

THALASSEMIA INTERMEDIA

Clara Camaschella,* Maria Domenica Cappellini^o

*Dipartimento di Scienze Biomediche e Oncologia Umana, sezione Clinica, Università di Torino, Ospedale S. Luigi Gonzaga, Orbassano CNR CII-CIOS Torino; ^oIstituto di Medicina Interna e di Fisiopatologia Medica, Università di Milano, Italy

ABSTRACT

Thalassemia intermedia is a clinical definition applied to patients whose clinical phenotype is milder than that of thalassemia major. Criteria used to define thalassemia intermedia including age at presentation, hemoglobin or fetal hemoglobin levels and transfusion independence, are unsatisfactory. The possibility of typing the molecular defect allows a retrospective analysis of patients and offers a new tool for the diagnosis of thalassemia intermedia. Nevertheless, because of several factors that interact in the disease expression, the β -genotype alone is not predictive of the phenotype in all cases. Although benign, the clinical course of thalassemia intermedia is characterized by several complications that can be prevented by an accurate follow-up. The conventional treatment of thalassemia intermedia remains controversial; it is hoped that recent advances in the pharmacological manipulation of hemoglobin switching will offer a therapeutic option in the future, at least to selected patients.

Key words: thalassemia intermedia

The definition *thalassemia intermedia* is a label applied to thalassemia patients characterized by a transfusion-independent clinical course of *intermediate* severity between thalassemia major and asymptomatic carriers.^{1,2} Since the definition of thalassemia intermedia is relative, it includes a wide range of clinically and genetically heterogeneous patients. The spectrum of disorders includes, at one end, patients who are able to produce approximately 6 g/dL of hemoglobin, show skeletal abnormalities and occasionally require transfusions and, at the other end, asymptomatic subjects with mild anemia and splenomegaly diagnosed by chance or during family studies.

The criterion of transfusion independency is unsatisfactory for defining thalassemia intermedia, since it varies among patients and is also related to medical trends. When a policy of starting high transfusion regimens in infancy for homozygous thalassemia patients was intro-

duced,^{3,4} some intermediate forms were probably enrolled in the program.

On the other hand, since the prognosis of thalassemia intermedia in terms of survival, quality of life, puberty and mature sexual function was considered better than that of patients regularly transfused,² some patients able to maintain high Hb levels only thanks to huge hemopoietic expansion and bone abnormalities were not enrolled in a regular transfusion program. This unfortunately resulted in not transfusing some patients who would have benefited from this treatment.

During the last decade several attempts have been made to improve definition of *thalassemia intermedia*. New clinical and molecular criteria are needed to establish prognosis and to offer different therapeutic options, at least to selected groups of patients. Moreover, this clarification becomes crucial to quantifying the prevalence of thalassemia intermedia patients in different countries for purposing of genetic counselling.

Correspondence: Clara Camaschella, Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, Ospedale S. Luigi Gonzaga, 10043 Orbassano (TO), Italy.

Acknowledgments: this work was supported in part by Progetto Nazionale Talassemia ISS, Rome and Murst 60%, Rome.

Received August 4, 1994; accepted October 25, 1994.

Problems in diagnosis

One of the major clinical criteria proposed for identifying patients with thalassemia intermedia is age at presentation. Some patients affected by very mild forms may be recognized in the second decade of life or even later, but usually the discrimination between thalassemia major and intermedia is a pediatric task. Modell and Berdoukas² have suggested that when homozygous patients present at two years of age with hemoglobin above 7 g/dL and do well clinically, it is wise to offer only supportive treatment and a proper follow-up. Some of these patients may indeed have thalassemia intermedia. Children who continue to grow normally at three years and do not develop evident bone changes without regular transfusions are definitely considered as thalassemia intermedia. Some of these patients develop hypersplenism, even severe, in following years and require splenectomy. Some may become transfusion dependent in adulthood. In our retrospective series of 165 thalassemia intermedia patients of Italian origin, 95% were diagnosed after two years of age.⁵ Forty-three percent had never been transfused, 30% were occasionally transfused during infections, pregnancy or surgery and 28% became transfusion dependent in adulthood. Figure 1 shows the age at first transfusion in this series of thalassemia intermedia patients.

Hemoglobin level at presentation and hemoglobin composition play an important role in the diagnosis of thalassemia intermedia. Although absolute Hb values cannot be established, usually patients presenting after two years of age have an average hemoglobin above 7.0 g/dL. In adult life some thalassemia intermedia patients who tolerate even lower hemoglobin levels well (Hb 6.0-6.5g/dL) may be encountered.

Hb F at diagnosis can be extremely variable, according to the genetic defect: β^0 homozygotes have almost 100% Hb F, whereas variable amounts of Hb A are present in β^+ homozygotes.

Due to the increased oxygen affinity of Hb F ($\alpha_2\gamma_2$), Hb composition has a remarkable effect on tissue hypoxia and on erythropoietin-medi-

ated erythropoiesis expansion. Hb F is remarkably variable among patients, ranging from 5 to 100%. No direct correlation exists between total Hb and Hb F levels, suggesting that different mechanisms are responsible for γ chain production. One mechanism is the preferential survival of F-cells, which operates when β chain synthesis is absent or very low. It is based on selection of cells intrinsically able to produce more γ chains and is of no use in compensating for a severe anemia.^{1,6} A second is an inheritable ability to produce more Hb F per cell (as occurs in $\delta\beta$ -thalassemia or *hereditary persistence of fetal hemoglobin*, also called HPFH) or to increase the number of F cells (as in the heterocellular type of HPFH).^{6,7} This mechanism is useful for partially correcting the degree of anemia since it reduces the α /non α chain imbalance within the red cell precursors, thus decreasing ineffective erythropoiesis.

Family studies often show the classic β -thalassemia trait in both parents. However, they are relevant in cases of coinheritance of α -thalassemia or atypical cases (silent forms of thalassemia or HPFH).

