leucocyte antigens was controlled with red cell washing, a procedure which wasted a large proportion of blood, and a series of filters which removed leucocytes and platelets at various stages prior to the transfusion.

With the progress of time, the initial concept of *hypertransfusion*, i.e. bringing the hemoglobin levels at normal or even supernormal levels in order to suppress the non-effective marrow activity gradually came to an equilibrium. This was a good step because the demands on blood were already difficult to meet and because the patients were unnecessarily loaded with enormous quantities of toxic iron. A good pre-transfusion hemoglobin level of 9.0 or 10.0 g/dL is now considered as adequate to ensure good growth and normal activity, especially in adult life.

Adequate transfusions did not solve the problem of thalassemia; they may have worsened it in a way. As the iron contained in the transfused erythrocytes could not be excreted, it was gradually deposited in various organs and caused damage through the abundant production of free oxygen radicals. The noxious potential of transfusional iron was noticed very early. Elaborate studies carried out by British pathologists already in the sixties reported the damage of several endocrine glands such as the parathyroid, the hypophysis, the gonads and others, as well as the catastrophe brought up by the metal in the liver and the heart. Inevitably, the whole internal medicine had to deal with the respective clinical pictures. Limited growth, all sorts of muscular cramps caused by the low serum calcium, absence of sexual maturation, liver failure with cirrhosis and finally cancer, as well as cardiac failure made worthless the advantages of longer survival and ended with death a few years later. Indeed, this was not a solution to the problem; the accumulation of iron had to be prevented and the iron overload had to be removed. In 1995, British authors have reported an additional reason for the iron overload; increased erythropoiesis, be it effective or non effective, increased the absorbance of dietary iron and could well be a factor aggravating the effects of transfusional iron. We now know that this is the effect of a vicious circle starting from the maturing erythroblasts with the synthesis of protein GDF15, which then inhibits synthesis of hepcidin and favors iron absorption; it took more than 30 years to understand this mechanism, but the problem was there from the very beginning and led to various specific diets with low iron content or ingestion of liters of tea in an effort to bind and prevent absorption of iron in the digestive system.

Neocyte transfusion went through a glorious but short period. Transfusing young red cells collected from several blood units by differential centrifugation meant longer transfusion intervals and less iron overload. But it was technically extremely cumbersome, demanded wasting a considerable amount of blood, had an increased risk of infection and was soon abandoned.

In the meanwhile, a novel way to measure iron overload was developed. An iron binding protein contained in the liver and other types of cells leaked into the plasma in a quantity proportional to the amount stored in the cells. Ferritin was a perfect indicator of iron overload and assays towards its measurement have soon emerged in practice, custom made but extremely difficult and time consuming at the beginning, and commercially available, easy and reliable to perform presently. This was an important technical step. Despite the various limitations and contradictory results which occasionally are reported, the fact remains that measurement of ferritin in the serum of the patients proved a reliable tool to assess the severity of the disease and monitor all therapeutic attempts. Establishing ferritin as an indicator of iron overload led to the appearance of numerous publications reporting various correlations with clinical data, pathological observations and, most of all, life expectancy. Ferritin levels showed a perfect correlation with the number of transfusions and high ferritin levels predicted shortened survival.

