Portatore e sindromi β-talassemiche

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DISCLOSURES

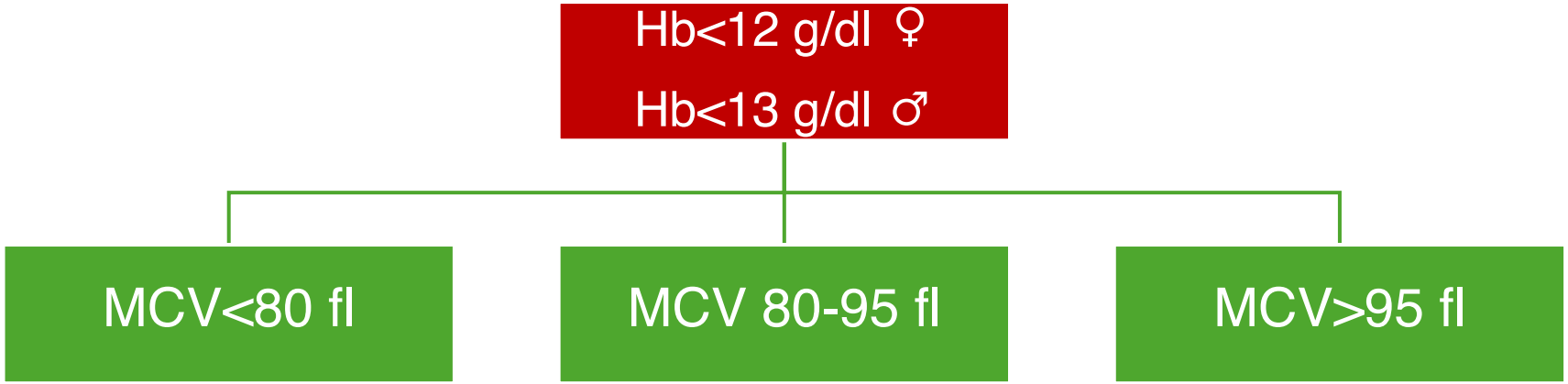
- Sanofi: honoraria for talks, advisory board, travel grants, research grant
- BMS: honoraria for talks, advisory board
- Vertex therapeutics: advisory board

DEFINIZIONE DI ANEMIA

Table 2. Haemoglobin cutoffs to define anaemia in individuals and populations

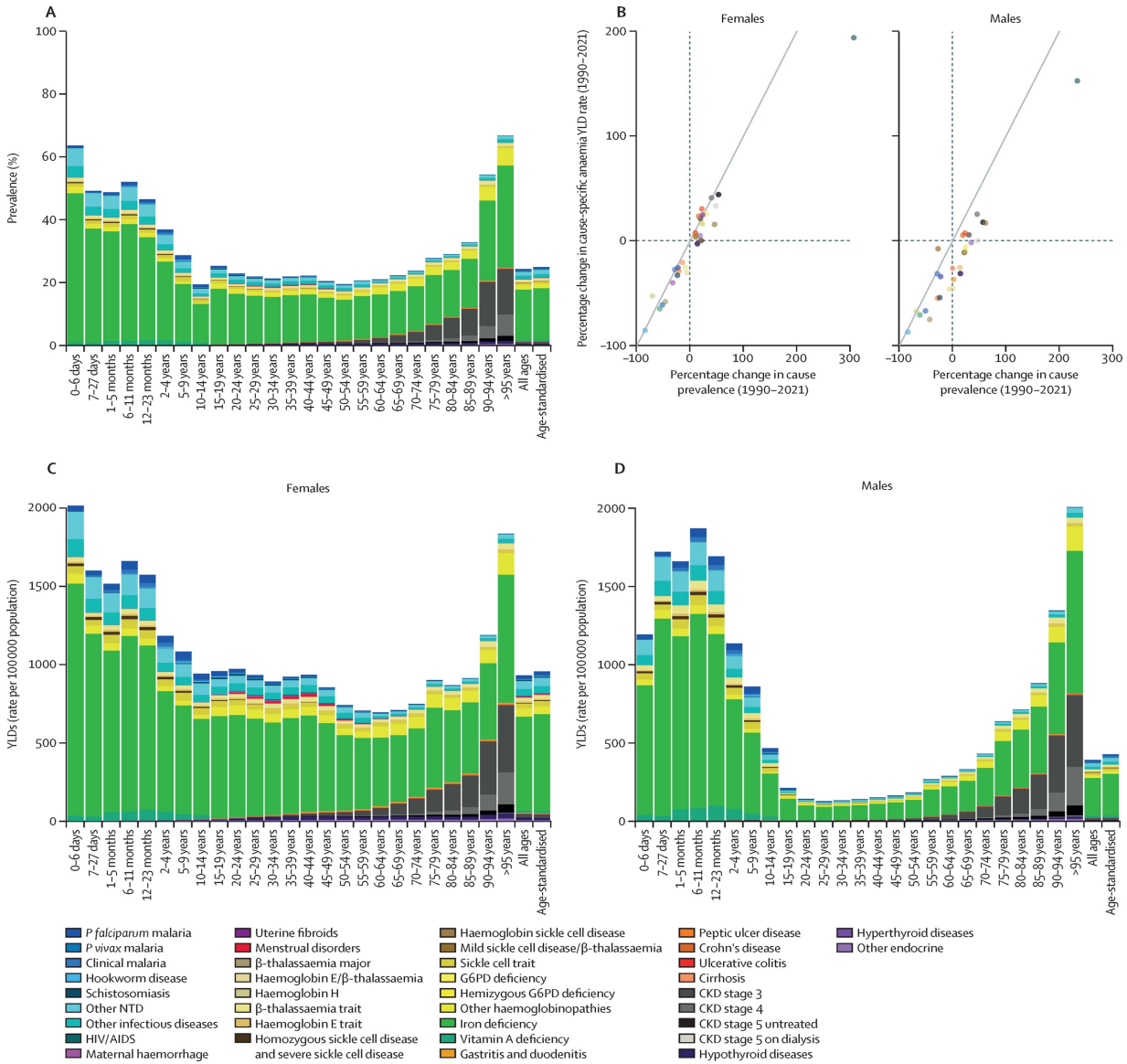
Population	Haemoglobin concentration (g/L)[a]
Children, 6–23 months	<105
Children, 24–59 months	<110
Children, 5–11 years	<115
Children, 12–14 years, nonpregnant girls	<120
Children, 12–14 years, boys	<120
Adults, 15–65 years, nonpregnant women	<120
Adults, 15–65 years, men	<130
Pregnancy	
First trimester	<110
Second trimester	<105
Third trimester	<110

[a] Based on 5th percentile.



WHO, Guideline on haemoglobin cutoffs to define anaemia in individuals and populations 2024

