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Benign Hematological Disorders

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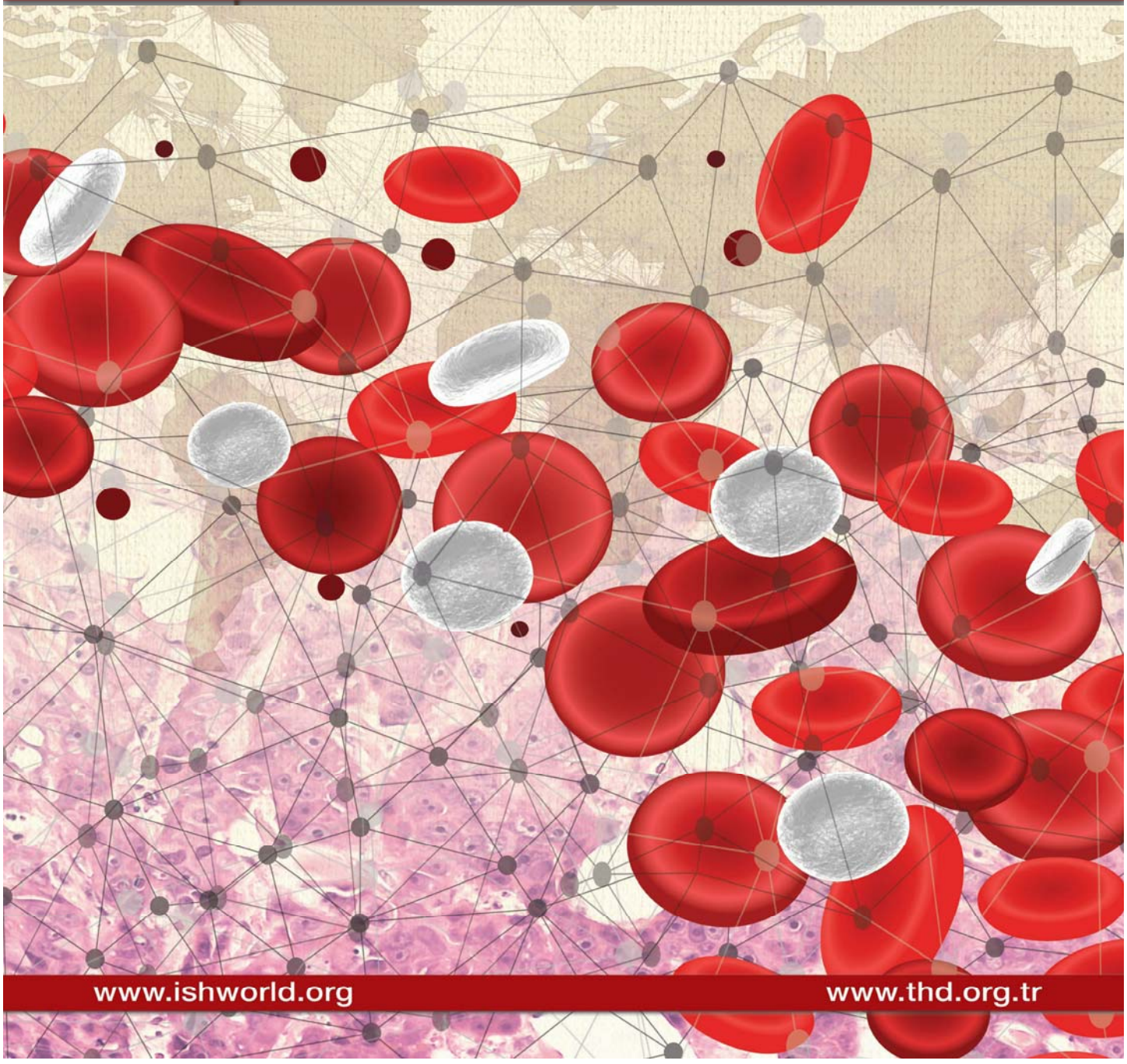
22-24 December, 2017

Papillon Ayscha Hotel Antalya, TURKEY



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Current Perspectives on Hematopoietic Stem Cell

Dr. Emin Kansu (Turkey)

Stem cell is defined as a primitive cell with a very high potential and infinite ability of self-renewal and differentiation into other cell types. Stem cells have unlimited potential for self-renewal to keep stem cell numbers and stem cell pool size constant life-long. These cells are clonal and have multilineage differentation features as well as engraftment potential to another host with complete reconstitution of hematopoiesis. Hematopoietic stem cells (HSC) have either symmetric or asymmetric divisions to keep their reserve constant and to differentiate to other cell types. HSCs can go to apoptosis when needed, enter into mitosis phase and also circulate in the peripheral blood at a constant rate. The most important and useful property of stem cells is that of self-renewal.

HSC is an adult stem cell derived from mesodermal stem cells (MSCs). MSCs as somatic stem cells also produce bone, muscle and cartilage stem cells. Adult HSCs have a long term self-renewal capacity and function as unipotent stem cells to produce erythroid, myeloid, lymphoid and megakaryocytic lineages. All these committed lineages are tightly regulated by hematopoietic growth factors, namely Erythropoietin (Epo), G-CSF /GM-CSF and Thrombopoietin (TPO) and IL-7. Current schema of hematopoiesis is shown in Figure – 1. Each committed HSC has a specific receptor on its surface for its respective ligand - hematopoietic growth factor **(**Figure-2).

HSCs are composed of a combination of heterogenous cells with different phenotypic characteristics. Mostly HSCs have a CD34 (+) and CD38(-) phenotype (Figure – 1). The stem cells or primitive progenitors from various tissues in adults share several phenotypic characteristics.

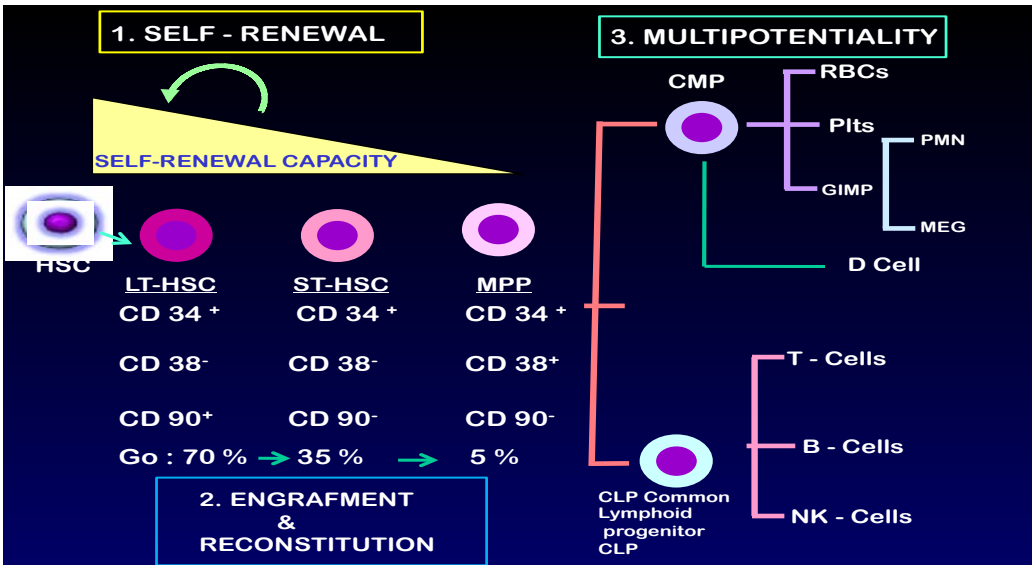

Figure – 1 . *Current schema of hematopoiesis with self-renewal capacity, multipotentiality and engraftment / reconstitution features*

During embryonic stages and fetal life, human HSCs start to appear in aorta- gonad-mesonephros (AGM), then switch to yolk sac stage for a very brief period followed by placental period and finally into the fetal bone marrow. During embryonic development, blood forming stem cells (HSCs) go from the yolk sac region to AGM. As HSCs divide some of the daughter cells remain as stem cells and continue dividing while others become committed to specific lineages. HSCs go into different stages of development in mouse and in human (Figures-2 , -3, -4 and -5). HSCs can be found in peripheral blood, bone marrow and cord blood.

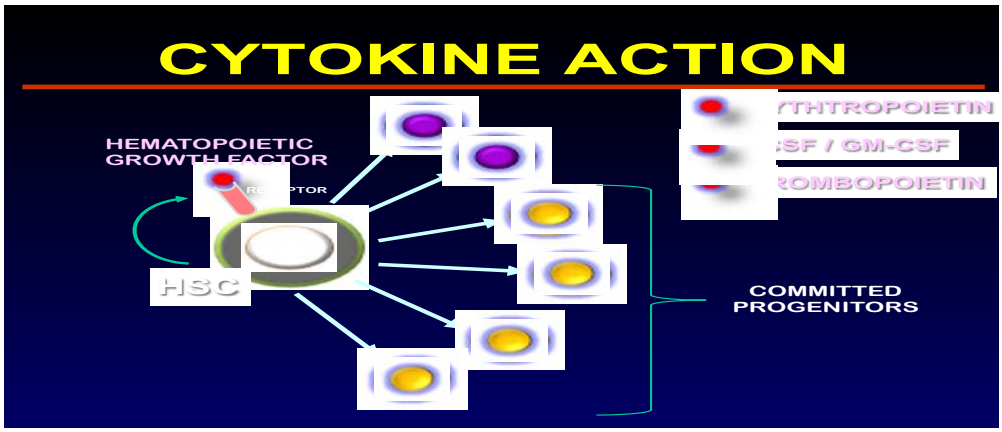

Figure – 2. *Action of hematopoietic growth factors (HGF) on hematopoietic stem cell. Each HGF binds its specific receptor on HSC and thru intracellular signaling pathways (i.e. Jak-Stat) and transcription factors activate genes for lineage commitment. Then, lineage committed stem cell produce progenitors which proceed to form mature blood cells (e.g. proerythroblasts to mature erythrocytes).*

Cellular decisions controlling HSCs development, self-renewal, and differentiation are thought to be regulated by the hematopoietic growth factors and cytokines that activate the expression of specific transcription factors.

In last three decades, we witnessed significant advances in our understanding of the cellular physiology and molecular regulation of hematopoiesis. At the heart of stem cell self-renewal and lineage commitment decisions lies lineage-specific transcription factors (TFs). HSCs share a number of TFs and surface markers, including SCL, GATA-2, C-kit, AA-4.1, CD34, Flit-3 ligand, Sca-1, VEGFR-1 and -2, only with the exception of CD45. Several transcription factors have been found to play critical roles in HSC physiology, including SCL (stem cell leukemia hematopoietic transcription factor), GATA-2, and Lmo-2, which are essential for primitive and definitive hematopoiesis, and AML-1 that is required for definitive hematopoiesis.

Recent progress has been made in elucidating the location and cellular components of the HSC **niche** in the bone marrow. The **niche** is perivascular, created partly by mesenchymal stromal cells and endothelial cells and often, but not always, located near trabecular bone. Niche plays a key role in quiescence, survival, growth, maintenance of HSCs in undifferentiated state and ultimately in differentiation of HSCs.

Two types of bone marrow **niche** have been defined; vascular niche and trabecular **niche.** The identity of the cells forming the HSC **niche** remains unclear. Previous studies have shown that osteolineage cells control the niche size and HSCs have been found preferentially localized in the endosteal region. However, haematopoiesis can be sustained in extramedullary sites and selective osteoblast depletion or expansion does not acutely affect HSC numbers.

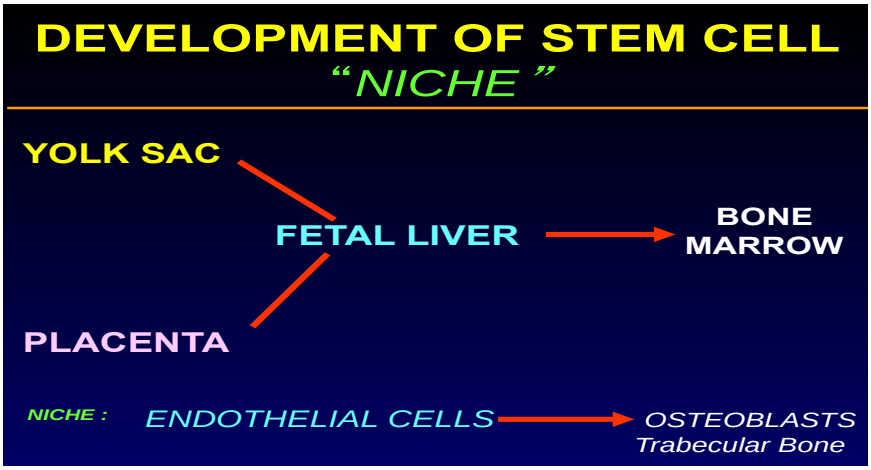

Figure – 3. *Development of HSCs at different sites in embryonic, fetal and adult life.*

HSCs have also been located preferentially in perivascular regions. During embryonic stages and fetal life, human HSCs start to appear in aorta- gonad-mesonephros (AGM), then switch to yolk sac stage for a very brief period followed by placental period and finally into the fetal bone marrow. During embryonic development, blood forming stem cells (HSCs) move from the yolk sac region to AGM. As HSCs divide some of the daughter cells remain as stem cells and continue dividing while others become committed to specific lineages. HSCs go into different stages of development in human and in mouse (Figures -2 , -3, -4 and -5).