In the evaluation of thalassemia intermedia and in the decision of whether to start transfu-

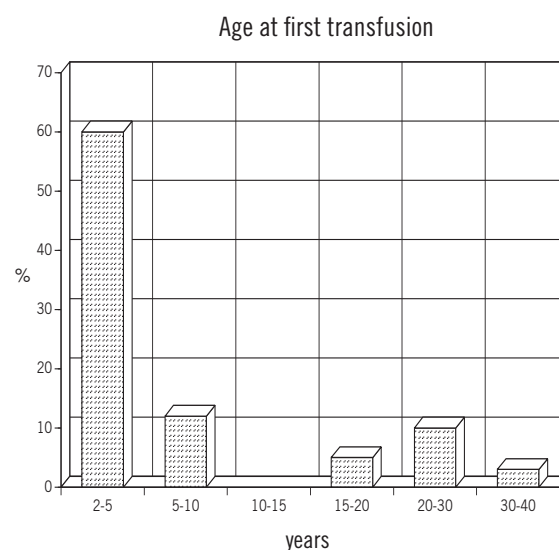


Figure 1. Age at first transfusion in a series of thalassemia intermedia patients (6).

sions in childhood, a major point could be the quantitation of erythropoietic expansion and of ineffective erythropoiesis. A simple index proposed to estimate bone marrow expansion was the width of the medullary cavity, as derived from X-rays by measuring the internal diameter of the second metacarpal bone. High values or recent increases were proposed as an indication to initiate transfusions.⁸ This index, however, is scarcely used in clinical practice. A tentative correlation of total Hb and Hb F levels with erythropoietic expansion was recently carried out by Galanello et al.,⁹ who evaluated serum erythropoietin and circulating transferrin receptor (TfR), which in the absence of iron deficiency provides the best estimate of total erythropoiesis.^{10,11} In untransfused patients with comparable degree of anemia, subjects with *high* (more than 40%) Hb F had significantly higher erythropoietin and TfR levels than those with *low* (less than 40%) Hb F. More extensive studies are needed to evaluate the usefulness of these functional determinations in categorizing thalassemia intermedia patients for the purpose of deciding whether to transfuse.

Recently, knowledge about the molecular pathology of β -thalassemia syndromes has permitted direct investigation of the β -globin gene mutations in thalassaemic patients, providing a new tool which can help in making the diagnosis.¹²

Molecular basis of thalassemia intermedia

The vast majority of patients with thalassemia intermedia have both β genes affected by inactivating mutations. A minority are carriers of a single β -thalassemia mutation.¹³ In Italy it can be estimated that 10-20% of β -thalassemia homozygotes, but only occasional heterozygotes, present the clinical phenotype of thalassemia intermedia. These proportions may vary in different populations.

The main genotypes occurring in thalassemia intermedia are summarized in Table 1.

Homozygosity or compound heterozygosity for mild mutations

Some β -thalassemia mutations allow a signifi-

cant residual production of normal β chains and are mild both *in vitro* and *in vivo*. The first description of homozygosity for a mild mutation was the *Portuguese* thalassemia,¹⁴ due to the homozygosity for the IVS I 6 C/T mutation. *In vitro* this substitution creates an alternative splice site which is rarely used so that significant amounts of normal β chains are produced.¹⁵ There is general agreement that this mutation is also mild *in vivo*. Heterozygotes for IVS I 6 C/T have less severe red cell alterations as compared to usual reference values.¹⁶ In the homozygous state the phenotype is usually that of thalassemia intermedia, although some patients with thalassemia major have been reported.¹⁷ On the contrary some compound heterozygotes for IVS I 6 C/T with a severe mutation are transfusion dependent.

A second group of mild mutations affects DNA sequences necessary for mRNA processing, i.e. promoter, polyadenylation signal, or CAP site. The most common mutations of this type in the Mediterranean area are -87 C/G and -101 C/T,¹⁸⁻²² both of which are associated with a mild decrease in β gene transcription. Transcriptional analysis of -87 C/G in Hela cells produces 20-30% residual β -activity.¹⁵ Similar experiments in MEL cells for -101 indicate a two- threefold decrease in promoter activity as compared to normal.¹⁸ Carriers of -87 C/G have larger and better hemoglobinized red cells¹⁶ than carriers of severe mutations, whereas -101 C/T

Table 1. Genotype combinations in thalassemia intermedia.

- | |
|---|
| 1. Homozygosity or compound heterozygosity for <i>mild</i> mutations |
| 2. Homozygosity or compound heterozygosity for <i>severe</i> mutations plus
- α -thalassemia
- heterocellular HPFH |
| 3. Compound heterozygosity for β -thalassemia and
- $\delta\beta$ -thalassemia
- deletion or non deletion HPFH |
| 4. Heterozygosity for β -thalassemia and triplicated α -locus |
| 5. Heterozygosity for a dominant form of β -thalassemia |

Table 2. Mild β -thalassemia mutations in Mediterraneans.

Mutation type	Ethnic group	Reference
-101 C→T	Turkish, Italian	(17,19)
-90 C→T	Portuguese	(24)
-87 C→G	Mediterranean	(28,22)
-87 C→T	German/Italian	(21, 23)
-86 C→A	Italian	(21)
Hb E cd 26 G→A	Mediterranean	(25)
Hb Knossos cd 27 G→T	Greek	(26)
IVS I 6 T→C	Mediterranean	(14)
IVS II 844 C→G	Italian	(20)
AATAAA→AATGAA	Mediterranean	(27)

carriers are hematologically normal.¹⁸ The combination of -101 C/T with a severe mutation produces a mild intermedia phenotype, just slightly more severe than the heterozygous condition.²⁰ The case of a single homozygote for -87 C/G has been described; the person was over 60, had a mild thalassaemic condition and enjoyed an almost normal quality of life.²³ Homozygotes for -101 C/T have not been reported; it is likely that they are hematologically normal. A C/T mutation at position -87 has also been described that affects CACCC box and causes approximately a 50% reduction of the activity in HeLa cells.²⁴ Other mild mutants occasionally found in Mediterranean countries are -90 C/T,²⁵ IVS2-844 C/G²¹ and two thalassaemic hemoglobinopathies: Hb E (GAG·AAG = 26 Glu·Lys)²⁶ and Hb Knossos (GCC·TCC = 27 Ala·Ser).²⁷ These substitutions create alternative splice signals that are rarely used. A polyadenylation mutant which changes AATAAA to AATGAA has been reported in Greeks.²⁸ A list of mild mutations found in the Mediterranean area is reported in Table 2.