ANEMIE MICROCITICHE



Hb<12 g/dl ♀

Hb<13 g/dl ♂

MCV<80 fl

- Anemia da carenza di ferro
- Talassemie
- Altre emoglobinopatie

→ **Storia familiare**

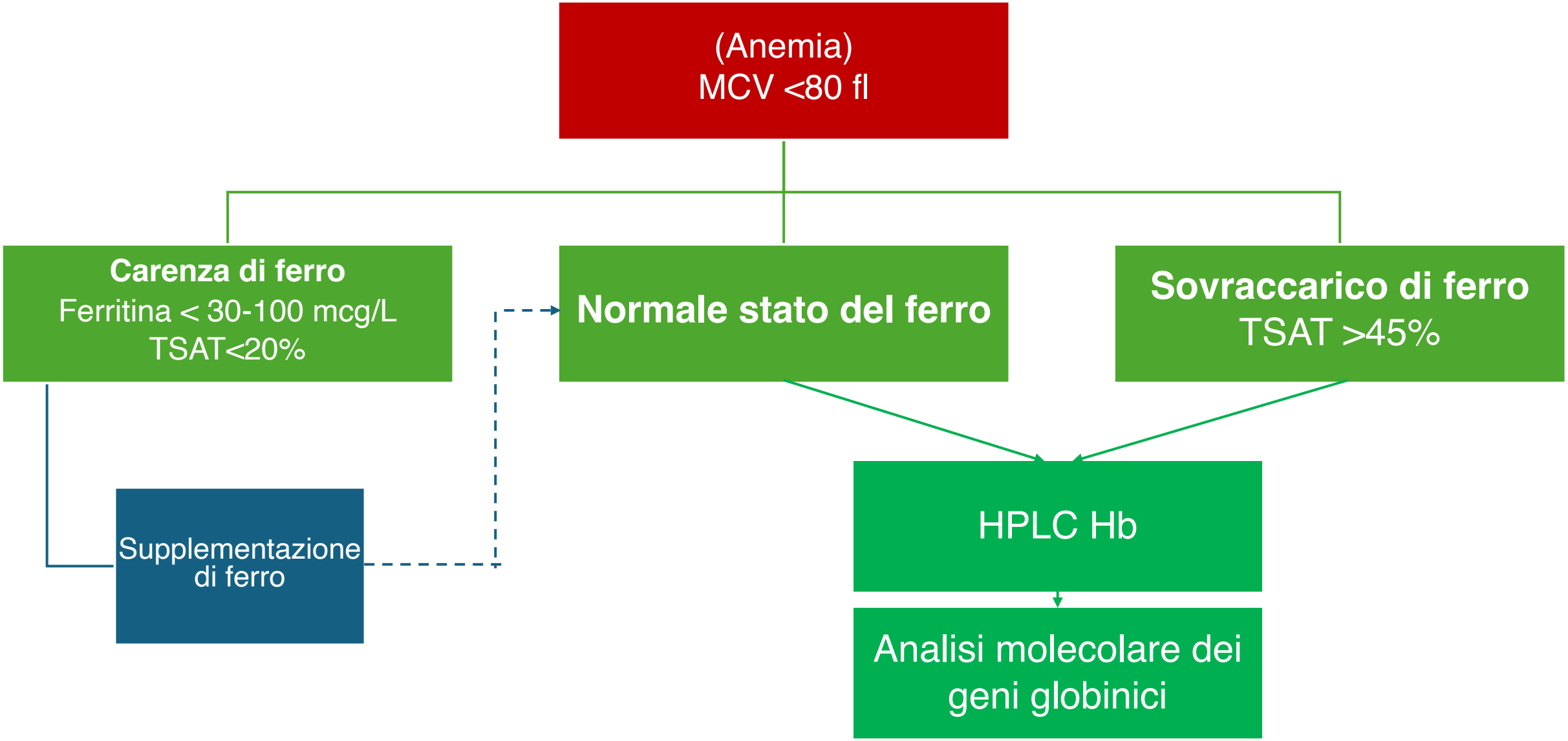
→**STATO DEL FERRO COMPLETO**

- Sideremia
- Transferrina
- Ferritina

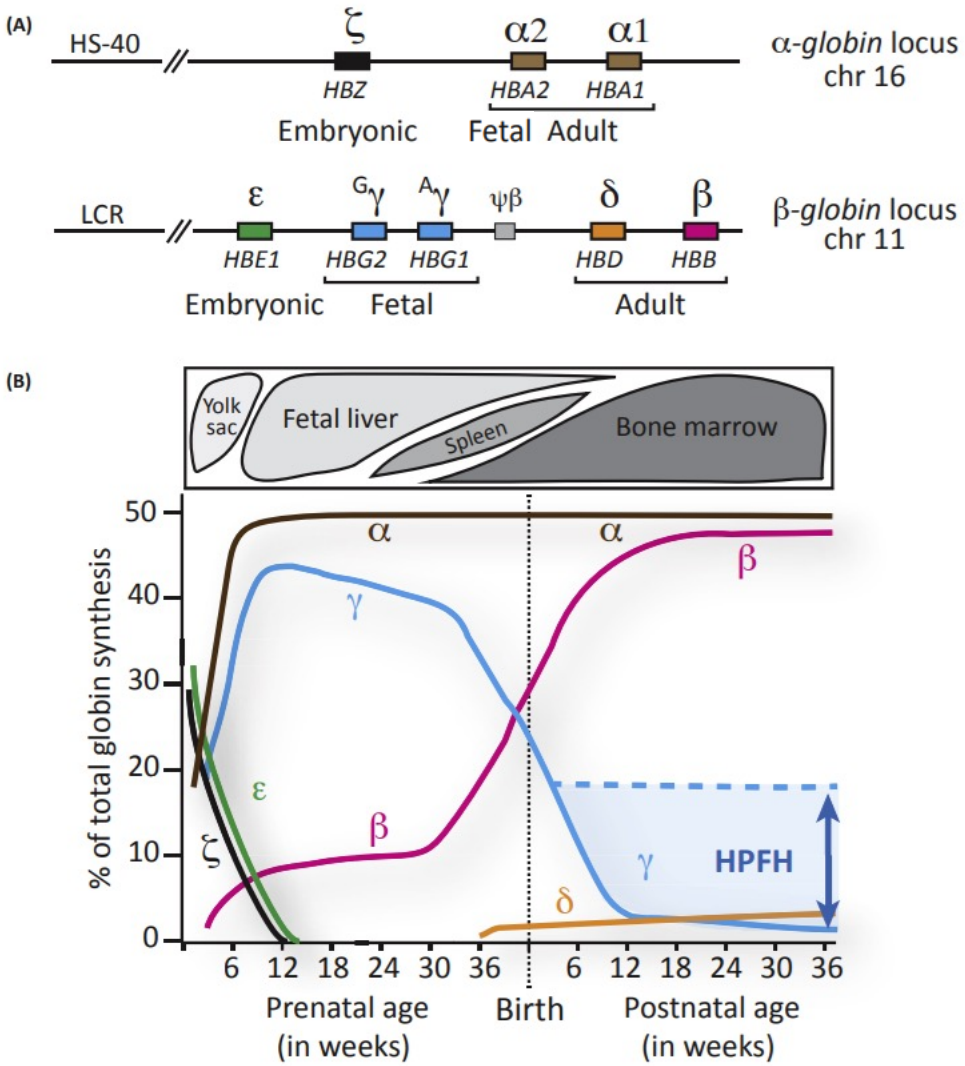
} Saturazione della transferrina

GBD 2021 Anaemia Collaborators, Lancet Haematol 2023

ANEMIE MICROCITICHE



EMOGLOBINA NELLE FASI DELLA VITA



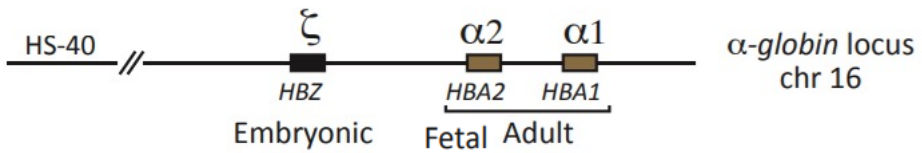
Bambino dopo 1° anno di vita e adulto

HbA ($\alpha_2\beta_2$) ~97%
HbA2 ($\alpha_2\delta_2$) 2.0-3.2%
HbF ($\alpha_2\gamma_2$) <1%

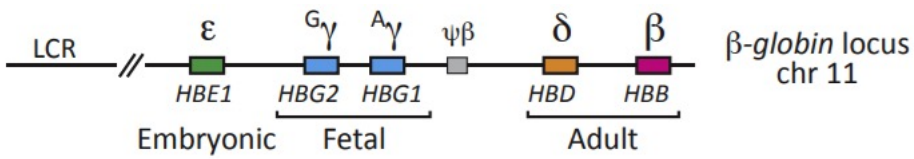
Modified from Wienert B, Trends Genet. 2018