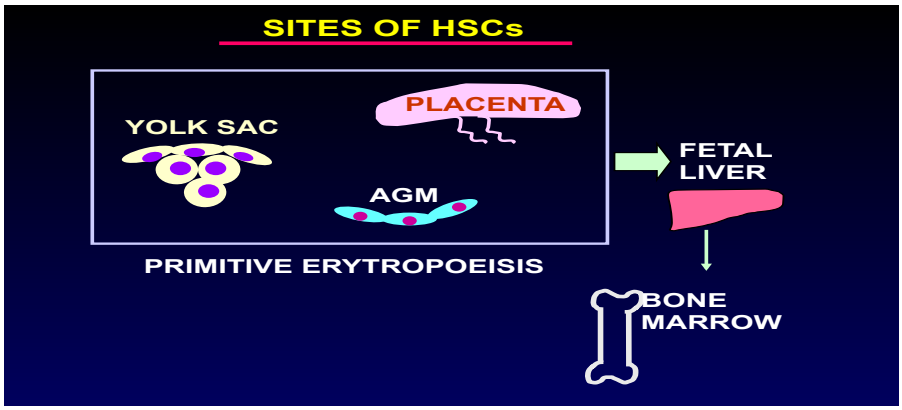

Figure – 4. *Sites of primitive and fetal hematopoiesis in humans. After birth, hematopoiesis is maintained in the bone marrow.*

It is becoming increasingly clear that bone marrow microenvironment has a critical biological function and can induce alterations in hematopoiesis. The cellular constituents forming the haematopoietic stem cell (HSC) **niche** in the bone marrow are mostly osteoblasts, endothelial and perivascular cells. It has been shown that mesenchymal stem cells (MSCs), identified using nestin expression, constitute an essential HSC niche component. HSCs at the **niche** site is directly regulated by the microenvironment. The vascular niche drives a robust self-renewal and expansion phase of HSCs.

HSCs located near reticular cells which express high levels of the chemokine CXCL12 (also called SDF-1) . However, the identity and function of these cells have not been clearly defined. The movement of HSCs may provide an insight into their **niche** because it is directly regulated by the microenvironment. HSC mobilization requires signals from the sympathetic nervous system (SNS) which under homeostasis lead to clock-controlled rhythmic oscillations of *Cxcl12* expression through the β_3-adrenergic receptor. Sympathetic fibres in the bone marrow are associated with blood vessels and adventitial reticular cells connected by gap junctions, thereby forming a structural network called the neuro-reticular complex at the **niche** site (Figure - 6). Both HSC and a **niche cell** are bound thru several ligands and receptors forming tight inter-cellular connections (Figure-7).
In recent years, studies are indicating that during aging, hematopoiesis may result of clonal selection at the level of the HSC. We may view the hematopoietic aging as a clonal selection in which a pool of HSCs that are heterogenous with respect to their self-renewal and differentiation capacities at birth are clonally selected over time. Several recent studies have strongly suggested that a significant proportion of HSCs in healthy aging individuals are likely to be derived from HSC clones that have acquired somatic mutations mutations that also are associated with hematologic malignancies, such as MDS and AML.

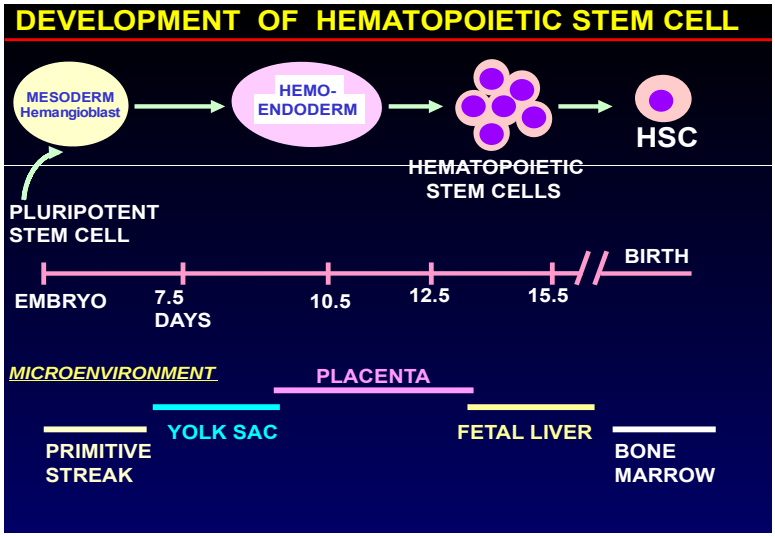

Figure - 5. *Developmental biology of HSCs in mouse.*

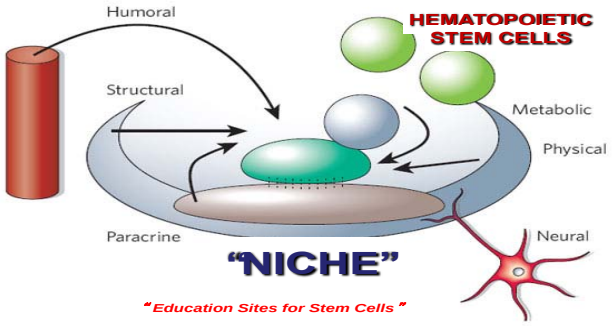

Figure – 6. *Schematic organization of bone marrow* ***niche*** *"Education site for a stem cell".*

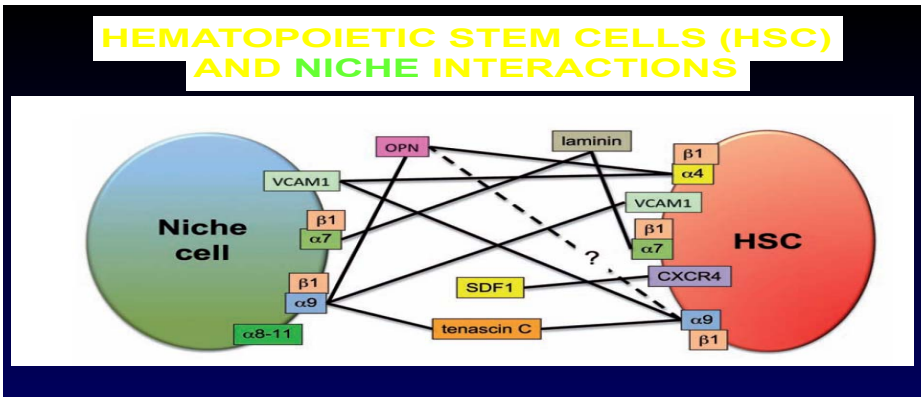

Figure - 7 *. Niche cells are connected to HSCs via several surface molecular receptors and ligands to keep them at the site.*

Hematologic cancers may often originate from the transformation of normal stem cells, similar signalling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include 'cancer stem cells' — rare cells with indefinite potential for self-renewal that drive tumorigenesis.

Allogeneic and autologous hematopoietic stem cell transplantation have shown that HSCs can cure several benign (Aplastic anemia, Fanconi's anemia,Storage diseases and hemoglobinopathies) and malignant hematological disorders (Acute and chronic leukemias, MDS).

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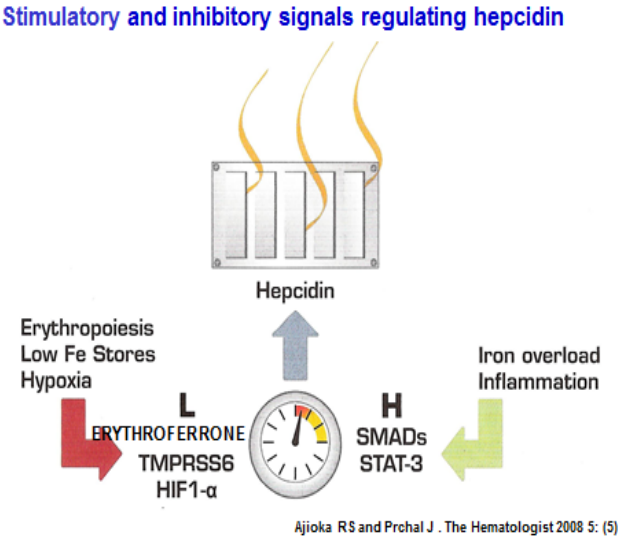
New Insights to Iron Metabolism and Iron Deficiency Anemia

Chaim Hershko (Israel)

Iron is the most abundant element on planet Earth contributing 35% to its mass. Earth's liquid iron core is instrumental in creating a magnetic field that surrounds Earth and shields the planet from harmful cosmic rays. Iron is a transition metal alternating between its oxidized ferric and reduced ferrous form and therefore essential for redox chemistry. Iron deficiency has serious consequences manifested not only in anemia but in abnormal mental and motor development in infancy, impaired work capacity, increased risk of premature delivery and increased maternal and infant mortality in severe anemia

Iron acquisition from the GI tract and its recycling from internal sources is regulated at the cellular and the systemic level. In the intestinal mucosa iron can be absorbed as heme iron or nonheme inorganic iron. Inorganic iron is first reduced by ferrireductase and then internalized by divalent metal transporter1 DMT1. For further transport into the circulation it combines with ferroportin, oxidized by ferroxidase and then bound to transferrin which takes it to its final destination. On the cellular level the production and activity of all of these proteins is enhanced by iron deficiency and offers protection from iron deficiency.

The discovery of hepcidin, the master iron regulatory protein by E Nemeth and T Ganz in 2004 revolutionized our understanding of iron homeostasis on the systemic level. Hepcidin is produced in the liver and is transported to all tissues in the body where it combines with ferroportin inhibiting the transport of iron from the cell to the circulation. Increased hepcidin inhibits iron import from the intestine, liver parenchyma and macrophages. Conversely, decreased hepcidin enhances iron absorption and release of iron from the liver and macrophages. Hepcidin production is adjusted to the iron requirements of the body. It is suppressed by low iron stores, hypoxia and increased erythropoiesis. Conversely it is stimulated by iron overload and inflammation (Figure 1).



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Inability to produce hepcidin to prevent iron overload is the genetic abnormality responsible for hereditary hemochromatosis

Causes of iron deficiency anemia (IDA)

If iron is so common in nature and in view of the elegant protective mechanisms against iron deficiency, why is iron deficiency the most common nutritional problem of the human race? The following conditions may cause iron deficiency in spite of effective protective mechanisms: (1) Increased nutritional requirements (infancy, pregnancy, adolescence); (2) deficiency of food iron (poor diet, malabsorption) and; (3) Increased blood loss (physiologic in fertile females or pathologic). The extensiveness of diagnostic workup should be adjusted to the clinical situation. In physiologic IDA the diagnostic workup is minimal. When there is reason to suspect pathologic blood loss such as in adult males and post menopausal females, complete GI workup is mandatory. However, conventional gastrointestinal workup including even capsule endoscopy, will fail to disclose the cause of unexplained or refractory iron deficiency in about 20 – 30 % of cases . In such patients the following additional diagnostic procedures are recommended:

Proposed diagnostic workup for unexplained or refractory iron deficiency anemia.

	H pylori	Autoimmune gastritis	Celiac disease	IRIDA
Screening	H pylori IgG antibodies or fecal antigen	Serum gastrin Anti-parietal Abs or Anti-intrinsic factor Abs	Tissue transglutaminase IgA abs	Suggestive history and clinical assessment
Advanced	Urease breath test Gastroscopy and biopsies (optional)	Gastroscopy and biopsies (recommended)	Duodenal biopsy, HLA screening for DQ2 or DQ8 genotypes	Sequencing of the TMPRSS6 gene *
Response to specific treatment	H pylori eradication	N.A.	Gluten-free diet	N.A.

* NCBI site of available laboratories for TMPRSS6 sequencing
http://www.ncbi.nlm.nih.gov/gtr/tests/?term5164656%5Bgeneid%5D.

Hershko C, Camaschella C, Blood. 2014 Jan 16;123(3):326-33.

If initial screening for H pylori, autoimmune gastritis, or celiac disease is positive, or if the history is suggestive of hereditary iron refractory iron deficiency anemia (IRIDA) , advanced studies are recommended according to the tests described in Table 1. Active H pylori infection in the population is very common and in most subject is harmless. However, when all other causes of iron deficiency are excluded, H pylori eradication will cure IDA in about 50% of patients. Autoimmune gastritis is encountered in 26% of women with treatment refractory IDA and is often associated with B12 deficiency and hypothyroidism. Celiac disease is found in 6% of unexplained IDA even without any clinical manifestations of gastrointestinal disease and responds very well to gluten free diet. Finally, IRIDA is a rare inherited disease caused by a mutation of the TMPRSS6 gene interfering with suppression of hepcidin in response to iron deficiency. In our experience, including the above 4 conditions in the diagnostic workup of unexplained iron deficiency, decreases the proportion of unexplained IDA to less than 7% of cases.

Recommendations for treating iron deficiency anemia

Iron is best absorbed in its ferrous form in a mildly acid medium. The recommended daily dose is 150 to 200 mg elemental iron. As side effects are dose related, dose reduction may improve tolerance. Expected response includes increased CHr within days, increased RDW, moderate or no reticulocytosis, and 50% correction of Hb deficit in 4 weeks. IV iron is indicated for the treatment of iron deficiency anemia when oral iron is inappropriate, ineffective or poorly tolerated. Novel parenteral iron preparations are much safer than iron dextran and up to 1000 mg can be administered at a single infusion. Hypersensitivity reactions to IV iron are rare but potentially life-threatening. Their management requires prompt recognition and grading of severity, together with meticulous monitoring and immediate treatment.

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Iron Overload in Thalassemias : Pathophysiology and Treatment
Yeşim Aydınok (Türkiye)

Pathophysiology of iron overload in regularly transfused and none or occasional transfused patients with thalassemia

The human body lacks a physiological mechanism for removal of excess iron. Body iron accumulation rate and distribution differ based on whether it develops as a consequence of regular transfusion regimen that occurs in thalassemia major (TM)[1], or due to increased intestinal iron absorption and release of recycled iron from reticuloendothelial system that occurs in thalassemia intermedia (TI)[2].

