

## Rare anemias in adolescents

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**Abstract.** Anemia can be the consequence of a single disease or an expression of external factors mainly nutritional deficiencies. Genetic issues are important in the primary care of adolescents because a genetic diagnosis may not be made until adolescence, when the teenager presents with the first signs or symptoms of the condition. This situation is relatively frequent for rare anemias (RA) an important, and relatively heterogeneous group of rare diseases (RD) where anaemia is the first and most relevant clinical manifestation. RA are characterised by their low prevalence (< 5 cases per 10,000 individuals), and, in some cases, by their complex mechanism. For these reasons, RA are little known, even among health professionals, and patients tend to remain undiagnosed or misdiagnosed for long periods of time, making impossible to know the prognosis of the disease, or to carry out genetic counselling for future pregnancies. Since this situation is an important cause of anxiety for both adolescent patients and their families, the physician's knowledge of the natural history of a genetic disease will be the key factor for the anticipatory guidance for diagnosis and clinical follow-up. RA can be due to three primary causes: 1. Bone marrow erythropoietic defects, 2. Excessive destruction of mature red blood cells (hemolysis), and 3. Blood loss (bleeding). More than 80% of RAs are hereditary, and about 20% remain undiagnosed but when their first clinical manifestations appear during childhood or adolescence, they are frequently misdiagnosed with iron deficiency. For this reason, RA are, today, an important clinical and social health challenge worldwide. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key Words:** Adolescence, Anemia, Erythrocytes

### Background

The World Health Organization (WHO) defines adolescents as the population in the age range of 10–19 years (1), a transition period between childhood and adulthood, characterized by rapid growth and development that offers a second and last chance for the catch up growth in life cycle. From a social point of view, adolescence is the period that prepares for the adults roles, whether for work or to start a family. Being a phase of important changes, it has received different names and some refer to adolescence as a time in which people go through an “identity crisis” (2). Adolescents of both sexes are among groups at risk for the development of anemia and the need for

further assessment of the etiology of anemia should be considered to design pragmatic intervention programs. During adolescence, multiple and multifaceted factors contribute to anemia, with iron deficiency being the most common cause. Evidence from the literature suggests that female adolescents, in a physiological state like menstruation or from low socio-economic status with macronutrient deficiencies, are the more prone to develop anemia (3). For this reason, anemia is, among the top leading causes of disability-adjusted life years lost in adolescents (4), and its cause has been found to be highly due to iron deficiency as demonstrated by the levels of hemoglobin concentration (Hb) and serum ferritin that have been always found to be significantly lower in females than in males. During adolescence the

need for iron increases from preadolescent level of 0.7–0.9 mg Fe/day to up to 2.2 mg 0Fe/day both among adolescent boys and girls. This increased iron requirement is attributable to peak pubertal development characterized by expansion of total blood volume, increase in lean body mass and the onset of menstruation in adolescent females (5). Iron need in females continue to remain high after menarche due to menstrual blood loss where the iron need averages about 20 mg of iron per month and it may also be as high as 58 mg in some individuals (6)

Under normal conditions, hemoglobin concentration (Hb) varies with age and sex and, therefore, the World Health Organization (WHO) considers that anemia exists when Hb concentration is less than 110g/L in children, 120g/L in young non-pregnant women, and 130 g/L in men. Fortunately, measurement of Hb is very affordable today, as all automated hematology analyzers perform it quickly, accurately, and inexpensively

Anemia adversely affects the cognitive performance, behavioral characteristics and physical growth of infants, preschool and school-age children. It also affects the immune status and morbidity from infections of all age groups and the use of energy sources by muscles. Hence, the physical capacity and work performance of adolescents and adults of all age groups are significantly affected (7). Among adolescents, anemia affects not only the present health status but can also have deleterious effects in later life

In clinical practice they may be some confusion between nutritional anemias and the anaemias that appear in the course of non-haematological or systemic diseases, also called secondary anaemias. This confusion is due to the fact that anaemia is not a disease but a clinical manifestation, and some rare diseases (RD) are associated with anaemia, moderate or severe. One example of this is hereditary telangiectasia (Rendu-Osler disease), where anaemia, due to iron deficiency, is, in general, the first sign of the disease during adolescence (7) Another important cause of misdiagnosis of anemia during the adolescence is the existence of hereditary RD associated with anemias as the most important clinical manifestation. These RD are called rare anemias (RA) because their prevalence in European population is lower than 5 cases per 10,000 individuals (8)