Homozygosity or compound heterozygosity for severe mutations

Some homozygous patients, in spite of having two mutations which fully inactivate both β -genes, have a mild phenotype or at least a late presentation of the disease.²⁹ In these cases

other genetic (or acquired) factors are responsible for the benign clinical course. Some of these interacting factors are linked to the β -cluster and others are not. The most well-known examples are α -thalassaemia and HPFH; others may play a role in stress erythropoiesis, such as -158 $^{\text{G}}\gamma$ C/T³⁰ or other polymorphic sites in the β -cluster (β -haplotypes), which have been associated with increased Hb F.

Determinants linked to the β -cluster

It is well known that the association of HPFH (both deletion and non deletion type) with β -thalassaemia gives rise to a mild thalassaemic disorder, since the high level of γ chains compensates for the absence of β chains.^{1,7} Even the association of a $\delta\beta$ -thalassaemia characterized by a moderate increase in the ability to produce Hb F and a β -thalassaemia mutant results in mild conditions.⁷ The association of heterocellular HPFH with severe homozygous β -thalassaemia is also a mild condition, in spite of 100% Hb F.³⁰

The C/T substitution at position -158 upstream from the $^{\text{G}}\gamma$ gene (Xmn I polymorphism) was originally related to high $^{\text{G}}\gamma$ and high Hb F in sickle cell anemia patients.^{30,32} Some β -thalassaemia homozygous patients with Xmn polymorphism have a benign clinical course.^{29,33,34} However, this polymorphism can also be found in thalassaemia major.^{6,29} The Hb F increase in thalassaemia intermedia subjects carrying this substitution is less documented than in sickle cell anemia. Also, a sequence variation (ATA insertion and a T deletion) at approximately 530 bp 5' to the β gene has been described as being able to bind a repressor protein.³⁵ This configuration was considered as a possible causal mutation in a thalassaemic individual without mutation in the structural sequences of the β -globin gene.³⁶ Some authors have suggested that specific configurations are associated with the ability to express more Hb F.³⁷ Other studies considered these variations to be neutral polymorphisms.^{13,38,39}

A region of the β -cluster with a major influence on globin gene expression is β -LCR.⁴⁰ Polymorphisms in the major domain of this region

(hypersensitive site 2 or HS2) have been implicated in the modulation of Hb F in sickle cell patients,⁴¹ and distinct configurations of a simple LCR HS2 repeat are in strong association with each of the major β^S haplotypes and are related to Hb F production.⁴² The role of polymorphic differences along the β cluster in modulating the Hb F increase in thalassemia intermedia is uncertain.

Determinants not linked to the β -cluster

α -thalassemia

The coinheritance of α thalassemia is able to ameliorate the clinical course of homozygous β -thalassemia patients.^{29,43,44} Deletion of two α genes is required to modify the excess of α chains in β^0 -thalassemia, since the deletion of a single gene has little effect on the phenotype in Sardinian β^0 patients.²⁹ Deletion of a single gene can ameliorate the phenotype in carriers of β^+ mutations.³³ A study of interacting α -thalassemia deletion mutants in continental Italy revealed that only a minority of the thalassemia major forms had concomitant α -thalassemia, but up to 19% of intermedia patients with severe mutations had α -thalassemia of the 3.7 type.⁶ We hypothesize that the co-inheritance of other α -thalassemia determinants which are less frequent in Italy, such as $-\alpha^{20,5}$, $\alpha^{5,2}$,⁴⁵ $\alpha^{5,2}-\text{Cal}$,^{46,47} plays a minor role in thalassemia intermedia.

Unknown factors

Some forms of heterocellular *hereditary persistence of fetal hemoglobin*^{33,48} and rare cases of β -thalassemia⁴⁹⁻⁵⁰ are due to still unknown determinants not linked to the β -cluster. These determinants could modulate Hb F or Hb A production as well as stimulate α -chain proteolysis.¹ Their role in thalassemia intermedia is difficult to assess at present and remains speculative.

Among the general population approximately 10% of people show high levels of F cells and increased levels of Hb F (up to 3%).^{7, 51,52} The interaction of heterocellular HPFH with homozygous β -thalassemia results in an improvement of the phenotype.³⁰ However, the

genetics of heterocellular HPFH is quite complex. Certainly some types are related to genes not linked to the β -cluster. There is evidence suggesting an X-linked inheritance.⁵¹⁻⁵³ Recently, a major candidate gene for heterocellular HPFH was identified⁵⁴ and mapped in a large Indian family on the long arm of chromosome 6.⁵⁵

At present the reason for the mild clinical course in a significant percentage of cases of thalassemia intermedia remains unexplained; in a recent study several patients were found to be homozygous or compound heterozygous for severe mutations without interacting α -thalassemia/HPFH, or to be heterozygotes with a normal number of α genes.⁶

It is likely that in the future a better understanding of the factors which play a role in regulating globin gene expression may shed some light on this problems.

Heterozygosity for β -thalassemia

Occasional patients with heterozygous β -thalassemia have a very mild thalassemia intermedia phenotype. Molecular biology studies in recent years have shown that these patients may be carriers of unusual genetic conditions, such as the interaction of triplicated a locus/heterozygous β -thalassemia or of *inclusion body* β -thalassemia.

Triplicated α locus/heterozygous β thalassemia

The importance of the chain imbalance in determining the severity of thalassemia is well exemplified by the interaction of triplicated α locus with heterozygous β thalassemia,⁵⁶⁻⁶⁰ which can determine a clinical phenotype of mild thalassemia intermedia.

Homozygosity for the triplication is definitely associated with a phenotype of thalassemia intermedia.^{56,61} In some cases heterozygosity for the triplication may result in a condition overlapping β -thalassemia traits.⁵⁻⁶⁰

Two major factors influence the disease phenotype: the type of β mutation (usually β^0 or β^+ severe mutations in thalassemia intermedia) and the ability of the affected chromosome to synthesize α chains. Perhaps even the proteolytic activity of the cell might play some role.

Although the triplicated α locus/ β -thalassemia interaction is considered rather rare, a systematic study of the incidence of this interaction in different populations is lacking.