Attempts to remove iron from the body have started in the fifties, when CIBA GEIGY in Basel reported that a siderophore extracted from a bacterial species, known as *streptomyces pillosus*, had an extremely strong affinity for iron and could be administered to humans, bind the iron which was not bound to transferrin, and be excreted in the urine. The chemical name of the compound was desferrioxamine. As desferrioxamine did not appear to cause any serious side effects, it has soon introduced for clinical trials in iron-laden thalassemic patients under the commercial name Desferal. The abundance of relevant reports cannot be summarized here; anyway, results were unanimously positive. Desferal could certainly eliminate excess iron; the only inconvenience was that it could not be absorbed from the gut and necessitated parenteral administration. Moreover, the amount of iron which was excreted following the intramuscular administration of the drug would never remove the huge amounts of deposited iron, the pharmacological reason being that the drug is rapidly passing into the urine and has a limited time margin to bind and carry out the available free plasma iron. This led to the concept of intravenous infusion of desferal, over several hours or even days, which was really effective but impossible to enter into common practice for obvious reasons. In addition, at least in the early steps of this approach, the abrupt mobilization of iron caused cardiac arrhythmias, often severe. The rationale for the parenteral infusion of desferal was that it could ensure high plasma levels over several hours; by that time we also knew that toxicity was caused of the free plasma iron and that it was this iron which should be constantly removed from the circulation. In addition, the amount of Desferal which could be administered intravenously was significantly larger than that supplied by intramuscular injections. The last route which was tried was subcutaneous administration. Desferal was not only well tolerated but ensured a gradual absorption, hence a longer presence in the plasma, the main inconvenience being that the volume of the required amounts of the drug at a tolerable dilution could not be administered in a bolus injection. This led to the development of various types of *infusors*, little balloons filled with desferal which was delivered slowly through a needle inserted in the abdominal wall, or various types of syringes, where a tiny electric motor pumped slowly the desferal solution through a fine needle into the subcutaneous tissues of the patients overnight. Application of the pump has not been as simple as it appears to day; in addition to the technical pitfalls which were gradually taken care of, convincing the patients to accept this overnight torture proved a difficult, often unrewarding procedure, for both patients, families and medical staff. On the other side, the effort was worthwhile, because its' effect on the patients who complied was magnificent. This was made clear in the numerous papers and communications which flooded the medical literature in the sixties leaving no doubt that subcutaneous overnight administration of desferrioxamine for long periods of time in several patients with transfusion dependent thalassemia, resulted in an important decrease of ferritin and an increase of their life expectancy.

In parallel, the need for an iron chelator which would be administered orally became more pressing. Patients demanded it; if parenteral therapy was so effective, then an equivalent oral agent had to be produced. Over the ensuing years, various iron chelating agents have appeared and vanished. DTPA, rhodotorulic acid, HBED, PIH and several others reached the point of uncontrolled clinical trials. However, none of them prove efficient enough. In the early eighties emerges the L1. This compound, chemically known as deferiprone has an unbelievable controversial story. It was synthesized in by Dr. Hider in the UK; then, Dr. Kontoghiorgis, a Cypriot chemist working with Dr Hider and later with Professor Hoffbrand gave it to iron laden, heavily transfused patients. The study was simple and had no clearcut criteria of effectiveness beyond iron excretion in the urine. However, the result was considered as important and was published in the British Medical Journal in 1987.

What followed this report cannot be easily abbreviated. As the Academic Laboratory could not supply the desired amount of deferiprone, various small pharmaceutical companies undertook its synthesis. The drug was thus administered to several groups of patients in India, Switzerland, Italy and elsewhere in an empirical way, and certainly, not in the form of a controlled clinical trial. A few of those studies were published; they all showed an increased iron excretion, occasionally a decrease of ferritin and no side effects which should prohibit administration of the drug, except for leucopenia and a few cases of agranulocytosis. It is true that the big pharmaceutical companies ignored the novel agent; they wanted to produce their own agents. A study carried out in the US on behalf of a big company using iron laden animals reported high toxicity and no effect. Deferiprone was about to disappear. But it did not; perhaps a letter signed by a large number of physicians involved in thalassemia helped avoiding its complete rejection.

In 1992 Olivieri and collaborators reported the efficacy of long term therapy with deferiprone in a patient with thalassemia intermedia and a significant iron load and confirmed its beneficial effects with regards to increasing iron excretion and decreasing serum ferritin and liver iron content. Later on, the Canadian based Apotex Company entrusted Olivieri with the conduction of a large scale well organized trial. It is very sad that this study had not a happy end because of the never proven liver toxicity. Be this as it may, the Apotex company finally obtained the necessary licences both from the European and the American Authorities and deferiprone entered the market in under the name Ferriprox.