SINDROMI TALASSEMICHE

Difetto quantitativo: ridotta sintesi di una o più catene globiniche



α-talassemie

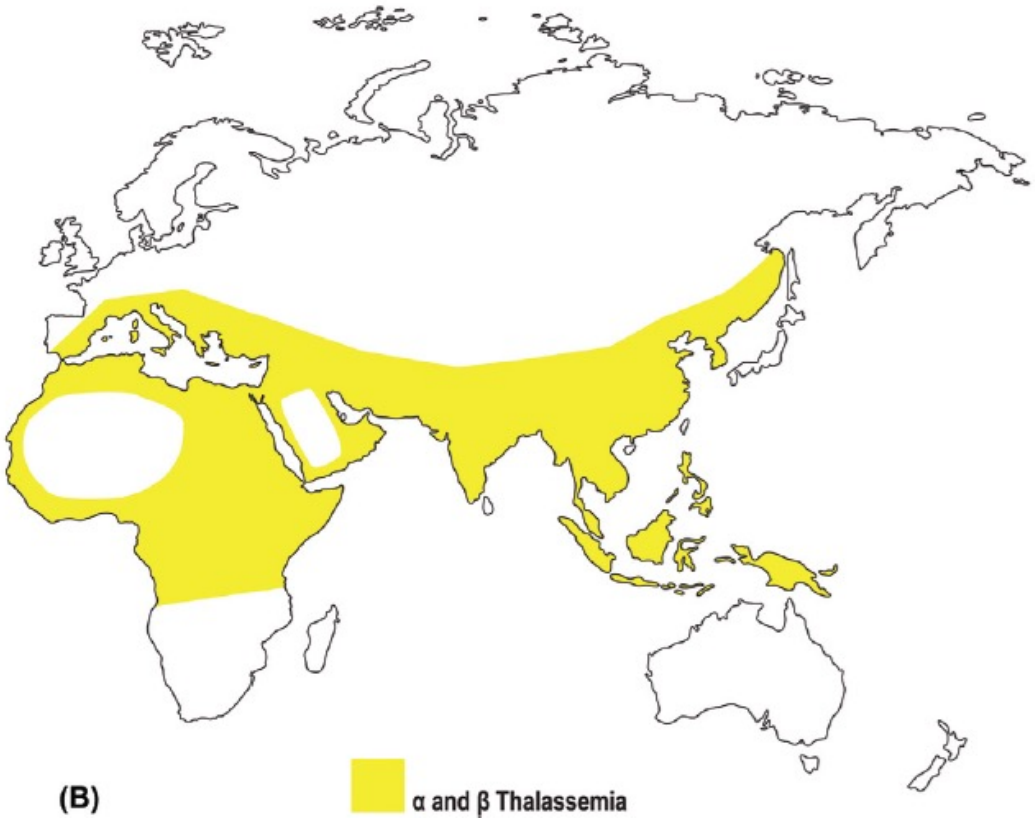


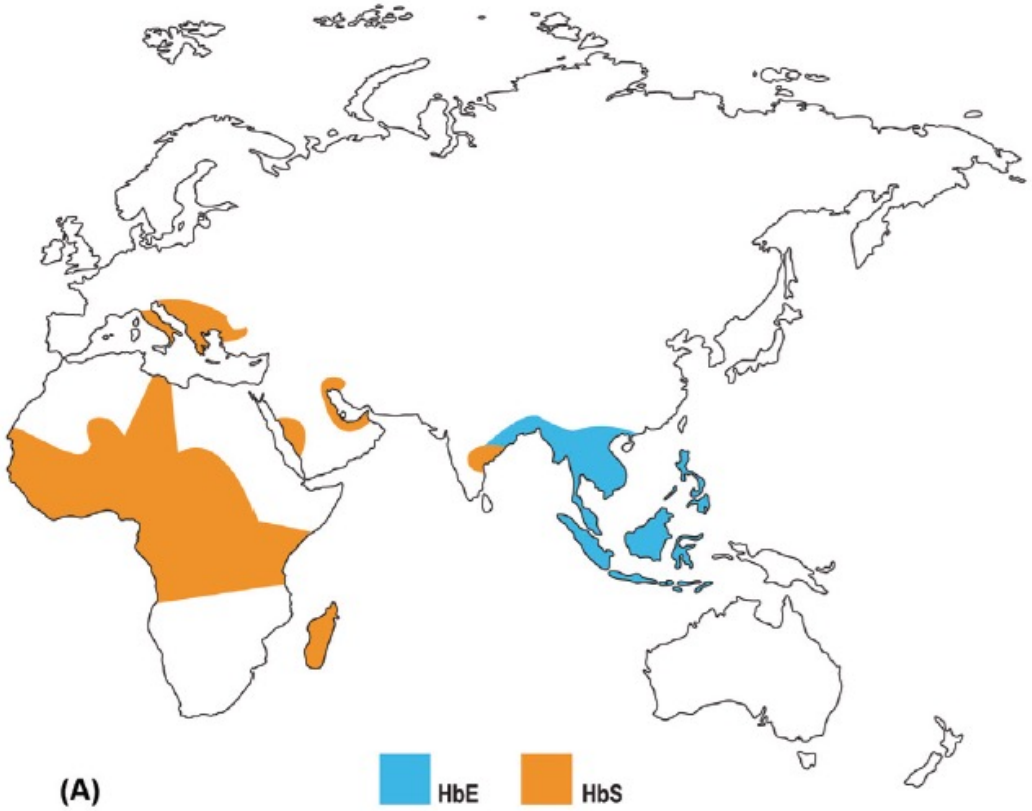
β-talassemie

δβ-talassemie

Modified from Wienert B, Trends Genet. 2018

EMOGLOBINOPATIE

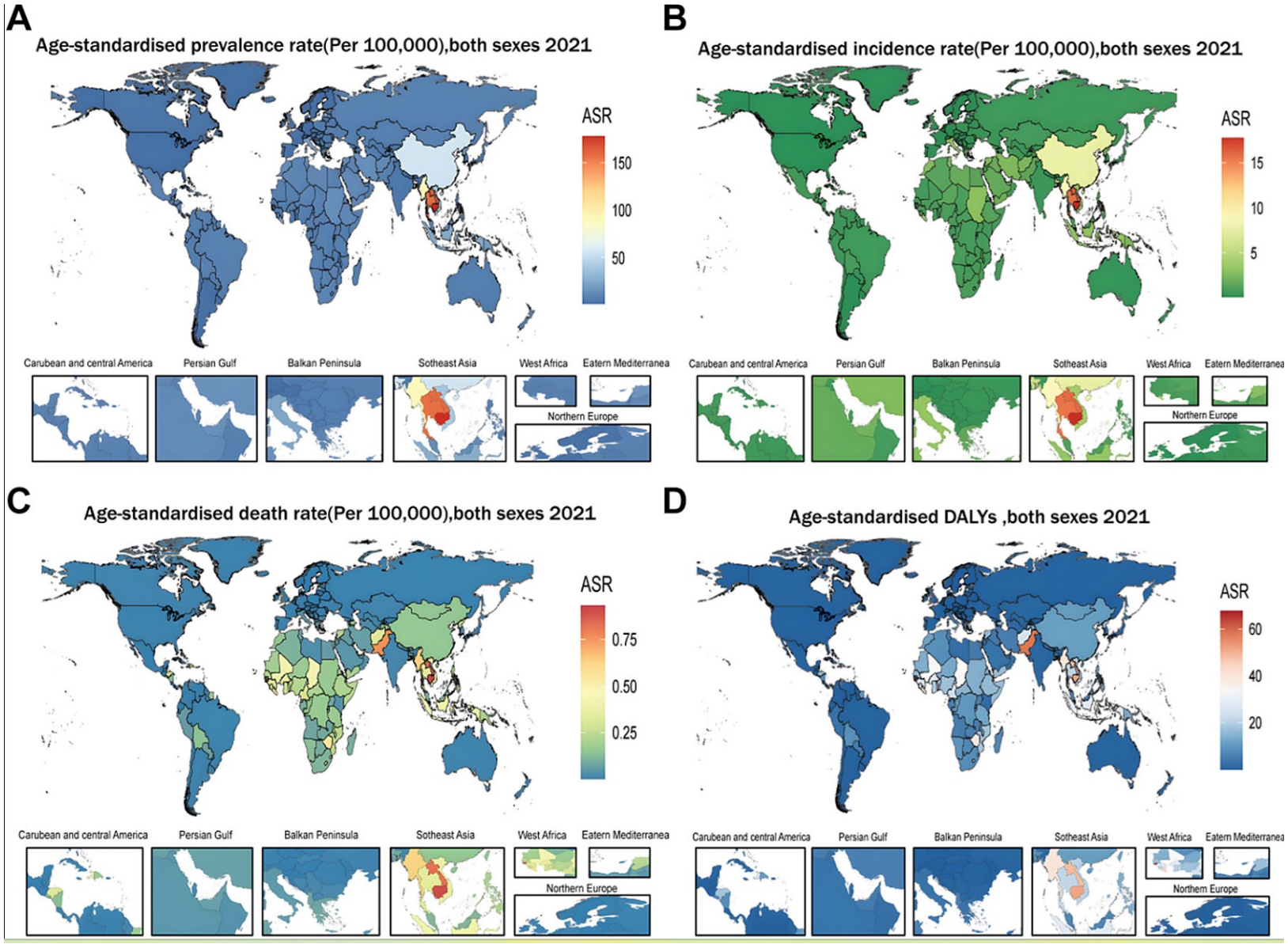




I differenti difetti dell'emoglobina possono coesistere in un soggetto dando quadri differenti di gravità

Weatherall DJ, Blood Reviews 2012

EPIDEMIOLOGIA - TALASSEMIE



Tuo Y, eClinicalMedicine 2024

EPIDEMIOLOGIA DELLE TALASSEMIE

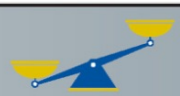
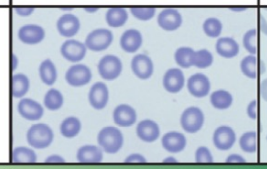
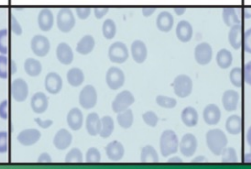
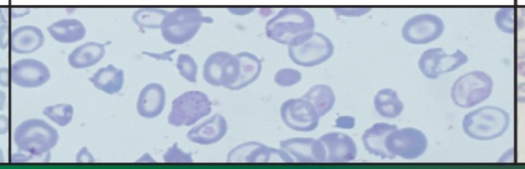
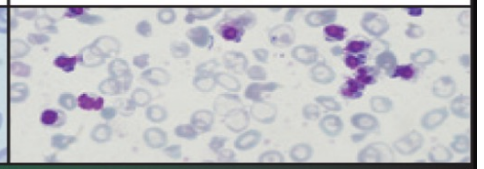
- Una delle più comuni patologie AR al mondo
- 1-5% di portatori al mondo
- Nel 2021: 1,310,407 casi al mondo

<u>Beta-talassemia</u>

- 60 mila nuovi nati con forme gravi al mondo ogni anno
- 3% di portatori al mondo e oltre 3 milioni di portatori sani in Italia, con punte di maggiore incidenza in Sardegna (12,9%), Sicilia (7-8%) e Puglia (5-8%)
- Prevalenza elevata nel Mediterraneo, Middle East, Sud-Est asiatico

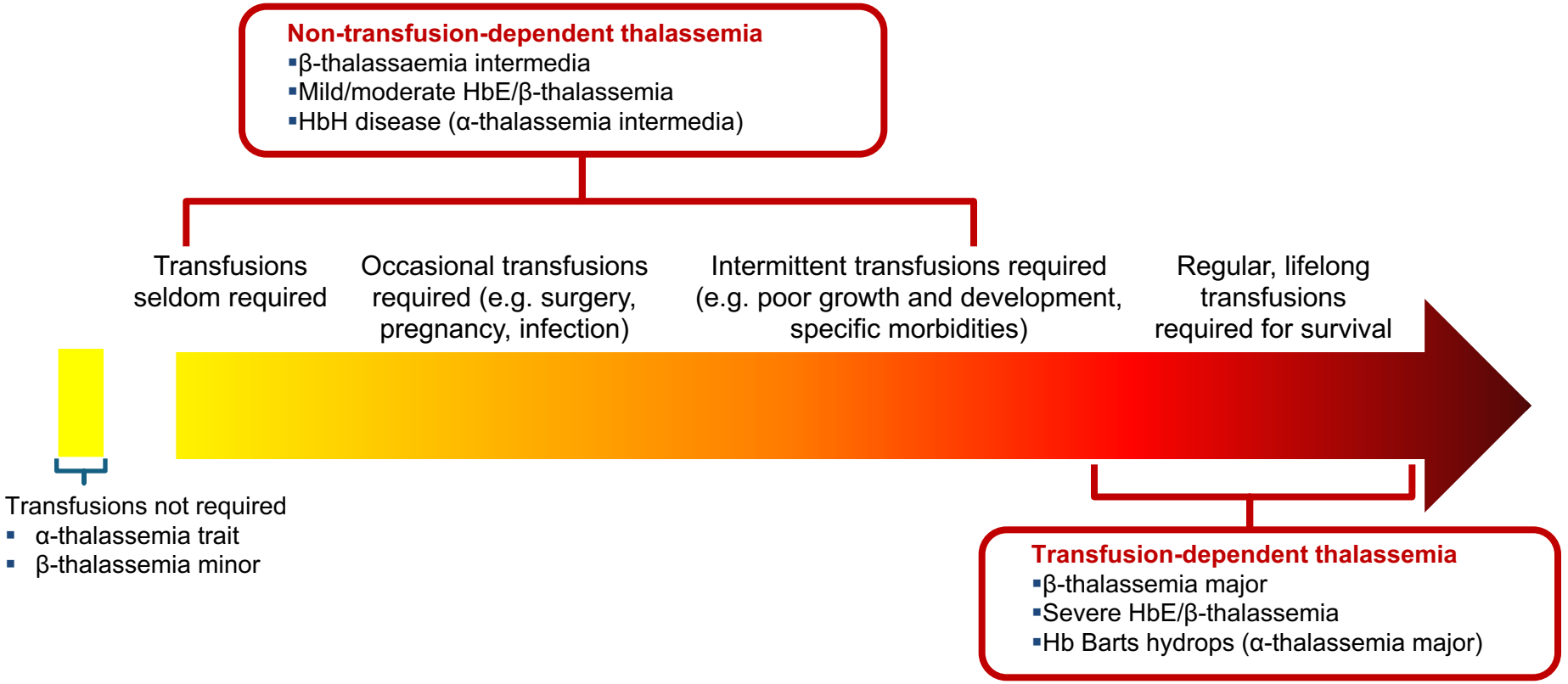
Tuo Y, eClinicalMedicine 2024
Modell, 2008

CLASSIFICAZIONE DELLE SINDROMI TALASSEMICHE

Globin chain balance					
Genotypes	β-thalassaemia	β/β^{silent}	β/β^{+}, β/β^{0}, β/β^{E}	Combination of βThal/β with α-gene multiplication $\beta^{silent}/\beta^{silent}$, β^{silent}/β^{+}, β^{silent}/β^{0}, β^{+}/β^{+}, β^{+}/β^{0}, β^{E}/β^{+}, $\beta E/\beta^{0}$, Combination of $\beta^{Thal}/\beta^{Thal}$ with either α-thalassaemia or increased fetal haemoglobin production	β^{+}/β^{+}, β^{+}/β^{0}, β^{E}/β^{+}, β^{E}/β^{0}, β^{0}/β^{0} ($\beta^{silent}/\beta^{silent}$, β^{silent}/β^{+}, β^{silent}/β^{0} with α-globin gene multiplication)
	α-thalassaemia	$-\alpha/\alpha\alpha$	$-\alpha/-\alpha$, $--/\alpha\alpha$	$--/-\alpha$, $-\alpha/\alpha^{ND}\alpha$, $\alpha^{ND}\alpha/\alpha^{ND}\alpha$, $--/\alpha^{ND}\alpha$	$--/-\alpha$, $\alpha^{ND}\alpha/\alpha^{ND}\alpha$, $--/\alpha^{ND}\alpha$, $--/--$
Haematological indexes					
Clinical phenotype		Normal		Mild Moderate	Severe
Transfusion requirements				Non-transfusion-dependent thalassaemia	Transfusion-dependent thalassaemia
Thalassaemia		Minor		Intermedia	Major