Dominant forms of thalassemia

Dominant forms of thalassemia or *inclusion body* thalassemia are determined by mutations in the β -globin gene which cause a thalassemia intermedia-like phenotype in the heterozygous state. Nucleotide substitutions described in these mutants produce either structurally highly unstable variants or prematurely truncated or elongated chains.⁶² The result is a highly unstable β -chain incapable of forming a tetramer with α chains. The unstable β -chains precipitate within the red cell precursors and give rise to large inclusions that result in a remarkable decrease of effective β chains.⁶³ The cell proteolytic system in these cases has to deal both with the unstable chain *per se* and with the consequent α chain excess. This process occurs not only within erythrocytes but within erythroblasts as well, resulting in a prevailing ineffective erythropoiesis. In contrast, in hemolytic anemia associated with unstable hemoglobins the damage occurs mostly in the circulating red cell. The pathophysiologic mechanism underlying these hemoglobinopathies strengthens the importance of the efficiency of cell proteolytic systems in dealing with excess chains.^{62,63} We might speculate that a deficiency in this machinery could play a role even in other thalassemia intermedia conditions.

These hemoglobinopathies are rare and often *de novo* mutations. A condition of this type should be suspected in patients with an intermedia phenotype but no positive family history.

A practical approach to interpreting molecular data in the diagnosis of thalassemia intermedia is shown in Figure 2.

Clinical problems

In thalassemia intermedia the chronic anemia itself might be responsible for several clinical problems. A relative folic acid deficiency is a common feature in untreated patients.² Hypersplenism due to spleen enlargement caused by

excessive red cell breakdown or red cell pooling and extramedullary hematopoiesis may exacerbate anemia. Iron overload is frequently recorded even in untransfused thalassemia intermedia patients because of ineffective erythropoiesis, peripheral red cell breakdown and increased intestinal iron absorption. This condition becomes evident mainly after the second or third decade of life, and the severity of overload varies from patient to patient. In agreement with previous reports,⁶⁴⁻⁶⁵ we observed that thalassemia intermedia patients who undergo splenectomy have significantly higher levels of transferrin saturation, ferritin and urinary iron excretion than their non splenectomized coun-

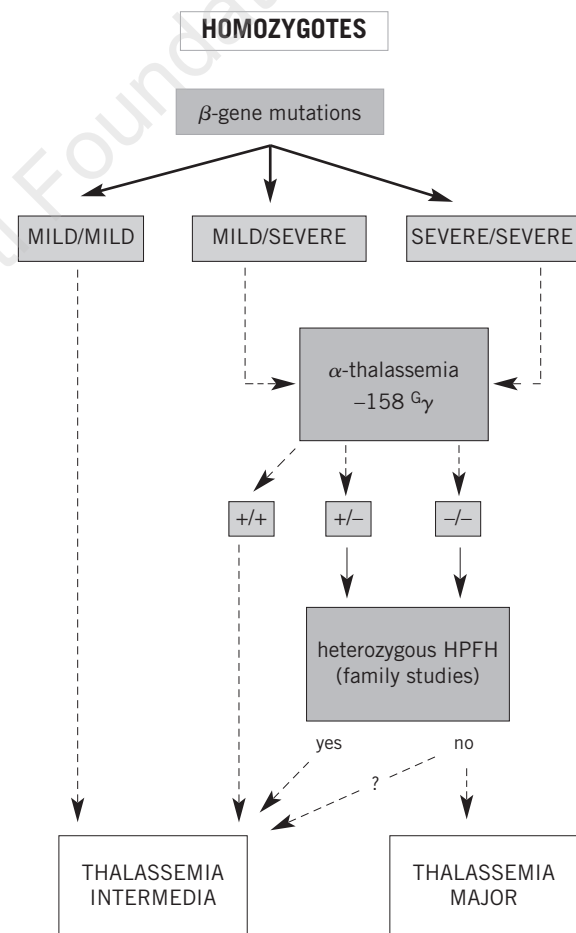


Figure 2. Practical approach to the investigating of thalassemia intermedia using molecular studies presently available. +/+ refers to the presence of both α -thalassemia and $-158 G_{\gamma} C \rightarrow T$ mutation; +/- to the presence of one factor; -/- to the absence of both.

terparts. However, these indirect parameters for measuring iron overload probably do not reflect hepatic iron because they do not correlate with the iron concentration measured in liver biopsy at the time of splenectomy.⁶⁶

Masses of heterotopic marrow often develop in patients as a result of continuous erythropoietic stress. Intrathoracic localizations are the most common, although extramedullary erythropoiesis has been described along the spinal cord and in parenchymal organs such as the liver.² Problems may arise from compression of the spinal cord or other organs and prompt treatment may be required.⁶⁷⁻⁶⁹ A large number of untransfused adult thalassemia intermedia patients present masses of heterotopic marrow at conventional radiology. In a long-term follow-up using CT scan and magnetic resonance imaging we showed that often these masses progressively reduce their functional activity because of fat substitution. In this case they do not require any type of therapy.⁷⁰⁻⁷¹

Leg ulcers are common in thalassemia intermedia. Several factors contribute to their pathogenesis: chronic anemia, reduced oxygen delivery to the distal regions, venous stasis and erythrocytes rheologic abnormalities.⁷²

Thrombotic events have been sporadically reported, suggesting a thrombotic tendency in thalassemia intermedia patients.⁷³ The mechanisms involved are not yet fully understood, although decreased levels of proteins C and S have been reported.⁷⁴⁻⁷⁵ A comprehensive study of coagulation and fibrinolysis in a subset of 30 thalassemia intermedia patients recently showed that there are signs of activated coagulation accompanied by secondary fibrinolysis in splenectomized thalassemia intermedia patients as compared to non splenectomized ones.⁷⁶

Treatment

Treatment of thalassemia intermedia should be conservative for as long as possible.^{2,77} Continuous folic acid supplementation is recommended in all thalassemia intermedia patients. Splenectomy is the first therapeutic approach to consider to correct anemia before starting regular transfusions. The age at splenectomy in tha-

lassemia intermedia is usually higher than in thalassemia major. During surgery it is useful to perform a liver biopsy to assess liver damage and iron deposition, and cholecystectomy when gallstones or sludges are present. Penicillin prophylaxis against pneumococcal infection is indicated in children but has not been demonstrated to be of clear value in adults. Early antibiotic treatment of non specific febrile illnesses in asplenic patients, while not supported by controlled trials, seems to be an acceptable compromise in adults.⁷⁸ Administration of a polyvalent pneumococcal vaccine is mandatory before splenectomy to avoid overwhelming infections.⁷⁹ Because of the demonstrated thrombotic tendency of these patients, thrombotic events must be guarded against during all surgical procedures by subcutaneous heparin administration.⁷⁶ On the other hand, the benefits of antiplatelet treatment have not been proved.