It is estimated that 100 ml of pure concentrated packed red blood cells (with a hematocrit of 100%) contain 108 mg of iron, which is approximately 35-100 times more than the daily requirement[3]. Patients receiving 2-4 Units of blood per month will have an accumulation of 5000-10000 mg of iron annually or 0.3-0.6 mg/kg per day. Such extreme iron efflux by repeated transfusions in patients with transfusion dependent thalassemia (TDT) results in overwhelming the carrying capacity of transferrin and the generation of harmful iron species, such as non-transferrin bound iron (NTBI) and labile plasma iron (LPI) that is cleared preferentially by the liver, myocardium and endocrine glands and that catalyses the formation of free radicals leading to oxidative damage in these tissues[4,5].

On the other hand, iron overload resulting from increased intestinal iron absorbtion has been recognized as an important clinical challenge in patients with non-transfusion dependent thalassemia (NTDT) beyond the ages of 10-15 years[6-7]. The key element in the pathophysiology of thalassemia is impaired α:β globin chains ratio in which the relative overproduction of alpha globins form insoluble hemichromes with eventual release of free iron leading to formation of reactive oxygen species in erythroid progenitors. It results in premature death of red cell within the bone marrow (called ineffective erythropoiesis) or peripheral circulation (called hemolysis) leading to anemia and low tissue oxygenation. Hypoxia inducible factor orchestrates the response to hypoxia by stimulating EPO leading to erythroid marrow expansion and transcription of ferroportin in the enterocytes contributing to increased iron absorbtion. Further, EPO exacerbates ineffective erythropoiesis (IE) and a variety of erythroid factors such as erythroferrone are produced by erythroid cells under condition of stress erythropoiesis and suppress Hepcidin synthesis in the liver in turn contribute to iron overload by upregulation of the transport of absorbed iron through the enterocytes basolateral membrane into the systemic circulation[8]. The accumulation of absorbed iron from intestine in NTDT patients may reach to 3-4 mg/day or as much as 1000 mg/per year[9]. An annual increase in liver iron concentration has been estimated as 0.38 ±0.49 mg Fe/g d.w. in a recent prospective study in NTDT[2].

Clinical consequences of iron overload

Iron overload becomes evident very early in the transfusion history. Transferrin saturation exceeds the normal range after 4–6 transfusions in newly diagnosed patients. From the available data from regularly transfused patients with thalassemia, it is clear that many patients develop cardiac, pancreatic and pituitary iron overload early in their transfusion history, if prevention of extra-hepatic iron accumulation by dynamic chelation management with timely dose tailoring based on changing body weight and surrogate markers of iron load would not be provided[10].

In contrast, in never or minimally transfused TI patients, MRI assessed liver (R2) and cardiac (T2*) iron demonstrated no evidence of cardiac iron overload, while there may be significant hepatic iron accumulation11 predisposing patients to develop fibrosis12 and hepatocellular carcinoma13. However, the use of frequent transfusion therapy within the wide severity range of TI likely predisposes to cardiac iron deposition as well. Therefore, iron levels should be regularly assessed and iron chelation therapy should be initiated where appropriate in NTDT patients.

Both TDT and NTDT patients are suffered from several complications related to iron overload unless iron stores are maintained at safe levels by appropriate iron chelation therapy (Table 1).

Table 1. Iron overload induced complications in TDT and NTDT

Complications	TDT	NTDT
Cardiovascular	Left ventricular failure, arrhythmia	Pulmonary hypertension, right ventricular failure, venous thromboembolism
Liver	Fibrosis, cirrhosis	Fibrosis, cirrhosis, hepatocellular carcinoma
Endocrine	Hypogonadism, growth failure, hypoparathyroidism, hypothyroidism, diabetes mellitus	

Objectives of iron chelation therapy in thalassemia

The goal of iron chelation has shifted from treating to preventing iron overload in order to achieve a normal pattern of complication-free survival and quality of life. The key concepts of optimal chelation therapy include timely initiation, close monitoring and continuous adjustment. Although the primary objective of iron chelation therapy is to maintain iron balance at safe levels, at all times, once iron is accumulated, the chelation therapy should be intensified appropriately to achieve a negative iron balance in order to accelerate unloading of tissue iron to safe levels during which suppression of LPI will be the key action that is necessary to avoid further organ toxicity and preserve organ functions[10,14].

Monitoring iron overload

Although the same tools that are available for the assessment of iron burden are used in both TDT and NTDT, monitoring of iron loading should be initiated after six to eight transfusions in newly diagnosed patients with TDT whereas it can be postponed to up to ten years of age by considering the slow kinetics of iron loading in NTDT.

Serum ferritin (SF) is the most commonly used measure for the diagnosis and monitoring of iron overload and still remains the only tool in many countries. Traditionally, iron chelation therapy is started when SF exceeds 1000 µg/L and maintenance of SF between 500-1000 µg/L may be associated with additional beneficial effects on complication-free survival in TDT[15,16].

It has demonstrated that liver iron concentration (LIC) can reliably measure total body iron stores[17]. Although, SF generally correlates with body iron stores, TM patients with identical SF show highly variable LIC[18]. Further, the studies in TDT have consistently demonstrated that the predictive value of SF trends to forecast changes in LIC was not strong enough [19,20]. In the modern management of iron overload, non-invasive quantification of LIC by MRI is considered the standard of care where available and may be used on patients as young as 4-5 years of age without sedation.

The ability of cardiac T2* MRI as a validated technique to assess myocardial siderosis has demonstrated little predictive value of LIC (as well as SF) for cardiac iron deposition in previously chelated patients[21,22] and has provided insights into the different kinetics of iron loading/unloading in liver and heart[23]. In fact, cardiac iron clearance was found to be nearly 4 times slower compared to hepatic iron removal[24]. By the light of these observations, monitoring cardiac iron by T2* MRI has been an indispensable measure to detect cardiac risks resulted from myocardial siderosis and to accordingly initiate an appropriate management strategy using iron chelation therapy in TDT. Cardiac T2* MRI may be deferred in well-chelated children until 8-10 years of age when they are able to undergo MRI without anaesthesia[25].

However, recent data has revealed that cardiac siderosis may occur even in younger children with high transfusion but poor chelation history[26]. Cardiac T2* of 20 ms and corresponding LIC of 1.16 mg/g dw are accepted as lower thresholds of normal[21,27]. Cardiac T2* of <20 ms has been associated with cardiac risk[21] and cardiac T2* of<10 ms has been strongly associated with heart failure and cardiac death[28]. Therefore, with the ability to recognize preclinical cardiac iron accumulation, clinicians were able to implement intensification of chelation therapy as a primary prevention strategy to save organ function in patients with TDT.

Table 2 summarizes the generally accepted SF and LIC thresholds for optimum management of iron chelation therapy in TDT and NTDT. Regardless of SF and LIC levels, cardiac T2* MRI should also be maintained above 20 ms which is considered to be a lower limit of normal myocardial iron.

Table 2. Recommended thresholds for iron chelation management in TDT and NTDT[29,30]

	Transfusion dependent thalassemia			Non-transfusion dependent thalassemia		
Metrics of iron stores	Start chelation	Maintain chelation	Stop chelation	Start chelation	Maintain chelation	Stop chelation
Serum Ferritin (µg/L)	≥1000	500-1000	NR	>800	300-800	<300
LIC (mg Fe/g dw)	>3	1.5-3.0	NR	>5	3.0-5.0	<3

LIC; Liver iron concentration, NR; not recommended

SF assessments at 3-6 weekly intervals can provide the most rapid feedback with respect to patients' adherence and response to chelation therapy. Particularly, SF response can help predict LIC response but a lack of SF response should be interpreted with caution and assessment of LIC should be prioritized for those with a lack of SF response[20]. However, whenever feasible, quantification of LIC is highly recommended in order to make appropriate chelation decisions and accurate conclusions regarding patients' adherence to chelation therapy[19]. Available guidelines recommend liver and cardiac iron examinations annually unless there is a clinical indication for more or less frequent assessments[29,30].

Iron Chelation Treatment

Prospective studies assessing the efficacy of iron chelation regimens have highlighted the importance of the rate of transfusional iron intake, the existing hepatic and extrahepatic (cardiac) iron burden, and the chelator dosing and regimen for appropriate management of iron overload. Iron chelators in clinical use are summarized in Table 3.

Table 3. Properties of iron chelators used in patients with transfusion-dependent thalassemia[31-34]

	Deferoxamine (DFO)	Deferiprone (DFP)	Deferasirox (DFX)	
Route	SC or IV infusion	Oral (tablet and syrup)	Oral dispersible	Oral film coated
Usual dose	20–60 mg/kg/day over 8–24 h	75 -100 mg/kg/day	20-40 mg/kg/day	14-28 mg/kg/day
Schedule	5–7 times weekly	3 times daily	Once daily	Once daily
Excretion	Urinary, with some fecal	Mainly urinary	Mainly fecal	Mainly fecal
Most frequent Adverse events	Injection-site reactions, Yersinia infections, HF hearing loss, Retinopathy, Poor growth, Allergy	GI AEs (nausea, vomiting, abdominal pain), increased ALT levels, arthralgia, neutropenia	GI AEs (diarrhea, vomiting, nausea, abdominal pain), rash, increased ALT levels, increased serum creatinine	
Warnings		Agranulocytosis, neutropenia	Renal toxicity, hepatic toxicity, GI hemorrhage	

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The Screening Programs and Pre-natal Diagnosis of Hemoglobinopathies: Where Are We in The Region?

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Hemoglobinopathies are distributed worldwide and are considered by the World Health Organization (WHO) to be a significant global health problem. The recent estimates suggest that at least 5.2% of the world population carry a significant hemoglobin gene variant (HbS, HbC, HbE, HbD,etc. β-thalassemia, α^0 thalassaemia) and so there may be ~270 million carriers worldwide[1]. Annual births with a major thalassemia including beta and alpha thalassemia major and severe HbE/beta thalassemia is approximately 68000 in all over the world whereas approximately 283 000 children are born with a sickle cell disorders most commonly in Africa. However, because of increased migration flow, hemoglobinopathies increase in Europe and North America[2-4].

World Health Organization (WHO) reported that 95% of affected births with thalassemia are born in the Middle East, South-East Asian and Western Pacific Region. Worldwide, transfusion is available for a small fraction of those who need it and most transfused patients die from iron overload because iron chelators are not widely available. It is therefore vital that international health agencies and governments of countries where the hemoglobin disorders occur at a high frequency become aware of the future extent of this problem and develop programs for their control and management[5].

Countries may be divided into 3 general categories: Endemic Mediterranean countries where long-established prevention programs have succeeded and specialized clinics are able to provide optimum treatment. The area of industrialized world where prevalence is increasing because of migration. These countries may provide control of disease but have problems in reaching immigrant groups that address language and social barriers. Finally, countries of the developing world where many of the governments do not yet recognize the hemoglobinopathies as an important health priority to develop control programs[5].

The Frequency and Distribution Pattern of β-Thalassemia Mutations in the Middle East / Gulf States

β-Thalassemia is endemic in all countries of the Middle East and constitutes major health problems in the region. The frequency of carriers varies from 1,7% to 9,0% among the countries of the region[6]. Further, the frequency shows wide regional variations and would rise to much higher rates in some areas (e.g. 10 % in Antalya province of Turkey[7] and 10-13% around the Caspian Sea and Persian Gulf regions of Iran[8]). The molecular basis of *β*-thalassemia clearly demonstrates the heterogeneity of this population. More than 50 mutations have been reported which are mostly of Mediterranean and Asian origin and reflect the geographical and historical background of the region. It is important to note that the most common β-Thalassemia mutations in the region are of β^0 or β^+-thalassemia associated with severe phenotype (Table 1).

Table 1.Comparison of the most common mutation frequencies among Middle East countries[6]

β-Thalassemia Trait	Prevalence		The most common mutations				
	Mean (%)	Range (%)	1	2	3	4	5
Bahrain[9,10]	3	2,0 - 5,4	-25bp del	Cd39	IVSI-5	IVSII-1	Cd44
Iran[8]	5	4,0 - 13,0	IVSII-1	IVSI-5	Cd8/9	IVSI-110	
Iraq[11]	4,6	-	IVSII-1	Cd44	Cd5	IVSI-1	Cd39
Gaza Strip[12]	4,3	3,3 – 7,9	IVSI-110	IVSI-1	Cd39	Cd5	IVSI-6
Jordan[13]	3	3,0 – 3,5	IVSI-110	IVSII-1	IVSII-745	Cd37	IVSI-1
Lebanon[6]	1,7	1,0 - 3,0	IVSI-110	IVSI-1	IVSI-6	IVSII-1	Cd5
Oman[14]	2	0,2- 3,9	IVSI-5	Cd44	Hb Dofar	25bp del	IVSII-1
Saudi Arabia[15]	3,4	-	IVSI-110	IVSII-1	Cd39	25bp del	IVSI-5
U.A.E[16]	6,9	-	IVSI-5	-25 bp del	Cd 8/9	IVS-II-1	Cd 39
Egypt[17]	9	-	IVSI-110	IVSI-6	IVSI-1	IVSII-848	IVSII-745
Turkey[18]	2,1	0,3 -10,0	IVSI-110	IVSI-6	IVSII-1	IVSII-745	IVSI-1

In general, consanguineous marriage, high fertility and birth rate together with the limited prevention programs and prohibited termination of affected pregnancies represent the main factors contributing to the relatively high incidence of hemoglobinopathies in the Middle East and South-East Asian countries[19].