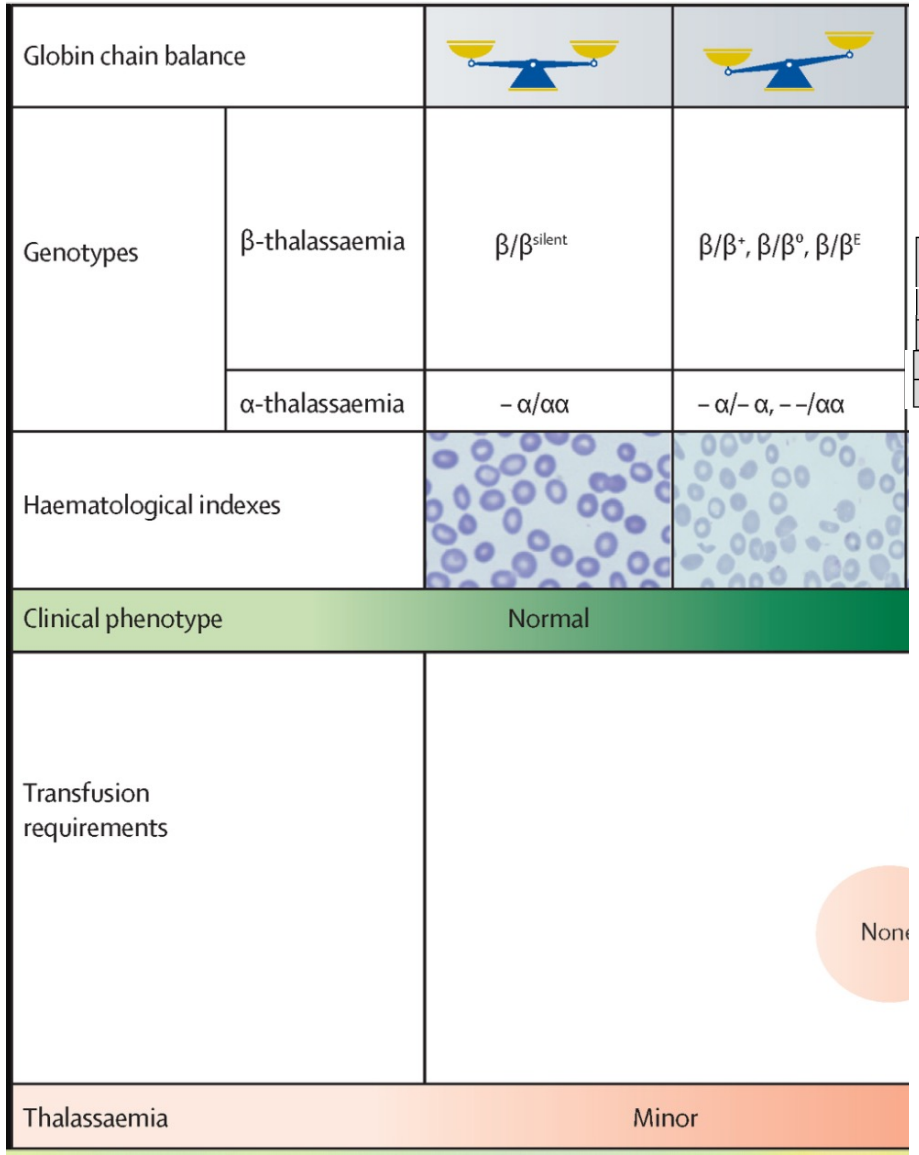
Kattamis A, Lancet 2022

CLASSIFICAZIONE DELLE SINDROMI TALASSEMICHE



Musallam KM *et al. Haematologica* 2013;98:833-844.

TRAIT BETA-TALASSEMICO



Hb A2 %	Hb F %	MCV fl	MCH pg	Difetto gene α	Difetto gene β	Difetto gene δ	Difetto gene γ (promoter)	Fenotipo
2.5 - 3.2	< 1.0	≥ 79	> 27	NO	NO	NO	NO	Normale
2.9 - 3.7	< 1.0	75 - 82	< 28	NO	β^{++}	NO	NO	Beta Tal. silenti
3.6 - 5.5	< 1.5	65 - 78	20 - 27	NO	β^{+}	NO	NO	Beta Tal.
4.1 - 6.5	< 1.5	60 - 72	18 - 26	NO	β°	NO	NO	Beta Tal.

Portatore di beta-talassemia

MCV<80 fl
MCH <27 pg
HbA2 >3.2%

Kattamis A, Lancet 2022

CASO CLINICO 1

F, 20 aa

ESAME EMOCROMOCITOMETRICO

Impedenziometria-fluorescenza

Globuli Bianchi	**7.17**		10e3/mmc	[4.8 - 10.8]
Globuli Rossi	**5.56**	*	10e6/mmc	[4.10 - 5.10]
Emoglobina	**11.4**	*	g/dL	[12.0 - 16.0]
Ematocrito	**35.8**	*	%	[36.0 - 46.0]
Volume Globulare medio	**64.4**	*	fl	[78.0 - 99.0]
Emoglobina corpuscolare media	**20.5**	*	pg	[25.0 - 35.0]
Conc. Hb corpuscolare media	**31.8**		g/dL	[31.0 - 37.0]
Indice di anisocitosi (RDW)	**16.7**	*	%	[11.5 - 14.5]
Piastrine	**306**		10e3/mmc	[130 - 400]
MPV	**11.7**		fl	[9.5 - 13.1]

FORMULA LEUCOCITARIA

Neutrofili	**3.91**		10e3/mmc	[1.50 - 6.50]
Linfociti	**2.41**		10e3/mmc	[1.20 - 3.40]
Monociti	**0.67**	*	10e3/mmc	[0.30 - 0.60]
Eosinofili	**0.13**		10e3/mmc	[0.10 - 0.80]
Basofili	**0.05**		10e3/mmc	[0.01 - 0.20]
Neutrofili %	**54.60**			
Linfociti %	**33.60**			
Monociti %	**9.30**			
Eosinofili %	**1.80**			
Basofili %	**0.70**			

Ferritina 35 mcg/L
TSAT 20%

Reticolociti 105000/mm^3

Indici di emolisi nella norma

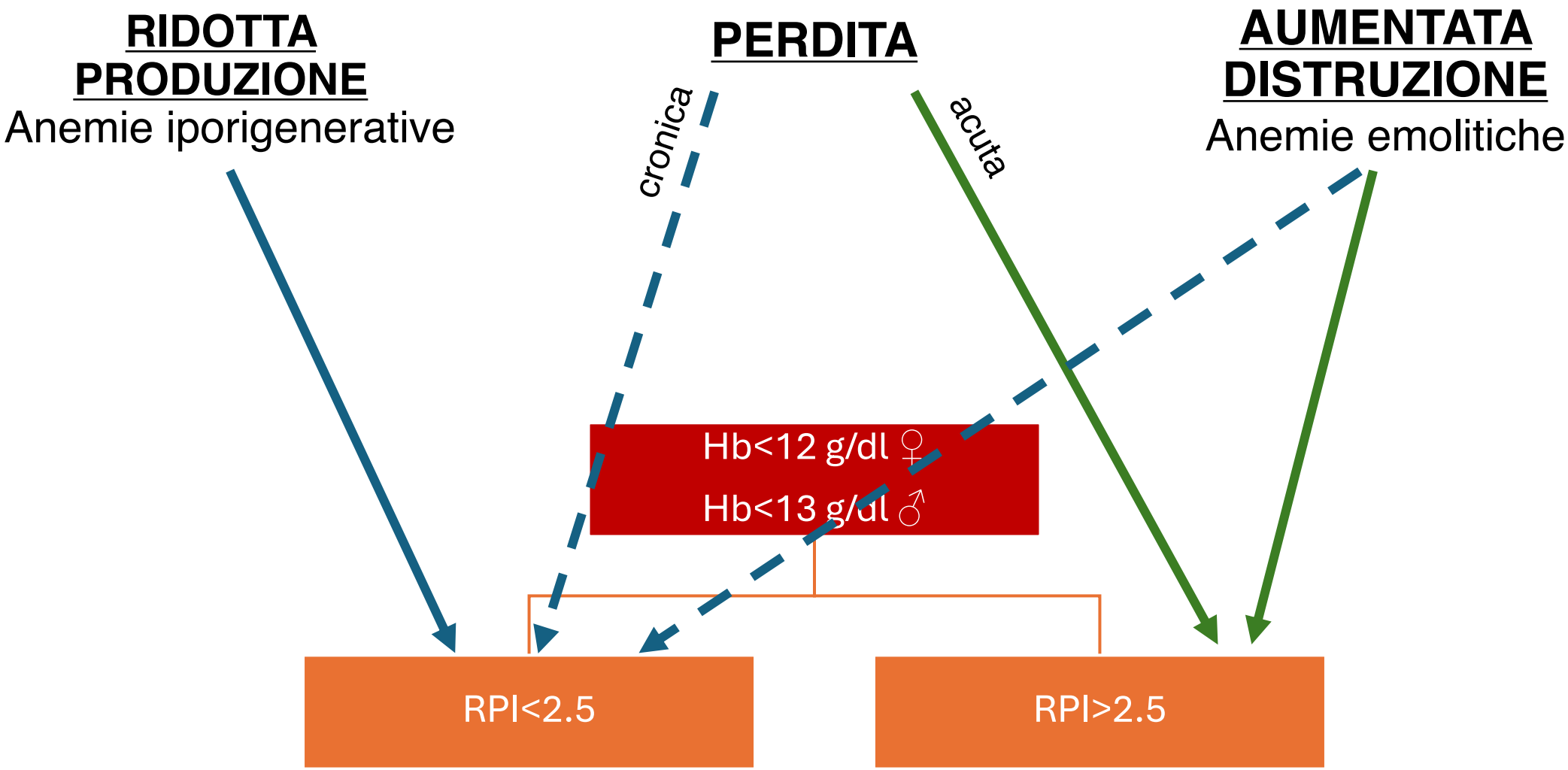
HPLC Hb

A2 6.3%. (v.n. 2-3.2 %)