Indications to transfuse include growth, sexual maturation, intercurrent infections, surgery, pregnancy, healing of leg ulcers. As a result of retrospective analysis of adult thalassemia intermedia patients,⁶ we suggest that in severe thalassemia intermedia genotypes the Hb level should be maintained around 10-11 g/dL by transfusions during infancy and adolescence in order to avoid bone deformities. The transfusion regimen in these cases should be reevaluated in adult life. When transfusions are started in adult life, it is suggested to maintain lower pretransfusional Hb levels (9-10 Hb g/dL) than for thalassemia major. It has been reported that for some patients starting transfusions late in life may result in the production of alloantibodies to red cells.⁸⁰⁻⁸¹ For this reason it is important to check compatibility accurately for Rh, Kell, Kidd and Duffy blood groups as well.

Chelation treatment with desferrioxamine (DF) is obviously indicated when a transfusion regimen is started. It is even indicated in untransfused patients when iron overload is documented.⁷⁶ Although there is no evidence of DF damage during pregnancy or breast-feeding, it is recommended to discontinue DF as soon as pregnancy is confirmed.

Leg ulcers are often refractory to conventional

treatment. Blood transfusions may favor ulcer healing, but often the ulcers recur after treatment is withdrawn. Very recently it has been reported that in a small number of patients a short treatment with new agents – such as recombinant erythropoietin or hydroxyurea (see below) – may be associated with a rapid improvement and even a healing of leg ulcers.^{82,83} Larger studies are needed to document the real effectiveness of these treatments.

When heterotopic marrow causes compression, small doses of radiotherapy (10-16 Gy) have been shown to relieve symptoms rapidly.^{68,84} In our experience and that of others, blood transfusions offer the same rapid effect of relieving compression.^{67,69}

New trends

Although bone marrow transplantation has been performed in patients with thalassemia intermedia, in view of the long survival of these patients with conventional treatment, this therapeutic approach should be evaluated case by case at present and cannot be proposed as standard therapy for all patients with a suitable marrow donor.^{85,86}

Thalassemia intermedia patients should potentially benefit more than their thalassemia major counterparts from pharmacological manipulation of the Hb switching proposed to increase Hb F. In the last few years it has been shown that several cytotoxic drugs – such as 5'-azacytidine (5'-Aza) and hydroxyurea (Hy) – are able to increase Hb F production in adult hemoglobinopathy patients.⁸⁷⁻⁸⁹ The mechanism of the Hb F increase was originally reported to be a direct effect of 5'-Aza on γ -globin gene methylation, but subsequently it was shown to be due prevalently to a selection of erythroid precursors able to produce Hb F. Hy has been shown to be the most effective drug in sickle cell anemia. In view of these results few cases of thalassemia intermedia have been treated and the results are still under evaluation.⁹⁰ Reversion of the Hb composition to a fetal pattern can also be achieved by other drugs such as butyrate^{91,92} and short chain fatty acids.⁹³ A short trial of continuous i.v. infusion of arginine butyrate at escalating doses in a small series of

patients affected by thalassemia or hemoglobinopathies demonstrated a remarkable increase in both total Hb and Hb F.⁹¹ Other preliminary experiences reported failure in some cases treated with the same drug.⁹⁴

Trials with recombinant human erythropoietin (Epo) have recently been started in selected patients. The rationale for this type of treatment is still controversial. Endogenous Epo levels in thalassemia intermedia are heterogeneous and in some cases inappropriate for the degree of anemia.^{95,96} The majority of patients treated with high-dose Epo (400-900 U/kg subcutaneously) three times weekly showed some degree of response such as an increase of total Hb, usually not of Hb F.^{97,98} This effect was ascribed to improvement of the erythropoietic activity of the bone marrow by Epo. The cost of this drug limits its use on a large scale.⁹⁹ A short-term treatment could be attempted in situations of severe anemia (e.g. following intercurrent infections) in alloimmunized patients who cannot tolerate blood transfusions.

Successful results have been obtained with combinations of Epo and Hy in some patients with sickle cell anemia.¹⁰⁰ Trials with the same combination and with an association of Epo and butyrate are under evaluation in thalassemia intermedia.

References

1. Weatherall DJ, Clegg JB. The thalassemia syndromes. 3rd ed. Oxford:Blackwell Sci Publ, 1981.
2. Modell B, Berdoukas V. Thalassaemia intermedia. In: The clinical approach to thalassemia. London:Grune & Stratton, 1984:242-55.
3. Piomelli S, Dsanoff S, Becker M, Lipera M, Travis S. Prevention of bone malformations and cardiomegaly in Cooley's anemia by early hypertransfusion regimen. *Ann NY Acad Sci* 1969; 165:427.
4. Propper RD, Button LN, Nathan DG. New approaches to the transfusion management of thalassemia. *Blood* 1980; 55:55-9.
5. Kazazian HH, Jr. The thalassemia syndromes: molecular basis and prenatal diagnosis in 1990. *Semin Hematol* 1990; 27:209-28.
6. Camaschella C, Mazza U, Roetto A, et al. Genetic interactions in thalassemia intermedia: analysis of β -mutations, α -genotype, γ -promoters and β -LCR hypersensitive site 2 and 4 in Italian patients. *Am J Hematol* 1994; in press.
7. Bunn HF, Forget BG. The thalassemias: molecular pathogenesis. In: Hemoglobin: molecular, genetic and clinical aspects. Philadelphia:WB Saunders Co, 1986:225-321.
8. Wood WG. Increased Hb F in adult life. In: Higgs DR, Weatherall DJ, eds. The haemoglobinopathies, Bailliere's Clin Haematol 1993; 6:177-213.