Implementation of population screening for hemoglobinopathies

Screening for hemoglobinopathies can be composed of full blood count (FBC) and high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) as less labor intense approaches compared to gel electrophoresis and a methods of choice for the initial screening of structural variants as well as accurate measurement of HbA_2 and HbF in population screening. Although in some HPLCs, HbA_2 would not be accurately quantified in the presence of HbS and separated from HbE in the presence of this abnormal hemoglobin, these obstacles are overwhelmed in recent HPLC technologies[20]. However, hemoglobinopathies are extremely heterogeneous disorders in which appropriately equipped laboratories with an experienced staff are required for correctly identifying at risk couples. Additionally, intensive education directed toward the health personnel and to the population at large is the most essential element for a successful prevention program[21].

Population based pre-marital screening is generally accepted by the countries where carrier frequency is high. Some countries like Iran prefer to screen male partner first and then female if male is carrier. This program aims to identify at risk couples before marriage and to offer counseling, thus providing them with the opportunity to separate or prenatal diagnosis and selective abortion within the fourth month of gestation[22]. Family studies of identified carrier can also be cost effective side- strategy. Couples who have been married before control programme has been established and still at reproductive age should also be screened. Currently, in a number of Middle East countries including Lebanon, Iran, Saudi Arabia, Tunisia, United Arab Emirates, Bahrain, Qatar, and Gaza Strip, the national premarital programs are mandatory and aimed at limiting carrier marriage[23-25]. In Turkey, a comprehensive hemoglobinopathy control programme comprising premarital screening, genetic counselling, and prenatal diagnosis and offering interruption of the pregnancy for the affected fetus has been implemented by law since 2003.

Although, the number of affected newborns per year demonstrated a trend towards a consistent decrease since 2009, we still continue to have babies with homozygous beta-thalassemia. In fact, a proportion of couples may still evade premarital screening or may not be identified as carriers due to laboratory or interpretation errors. Further, prenatal diagnosis may not be offered to or accepted by the at-risk families. The auditing all components of the programme carefully and applying appropriate corrective measures should be continued to further improvement in the achievement[26].

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Thalassemia Traits Difficult to Diagnose and The Significance in Marrital Screenings

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Most β-thalassemia carriers, have mild anemia with microcytosis (MCV <80 fL), hypochromia (MCH <27 pg) and elevated HbA2 (>3.5 %). However there are rare phenotypes who do not fit to this common phenotype and this can impact upon premarital screenings, appropriate genetic and family counseling.

Most β-thalassemia mutations are single nucleotide mutations, small insertions or deletions. Certain β+-thalassemia mutations, such as promoter nucleotide –101 C>T (HBB:c.–151C>T) mutation cause a silent carrier phenotype. Carriers of these "silent" mutations have minimal or no abnormalities in Hb, MCV, MCH and HbA2. On the other hand, patients who are compound heterozygous with either β+- or β0-thalassemia mutation can present as β-thalassemia intermedia with occasional transfusions requirements.

Carriers of the same β-thalassemia mutations might have very different blood counts. For example, among 35 carriers of β-globin gene translation initiation codon ATG mutations, Hb was found to range between 7.7 and 12.9 g/dL (mean, 10.4) and MCV ranged between 48.5 and 76.0 fL (mean: 56.4).
Beta-thalassemia carriers may also have concomitant α-thalassemia mutations. In this situation, these individuals have milder hematological findings due to improved imbalance in α-/β-globin chain ratio. This condition may be problematic during premarital screening. If this individual gets married to a β-thalassemia carrier, their offspring may have β-thalassemia major pheneotype. ITherefore, co-inheritance of both β- and α-thalassemia mutations is important for pre-marrital counseling.
Conversely, β-thalassemia carriers who have inherited α-globin gene triplication or quadriplication may present with β-thalassemia intermedia phenotype.
KLF1 gene mutations have been reported be associated with a borderline high HbA2 levels and can cause problem in β- thalassemia screening programs.
Concomitant inheritance of variant δ-globin chains, δ-thalassemia mutations and rare deletion involving the δ-globin gene may cause β-thalassemia carriers to have normal HbA2 and might confound the correct diagnosis of β-thalassemia carriers.
In patients with iron deficiency, HbA2 has been reported to be lower, but not below the 3.5% cut-off.
Other problematic conditions are dominant thallasemias, β-thalassemias caused by large deletions, loss of heterozygosity and mosaic segmental uniparental isodisomy.

New Therapeutic Approaches in Thalassemia

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The conventional management of thalassemia is blood transfusion, management of iron overload and monitoring for the disease and iron chelators complications. Stem cell transplantation (SCT) from a matched sibling donor was the only curative way for the management of this disease. There is improvement in the results of gene therapy and new advances in SCT.

New therapeutic approaches have emerged either potentially curative as SCT from a haploidentical or matched unrelated donors or by gene therapy. Novel drugs adjuvants to conventional therapy to improve endogenous erythropoiesis, decrease transfusion and chelation requirements are emerging. The use of modified activin receptor II fusion proteins (Sotatercept and Lusptercept) to enhance late stage erythropoiesis by acting as a ligand traps for members in the transforming growth factor B-superfamily. The aim of this therapy is to increase hemoglobin levels in both non-transfusion dependent thalassemia (NTDT) and transfusion dependent thalassemia (TDT) and to decrease the transfusional requirements in TDT. A positive effect on iron overload is mediated by the expected improvement in ineffective erythropoiesis and reduction in transfusion iron load. Clinical application of Jack 2 Kinase inhibitor in thalassemia based on improving the balance between proliferation and differentiation can improve the ineffective erythropoiesis. It reduces the spleen size in clinical trials which, in TDT, may reduce the transfusion requirements. Long acting hepcidin analogs (minihepcidin) have been developed to suppress iron absorption and are currently being investigated. Advances in iron chelation therapy to increase the patients' compliance will control the iron overload and improve the survival of the patients. A recent phase II study in TDT has shown the efficacy and safety of film-coated tablets of Deferasirox. It proved a greater patient satisfaction, compliance and palatability compared to the dispersable tablet formulation.

Conclusion: Options for cure of thalassemia by gene therapy and extended stem cell donor for transplantation are encouraging. New emerging therapeutics for the management of thalassemia is promising for better life and survival of the patients.

Therapeutic targets in sickle cell disease

Miguel Abboud (Lübnan)

Therapeutic targets in sickle cell disease

The treatment of patients with sickle cell disease has relied mainly on supportive and preventive care. These strategies, such as the prophylactic use of penicillin, prompt attention and management of febrile episodes and use of transfusions and antibiotics for acute chest syndrome have resulted in the increased survival and wellbeing of children with SCD. Outside the realm of supportive care hydroxyurea remains the only approved drug which will impact the pathophysiology of sickle cell disease. Its use has led to significant improvement in the clinical condition of patients with the disease, decreased mortality in both adults and children and has impacted end organ dysfunction. Hydroxyurea nonetheless has its limitations and significant concerns remain about potential toxicity and impact on male reproduction. Recently new understanding of the pathophysiology of sickle cell disease has led to development of new drugs that will target the disease process are being developed. These processes included.1. Induction of fetal hemoglobin Adhesion mediated by selectins 3. Hemoglobin polymerization 4. Hemolysis and NO scavenging 5. Increased reactive oxygen species and heme, 6. Inflammation and 6. Platelet activation. These mechanisms and drugs will be discussed in addition to a brief description of current gene therapy trials.

1. Induction of fetal hemoglobin

Fetal hemoglobin (HbF) is a potent inhibitor of the polymerization of sickle hemoglobin (HbS). In vitro HbF prolongs the delay time required for a critical HbS polymer to form. It has long been known that sickle cell disease patients with higher fetal hemoglobin levels have fewer pain crises, fewer complications and longer survival. Hydroxyurea, the only drug approved for the treatment of SCD, acts in part by increasing HbF levels. The silencing of the gamma globin genes through DNA methylation if reversed could lead to major increments in HbF. Demethylating gents such a azacytidine and decitabine have been studied and shown to increase HbF. More recently an oral formulation of decitabine combined with tetrahydrouridine is being studied with promising results. Interesting results were also seen with metformin. In addition to pharmacologic approaches gene editing approaches to increase HbF will be discussed.

2. Inhibition of cellular adhesion

Cellular adhesion of both erythrocytes and neutrophils (Figure 1) are important for the initiation of vaso-occlusion. Selectins are surface adhesion molecules that play a critical role in sickle RBC adhesion. Inhibition of P selectin by crizanluzomab was recently shown to be effective in preventing vaso-occlusive crises. Other compounds including rivipansel and sevuparin are currently being investigated for their potential role in curtailing painful crises once vaso-occlusion has started.

3. Inhibition of HbS Polymerization

Polymerization of HbS is the process that initiates vaso-occlusion (Figure 2). It has been shown in vitro that this can be prevented by increasing the affinity of HbS to oxygen. Use of compounds that increase oxygen affinity and shift the oxygen dissociation curve to the right have been shown to prevent sickling in vitro and prolong the survival of sickle mice exposed to hypoxia. An oral agent GBT 440 has recently entered clinical trials

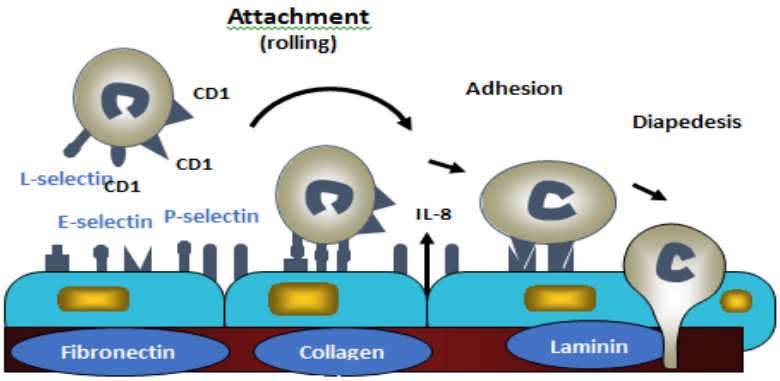


Figure 1

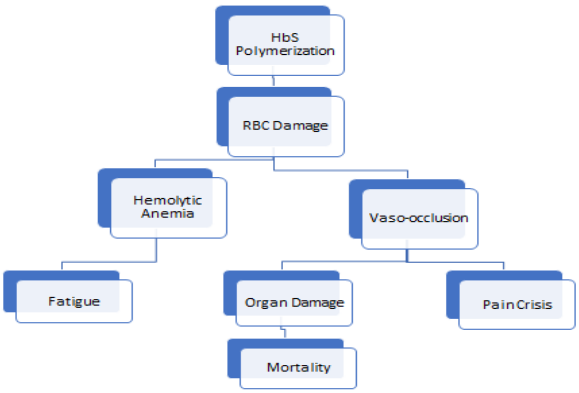
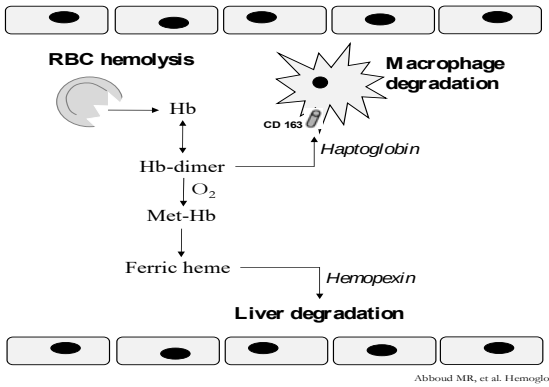


Figure 2

4. Hemolysis and NO Scavenging and Reactive oxygen

Nitric oxide depletion (figures 3a and 3b0 may play and important role in the pathophysiology of sickle cell disease. The use of arginine, the precursor of NO, has been shown to be effective in small trials in curtailing pain and decreasing opiate use. Rcenletly L Glutamine, which effects the intracellular oxidative environment by repleting glutathione and NADPH was approved for the prevention of vaso-oclussive episodes in patients with SCD.

Hemolysis and vasculopathy (1)



Abboud MR, et al. Hemoglobin. 2009;33 Suppl 1:S93-S106..

Hemolysis and vasculopathy (2)

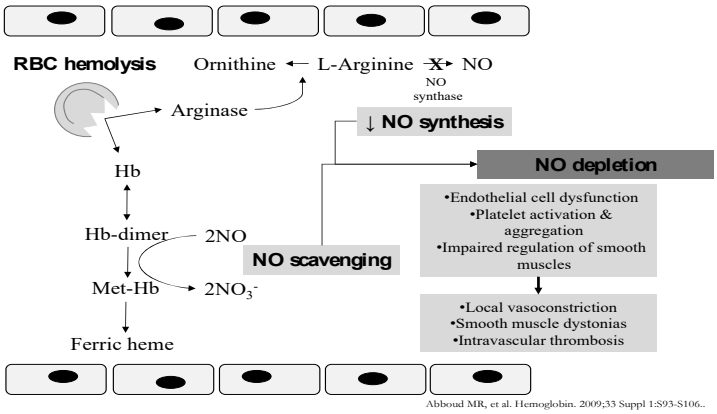


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Figure 3a and 3b

Studies involving anti-inflammatory agents and antiplatelet agents will also discussed as well as gene therapy approaches.

Microangiopathic Hemolytic Anemias (MAHAs): Differential diagnosis and management

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Microangiophatic hemolytic anemias (MAHAs) are a heterogeneous group of disorders affecting children and frequently young adults with an either congenital or acquired manner. MAHA is a descriptive term for non-immune hemolysis (a.k.a Coombs-negative hemolysis) resulting from intravascular red blood cell fragmentation that causes formation of schistocytes on the peripheral smear. Abnormalities in the microvasculature are frequently observed. Additionally, intravascular devices such as a prosthetic heart valves or intracardiac devices may also cause MAHA. Characteristic laboratory data include a negative direct antiglobulin (Coombs) test (DAT), an increased lactate dehydrogenase (LDH), increased indirect bilirubin, and low haptoglobin. When associated with microvascular thrombi and thrombocytopenia is also observed, a thrombotic microangiopathy (TMA) should be considered.