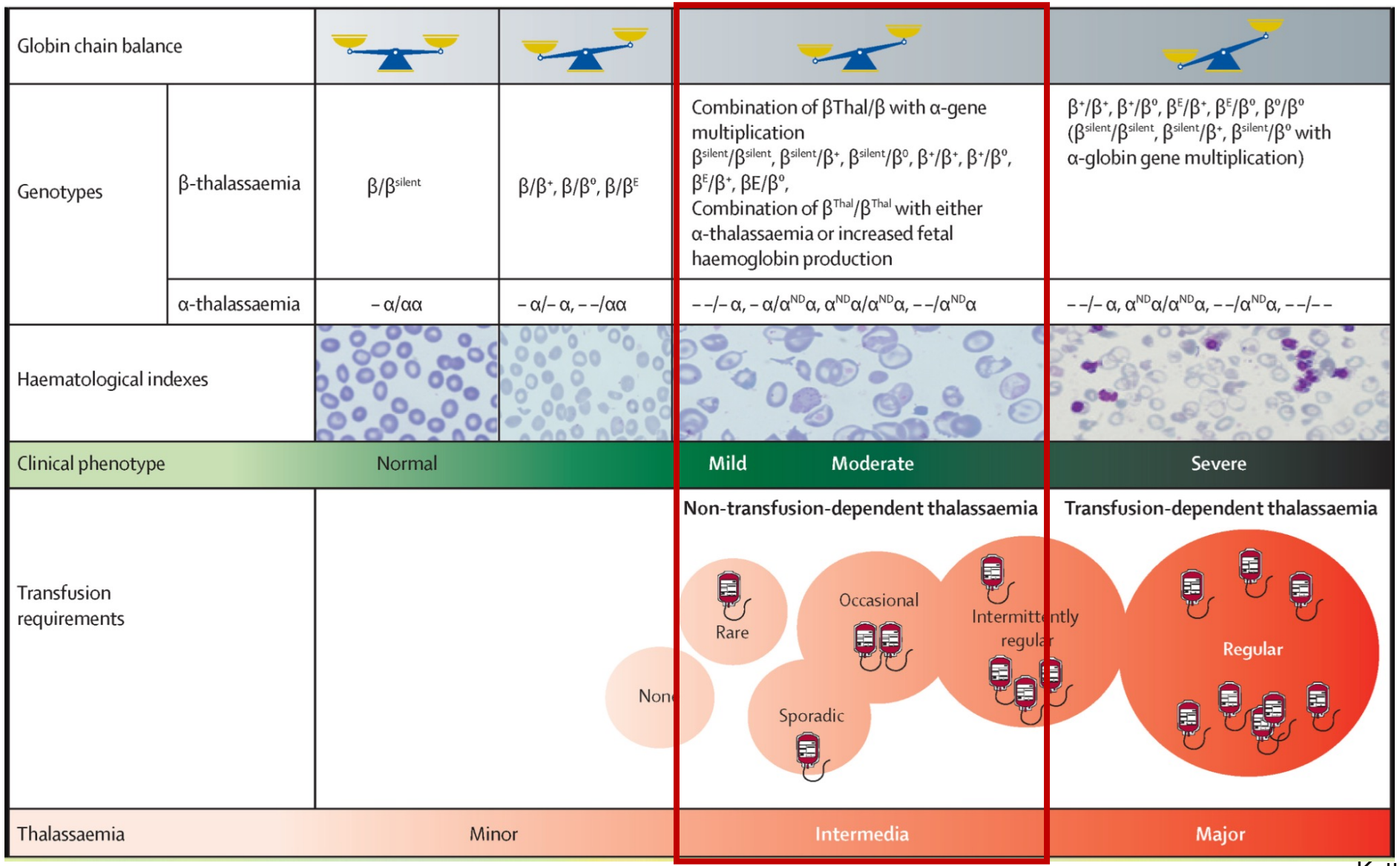
HbF 1.0% (v.n. <1%)

Assenza Hb anomale

CLASSIFICAZIONE FUNZIONALE: INDICE DI PROLIFERAZIONE RETICOLOCITARIA



BETA-NTDT



Kattamis A, Lancet 2022

CASO CLINICO 2

M, 50 aa, splenomegalia

EMATOLOGIA

ESAME EMOCROMOCITOMETRICO

Globuli Bianchi	7.32		10e9/L	[4.8 - 10.8]
Globuli Rossi	6.08	*	10e12/L	[4.50 - 5.30]
Emoglobina	11.8	*	g/dL	[13.5 - 17.5]
Ematocrito	38.3	*	%	[41.0 - 53.0]
Volume Globulare medio	63.0	*	fl	[80.0 - 94.0]
Emoglobina corpuscolare media	19.4	*	pg	[25.0 - 35.0]
Conc. Hb corpuscolare media	30.8	*	g/dL	[31.0 - 37.0]
Indice di anisocitosi (RDW)	19.6	*	%	[11.5 - 14.5]
Piastrine	126	*	10e9/L	[130 - 400]

FORMULA LEUCOCITARIA

Neutrofili	4.62		10e9/L	[1.50 - 6.50]
Linfociti	1.82		10e9/L	[1.20 - 3.40]
Monociti	0.66	*	10e9/L	[0.30 - 0.60]
Eosinofili	0.18		10e9/L	[0.10 - 0.80]
Basofili	0.04		10e9/L	[0.01 - 0.20]
Neutrofili %	63.10			
Linfociti %	24.90			
Monociti %	9.00			
Eosinofili %	2.50			
Basofili %	0.50			
NRBC (eritroblasti)	0.03		10e9/L	
NRBC% (eritroblasti)	0.40			

RETICOLOCITI

Reticolociti	0.226	*	10e12/L	[0.02 - 0.10]
Reticolociti %	3.720	*		[0.80 - 3.00]

CHIMICA CLINICA

BILIRUBINA TOTALE	SIERO	2.39	*	mg/dL	[0.12 - 1.10]	DPD
BILIRUBINA DIRETTA	SIERO	0.90	*	mg/dL	[0.00 - 0.30]	Jendrassik
LDH	SIERO	358	*	U/L	[135 - 225]	IFCC l-p
FERRO	SIERO	209	*	µg/dL	[59 - 158]	Ferrozina

PROTEINE SPECIFICHE

FERRITINA	SIERO	390		µg/L	[30 - 400]	Ecla
TRANSFERRINA	SIERO	184	*	mg/dL	[200 - 360]	Immuno turbidimetri
APTOGLOBINA	SIERO	<20		mg/dL	[30 - 200]	Immuno turbidimetri

TSAT 79%

EMATOLOGIA

Esami eseguiti presso il Laboratorio di Ematologia(LD1)

INDAGINI PER LA TALASSEMIA E LE EMOGLOBINOPATIE

Globuli Rossi	**6.08**	10e6/mmc
Emoglobina	**11.8**	g/dl
Ematocrito	**38.3**	%
Volume Globulare medio	**63.0**	fl
MCH	**19.4**	pg
MCHC	**30.8**	g/dl

Separazione cromatografica Hb (HPLC) :

HbA2:	5.1	%	[2.0 - 3.2]
HbF:	1.8	%	< 1

Hb ANOMALE: ASSENTI

CONCLUSIONI: Eterozigosi per Beta-talassemia; opportuno estendere le indagini per la talassemia e le emoglobinopatie ai familiari e all'eventuale partner.

VITAMINE

ACIDO FOLICO (Folati)	*SIERO*	**> 20**	µg/L	[4.6 - 18.7]	Eclia
VITAMINA B12	*SIERO*	**373**	ng/L	[191 - 663]	Eclia

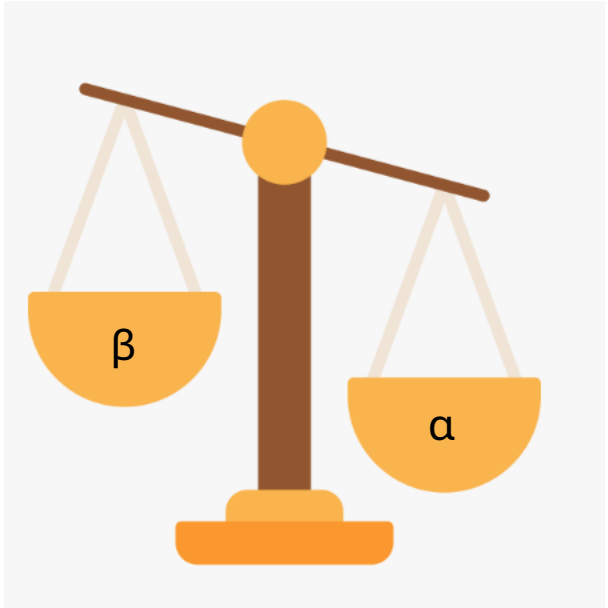
INDAGINI MOLECOLARI PER TALASSEMIA ED Hb-PATIE

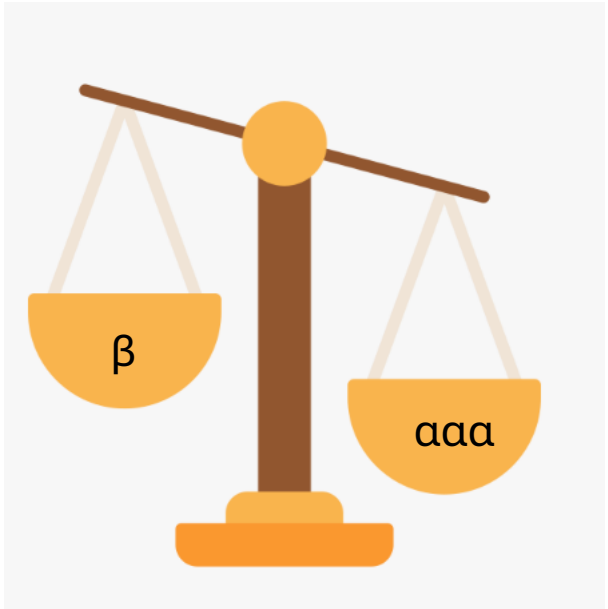
Identificativo Campione : TH 466/18

Indicazioni all' analisi :	Tipizzazione molecolare in soggetto con sospetta Talassemia Intermedia
Materiale pevenuto:	SANGUE INTERO — Materiale utilizzato : DNA estratto da leucociti del sangue periferico
Tecniche Utilizzate :	ß-Talassemie: Sequenziamento diretto delle seguenti regioni del gene HBB (NM_000518.4): da c.-238 (-188 5'UTR) a c.315+90 (IVS II-90); da c.316-207 (IVS II-645) a c.*174 (+1600 3'UTR)
	MLPA 127Kb (Cluster a-globinico) probemix P140-C1 HBA
Risultati:	**E' stato eseguito lo studio dei geni alfa globinici mediante tecnica MLPA, tale indagine ha evidenziato la presenza della triplicazione dei geni alfa globinici.** **Presenza in eterozigosi della mutazione c.118C>T (CODON 39) del gene beta globinico.**
Interpretazione :	**Tale genotipo può essere associato ad un fenotipo di talassemia intermedia il cui quadro clinico può essere particolarmente eterogeneo da caso a caso.** **Si consiglia consulenza genetica.**