9. Sbyrakis S, Karagiorga-Lagana S, Voskaki I, Efthimou H, Karaklis A. A simple index for initiating transfusion treatment in β -thalassaemia intermedia. *Br J Haematol* 1987; 67:479-85.
10. Galanello R, Barella S, Turco MP, et al. Serum erythropoietin and erythropoiesis in high and low fetal hemoglobin β -thalassaemia intermedia patients. *Blood* 1994; 83:561-5.
11. Kohgo Y, Niitsui Y, Kondo H, et al. Serum transferrin receptors as a new index of erythropoiesis. *Blood* 1987; 70:1955-9.
12. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 1990; 75:102-7.
13. Thein SL. β -Thalassaemia. In: Higgs DR, Weatherall DJ, eds. *The haemoglobinopathies*. Bailliere's Clin Haematol 1993; 6:151-75.
14. Tamagnini GP, Lopes MC, Castanheira ME, Wainscoat JS. β^+ thalassaemia Portuguese type: clinical, haematological and molecular studies of a newly defined form of β -thalassaemia. *Br J Haematol* 1983; 54:189-200.
15. Treisman R, Orkin SH, Maniatis T. Specific transcription and RNA splicing defects in five cloned β -thalassaemia genes. *Nature* 1983; 302:591-6.
16. Rosatelli C, Leoni GB, Tuveri T, et al. Heterozygous β -thalassaemia: relationship between the hematological phenotype and the type of β -thalassaemia mutation. *Am J Hematol* 1992; 39:1-4.
17. Chehab FF, der Kaloustian V, Khouri FP, Deeb SS, Kan YW. The molecular basis of β -thalassaemia in Lebanon: application to prenatal diagnosis. *Blood* 1987; 69:1141-5.
18. Diaz-Chico JC, Yang KG, Stoming TA. Mild and severe β -thalassaemia among homozygotes from Turkey: identification of the types by hybridization of amplified DNA with synthetic probes. *Blood* 1988; 71:248-53.
19. Rosatelli MC, Oggiano L, Leoni GB, et al. Thalassaemia intermedia resulting from a mild β -thalassaemia mutation. *Blood* 1989; 73:601-5.
20. Ristaldi MS, Murru S, Loudianos G, et al. The C \rightarrow T substitution in the distal CACCC box of the β -globin gene promoter is a common cause of silent β thalassaemia in the Italian population. *Br J Haematol* 1990; 74:480-6.
21. Murru S, Loudianos G, Deiana M, et al. Molecular characterization of β -thalassaemia intermedia in patients of Italian descent and identification of three novel β -thalassaemia mutations. *Blood* 1991; 77:1342-7.
22. Meloni A, Rosatelli C, Faà V, et al. Promoter mutations producing mild β -thalassaemia in the Italian population. *Br J Haematol* 1992; 80:222-6.
23. Camaschella C, Alfano A, Gottardi E, et al. The homozygous state for the -87 C \rightarrow G β^+ thalassaemia. *Br J Haematol* 1990; 75:132-8.
24. Kulozik AE, Bellan-Koch A, Bail S, Kohne E, Kleihauer E. Thalassaemia intermedia: moderate reduction of β -globin gene transcriptional activity by a novel mutation of the proximal CACCC promoter element. *Blood* 1991; 77:2054-8.
25. Faustino P, Osorio-Almeida L, Barbot J, et al. Novel promoter and splice junction defects add to the genetic clinical or geographical heterogeneity of β -thalassaemia in the Portuguese population. *Hum Genet* 1992; 89:573-6.
26. Orkin SH, Kazazian HH Jr, Antonarakis SE, Ostrer H, Goff SC, Sexton JP. Abnormal RNA processing due to the exon mutation of βE globin gene. *Nature* 1982; 300:768-70.
27. Orkin SH, Antonarakis S, Loukopoulos D. Abnormal processing of β Knossos RNA. *Blood* 1984; 64:311-3.
28. Jankovich JL, Efremov GD, Petkov G, et al. Two novel polyadenylation mutations leading to β^+ -thalassaemia. *Br J Haematol* 1990; 75:122-6.
29. Galanello R, Dessi E, Melis MA, et al. Molecular analysis of β^0 -thalassaemia intermedia in Sardinia. *Blood* 1989; 74:823-7.
30. Gilman JG, Huisman THJ. DNA sequence variation associated with elevated fetal G γ globin production. *Blood* 1985; 66:783-7.
31. Cappellini MD, Fiorelli G, Bernini LF. Interaction between homozygous β^0 -thalassaemia and the Swiss type of hereditary persistence of fetal haemoglobin. *Br J Haematol* 1981; 48:561-72.
32. Labie D, Pagnier J, Lapoumeroulie C, et al. Common haplotype dependency of high G γ globin gene expression and high Hb F in β -thalassaemia and sickle cell anemia patients. *Proc Natl Acad Sci USA* 1985; 82:2111-4.
33. Thein SL, Wainscoat JS, Sampietro M, et al. Association of thalassaemia intermedia with a β -globin gene haplotype. *Br J Haematol* 1987; 65:367-73.
34. Winichagon P, Thonglairoam V, Fucharoen S, Wilairat P, Fukumaki Y, Wasi P. Severity differences in β -thalassaemia/haemoglobin E syndromes: implication of genetic factors. *Br J Haematol* 1993; 83:633-9.
35. Berg PE, William DM, Qian RL, et al. A common protein binds to two silencers 5' to the human β -gene. *Nucl Acid Res* 1989; 17:8833-5.
36. Semenza GL, Delgrosso K, Poncz M, et al. The silent carrier allele: β -thalassaemia without a mutation in the β -globin gene or its immediate flanking regions. *Cell* 1984; 39:123-8.
37. Ragusa A, Lombardo M, Beldjord C, et al. Genetic epidemiology of β -thalassaemia in Sicily: do sequence 5' to the Gg gene and 5' to the β gene interact to enhance Hb F expression in β -thalassaemia? *Am J Hematol* 1992; 40:199-206.
38. Wong SC, Stoning TA, Efremov GD, Huisman THJ. High frequencies of a rearrangement (+ATA; -T) at -530 to the β -globin gene in different populations indicate the absence of a correlation with a silent β -thalassaemia determinant. *Hemoglobin* 1989; 13:1-5.
39. Dimovski AJ, Adakile AD, Divoky V, Baysal E, Huisman THJ. Polymorphic pattern of the (AT) \times (T)y motif at -530 5' to the β -globin gene in over 40 patients homozygous for various β -thalassaemia mutations. *Am J Hematol* 1994; 45:51-7.
40. Grosveld F, Blom van Assendelf G, Greaver DR, Koliass G. Position independent, high level expression of the human β -globin gene in transgenic mice. *Cell* 1987; 31:975-85.
41. Oner C, Dimovski AJ, Altay C. Sequence variations in the 5' hypersensitive site-2 of the locus control region in βS chromosomes are associated with different levels of fetal globin in hemoglobin S homozygotes. *Blood* 1992; 79:813-9.
42. Perichon B, Ragusa A, Lapoumeroulie C, et al. Interethnic polymorphism of the β -globin locus control region (LCR) in sickle cell anemia patients. *Hum Genet* 1993; 91:464-8.
43. Wainscoat JS, Kanavakis E, Wood WG. Thalassaemia intermedia in Cyprus: the interaction of α and β -thalassaemia. *Br J Haematol* 1983; 53:411-6.
44. Antonarakis SE, Kang J, Lam VMS, Tam JWO, Li AMC. Molecular characterization of the β -globin gene mutations in patients with β -thalassaemia intermedia in South China. *Br J Haematol* 1988; 70:357-61.
45. Lacerra G, Fioretti G, De Angioletti M, et al. (α) $\alpha^{5.3}$: a novel α^+ -thalassaemia deletion with the breakpoints in the α_2 -globin gene and in close proximity to an Alu family repeat between the α_2 and α_1 globin genes. *Blood* 1991; 78:2740-6.
46. Fortina P, Dianzani I, Serra A, et al. A newly characterized α -thalassaemia-1 deletion removes the entire α -like globin gene cluster in an Italian family. *Br J Haematol* 1991; 78:529-34.
47. Fortina P, Parrella T, Sartore M, et al. Interaction of a rare illegitimate recombination event and a poly A addition site mutation resulting in a severe form of α thalassaemia. *Blood* 1994; 83:3356-62.
48. Gianni AM, Bregni M, Cappellini MD, et al. A gene controlling fetal hemoglobin expression in adults is not linked to the non- α globin cluster. *EMBO J* 1983; 2:921-5.
49. Murru S, Loudianos G, Porcu S, et al. A β -thalassaemia phenotype not linked to the β -globin cluster in an Italian family. *Br*