## Adolescence and the rare anemias

For many years, the RA have been underestimated by public health providers, due to its frequent misdiagnosis with iron deficiency anaemia, the most frequent cause of anaemia worldwide. More than 80% of RAs are hereditary, but in about 20% of patients, the underlying cause of the anemia remains still unknown. The importance of RA was recognised for first time by the European Commission that, in 2020, approved through the DG-SANCO the co-finance of the project: European Network for Rare and Congenital Anaemias (ENERCA). Interestingly, this project started shortly before the establishment, in 2004, of the High Level Group (HLG), that brought together experts from all the Member States (MS) in several areas of RD expertise (9). For RA, this was a great advantage, because it facilitated the progressive development of ENERCA Project in parallel to the development of the different HLG areas of action: (a) Patient safety and quality of care, (b) Health impact assessment and health systems, (c) Health technology assessment, (d) European workforce for health professionals, e) European reference networks, (f) Information and e-health and more recently, (g) Cross-border healthcare purchasing and provision. More recently, in 2016, the creation of the European Reference Network (ERN) for Rare Hematological Diseases (RHD), or EuroBloodNet has provided ENERCA sustainability by expanding, and implementing, all its health services to oncological and non-oncological RHD (10)

Unfortunately, for many RAs the treatment is still palliative and in general based on blood transfusions, the administration of iron chelators and erythropoiesis stimulators or splenectomy. During the last five years, genetic therapy for some RA (thalassemia and pyruvate-kinase deficiency) has significantly progressed, but, up to now, the only curative option for very severe cases is the hematopoietic stem cell transplantation (HSCT). This treatment is not always advisable given the high morbidity associated with the intervention and the frequent absence of related donors.

Hereditary hemolytic anemia (HHA) is a relatively frequent cause of anemia during childhood and adolescence. Some hereditary RAs, however, are more frequently diagnosed during neonatal period or early

childhood as for example sickle-cell disease (SCD) and erythropoietic bone marrow failure. In general these RAs are diagnosed at an early age but chronic anemia persists during the adolescence and beyond, during the adult life. Among the most frequent hereditary RAs that are diagnosed during adolescence are the Congenital Dyserythropoietic Anemias (CDA), the Hereditary Hemolytic Anemias (HHA) due to membranopathies and enzyme defects, and the Hemoglobinopathies mainly due to thalassemia (Thal). CDA and HHA are clinically highly heterogeneous and characterized by chronic anemia of variable degree, associated with a complex, and often unexplained genotype-phenotype correlations. Both genetic disorders are caused by mutations in more than 70 genes controlling red blood cell (RBC) production (erythropoietic defects) and structure (RBC defects). Mutations in these genes lead to alterations in hemoglobin (Hb) levels, RBC differentiation and proliferation, cell membrane structure, and defective activity of erythrocyte enzymes (11,12).

### Congenital Dyserythropoietic anemia (CDA)

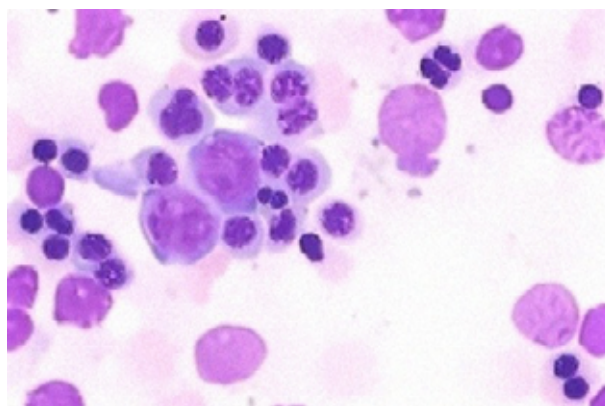
The bone marrow erythropoietic failure (BMEF) and can be congenital or acquired, both quantitative (selective RBC aplasia or erythroblastopenia) or qualitative (maturation disorders or dyserythropoiesis). The vast majority of BMEF are the consequence of differentiation–proliferation pathways defects that can appear at the different steps through the erythroid lineage leading to a decrease of effective erythropoiesis and anemia. Hereditary causes of BMEF include congenital bone marrow aplasia (Fanconi anemia), selective erythroblastopenia (Diamond-Blackfan anemia) and congenital dyserythropoietic anemia (CDA). Diamond-Blackfan anemia (DBA) is defined by macrocytic moderate or severe anemia that can occur in association with hyporegenerative bone marrow and physical abnormalities such as craniofacial anomalies and defects of the upper limb and hand, which mainly include the thumb. This disease shows mutations in 20 genes for ribosomal proteins of a total of 80 genes that encode the complete ribosome (13).

Congenital dyserythropoietic anemia (CDA) is the clinical manifestation of an heterogeneous group of erythropoietic defects that include medullar abortion of erythroblasts before maturing to reticulocytes (dyserythropoiesis), and significant changes of RBC morphology. Clinically, CDA presents with anemia, generally macrocytic, and iron overload. To date, five clinical forms of CDA have been described: CDA I, CDA II, CDA III, CDA IV, and CDA with thrombocytopenia (14).