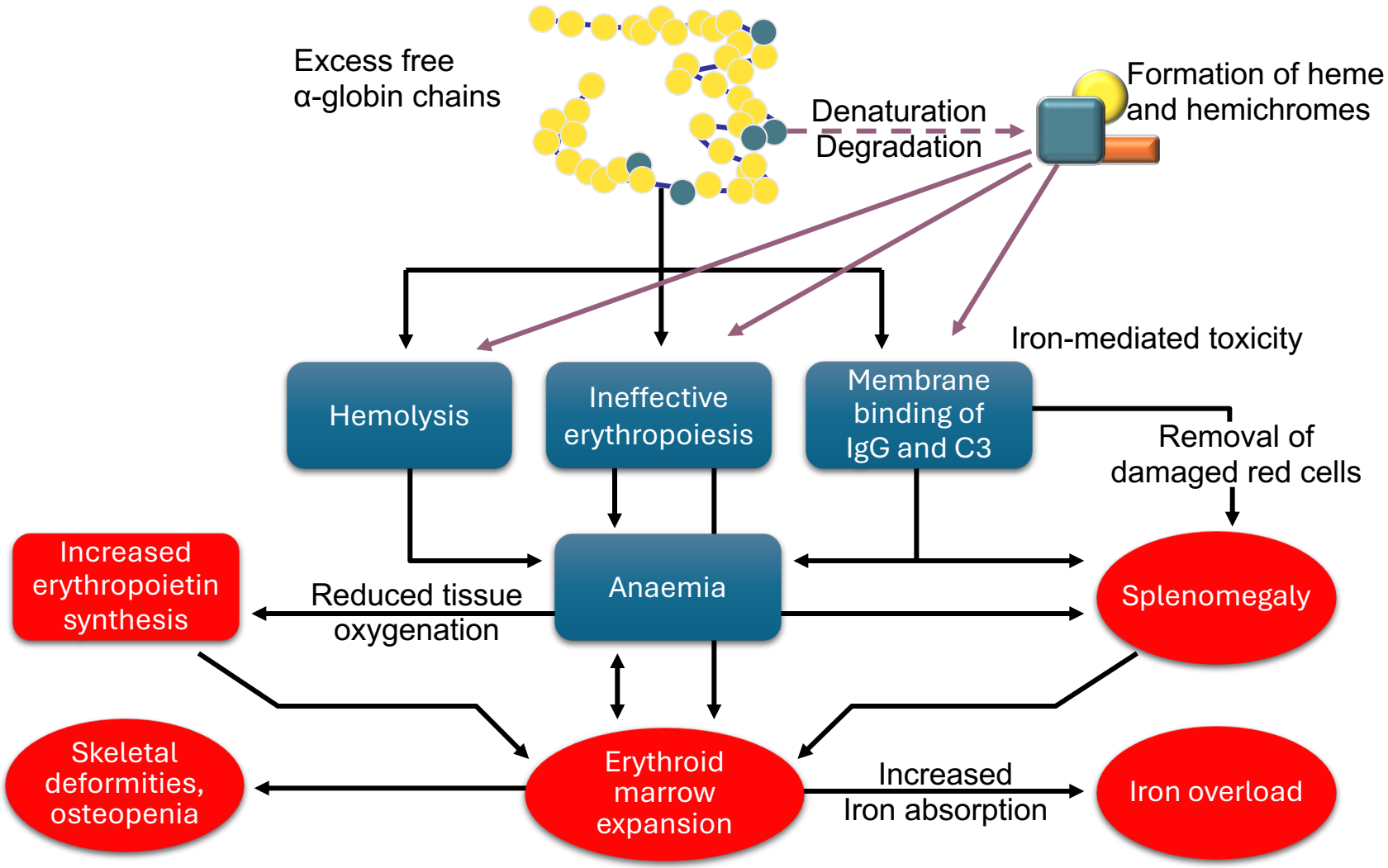
Riferimento numerazione sequenza NM_000518.4-per Beta,sequenza NM_000558.4 per alfa, sequenza NM_000519.3 per Delta, sequenza NM_000184.2 e NM_000559.2 per gamma Nomenclatura secondo HGVS V 15.11 (in parentesi nomenclatura tradizionale) Il Laboratorio partecipa al "Progetto nazionale per la standardizzazione e l'assicurazione di qualità dei test genetici"promosso dall' Istituto Superiore di Sanità

REFERTO IN OTTEMPERANZA ALLA LEGGE 196/063 SULLA PRIVACY





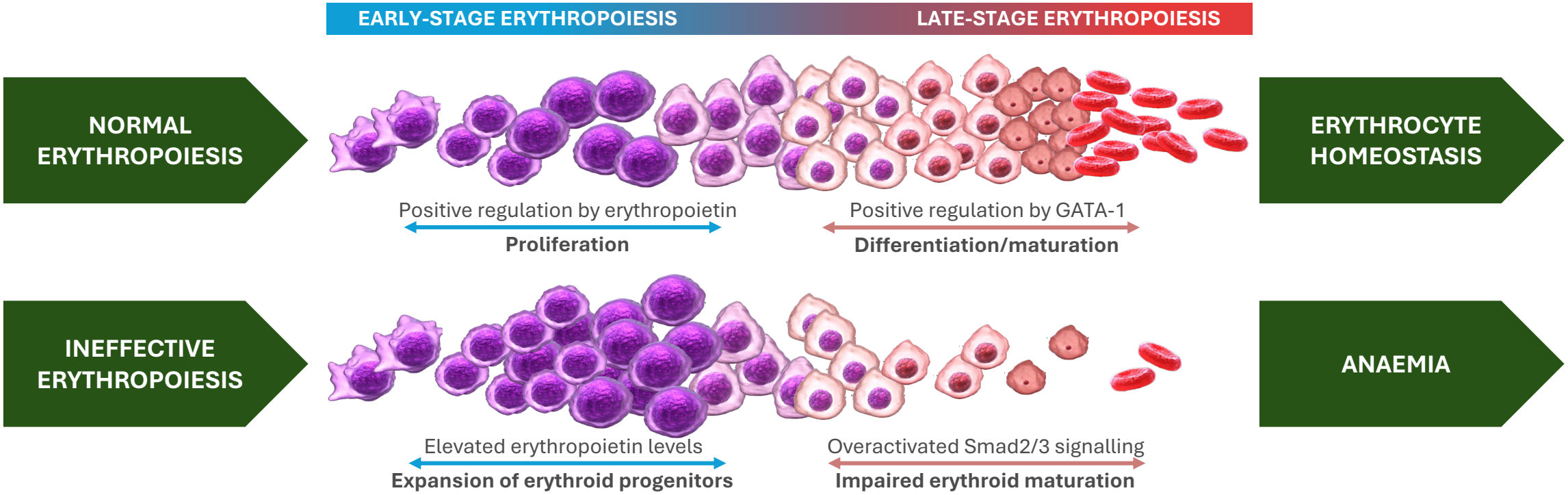
FISIOPATOLOGIA



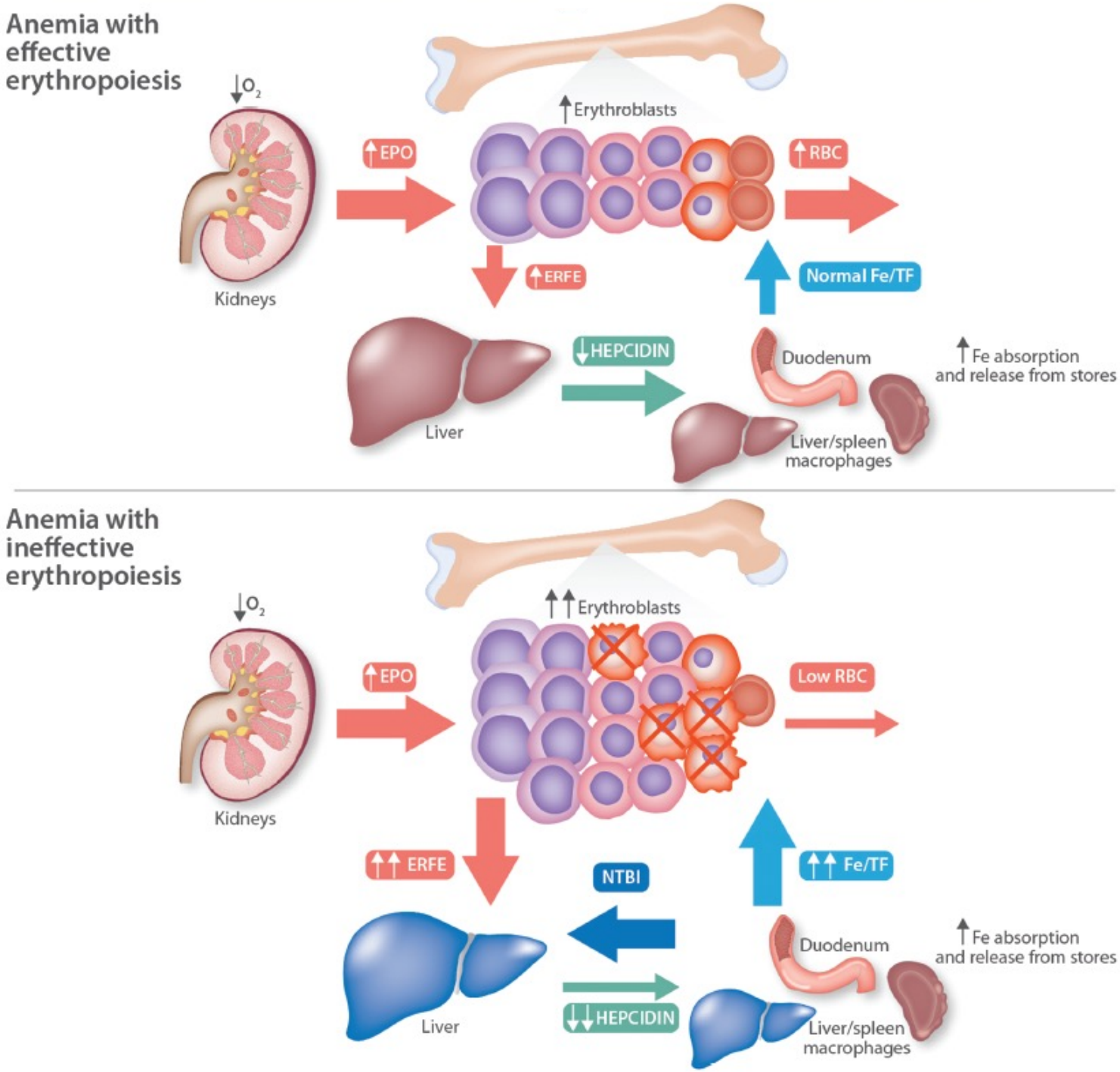
Olivieri NF, et al. N Engl J Med. 1999;341:99-109.

ERITROPOIESI INEFFICACE

Ineffective erythropoiesis is marked by increased proliferation of erythroid progenitors, increased apoptosis of erythroblasts, and impaired maturation of erythroblasts: anaemia is the primary clinical manifestation[1]