- J Haematol 1992; 81:283-7.
50. Thein SL, Wood WG, Wickramasinghe SN, Galvin MC. β -thalassemia unlinked to the β -globin locus in an English family. *Blood* 1993; 82:961-7.
 51. Miyoshi K, Kaneto Y, Kawai H, et al. X-linked dominant control of F-cells in normal adult life: characterization of the Swiss type of hereditary persistence of fetal hemoglobin regulated dominantly by gene(s) on X-chromosome. *Blood* 1988; 72:1854-60.
 52. Sampietro M, Thein SL, Contreras M, Pazmany L. Variation of Hb F and F-cell number with the $^6\gamma$ Xmn I (C \rightarrow T) polymorphism in normal individuals. *Blood* 1992; 79:832-3.
 53. Chang YC, Smith KD, Moore RD, Serjeant G, Dover GJ. The X-linked F cell production locus: genetic mapping and role in fetal hemoglobin production. Ninth Conference on Hemoglobin Switching Rosario Resort, Orcas Island, Washington, June 10-15, 1994.
 54. Thein SL, Sampietro M, Rohde K, et al. Detection of a major gene for heterocellular hereditary persistence of fetal Hemoglobin after accounting for genetic modifiers. *Am J Hum Genet* 1994; 54:214-28.
 55. Craig JE, Fisher C, Rochette J, et al. Localization of a major gene for heterocellular HPPH to a region of chromosome 6q. Ninth Conference on Hemoglobin Switching Rosario Resort, Orcas Island, Washington, June 10-15, 1994.
 56. Galanello R, Ruggeri R, Paglietti E, Addis M, Melis MA, Cao A. A family with segregating triplicated α -globin loci and β -thalassemia. *Blood* 1983; 62:1035-40.
 57. Sampietro M, Cazzola M, Cappellini MD, Fiorelli G. The triplicated α -gene locus and heterozygous β -thalassemia: a case of thalassemia intermedia. *Br J Haematol* 1983; 55:61-70.
 58. Camaschella C, Bertero MT, Serra A, et al. A benign form of thalassemia intermedia may be determined by the interaction of triplicated α locus and heterozygous β -thalassemia. *Br J Haematol* 1989; 66:103-7.
 59. Kulozik AE, Thein SL, Wainscoat R, et al. Thalassemia intermedia: interaction of the triple α -globin gene arrangement and heterozygous β -thalassemia. *Br J Haematol* 1987; 66:109-12.
 60. Kanavakis E, Metaxotou-Mavromati A, Kattamis C, et al. Thalassemia intermedia: interaction of the triple α -globin gene arrangement and heterozygous β -thalassemia. *Br J Haematol* 1987; 66:109-12.
 61. Kazazian HH, Jr, Boehm CD. Mutation analysis in the clinics. In: Mutations in the human genome. May 13-14, 1993. Turin: Orta S. Giulio MAF, 1993:28-9.
 62. Thein SL, Hesketh C, Taylor P, et al. Molecular basis for dominantly inherited inclusion body β -thalassemia. *Proc Natl Acad Sci USA* 1990; 87:3924-8.
 63. Kazazian HH Jr, Dowling CE, Hurwitz RL, Coleman M, Stopeck A, Adams III JG. Dominant thalassemia like phenotypes associated with mutations in exon 3 of the β -globin gene. *Blood* 1992; 79:3014-8.
 64. Pootrakul P, Vougsmasa V, Laongpanich P, Wasi P. Serum ferritin levels in thalassemias and the effect of splenectomy. *Acta Haematologica* 1981; 66:244-50.
 65. Pootrakul P, Kitcharoen K, Yansukon P, et al. The effect of erythroid hyperplasia on iron balance. *Blood* 1988; 71:1124-9.
 66. Fiorelli G, Fargion S, Piperno A, Battafarano N, Cappellini MD. Iron metabolism in thalassemia intermedia. *Haematologica* 1990; 75:89-95.
 67. Camaschella C, Saggio G, Pich PG, et al. Thoracic extramedullary hematopoiesis in thalassemia intermedia during adult life: report of 9 cases. *Haematologica* 1981; 66:613-26.
 68. Angelucci E, Baronciani D. Extramedullary bone marrow tumor in thalassemia. *Haematologica* 1994; 79:393-4.
 69. Mancuso P, Zingale A, Basile L, Chiaramonte I, Tropea R. Cauda equina compression syndrome in a patient affected by thalassemia intermedia: complete regression with blood transfusion therapy. *Childs Nerv Syst* 1993; 9:440-1.
 70. Martin J, Palacio A, Petit J, et al. Fatty transformation of thoracic extramedullary hematopoiesis following splenectomy: CT features. *J Comput Assist Tomogr* 1990; 14:477-8.
 71. Nastri G, Cappelletti M, Cappellini MD, Bonelli N, Balzani A, Fiorelli G. Localizzazioni toraciche dell'eritropoiesi extramedullare. *Radiol Med* 1992; 84:576-81.
 72. Gimmon Z, Wexler MR, Rachmilevitz EA. Pathogenesis of juvenile leg ulcers in β -thalassemia major and intermedia. *Plastic Reconstr Surgery* 1982; 69:320-3.
 73. Yashar VBB, Barenholz Y, Hy Am F, Rachmilevitz E, Eldor A. Phosphatidylserine in the outer leaflet of red blood cells from β -thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. *Am J Hematol* 1993; 44:63-5.
 74. Eldor A, Lellouche F, Goldfarb A, Rachmilevitz EA, Maclouf J. *In vivo* platelet activation in β -thalassemia major reflected by increased platelet thromboxane urinary metabolites. *Blood* 1991; 77:1749-53.
 75. Eldor A, Krausz Y, Atlan H, et al. Platelet survival in patients with β -thalassemia. *Am J Hematol* 1989; 32:94-9.
 76. Coppola R, Cappellini MD, Bottasso B, et al. Hypercoagulability in patients with thalassemia intermedia. Congresso Nazionale della Società Italiana di Studio Emostasi e Trombosi. Lanciano, 26-30 giugno 1994.
 77. Fosburg MT, Nathan DG. Treatment of Cooley's anemia. *Blood* 1990; 76:435-44.
 78. Styrt B. Infection associated with asplenia: risk, mechanisms and prevention. *Am J Med* 1990; 88:33N-42N.
 79. Lortan JE. Management of asplenic patients. *Br J Haematol* 1993; 68:566-9.
 80. Rebullia P, Modell B, for the CooleyCare programme. Transfusion requirements and effects in patients with thalassemia major. *Lancet* 1991; 337:277-80.
 81. Michail-Merianou V, Pamphili-Panousopoulou L, Piperi Lowes L, Pelegrinis E, Karaklis A. Alloimmunization to red cell antigens in thalassemia: comparative study of usual versus better-match transfusions programmes. *Vox Sang* 1987; 52:95-8.
 82. Politis C, Fragatou S, Cozza G. Recombinant human erythropoietin induced rapid and complete healing of chronic leg ulcers in two patients with thalassemia intermedia. In: New trends in therapy for hemoglobinopathies and thalassemias, Paris, September 19-22, 1994.
 83. Di Palma A, Olivieri N, Sprocati M, Tenan R, Vullo C. Rapid healing of chronic leg ulcers in patients with thalassemia during treatment with hydroxyurea. In: New trends in therapy for hemoglobinopathies and thalassemias, Paris, September 19-22, 1994.
 84. Papavasiliou C, Sandilos P. Effect of radiotherapy on symptoms due to heterotopic marrow in β -thalassemia. *Lancet* 1987; i:13-4.
 85. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med* 1990; 322:417-21.
 86. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in adult thalassemia. *Blood* 1992; 80:1603-7.
 87. Ley TJ, DeSimone J, Anagnou NP, et al. 5-azacytidine selectively increases γ -globin synthesis in a patients with β -thalassemia. *N Engl J Med* 1982; 307:1469-75.
 88. Ley TJ, DeSimone J, Noguchi CT, et al. 5-azacytidine increases γ -synthesis and reduces the proportion of dense cells in patients with sickle cell anemia. *Blood* 1983; 62:370-80.
 89. Rodgers GP, Dover GJ, Schechter AN, Nienhuis AW. Hematologic response of patients with sickle cell disease to treatment with hydroxyurea. *N Engl J Med* 1990; 322:1037-45.
 90. Reported at the Ninth Conference on Hemoglobin Switching