Definition of MAHA and distinction from TMA is important. Generally it may be accepted that all MAHAs are not caused by a TMA, but nearly all TMAs cause MAHA and thrombocytopenia. Although TMA is a pathologic diagnosis made by biopsy, it is commonly implied from the observation of MAHA and thrombocytopenia in the appropriate clinical setting.

Many systemic disorders can cause MAHA and thrombocytopenia. Some of the more common and well-described conditions include pregnancy complications such as preeclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), severe hypertension, systemic infections (bacterial, viral, fungal or rickettsial), systemic malignancies, rheumatic disorders, hematopoietic stem cell transplantation or organ transplantation, disseminated intravascular coagulation and severe vitamin B12 deficiency. The principal disorders that may cause MAHA with or without thrombocytopenia are summarized in Table 1.

The initial evaluation should be focused on confirming that the patient has true microangiopathic hemolytic anemia (MAHA) with or without thrombocytopenia, and excluding systemic disorders that may manifest with these findings, based on a consideration of presenting findings and likely causes.

The important laboratory tests that must be performed for all patients include a whole blood count (WBC), to assess the degree of anemia and thrombocytopenia; lactate dehydrogenase (LDH), to estimate the severity of hemolysis, and serum creatinine levels, to determine the severity of renal dysfunction. All patients with MAHA and thrombocytopenia without an obvious systemic illness that may explain these findings should have their ADAMTS13 activity evaluated for the differential diagnosis of thrombotic thrombocytopenic purpura (TTP). Though, ADAMTS13 activity measurements may take several days, and patients with a clinical possibility of TTP require urgent

therapy with plasma exchange therapy (PEX). Treatment should be commenced on the clinical probability and be reevaluated or supported after the ADAMTS13 activity results.

All patients with MAHA and thrombocytopenia without an obvious systemic illness and also who have had severe abdominal pain with diarrhea, should have a stool culture for enterohemorrhagic *Escherichia coli* (EHEC). *Shigella dysenteriae* is a more common cause of TMA in Asia but is not as such a common cause in America or Europe.

All patients with MAHA with thrombocytopenia who have negative testing for TTP and ST-HUS should be tested for cobalamin C deficiency-mediated TMA using measurement of serum homocysteine and/or methylmalonic acid (MMA). The role for testing complement regulation by measuring complement proteins (C3 and C4, CH50), antibodies to complement proteins, or complement gene mutations are unclear. Decreased levels of complement factors or the presence of anti-complement factor H (CFH) antibodies may be helpful in suggesting a complement-mediated TMA; however, normal complement levels do not exclude the possibility of a complement-mediated TMA

Table 1. Disorders that may cause MAHA with/without thrombocytopenia

Disorders	**Distinctive Features**
Systemic Infections	Bacterial, viral and fungal etiologies. Renal failure, high fever may suggest sepsis and severe infection rather than a primary TMA syndrome
Systemic Malignancies	Microvascular thrombi may cause similar lesions with TMA syndromes. Older age, constitutional symptoms and leukoeryhtroblastic peripheral smear are distinctive
Severe hypertension	Renal TMA syndrome caused by hypertension may not be differentialized from TTP.
Pregnancy related syndromes	Severe preeclampsia and HELLP syndrome whic improve spontaneously after delivery is distinctive.
Systemic lupus erythematosus	May mimic TTP and other TMA syndromes and no spesific distinctive feature is observed
Drug reactions	Several drugs are reported as the cause of drug related TMA syndromes either immune mediated or dose depended.
Stem cell transplantation	A TMA syndrome, unresponsive to plasma exchange is observed
Complement regulation abnormalities	Genetic abnormalities regarding complement regulation are distinctive

The major challenge in the management of MAHAs include the assessment of the need of urgent interventions including PEX and anti-complement therapy. PEX is an established treatment for TTP though the benefit of PEX in other TMA syndromes are not as clear. As requiring specialized therapeutic apheresis center as well as a replacement of a central line, patients with a possible diagnosis of TTP should be transferred to a special and capable center. The decision to start anti-complement therapy should be based on strong suspicions for complement mediated TMA and also in a rapid manner, preferably within 24 to 48 hours. For other possible differential diagnosis such as drug induced TMA, discontinuation of the implicated drug and for cobalamin deficiency mediated TMA, high dose cobalamin with folinic acid should be started. Remaining TMAs and MAHAs without thrombocytopenia, primary management is supportive care including red cell transfusions.

Emerging Therapeutic Options and Strategies in Hemophilia: Novel Factor Concentrates

Kaan Kavaklı (Turkey)

Hemophilia is a life-longer and X-linked genetical coagulation disorder. Currently about 20.000 people have hemophilia in the USA and 6.000 patients in Turkey. Most of them are factor VIII deficiency, hemophilia-A. The main treatment for hemophilia is called replacement therapy. Clotting factor concentrates may be plasma-derived or recombinant. In severe hemophilia-A (FVIIII <1%), prophylaxis is gold standart therapy for preventing hemophiliac arthropathy.

Despite multiple therapeutic options, intra-venous way of therapy, cost of treatment and inhibitor development are main pitfalls of current hemophilia care and therapy. Especially burden for intra-venous way problems are major unmet need for hemophilia.

Several technologies are being implemented to advance hemophilia treatment. First of all extended half-life (EHL) factor concentrates are now available in Western countries. Thanks to these agents once or twice weekly regimens are possible for adult or children patients in prophylaxis for hemophilia-A. For hemophilia-B is progression is much more better as in every one or two weeks of infusions. Unfortunately EHL-products are not yet available in Turkish market even though we have familiarized them by clinical trials for five years. We can use this products twice weekly in stead of thrice weekly for small children. We have some adults in trials who able to treated only once weekly for routine prohylaxis. More higher trough levels are possible with EHL-products with same infusion frequency during prophylaxis.

However, main actors for future seems to be subcutaneous way agents. Interestingly, these products are not replacement agents and they are small molecules which produce enhancement of thrombin. Nowadays three novel molecules are in clinical trials in different stages. Emicizumab (ACE-910), a monoclonal antibody mimics FVIII in the coagulation system is recently approved by FDA in the USA for hemophilia patients with inhibitors (HemLibra, Roche). This molecule is the first approved monoclonal antibody for hemophilia field.

Other two agents work with inhibition of some anti-coagulation proteins in the blood. Concizumab works to inhibite of TPFI. So it's mechnanism of action is with Anti-TFPI effect (Novo Nordisk, Denmark). Fitusuran is either anti-antitrombin molecule but very different product as well. It is not monoclonal antibody. It's technology depends on RNA interferance approach (Alnylam Ther, USA).

Subcutaneous way is main advantage of Emicizumab, Concizumab and Fitusuran. Interestingly, Emicizumab is used by in every one week and Fitusuran is used in every one month of intervals. All agents developed for routine prophylaxis. They have no need for intra-venous injections. In near future, these agents will be used in routine prophylaxis programs in hemophilia-A and B. More interestingly, these products may be used in all factor deficiencies with or without inhibitors. Only Emicizumab is not efficient for hemophilia-B due to it's normal FIX need in the mechanism.

No doubt, these new SC agents have some disadvantages. First of all, they are not efficient for breakthrough bleedings. So, we will still need factor concentrates again for treating bleedings and for perioperative management of patients.

In summary, currently plasma-derived or recombinant products are available in Turkey. We have used these products for years. However EHL-products will come to Turkish market in a year. With these products, we can use more infrequently prophylactic infusions and much more higher trough levels for FVIII or FIX activity. No doubt, main game-changer agents will be subcutaneous way products. Emicizumab, Concizumab and Fitusuran will be main actors for prohylaxis in the future of hemophilia.

Gene therapy trials have reached phase-3 either in hemophilia-B (Spark Ther, USA) and hemophilia-A (Biomarin Ther, USA) after long-term experimental studies. Both technology use adenovirus transmitted vector system. However next game-changer procedure for gene therapy will be "Gene Correction" models. Nowadays experimental studies were completed with success and clinical trials in humans will be started. CRISPR (Cas-9) and Zinc Finger Nucleaz (ZFN) technologies are current procedures for gene correction approachs. The most negative point of gene therapy is the cost. The estimation of one patient's cost in gene therapy is close to one million dollars. So, in future we will have significant difficulties to reach gene therapy even though it is feasible for routine practise.

Risk Factors and Clinical Management of Inhibitor Development in Hemophilia

Ayşegül Ünüvar (Turkey)

The development of an inhibitor against factor VIII (FVIII) or IX (FIX) is a serious complication of hemophilia results from repeated factor infusions. “Inhibitors” in hemophilia refer to IgG antibodies that neutralize clotting factors. In the last years, inhibitors are considered the most severe treatment-related complication in hemophilia. Inhibitor development in patients with haemophilia A (HA) is not only an extremely challenging complication of replacement therapy, but also very costly in terms of financial resources.

The presence of a new inhibitor should be suspected in any patient who fails to respond clinically to clotting factors, particularly if he has been previously responsive. In this situation, the expected recovery and half-life of the transfused clotting factor are severely diminished.

The cumulative incidence (i.e., lifetime risk) of inhibitor development in severe hemophilia A is in the range of 20–30% often within the first 20-50 exposure days (EDs), approximately 5–10% in moderate or mild disease. Inhibitors are much less frequently encountered in hemophilia B, occurring in less than 5% of affected individuals

In severe hemophilia A, the median age of inhibitor development is 3 years or less in developed countries. In moderate/mild hemophilia A, it is closer to 30 years of age, and is often seen in conjunction with intensive FVIII exposure with surgery.

In severe hemophilia, inhibitors do not change the site, frequency, or severity of bleeding. In moderate or mild hemophilia, the inhibitor may neutralize endogenously synthesized FVIII, thereby effectively converting the patient’s phenotype to severe. In all cases, inhibitors render treatment with replacement factor concentrates difficult. Patients on clotting factor therapy should therefore be screened for inhibitor development.

Confirmation of the presence of an inhibitor and quantification of the titer is performed in the laboratory, preferably using the Nijmegen-modified Bethesda assay. For children, inhibitors should be screened once every 5 EDs until 20 EDs, every 10 EDs between 21 and 50 EDs, and at least two times a year until 150 EDs. For adults with more than 150 EDs, apart from a 6–12 monthly review, any failure to respond to adequate factor concentrate replacement therapy in a previously responsive patient is an indication to assess for an inhibitor.

Inhibitor measurement should also be done in all patients who have been intensively treated for more than 5 days, within 4 weeks of the last infusion. Inhibitors should also be assessed prior to surgery or if recovery assays are not as expected, and when clinical response to treatment of bleeding is sub-optimal in the postoperative period.

Patients with inhibitors divide into two groups; *low responder (LR) patients* (anamnestic response to FVIII/FIX or inhibitor titer is always (persistently) less than 5 B.U.) or *high responder (HR) patients* (inhibitor titer ≥5 B.U.).

High responding inhibitors tend to be persistent. If not treated for a long period, titer levels may fall or even become undetectable, but there will be a recurrent anamnestic response in 3–5 days when challenged again with specific factor products. Some low titer inhibitors may be transient, disappearing within 6 months of initial documentation, despite recent antigenic challenge with factor concentrate.

Very low titer inhibitors may not be detected by the Bethesda inhibitor assay, but by a poor recovery and/or shortened half-life (T-1/2) following clotting factor infusions.

Risk Factors for Inhibitor Development

Inhibitor development is a multifactorial disease. Risk factors for inhibitor development can be patient-related (severity of hemophilia, FVIII gene mutation, family history of inhibitor, ethnicity, polymorphisms in immune-response genes) and/or treatment-related (number of exposure days, intensity of treatment, age at first exposure, type of FVIII concentrate, current infection or inflammatory state). A large part is explained by genetics, but environmental factors also contribute to its occurrence. It has been established that the molecular factor VIII gene defect is a strong individual predictor of inhibitors. Patients with a single nucleotide missense mutation have a lower risk for inhibitor formation, while the risk is high in patients with major gene deletions. Afro-Americans were shown to have a higher likelihood to develop inhibitors. This finding, which might be related to different HLA patterns, still awaits confirmation. Recently, polymorphisms in the immune-regulating genes coding for interleukin 10 (IL-10), tumor necrosis factor-alpha (TNF-alpha), and cytotoxic T-lymphocyte associated protein-4 (CTLA-4) were found to be associated with inhibitor development.

The genetic risk factors cannot be modified, so that the efforts of investigators have been concerted on the possible prevention of non-genetic factors. These include age at first exposure to factor VIII, vaccination, infection, surgery and intensive treatment (immunologically “Danger Signals”). The type of therapeutic molecule used, plasma-derived FVIII (pdFVIII) or recombinant FVIII (rFVIII) remains the most controversial risk factor for the development of inhibitors in haemophilia patients. A recent randomized trial, the Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET), showed a higher risk of inhibitor development with recombinant factor VIII (rFVIII) than plasma-derived concentrates (pd-FVIII). The retrospective Concerted Action on Neutralizing Antibodies in severe hemophilia A (CANAL) study and the prospective Research of Determinants of Inhibitor Development (RODIN) study which investigated the relationship between inhibitor development and treatment-related risk factors in previously untreated patients (PUPs) with severe hemophilia A reported a higher inhibitor rate in patients treated for at least 5 consecutive days than in those receiving only 1–2 days of treatment. This was particularly evident with higher daily doses (>50 IU/kg) and when the first treatment was used for surgery rather than for bleeds or prophylaxis. The only effective preventive strategy seems now to be based, at least when feasible, on avoiding early peak treatment periods.