Meanwhile, the search for other, possibly better forms of iron chelators continued. In 2005 Novartis had a novel chelator approved by the FDA; this had the scientific name Deferasirox and was marketed as Exjade. Exjade had its own advantages: it could be taken once a day as liquid preparation, had a long plasma life, had fewer side effects including the serious threat of agranulocytosis, and was very effective at the prescribed doses. Because of these advantages the use of Exjade has rapidly expanded all over the thalassemia world and is one of the most commonly used iron chelators presently, the main drawback being its high price.

As expected, the search for other iron chelators has not stopped here and novel compounds appear in the horizon. Of interest, recent advances in deciphering the iron metabolism, which will be dealt with by the next lecturers, approach this matter from other points of view.

The number of publications which appeared over the last twenty years with regards to the specific properties, the iron removal potential, and the side effects of the above chelators is innumerable; indeed, we have witnessed serious debates and controversies, various personal beliefs and commercial competition, and repetitive results and publications. But, admittedly, many of these studies were carried out with strictly controlled ways, evaluated large numbers of patients with accurate statistical methods, and measured the efficacy of each chelator or mode of administration using modern and reliable techniques.

At the technical level, a major step has been the correlation of serum

ferritin to the liver iron content chemically assessed in liver specimens obtained by biopsy. Further correlation of this finding with a series of imaging techniques, such as the Squidd, and later the computed axial tomography and magnetic resonance studies, allowed the evaluation of the iron removal potential of several iron chelators in numerous clinical trials and provided a more precise assessment of the efficiency of each one of them at the clinical level.

Along the same line goes the ever accumulating information relating iron overload to cardiac disease, which now can be assessed not only clinically or by myocardial biopsy, but also through measuring the ejection fraction and other components obtained by echocardiography, or by exploiting the paramagnetic properties of iron and applying programs which allow its quantification in the myocardial tissue by MRI. An important observation in this field was the recognition that, occasionally, the deposition of iron in the myocardium may largely exceed that of the liver, implying that ferritin levels may be misleading in a few instances.

Using the above and other techniques confirmed that good compliance to the above drugs, taken solely, in sequence, or in combination, was able to significantly decrease the ferritin levels in patients with severe iron overload, and gradually improve the associated dangerous liver or cardiac failure. Moreover, good compliance to therapy allowed younger patients to secure a perfect iron balance, thereby preventing the accumulation of transfusional iron, whose chronic deposition is catastrophic through the continuous release of free radicals.

During the last years other equally important interventions have taken place. In addition to removing the toxic effects of iron on the endocrine system, a series of careful studies of the levels and rhythm of secretion of a number of sex hormones led to sophisticated ingenious replacement therapies, which have allowed a better growth, sexual development and, above all, child bearing, a blessing for the couples where one or both partners had thalassemia.

Moreover, studies of bone metabolism in thalassemic patients revealed not only the underlying pathogenetic mechanisms but they also paved the way for the application of novel agents, such as the various phosphonates and others, which could reinforce the bone structure and prevent the frequent, often unjustified, fractures.

With the progression of time and compliance to the above therapies, the quality of life of thalassemic patients started improving. The patients now display normal growth and a normal appearance, they can participate in all sports of athletics, they can marry and have children, work regularly, attend all kinds of studies and become good professionals including medical doctors. For the younger generation of thalassemics, severe hepatitis, liver damage and cancer, cardiac arrhythmias and heart failure are not more frequent than in matched individuals without thalassemia and life expectancy tends to equal that of normal population. No doubt, the price is very high; regular transfusions, continuous iron chelation therapy, vaccines, hormones, phosphonates and other medications cannot be stopped and require a lot of courage, patience and strict compliance. But it is worthwhile! The dreadful picture of sick children fifty years ago cannot compare with the picture of the thalassemic patients to-day: smiling, beautiful, active and happy children, keen to absorb whatever good life can offer to them. A previously fatal disease has now changed to a chronic tolerable illness.