- Rosario Resort, Orcas Island, Washington, June 10-15, 1994.
91. Perrine SP, Ginder GD, Faller DV, et al. A short term trial of butyrate to stimulate fetal globin gene expression in the β -globin disorders. *N Engl J Med* 1993; 328:81-6.
 92. Blau A Costanoulakis P, Shaw CM, Stamatoyannopoulos G. Fetal hemoglobin induction with butyric acid. Efficacy and toxicity. *Blood* 1993; 81:529-37.
 93. Liakopoulou E, Blau CA, Li Q, et al. Fetal hemoglobin induction using short chain fatty acids. Ninth Conference on Hemoglobin Switching Rosario Resort, Orcas Island, Washington, June 10-15, 1994.
 94. Olivieri NF. Results of trials with intravenous arginine butyrate and recombinant human erythropoietin in thalassemia and sickle cell diseases. Ninth Conference on Hemoglobin Switching Rosario Resort, Orcas Island, Washington, June 10-15, 1994.
 95. Dore F, Bonfigli S, Gaviano E, et al. Serum erythropoietin levels in thalassemia intermedia. *Ann Hematol* 1993; 67:183-6.
 96. Barosi G. Control of erythropoietin production in man. *Haematologica* 1993; 78:77-9.
 97. Olivieri NF, Freedman MH, Perrine SP, Dover GJ, Sheridan B. Trial of recombinant human erythropoietin: three patients with thalassemia intermedia (letter). *Blood* 1992; 15:3258-60.
 99. Rachmilewitz E, Goldfarb A, Dover G. Administration of erythropoietin to patients with β -thalassemia intermedia. A preliminary trial. *Blood* 1991; 78:1145.
 99. Rodgers GP, Dover GJ, Uyesaka N, Noguchi CT, Schechter AN, Nienhuis AW Augmentation by erythropoietin of the fetal hemoglobin response to hydroxyurea in sickle cell disease. *N Engl J Med* 1993; 328:73-80.
 100. Barosi G, Cazzola M, De Vincentiis A, Grossi A, Tura S. Guidelines for the use of recombinant human erythropoietin. *Haematologica* 1994; 79:526-33.