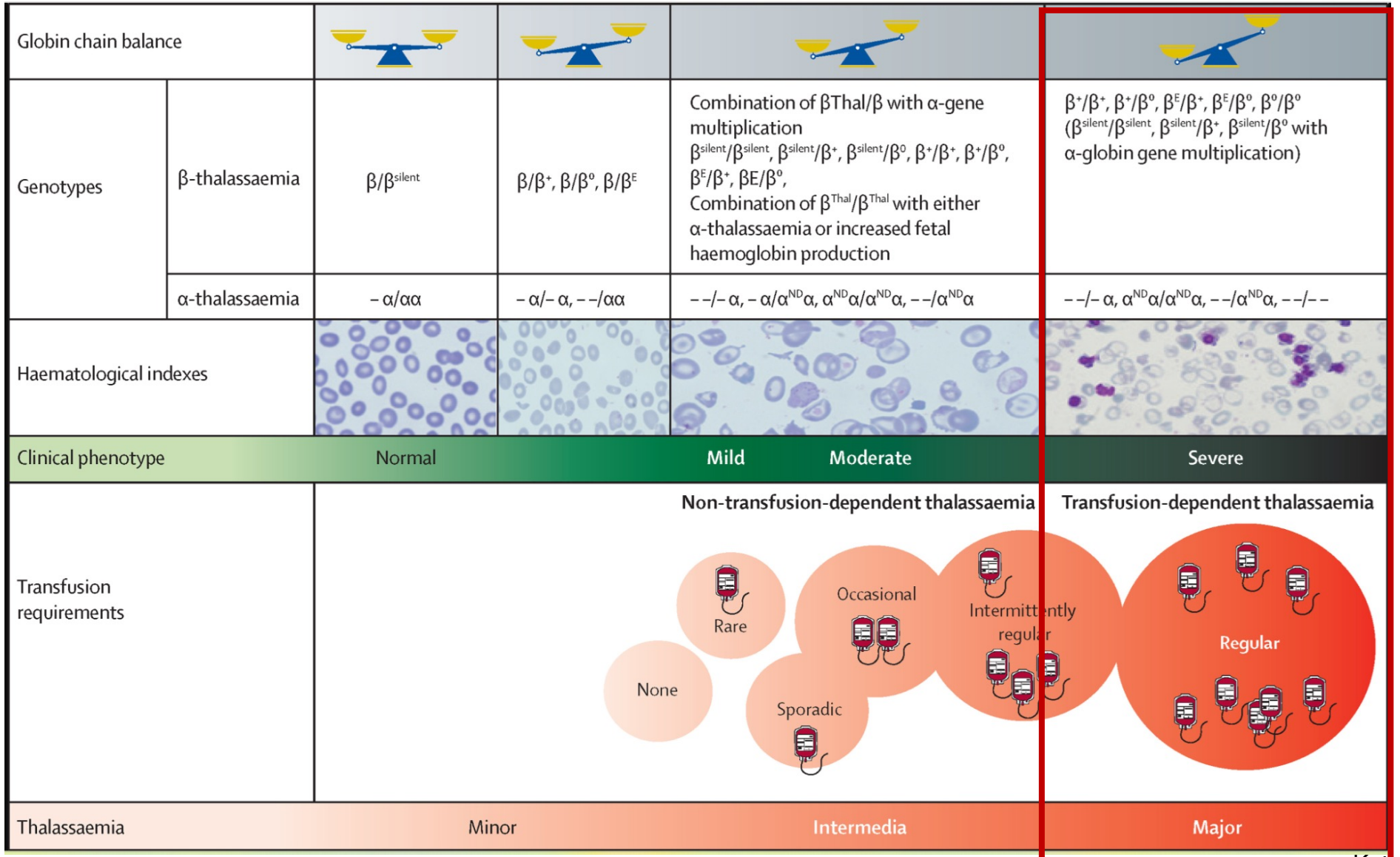


Oikonomidou PR, Rivella S. Blood Rev. 2018;32:130–43.
Figure adapted with permission from Valent P, et al. Haematologica. 2018;103:1593–603 and Verma A, et al. J Clin Invest. 2020;130:582–9.



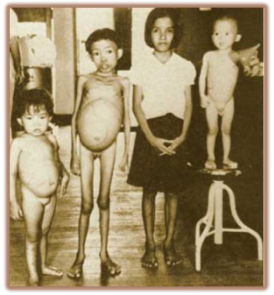
Coffey R, HemaSphere 2018

CLASSIFICAZIONE DELLE SINDROMI TALASSEMICHE



Kattamis A, Lancet 2022

1880: Cardarelli
1884: Somma



1925: Cooley description
1925: Rietti
1928: Greppi

1935: Miceli

1940–1950:
Caminopetros, Silvestroni, Bianco
Hb abnormalities, hereditary pattern

1949–1960: Pauling: Hb structure
HbS-Mendelian transmission
Ceppellini: HbA_2

1960–1970:
Weatheral and Clegg
Hb chain synthesis

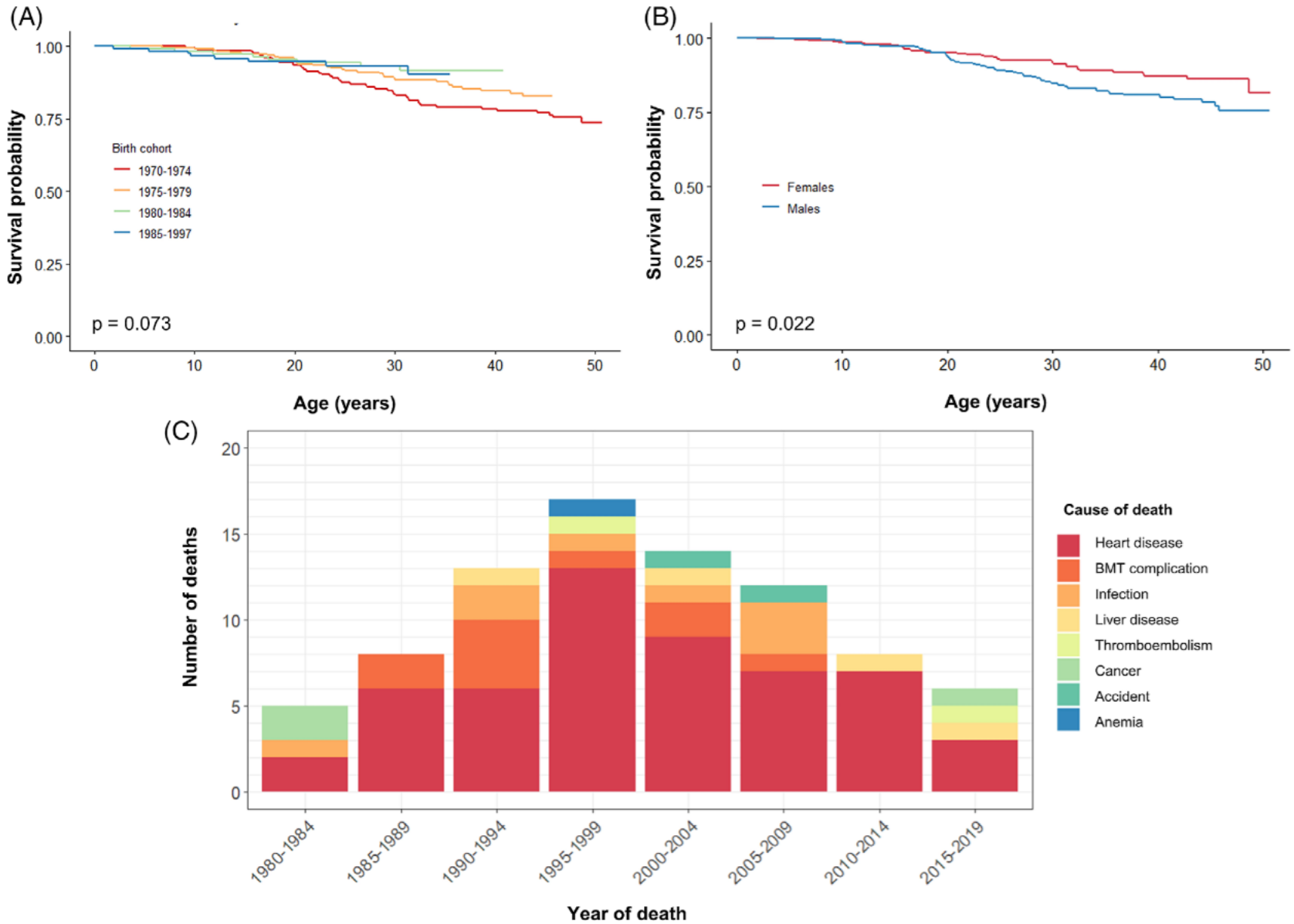
1970–1980: transfusional therapy
Iron chelation: deferoxamine

1980–2000:
Prenatal diagnosis (Kan)
Bone marrow transplantation (Lucarelli)

2000... new oral iron chelators

Present:
Gene therapy

SOPRAVVIVENZA BETA-THALASSEMIA IN ITALIA



Forni GL, Am J Hem 2023

NUOVE SFIDE

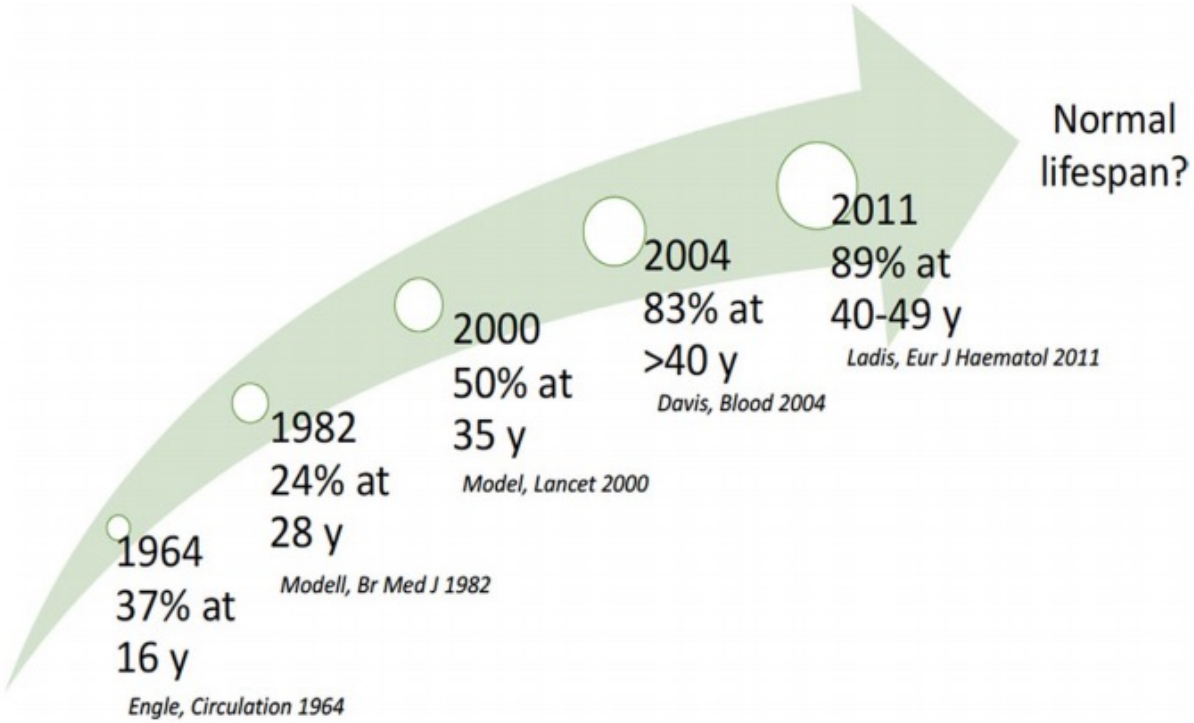
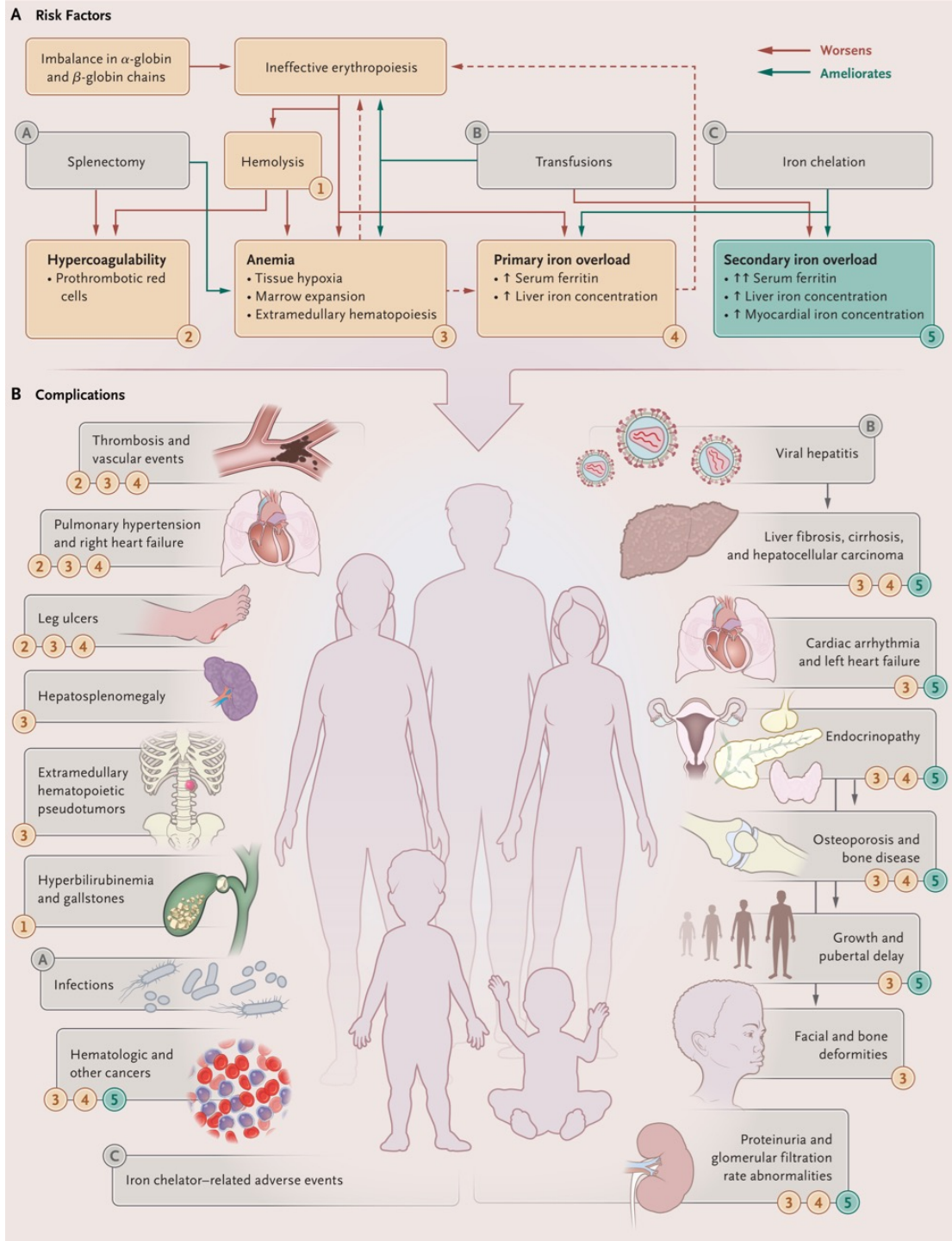