Management of Bleeding For Patients with Inhibitors

Once an inhibitor develops, treatment of bleeding episodes is difficult (particularly in high responder patients), since they do not respond to FVIII therapy, because the inhibitor rapidly blockade the infused FVIII.

Management of bleeding in patients with inhibitors must be in consultation with a center experienced in their management. Choice of treatment product should be based on titer of inhibitor, records of clinical response to product, and site and nature of bleed.

Patients with a low-responding inhibitor may be treated with specific factor replacement at a much higher dose, if possible, to neutralize the inhibitor with excess factor activity and stop bleeding. Patients with a history of a high responding inhibitor but with low titers may be treated similarly in an emergency until an anamnestic response occurs, usually in 3–5 days, precluding further treatment with concentrates that only contain the missing factor.

For HR patients, bypassing agents remain the mainstay of therapy for management of bleeding. Recombinant factor VIIa (rFVIIa) and activated prothrombin complex concentrates (aPCC) are similarly effective in populations of patients with hemophilia and inhibitors; however, individuals may show a better response to one agent over another.

The prophylactic use of bypassing agents is an important tool to reduce morbidity in patients before they undergo immune tolerance induction (ITI) and in those with persistent high titer inhibitors. Recent studies have shown that prophylaxis with bypassing agents can reduce bleeding episodes by 50-80%, but cost and lack of convenience remain barriers.

Porcine factor VIII has been effective in halting bleeding in some patients. The plasma-derived preparation is being superceded by a recombinant porcine factor VIII concentrate currently in clinical trials.

Although there has been interest in the use of immunosuppressive therapies in patients with inhibitors, their role is not yet defined, and there is no consensus as to whether they have a place in the management of these patients.

Allergic reactions in patients with hemophilia B

Up to 50% of hemophilia B patients with inhibitors may have severe allergic reactions, including anaphylaxis to FIX administration. Such reactions can be the first symptom of inhibitor development. For this reason, newly diagnosed hemophilia B patients should be treated in a clinic or hospital setting capable of treating severe allergic reactions during the initial 20 treatments with FIX concentrates. Reactions can occur later, but may be less severe.

Immune Tolerance Induction

Antibody (inhibitor) eradication is the ultimate goal of inhibitor management. The sole clinically proven strategy for achieving antigen-specific tolerance to factor VIII (FVIII) or factor IX (FIX) is immune tolerance induction (ITI). In patients with severe hemophilia A and good risk profiles, eradication of inhibitors is often possible by ITI therapy. Possible mechanisms by which tolerance is induced by ITI include inhibition of B-cell memory and induction of T-cell anergy, anti-idiotypic antibodies or suppressor T cells. Before ITI therapy, high-responding patients should avoid FVIII products to allow inhibitor titers to fall and to avoid persistent anamnestic rise.

Several ITI regimens have been used in retrospective cohort studies and have been shown to be successful in 50–80% of patients, and the time to immune tolerization ranges from just over a 1 month to more than 2 years.

Moreover, several retrospective ITI registries have identified patient- and treatment-related variables which could have an impact on ITI outcome or on the time interval required to eradicate the inhibitor. However, despite long clinical experience and considerable efforts, the predictors of ITI outcome and the optimal ITI regimen are still debated due to the paucity of evidence from large, rigorous prospective studies and conflicting data from the registries. Specifically, the issues of optimal FVIII dose and administration intervals remain controversial.

Several ITI regimens have been described since ITI was first reported by Brackmann and Gormsen in 1977. The original *"Bonn Protocol-high dose ITI"* developed by Brackmann and his colleagues used FVIII in a dose of 100 units/kg twice a day with activated prothrombin complex concentrate (50 units/kg/dose twice a day) until the inhibitor titer fell to less than 1 B.U. and then, FVIII was increased to 150 units/kg/dose twice a day without aPCC. This regimen was continued until a normal FVIII recovery and half-life was demonstrated. Following that, most patients returned to an episodic treatment. Then "procedure 2" was developed, aPCC was used only to treat acute bleeding episodes during ITI, and they applied FVIII in a dose of 150 units/kg twice a day. After "Bonn Protocol", several others [Malmö protocol, Dutch (low-dose protocol), intermediate-dose protocols] have reported new protocols with varying doses and schedules with success (Table 1) and have demonstrated similar efficacy.

Table 1. The results of immune tolerance protocols

	Success rate (%)	Inhibitor elimination time (month)
Bonn protocol	92	14
Kasper	75	3
Malmö protocol	80	1
Dutch*	87	12
Gruppo	63	24

*Success was defines as inhibitor titer less than 2 BU, and FVIII recovery is at least 50% of normal

In 1988, Professor Nilsson et al *(Malmö Protocol)* reported that using protein A sepharose extracorporeal adsorption for rapidly decreasing the high titer inhibitors (≤10 BU) followed by FVIII in high doses every 8 or 12 hours to maintain FVIII level higher than 30%. Intravenous immune globulin and cyclophosphamide were used in addition. After eradication of inhibitor, FVIII is given prophylactically two to three times a week.

In the Netherlands *(Dutch protocol)*, a regimen of 25 U/kg FVIII given every other day was reported successfully with a different definition of success. Gruppo et al used FVIII once weekly, and success rate was 63% in a median time of 24 months.

An international trial (I-ITI) randomized good risk severe HA subjects with HR inhibitor from 55 participating centers to receive either 200 IU/kg/day or 50 IU/kg thrice weekly FVIII for up to 33 months and demonstrated an approximately 70% overall success rate. The time to negative inhibitor titer and normal FVIII recovery was shorter in the high-dose group, although both dosing groups had similar rates of tolerance.

Successful ITI has been defined by consensus groups and similar definitions have been used in clinical trials. Broadly, tolerance to FVIII is demonstrated when an inhibitor is no longer detected (negative Bethesda assay) and anormal pharmacokinetic response to FVIII infusion is observed. A recovery of 66% of expected and a half-life of >6 hours have been considered sufficiently normal pharmacokinetic responses to characterize complete tolerance, although some have argued that a longer half-life (>7 hours) should be the goal. Partial tolerance is typically defined as an inhibitor titer <5 BU/mL and the ability to use FVIII to prevent and treat bleeding despite a recovery <66% and/or half-life <6 hours. Failure of tolerance (the absence of partial or complete tolerance) can be more difficult to identify. In general, failure to fulfil the criteria for complete or partial response within at least 9 months of ITI treatment (the lack of at least 20% decrease in inhibitor titer over a 6-month period after the first 3 months of ITI).

There are many variables could be effect the success of ITI such as inhibitor titer at onset of ITI (<10 BU/mL), historical peak inhibitor titer (<200 BU/mL), maximum titer during ITI (<200 BU/mL), interval between first inhibitor and ITI (<5 years), age at onset of ITI (<8 years), use of FVIII dosage, interruption during ITI (< 2 weeks), and genetic variability.

In particular, it has been postulated that vonWillebrand factor (VWF) containing FVIII concentrates may improve the ITI success rate in some patients with inhibitors, especially those with the following poor prognostic characteristics: a historical peak inhibitor level >200 BU/mL, an inhibitor level >10 BU/mL at the start of ITI, age >5 years or interval of >24 months between inhibitor detection and start of ITI. In vitro experimental data and animal studies suggest that VWF decreases potential FVIII immunogenicity by epitope masking and protection from endocytosis by dendritic cells. Thus, increasing plasma concentration of VWF could decrease the amount of FVIII (antigen) presented to T lymphocytes, thereby reducing T-cell activation. The immunoprotective effect of VWF on FVIII may consequently have a positive impact on ITI. However, in children undergoing ITI for the first time, in keeping with expert recommendations, the same product associated with inhibitor development should be preferred. VWF/FVIII concentrates may be used for ITI in children with a previous ITI failure.

In addition, the probability of inhibitor recurrence is significant in the first 5 years after ITI and is associated with an FVIII recovery of < 85% at the end of ITI and the use of concomitant immune modulation therapy during ITI but not adherence to post-ITI prophylaxis. These findings suggest that after ITI, patients should be carefully monitored for inhibitor recurrence, particularly those who have received immune modulation such as rituximab.

Response to ITI may be less favorable in patients with moderate/mild hemophilia. Experience with ITI for hemophilia B inhibitor patients is limited. The principles of treatment in these patients are similar, but the success rate is much lower, especially in persons whose inhibitor is associated with an allergic diathesis. Hemophilia B inhibitor patients with a history of severe allergic reactions to FIX may develop nephrotic syndrome during ITI, which is not always reversible upon cessation of ITI therapy. Alternative treatment schedules, including immunosuppressive therapies, are reported to be successful.

In conclusion, inhibitor formation is a major problem. The process of inhibitor eradication through immune tolerance therapy is the standard of care and optimal long-term strategy for prevention of future bleeds and restoration of factor efficacy. There is clearly a need for cooperative multicenter studies determining which regimen is the most effective in hemophilia A patients with inhibitors. In addition, for the group of patients who fail to respond to ITI or have hemophilia B, new and improved tools are needed. Nonetheless, there are several novel therapies in development or active clinical trials that may potentially lessen the burden of disease and reduce bleeding risk in hemophilia patients with inhibitors.

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Hematologic Manifestations and Complications of Gaucher Disease

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Gaucher disease is a lysosomal storage disease characterised by a genetic distruption in the metabolic breakdown of glucocerebroside due to lack of glucocerebrosidase enzyme. Gaucher disease was first described by Philippe Gaucher in 1882 and is the most common lipid storage disease. The mode of inheritance is autosomal recessive with an elevated inheritance in Ashkenazi Jews. So far, more than 500 different mutations have been described in the glucocerebrosidase gene which is located on chromosome 1. The former classification of Gaucher disease spanned 3 types; type 1, 2 and 3 according to the time of the first manifestation and involvement of the central nervous system. However, this classification was abandoned because some patients may present with intermediate phenotypes. The natural course is characterised by complex multiorgan involvement which causes a diagnostic challenge. Glucocerebroside accumulates in macrophages (Gaucher cells) in the spleen, liver, bone marrow and in other organs causing a wide spectrum of signs and symptoms. As anemia, thrombocytopenia and splenomegaly are the most common presenting features, many patients are initially consulted by hematologists. Data from the International Gaucher Registry 2008 showed that splenomegaly and thrombocytopenia are present at diagnosis in 86% and 60% of patients respectively. Other common findings are; hepatomegaly, anemia, bleeding, and bone pain. Former type 2 and 3 patients may have neurological symptoms. Recent studies have corroborated that the incidence of multiple myeloma and Parkinson's disease is increased in Gaucher disease. A recent study also suggested that there is an increased risk of cancer and hematological malignacies. The earlier the disease presentation as in early childhood, the more severe the glucocerebroside accumulation and course of the disease. The diagnostic gold standard is still the measurement of reduced activity of glucocerebrosidase in leukocytes. In laboratory analysis, elevated values of chitotriosidase, tartrate resistant acid phosphatase, angiotensin converting enzyme (ACE) and serum ferritin are found. Since the diagnosis of Gaucher disease is associated with an expensive treatment, the finding of decreased glucocerebrosidase activity should be complemented by molecular genetic testing. Genotypic phenotypic association is unfortunately weak. Genetic diagnosis is possible during pregnancy using amniocentesis or chorionic villus sampling. Gaucher disease is the first disease treated with a specific enzyme. The aims of therapy are resolution of hepatomegaly, avoidance of splenectomy, normalisation of hematological values, improvement of quality of life, prevention of bone complications and increase in life expectancy. Glucocerebrosidase was first isolated from the human placenta in 1977. Later, alglucerase was replaced by the genetically engineered imiglucerase. Enzyme replacement therapy (ERT) is the gold standart of treatment. Recently, two additional recombinant substances have been approved; velaglucerase derived from human cell line, and taliclucerase derived from a carrot cell line. Another approach to therapy is inhibition of synthesis of the stored substance by substrate inhibitors. Since 2002, miglustat has been licenced for Gaucher disease when ERT is unsuitable. Very recently, eliglustat has been approved for substrate reduction therapy (SRT). The advantage of SRT over ERT is the oral route of application. There is still no effective treatment of central nervous complications in neuropathic forms of Gaucher disease.

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Disseminated Intravascular Coagulation

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Disseminated intravascular coagulopathy (DIC) is an acquired syndrome triggered by various causative conditions including infection, malignancy, trauma, burns, obstetric complications among many others, which lead to uncontrolled and disseminated intravascular activation of coagulation and fibrinolysis.

Depending on the underlying physiopathological mechanisms the clinical phenotype of the DIC differs to be either of “bleeding type”, or the “thrombotic type” or a combination of those. The course of the disease may be acute which is usually symptomatic or chronic, which usually presents with thrombotic disease or remain asymptomatic with laboratory findings only.

There are no specific laboratory tests for the definite diagnosis or exclusion of DIC. Among the pathologic laboratory findings the most useful and widely utilised ones include, complete blood count, blood film, prothrombin time (PT), activated partial thromboplastin time, thrombin time, fibrinogen and fibrin degradation products (FDP) or D-dimer. Blood chemistry might be helpful for identifying the severity of the condition or the co-morbidities.

To facilitate the diagnosis several scoring systems have been proposed. Of those the most widely used one is that of the International Society for Thrombosis and Haemostasis. This scoring system indicates a diagnosis of DIC using platelet counts, PT, fibrinogen and D-dimer (or the FDP).

Management of DIC requires primarily immediate treatment of the causative underlying clinical condition. All the other interventions are supportive and mainly include replacement of deficient blood components in bleeding patients, and rarely usage of heparin in patients with thrombosis and appropriate clinical status.