Is this the end of the story? Certainly no; there are still many issues to be addressed and almost all components of the reported management need innovation and development. The final step, *i.e.*, cure of thalassemia, is still far away, but not impossible. A session of this Conference will focus in this matter, and bone marrow transplantation, gene therapy and other molecular approaches will be presented in detail.

Changing thalassemia from a fatal condition to a tolerable disease has been supported by several factors. Perseverance of the patients to improve their own situation is a major one. We must acknowledge that most clinical trials were carried out on patients who accepted to receive one or another medication in varying dosages and under strict rules,

and then undergo innumerable blood examinations, imaging procedures and painful biopsies in order to extract novel useful information. This contribution was never followed by any kind of remuneration; it was a generous offer of the thalassemic patients to their thalassemic colleagues, in the firm belief that their action would help improving the overall situation. Within this context, the anonymous patient contribution cannot be overemphasized.

Next to praise come the medical and paramedical staff who take care of these patients; treating thalassemia is a demanding procedure because it is repetitive, tedious, chronic and rarely only does it provide the desired satisfaction. Approaching the patients and consistently following them over the years demands dedication, a strong faith in the value of this monotonous task, a close doctor-patient relation, and lot of compassion and patience. It is this group of anonymous medical staff who have contributed in changing the picture of thalassemia and deserve a lot of gratitude and recognition.

Adequate resources have been instrumental for the change I just referred to. Clearly, this has occurred mostly in countries which were in position to support the disproportionately huge expenses associated with the optimal treatment of thalassemic patients, such as Italy, Greece, Cyprus, Southern France, the United Kingdom, the United States and a few other, where the number of patients is relatively small. Regrettably, in many other countries with high incidence of thalassemia, management of the patients to-day is far from being acceptable.

How can this be envisaged? I strongly believe that, until a curative therapy is implemented, the only solution is to prevent the birth of more newborns with the disease, by either premarital counseling, or by prenatal diagnosis, or, hopefully soon, by preimplantation or even preconceptional diagnosis. In fact, even in the developed countries where the great change in the management of thalassemia has occurred, nothing would have happened if the number of new patients would con-

tinue to increase. There are now several formal publications regarding the ever increasing cost of the optimal treatment of thalassemia and there is no hope that this will decrease in the middle of the present financial crisis. Meeting the necessary expense for total management in countries with thousands of thalassemic babies born each year is virtually impossible; therefore, improving their management will never occur. On the contrary, if people, physicians, clergy and authorities realize this impasse and promote prevention of thalassemia, all available resources will be re-directed to improving the management of the children who are now surviving and provide them with optimal therapy, relief, activity and a smile.

Antonio Cao has significantly contributed in all these fields. As Professor of Pediatrics in Cagliari he was involved in thalassemia already in 1974, when he organized and directed the Institute of *Clinica e Biologia dell'Età Evolutiva* which, in association with the *Primary Regional Hospital for Microcythemia* provided high level care to hundreds of Sardinian thalassemic children. Antonio also established prenatal diagnosis of thalassemia in Sardinia which significantly reduced the yearly number of affected newborns and made significant scientific contributions, such as the molecular characterization of various types of α , β , and δ -thalassemia in Sardinia and elsewhere, numerous studies of the genotype-phenotype correlation, identification of several new transcription factors active on the β -globin genes and extensive linkage studies among Sardinians which identified nucleotide sequences across the human genome which promote β -globin chain synthesis and produce hemoglobin F.

Antonio was a hard worker and a good companion; he has created a school of dedicated collaborators and left his mark on several academic issues in Italy. His memory will remain vivid across the thalassemic world.

Oral presentation

Oral presentation is available online