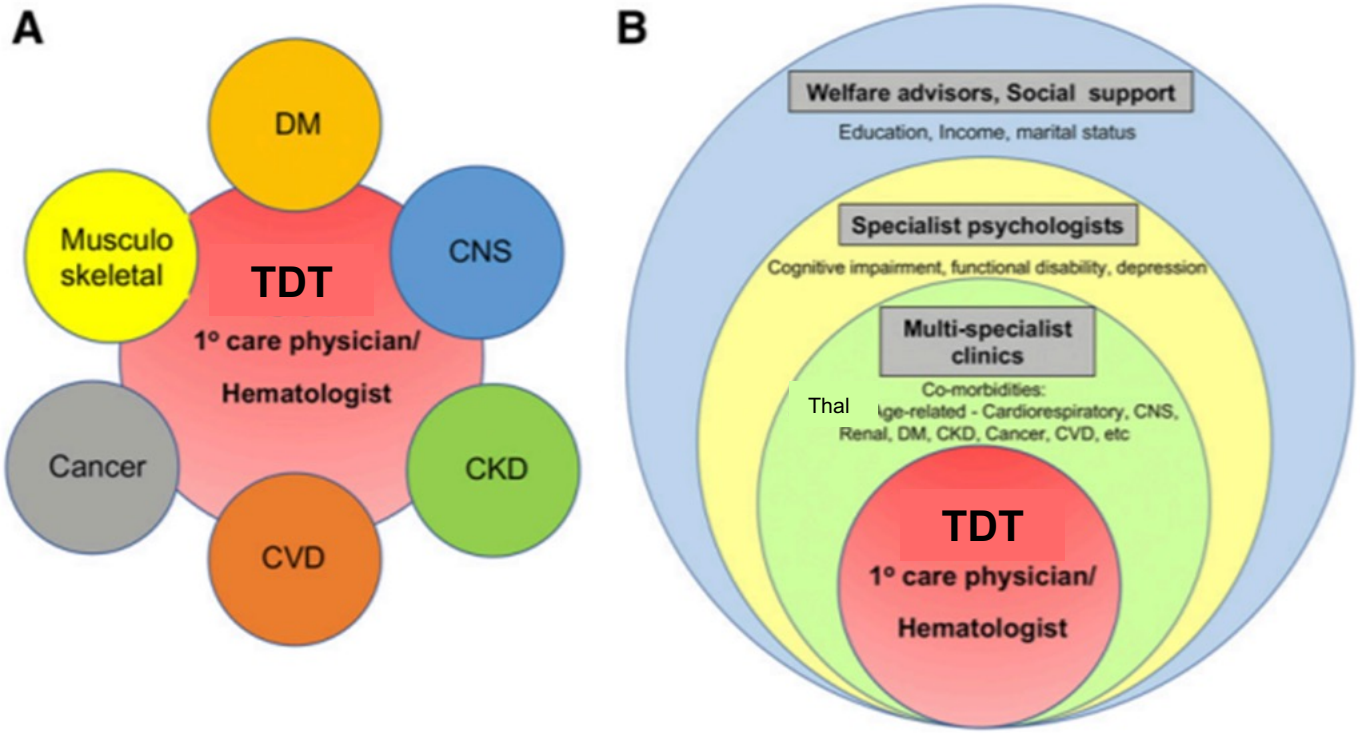
FIGURE 1 Evolution of thalassaemia patients' survival over the past decades according to selected publications [Colour figure can be viewed at wileyonlinelibrary.com]

Farmakis D, Eur J Haematol 2020



AT Taher et al. N Engl J Med 2021

NUOVO APPROCCIO



Modified from: Howard J, Hematology Am Soc Hematol Educ Program 2019

HBE

- Variante emoglobinica frequente in Asia
- Instabile, sintetizzata con minor efficienza
- Sostituzione di una base al codon 26 del gene beta-globinico (GAG-AAG) con sostituzione aa lisina→ac. glutammico

Table 1. Hematological data and clinical picture of subjects with HbE with different kinds of α-globin gene interactions

Hb E	α-Globin gene	Hb (g/dl)	MCV (fl)	Hb typing	Hb E (%)	HbBart's (%)	Hb F (%)	Clinical
Hb E heterozygote	αα/αα	12.8 ± 1.5	84 ± 5	EA	29 ± 2.3	-	0.9 ± 0.7	Normal
Hb E homozygote	αα/αα	10.6 ± 1.2	65 ± 3	EF	88 ± 2.6	-	3.6 ± 1.6	Normal
Hb E β thalassemia	αα/αα	7.1 ± 1.4	59 ± 3	EF	58 ± 9.5	-	38 ± 11.7	Mild to severe disease

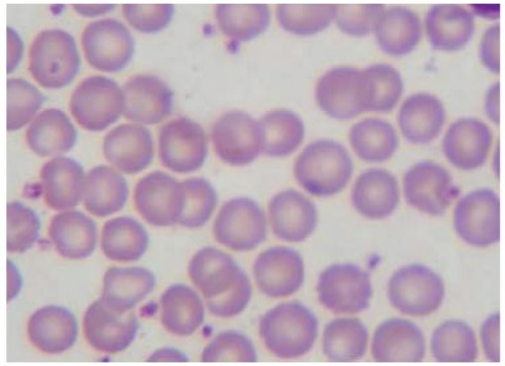


Figure 1. The peripheral blood film in hemoglobin E trait showing normal red cell morphology.

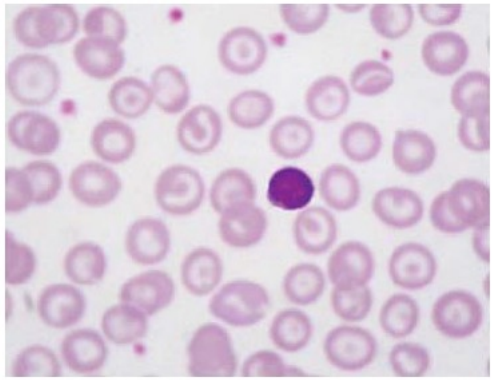


Figure 2. The peripheral blood film in the homozygous state for hemoglobin E showing large numbers of target cells.

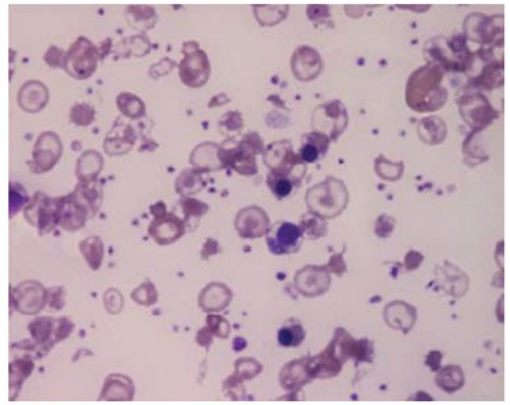


Figure 3. The peripheral blood film in hemoglobin E β thalassemia after splenectomy showing numerous nucleated red cells and a high platelet count.

Fucharoen S and Weatherall D, Cold Spring Harb Perspect Med 2012

ELEVATI VALORI DI HBF

- NTDT e TDT
- Persistenza ereditaria di HbF (HPFH), spesso senza segni clinici e di laboratorio
- neoplasie midollari
- stress eritropoietico
- trattamento con agenti citotossici (es. idrossiurea)
- δβ talassemia: livelli di HbA2 sono in genere normali/bassi e i livelli di HbF sono aumentati (5-20%)

TAKE HOME MESSAGES

- Patologia frequente e in continua evoluzione dal punto di vista epidemiologico
- Inquadramento: emocromo completo, reticolociti, stato del ferro, indici di emolisi, B12, folati, HPLC Hb
- Red flags: anemia microcitica/microcitosi, poliglobulia, reticolocitosi, alterazione indici di emolisi, alterata HbA2
- Concomitanti difetti qualitativi e quantitativi possono coesistere e impattare sul quadro clinico
- Il classico quadro clinico è in costante evoluzione con nuove sfide per gli operatori sanitari ed i pazienti

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