Attempts for replacing the missing natural anticoagulants (protein C, antithrombin and thrombomodulin) to oppose the prothrombotic action of the excess thrombin have all failed to significantly improve the survival rates in patients affected by DIC.

When and Whom to Screen for Hereditary Thrombophilia?

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Thrombophilia can be defined as the tendency to develop thrombosis, and includes acquired and hereditary disorders. Acquired thrombophilic risk factors such as obesity, pregnancy, smoking, diabetes, and cancer are quite common in population. Although a familial tendency of thrombosis has been recognized more than a century ago, the first evidence of inherited thrombophilia was reported in 1965 as antithrombin (AT) deficiency.[1] Later, protein C (PC) deficiency[2] and protein S (PS) deficiency[3] were described in families with thrombosis in 1981 and 1984, respectively. These three mutations are quite rare in the normal population (Table 1), and are responsible only for 1-4% of familial venous thromboses. After the discovery of factor V Leiden (FVL) mutation (*F5 G1691A*) in 1994[4] and the prothrombin gene (PG) mutation (*F2 G20210A*) in 1996[5], hereditary thrombophilia screening became very popular. Population studies clearly showed that hereditary thrombophilia increases the risk of venous thromboembolism (VTE), and has no effect on the development of arterial thrombosis.

In animal and human studies, many other gene variations are suggested to increase the risk of thrombosis, some of which are very rare. For example, homozygous cystathionine beta synthase mutation causes homocystinuria, which is characterized by both arterial and venous thromboses, skeletal deformities, mental retardation and dislocation of the ocular lens. Thrombotic events start in the first or second decade of life in affected individuals. The estimated frequency of homocystinuria varies from 1/45.000 to 1/1000.000 in different populations.[6] Another example is methylene tetrahydrofolate reductase (MTHFR) variations that are very common in Caucasian populations. The MTHFR enzyme plays a role in the conversion of homocysteine to methionine. It has been suggested that genetic variations in the *MTHFR gene (*C677T and *A1298C*) may impair or inactivate the enzyme, and results in higher plasma levels of homocysteine, especially in individuals who also have folate deficiency. Both genetic variations are very common[7], for instance, approximately 25% of Hispanics and 10-15% of Caucasians are found to be homozygous for *MTHFR* C677T. Although early studies suggested that the MTHFR deficiency might be associated with venous and arterial thrombosis by causing hyper-homocysteinemia, more recent analysis has found no association. *MTHFR* genotyping is not recommended by current guidelines as part of thrombophilia screening.[7-11]

Homozygous forms of FVL or PG mutations, double heterozygotes for both FVL or PG mutations, and AT deficiency are classified as 'high-risk thrombophilia'; heterozygous forms of FVL or PG mutations, PC and PS deficiencies are defined as 'low-risk thrombophilia'.

Table 1: Inherited thrombophilia: Prevalence and the risk of thrombosis [12]

Thrombophilia	Prevalence	RR of initial VTE	RR of recurrent VTE	RR of initial VTE during pregnancy
FVL-heterozygous	2-7%	3.48-5.51	1.1-1.8	8.3
FVL-homozygous	0.06-0.25 %	6.79-19.29	1.8	34.4
PGM-heterozygous	1-2 %	2.25-3.48	0.7-2.3	6.8
PGM-homozygous	rare	2.19-20.72	Unknown	26
Double (FVL+PGM) heterozygous	0.1%	1.13-5.04	2.7	Unknown
PC deficiency	0.2-0.5%	10	1.8	4.8
PS deficiency	0.1-0.7%	9.6	1.0	3.2
AT deficiency	0.02%	10-30	2.6	4.7

Who should be tested for hereditary thrombophilia?

Testing for thrombophilia is a procedure that is expensive, requires experienced laboratory personnel, and increases laboratory work-load. Clinical benefits and usefulness of the thrombophilia testing are also limited. Since approximately 10% of the population will carry either FVL or PG mutations, unselective thrombophilia screening may cause unnecessary treatments, insurance limitations, and psychological harm.

Studies investigating the natural course of VTE and pregnancy complications in patients with inherited thrombophilia yielded controversial results, and this uncertainty has also been reflected in current guidelines.[7-11] In general, testing for thrombophilia is recommended in patients in which VTE develops before 40 years of age, patients with unprovoked and/or recurrent venous thrombosis, in patients with family history of thrombosis (especially family history with unprovoked and recurrent VTE), in patients with unusual site thrombosis (e.g. cerebral vein thrombosis, abdominal vein thrombosis), and in patients with purpura fulminans or vitamin K antagonist-related skin necrosis. [7-11]

Thrombophilia testing is not recommended in those with arterial thrombosis including children who have had a stroke. Thrombophilia testing should be done in selected patients with upper extremity VTE and retinal vein thrombosis. Upper extremity VTE is usually associated with central venous catheters or throracic outlet syndrome, thus hereditary thrombophilia should be screened in patients with no obvious reason for upper extremity thrombosis. Meta-analyses showed that retinal vein thrombosis was mostly associated with systemic diseases such as hypertension, diabetes, and hyperlipidemia; FVL and PG mutations were only weak risk factors. Current guidelines do not recommend to do routine thrombophilia testing for patients with retinal vein thrombosis.[11,13]

Inherited thrombophilia increases the risk of VTE during pregnancy, oral contraceptive use, and hormone replacement therapy. According to thrombophilia guidelines, the clinical features should be considered first when assessing the risk of thrombosis associated with pregnancy. Individual risk factors (obesity, advanced age, smoking history, co-morbidities), history of thrombosis in the patient and her family, the presence of a risk factor for previous thrombotic event (provoked by estrogen or pregnancy, provoked by a transient risk factor, or unprovoked), history of recurrent thrombosis, and pregnancy related risk factors (hyperemesis gravidarum, pre-eclampsia, intra-uterine growth retardation, postpartum hemorrhage, post-partum infections, emergency Cesarean section) are evaluated. If there is no clinical risk factor, no history of thrombosis or family history of thrombosis; anticoagulant prophylaxis is not recommended during antepartum period, but can be considered for pregnant women with high-risk thrombophilia during postpartum period. [14-16] A recent study however showed that unselected women with high-risk thrombophilia had an increased risk of gestational VTE regardless of a family history of thrombosis. Authors suggested that all women with high-risk thrombophilia should receive antepartum thromboprophylaxis. [17]

It is not possible to screen thrombophilia in all women planning to take oral contraceptives or estrogen containing drugs. Women who have a history of thrombosis, or strong family history of thrombosis should not use oral contraceptives or estrogen replacement therapy, regardless of thrombophilia status as a negative thrombophilia test will not exclude an increased risk for VTE. If a woman develops VTE during oral contraceptive use and thrombophilia test shows a positive result, oral contraceptives should be stopped. Anticoagulant therapy should be continued according to clinical requirements, though it is not necessary to continue anticoagulation indefinitely following cessation of oral contraceptives in these patients.[11,14,15]

When and how should be tested for hereditary thrombophilia?

Different 'loss of function' mutations are described for protein C, protein S and AT deficiencies which create some difficulties in the diagnosis of such deficiencies. Genetic analyses of involved genes are expensive and can be done in specialized laboratories. Tests describing the activity of the molecule (functional tests) are used in the screening of PC and AT deficiencies. Laboratory testing for PS is much more complicated, usually both free PS antigen and PS activity assays are required. The levels and activities of PC, PS and AT are greatly affected by different conditions including pregnancy, systemic diseases (nephrotic syndrome, liver disorders, inflammatory disorders, infections), drugs (anticoagulants, oral contraceptives), acute phase of thrombosis (consumptional deficiency), and even by bed-rest. Tests for PC, PS, and AT should be done preferably after cessation of anticoagulant therapy, since the initial VTE management will not change, regardless of thrombophilia results. Given vitamin K antagonists inhibit the activities of PC and PS, these drugs should be stopped for 1-2 weeks. Unfractionated or low-molecular heparins use AT for their activities, thus functional tests should be done after cessation of heparins according to their half-lives. Oral direct thrombin inhibitors and direct FXa inhibitors may affect coagulation-based tests, and should be withheld for minimum 2-3 days. [8, 11,14]

FVL and PG mutations are single point mutations, and are detected by polymerase chain reaction-based molecular genetic methods. Peripheral blood leukocytes are used as a DNA source, and testing is reliable in any clinical situation except severe leukopenia.

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Acquired Aplastic Anemia

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Acquired idiopathic aplastic anemia is defined as marrow aplasia without any specific reason. Camitta's criteria (Blood 1976;48:63) are used to diagnose and grade severity of aplastic anemia. Although no specific routine test has been developed to show its autoimmune nature, response to anti-T lymphocyte globulin (ATG) indirectly prove this pathogenesis.

The differential diagnosis of aplastic anemia include constitutional forms of marrow aplasia such as Fanconi's aplastic anemia and disorders of telomer biology, hypocellular myelodysplastic syndrome (MDS) and some syndromic forms of marrow aplasia like transfusion-associated graft-versus-host disease (GVHD). -7/del(7q), -5/del(5q) and presence of dysplastic myeloid and/or megakaryocytic precursors are in favour of MDS. Routine testing for Fanconi's aplastic anemia in relatively younger aplastic anemia patients and testing for telomeropathies in case of predictive additional morbidities such as leukoplakia, nail dyskeratosis, pulmonary fibrosis and cryptic cirrhosis are necessary.

Cure, refractory disease, death, and transformation to other marrow disorders are possible consequences during course of aplastic anemia. Paroxysmal nocturnal hemoglobinuria (PNH), MDS and acute myeloid leukemia (AML) may develop as secondary marrow disorders.

Many different risk factors has been described for prediction of prognosis. Age is the most important determinant of treatment response. Large population cohort data have showed that ≤40 years patients who are treated with first line allogeneic stem cell transplantation constitute the best prognostic subgroup (Int J Hematol 2016;104:168). On the other hand first line transplantation in >40 patients is associated with the worst prognosis. Therefore immunosupression with ATG and cyclosporin is preffered as first line treatment in older patients. Addition of G-CSF to this regimen does not provide significant benefit (Blood 2011;117:4434). Unnecessary use of corticosteroids may lead to acquired Cushing's disease and an increased fungal infection risk (Hematology Am Soc Hematol Educ Program 2013;2013:76)

Important developments in aplastic anemia during last 10-15 years can be summarized as below:

It was possible to track PNH clones in aplastic anemia patients by means of flourescent aerolysin-based flow cytometry. Now it is also possible to track which patient will develop MDS/AML by means of next-generation sequencing methods (Blood 2016;128:337).

Studies of telomer biology in aplastic anemia shed light on some important facts: Although rare some telomer biology disorders (other than the classical example of dyskeratosis congenita) may lead to aplastic anemia phenotypically indistinguishable from idiopathic aplastic anemia (Lancet 2002;359:2168, Blood 2003;102:916, N Engl J Med 2005;352:1413). Telomer length is an independent prognostic factor in idiopathic aplastic anemia (JAMA 2010;304:1358). Studies of telomer biology in aplastic anemia helped to define a new disease category, telomeropathies.

Horse AG is better that rabbit ATG in first line treatment setting (Exp Hematol 2006;34:826, N Engl J Med 2011;365:430).

Peripheral blood as stem cell source is associated with more chronic GVHD and worse outcome after stem cell transplantation (Blood 2007;110:1397).

Cyclophosphamide±ATG is the preffered conditioning regimen for stem cell transplantation. Addition of fludarabine may decrease GVHD and increase success rate especially in relatively older patients (Haematologica 2009;94:1312, Biol Blood Marrow Transplant 2011;17:717, Blood 2011;118:2351)

Eltrombopag may restore trilineage hematopoiesis in refractory severe aplastic anemia (N Engl J Med 2012;367:11, Blood 2014;123:1818). Now has been investigated in first line treatment setting in addition to ATG and cyclosporin (Blood 2015;126:Abstract LBA-2).

Congenital Dyserythropoietic Anemia:

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Congenital Dyserythropoietic Anemia's (CDA) are a group of rare genetic disorders characterized by anemia with ineffective erythropoiesis, jaundice, hepatomegaly, splenomagaly, cholelithiasis, extramedullary hematopoiesis and iron overload, and most importantly distinctive morphologic abnormalities in erythroid precursors on bone marrow examination. Anemia is due to ineffective erythropoiesis as well as non-immune hemolysis. The reticulocyte count is relatively low for the degree of anemia (1).

There are 4 types of CDA and also there are variants. The most common type is CDA Type II. There are only few patients reported from Type IV and variants.

CDA has to be in the differential diagnosis of any patient who is thought to have non-immune hemolytic anemia. Anemia could be very severe causing hydrops fetalis, or very mild that CDA cannot be diagnosed until adult ages. The mode of inheritance is autosomal recessive for CDA Type I and II and autosomal dominant for CDA Type III and IV. Although there are some unique features of every type of CDA they do share most of the clinical properties. Almost all types have mild to moderate anemia, inadequate reticulacyte response, anisopoikilocytosis, basophilic stippling of red cells, and nucleated erythrocytes on the peripheral blood smear, indirect hyperbilirubinemia, increased LDH, AST, decreased haptoglobulin, hypercellular bone marrow with dyserythropoiesis. Other specific features would be listed under the CDA type headings. The differential diagnosis has to be made between thalassemia syndromes, hemoglobin C, certain unstable hemoglobins, hereditary sideroblastic anemias, hereditary persistence of fetal hemoglobin, red blood cell membrane defect, vitamin B12 or folate deficiency, iron deficiency, alcohol abuse, liver disease, heavy metal poisoning, and MDS. Diagnosis is made with clinical and laboratory features of hemolytic anemia, the bone marrow findings (light microscopy and electron microscopic findings) and genetic testing. Treatment is symptomatic in all forms. Some patients might require red blood cell transfusions during neonatal period but expected to have a better clinical course later on. Splenectomy leads to a moderate increase in hemoglobin and may be an option for transfusion dependent patients. Cholecystectomy may be necessary for gall stones. Patients should be monitored for iron overload and treated as necessary (2). The characteristic and some of the distinctive features of CDA Types I-IV are shown in Table I (3-21). Some forms of CDA cannot be classified as any of the CDA types described in table I. Some of these are reported as a part of their syndromic features i.e. Majeed Syndrome, mevalonic aciduria (22,23). They are extremely rare and reported in only couple of families.

Table I

Type	**I**	**II**	**III**	**IV**
Inheritance	Otosomal Recessive	Otosomal Recessive	Otosomal Dominant	Otosomal Dominant
Gene mutated	Codanin-I	SEC23B	KIF23MKLP1	KLF1
Red cell size	Macrocytic	Normocytic	Macrocytic	Normocytic
Bone marrow findings	Megaloblastic, binucleated (2%-5%), incompletely divided celss with thin chromatin bridges	Binucleated erythroblasts with 2 nucleus equal to with other (10%-40%)	Bi or multinucleated erythroblasts (gigantoblasts) (10%-40%)	Binucleate forms, nuclear budding, rare karyorrhexis
Additional features	Congenital abnormalities	Acid serum hemolysis (Ham test) positive	Intravascular hemolysis, less iron overload	Elevated hemoglobin F

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Clinical and Laboratory Findings Suggestive of Hereditary Bone Marrow Failures

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Patients with inherited bone marrow failure syndromes are usually diagnosed after development of the hematological findings, including bone marrow failure, myelodysplastic syndrome (MDS) and leukemia. However these patients may have additional disease-specific congenital anomalies and comlications that may develop during the course. The family history of these patients may reveal increased propensity to certain cancers.

Although, 20% of Fanconi anemia patients may have no physical anomalies, there are several non-hematological findings in these patients that may be suggestive for Fanconia anemia, including short stature, cafe au lait spots, radial ray anomalies, microcephaly, microphtalmia, renal anomalies, including horse-shoe kidney and pelvic kidney, cardiac anomalies etc. These patients have a high risk of endocrinological abnormalities including diabetes mellitus, insulin resistance, GH deficiency, hypothroidism, hypogonadism etc. There is increased overall risk of any cancer of 20-50 fold. AML risk is increased 300-800 fold, squamous cell cancers in the head and neck region increased 200-800 fold, esophageal cancer increased 1300-6000 fold, vulvar cancer 500-4000 fold and MDS 5000 fold. The patients with Fanconi anemia develop these cancers at an earlier age compared to de-novo cancer patients. Besides these patients develop severe and prolonged hematological toxcities with chemotherapies. Any patient who was diagnosed with aplastic anemia, MDS or AML should be screened for Fanconi anemia. A high MCV without any identifiable cause should prompt a possible diagnosis of Fanconi anemia.

Patients with dyskeratosis congenita (DKC) may present in various conditions and age of diagnosis may range from infancy to adulthood. A DKC variant namely, Hoyeraal Hreidarsson variant, may presemt with cerebellar aplasia, microcephaly and early onset bone marrow failure. Some of the patients are diagnosed with the apperance of dermatological and oral findings, including dysplastic nails, lacy reticular pigmentation and oral leukoplakia; wheras some others are diagnosed after apperance of cytopenia or pancytopenia. Pulmonary fibrosis and hepatopulmonary syndrome are other potential presentations that may appear in adulthood. Rare presentations include strictures in lacrimal ducts, esophagus or urethra, early gray hair, and early hair loss. Patients are prone to develop MDS, AML, lymphomas, squamous cell carcinomas in the head-neck region or anogenital region. Besides DKC patients have increased propensity to develop stomach, lung, esophagus and skin cancers.

Diamond-Blackfan anemia (DBA) is another rare inherited bone marrow failure syndrome. The patients usually develop severe anemia before 1 year of age. The physical abnormalities that may accompany to anemia are thumb abnormalities including triphalangeal thumb, short stature, hypertelorism, epichantal folds, strabismus, glaucoma, cataract and congenital heart defects. Recently, a DBA-like phenotype has been described due to biallelic mutations in *CECR1* gene, encoding ADA2 enzyme. These latter patients have propensity to stroke and the inheritance pattern is autosomal recessive. Besides, erythrocyte ADA levels are either normal or decreased in *CECR1* patients, contrary to classical DBA patients who have high erythrocyte ADA levels.

Patients with DBA also have propensity to develop malignancies, but compared to patients with Fanconi anemia, they develop malignancies at 3rd to 4th decade, earlier than Fanconi anemia patients. Colon, lung, stomach, gynecological cancers, osteosarcomas, MDS and AML are the potential malignacies that may develop in patients with DBA. Disease is usually autosomal dominantly inherited and incomplete penetrance is a feature. The parent of a classical DBA patient may have no anemia, but only short stature and may develop MDS later in life. So family genetic work-up may help to follow these genetic carriers of DBA.
Several other inherited bone marrow failure syndromes including, *Shwachman–Diamond* syndrome , TAR syndrome, congenital amegakaryocytic thrombocytopenia, congenital neutropenias will also be outlined in this topic. The patients with inherited bone marrow failure syndromes may present with different phenotypes. Furthermore, these disease are rare, some of them are difficult to diagnose and follow-up, including the cancers during the course, requires experience.

Stem Cell Transplantatıon in Fanconi` s Anemia

Duygu Uçkan Çetinkaya (Türkiye)

Allogeneic HSCT has a critical role in management of patients with FA by correcting hematological abnormalities. Definite indications for HSCT in FA are severe bone marrow failure and MDS/AML evolution. Additionally, patients in whom poor risk cytogenetic features are detected in the routine yearly (or 6 monthly) testing are transplanted before development of malignant hematopoiesis.
HSCT procedure in FA carries specific considerations due to the DNA repair defect, chromosomal instability and related pathophysiology making them susceptible to the unfavorable effects of the conditioning regimen, inflammatory complications and oxidative stress. Therefore the classical doses of the conditioning regimens (chemo and/or radiotherapy) have led to dismal results in transplanted patients in the past with increased risk of complications including regimen related toxicity, graft failure and graft versus host disease (GVHD). In the last 2 decades, with better understanding of disease pathophysiology and stem cell biology in FA patients, modifications made in the conditioning regimens (e.g.about50-90% reduction in cyclophosphamide dosing) has dramatically improved survival after HSCT. Transplantation from young donor (<10yr) before development of MDS/AML, omitting radiation and inclusion of fludarabine in the preparative regimen has been associated with best outcomes approaching 95% survival. Additional favorable factors have been listed as, none or limited number of malformations, normal pre-transplant liver function, platelet count within acceptable levels, and lack of androgen use. Pre-transplant opportunistic infections and transfusion history (>20) have been reported to be associated with poor outcome in alternative donor transplants. The results of cord blood transplantation in FA is inferior to bone marrow transplantation but has been improving by the use of fludarabine, better HLA matching and higher cell doses. Haploidentical transplantation should only be performed in the context of active clinical trials, or in experienced centers. Gene therapy by the use of gene corrected CD34 positive cells is an experimental approach, efficacy/safety not confirmed in human studies.

In FA patients, bone marrow failure is permanently corrected with HSCT and the risk of AML is greatly reduced due to establishment of donor hematopoiesis. However, the risk of secondary malignancy (oropharyngeal, skin, head and neck) remains high due to the persisting DNA repair defect in non-hematopoietic tissues not corrected with HSCT, in fact, with additional contribution of the chemotherapeutic agents in the conditioning regimen. The risk of secondary cancer after HSCT is increased in patients transplanted at age >10, in those with clonality, by the use of peripheral blood stem cells as stem cell source and by the presence of chronic GVHD.

In FA,the main HSCT complications, particularly before switching to fludarabine containing regimens were regimen related toxicity, graft failure and severe GVHD, all of which seem to be controlled by recently modified HSCT practice. Apart from the changes in the conditioning regimen availability of high resolution HLA typing, better graft manipulation techniques and improvements in supportive care are also factors contributing to better outcome. Increased understanding of susceptibility of patients to oxidative stress and inflammation has led to implementation of better control measures.

The timing of HSCT needs special evaluation in FA. Transplant outcome is significantly worse in patients transplanted after development of AML with overall survival rates below 40%. The risk of developing MDS/AML has been reported to be 33% by 40 years of age and HSCT prevents progression. Decision to perform HSCT in patients with FA should be individualized.In standard risk patients (<18 yr, good organ functions, absence of MDS/AML) HSCT is recommended prior to development of persistent and severe cytopenia in order to avoid transfusions and opportunistic infections. HSCT is also indicated in patients with advanced MDS/AML in spite of the lower survival rates. On the other hand, preemptive HSCT is recommended for patients with mutations involved in rapid progression to MDS/AMLand/or poor survival (e.g.biallelic FANCD1/BRCA2 mutation).

On the basis of these points, patient management and decision issues in FA is outlined as;

- Management of BM failure: FA patients with mild cytopenia may not develop BM failure *(about 10% of patients don`t)* till advanced ages > 40. Therefore HSCT is not offered for those patients. *(If transplanted, these patients with cellular marrow will need more intense conditioning that may be associated with increased toxicity*). Yearly BM aspiration and cytogenetic analysis is recommended. In patients with moderate BM failure *(ANC 500-1000, platelet 30-50000, Hgb 8-10)* and declining blood counts HSCT from an HLA matched related donor or closely matched unrelated donor is indicated. If blood counts are stable, patients may be followed closely by CBC and by BM cytogenetics. Patients with severe BM failure (ANC<500, platelet <30000, Hgb<8) and/or transfusion dependence, HSCT is indicated with the best available donor (HLA matched related or closely matched unrelated).

In patients without an available donor or in the presence of pre-existing organ dysfunction androgen therapy may be used to improve blood counts; however, hepatic side effects should be considered, particularly if HSCT will be an option in time.

Another options are, cord blood or haploidentical HSCT, or investigational therapies such as gene therapy.

- Management of/ follow up for clonal hematopoesis: Allogeneic HSCT is indicated in patients with MDS/AML or in those who have poor risk cytogenetic features.

In patients with morphological features of leukemia, multilineage dysplasia, or in those with poor risk cytogenetic features (-7,+3q) even in absence of abnormal morphology, immediate HSCT is needed. In general patients undergo HSCT without prior chemotherapy. Those with AML, or MDS with excess blasts (BM blast 10-15%) a single course of dose reduced FLAG prior to HSCT may be applied. However, chemotherapy course(s) before HSCT is a controversial issue.

The risk of clonal hematopoesis is high in FA. In patients who are not eligible for immediate transplant, yearly or 6 monthly BM examination with cytogenetics is performed. G banding of minimum 20 metaphases is evaluated to detect acquired chromosomal aberrations. Specific aberrations associated with transformation to MDS (1q+,3q+,-7,-7q) can be detected by FISH, and/or whole genome SNP array with copy number analysis may be used.

Among cytogenetic abnormalities, some, e.g. 1q+, del(20q),del(5q) are not definitely associated with poor outcome. These patients may be followed closely with BM examinations to detect stability or unstability of the abnormal clone.

Presence of biallelic mutations of BRCA2 (FANCD1) is a challenging issue due to very high risk of MDS/AML without BM failure. Due to the 80% of leukemia risk by age 10 years, preemptive HSCT is usually the preferred option in these patients.

- Stem cell source and donor issues: Bone marrow is the preferred stem cell source rather than peripheral blood stem cells or cord blood. Donor selection is also a critical issue. FA patients with mosaicism may be asymptomatic, there may be incomplete penetrance of FA-associated anomalies, or disease manifestations may present late. Therefore sibling and related donors should undergo chromosomal breakage testing and/ or genetic analysis before appointed as HSCT donor.
- Long term complications and follow up: With increasing transplant success in the last 2 decades the main problem in FA patients has emerged as long term complications, among which solid tumors appear as the most challenging one. FA patients are prone to head and neck squamous carcinoma, skin, liver, anogenital tumors, breast and other cancers. This risk is increased after transplantation due to the use of conditioning regimens in spite of the lowered doses. Treatment of the solid tumor may also be challenging and reduction of chemotherapy doses will be needed.

Patients are prone to all other long term problems including endocrinologic, musculoskeletal, metabolic and other systems` complications. Establishment of a multidisciplinary team, the use of screening tools, self assessment and systematic approach for follow up is extremely important.

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Long-Term Follow-up of Pediatric Stem Cell Transplant Patients

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Over the past five decades there has been considerable progress and success in the field of hematopoietic stem cell transplant (HSCT) in children due to advances in technology and supportive care techniques, leading to significant improvements in transplant outcomes for both malignant and nonmalignant indications. Also, newly emerging indications for transplantation, introduction of newer graft sources (eg, umbilical cord blood) and different transplantation techniques (eg. MUD, haploidentical HSCT) have also contributed to an increase in the number of HCT survivors. These survivors are at risk for developing late complications secondary to pre-, peri-, and post transplantation exposures and risk factors, which must be followed-up and treated whenever needed with proper interventions. This issue is complicated by the wide age range of children receiving HSCT. The program for follow-up of these children must be planned for covering all developmental stages of childhood and after they become adults as all these periods of life have different sensitivities to this very intensive treatment and can result in different sets of complications. This can be facilitated by a dedicated "long-term-follow-up (LTFU)" clinic that provides lifelong care for BMT survivors. Guidelines for screening and preventive practices for HCT survivors arebeing published.

This session will be concentrated on subjects like, After BMT in childhood, what are the main late effects of majör concern?, How will we follow these patients for both adaptation to normal life and the treatment of late, longterm effects?

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