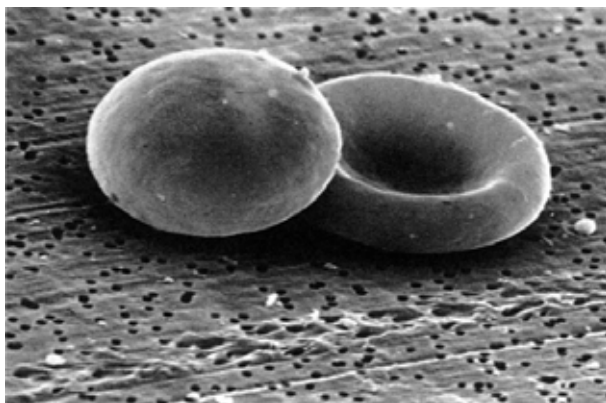
The most common forms are CDA types I and II which are caused by bi-allelic mutations in the *CDAN1/CDIN1* genes for CDAI and in the *SEC23B* gene for CDAII. CDA I and CDA II have an autosomal recessive hereditary pattern whereas CDA III and CDA IV are autosomal dominant and CDA with thrombocytopenia has an X chromosome inheritance (15). In general, the diagnosis of CDA is based on the examination of the bone marrow morphology and for many years erythroid hyperplasia associated with binuclearity or late erythroblast multinuclearity has been considered the diagnostic clue for CDA II (Figure 1)

### Membranopathies

RBC membranopathies are due to structural or functional defects in the RBC membrane proteins. In general, they are inherited with a dominant autosomal character, but there are forms that are transmitted



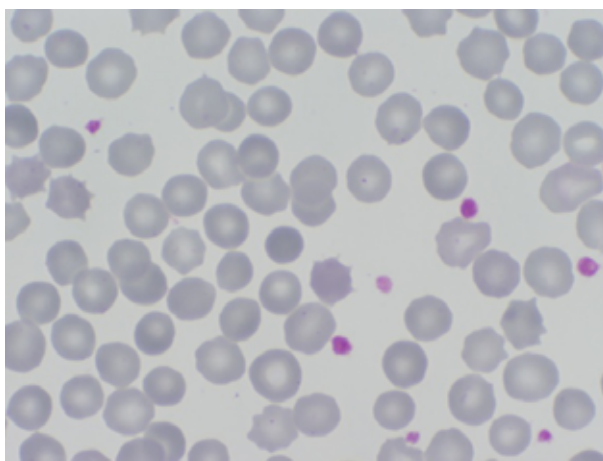
**Figure 1.** Bone marrow picture of a patient with congenital dyserythropoietic anemia type II (CDA II)



**Figure 2.** Red Blood Cells observed with scanning electron microscope (SEM). Left: spherocyte. Right: normal red blood cell

recessively. The main effects of these genetic abnormalities are a lowered RBC deformability and decreased RBC survival (16). RBC membranopathies are classified into three main groups: hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and hereditary stomatocytosis (HSt). In all the cases altered membrane structural and functional organization can be found.

**Hereditary spherocytosis** (HS; OMIM 182870, 182900, 270970, 612653, 612690) is the most frequent form of HHA in Caucasians. The clinical manifestations can range from symptom-free carriers to patients with severe hemolysis. In the most severe cases, clinical manifestations can appear during neonatal period, but in general they appear more frequently during childhood and adolescence. HS is due to a defect in the proteins of the membrane skeleton that produce vesiculation and partial loss of the same with the subsequent decrease of the surface/volume ratio (S/V) and the appearance of spherical RBCs or spherocytes (Figure 2). In HS the mutations that cause spherocytosis have been found in five genes that encode proteins that are involved in interactions between the cytoskeleton and the RBC phospholipid bilayer: SPTB (Beta-spectrin), ANK1(Ankirin), SLC4A1 (Band 3) , SPTA1(Alpha-Spectrin) and EPB42 (Band 4.2). Hemolysis occurs almost exclusively in the spleen, so hemolytic anemia is usually associated with splenomegaly, intermittent jaundice and premature formation of gallstones (Cholelithiasis). In some cases,



**Figure 3.** Peripheral blood smear stained with MGG from a patient with hereditary spherocytosis. A typical spherocyte can be observed

a transient erythroblastopenia due to concomitant parvovirus B19 infection, or folic acid deficiency can appear (17) .The diagnosis of HS is essentially based on the presence of circulating spherocytes (Figure 3) but in addition to the classical triade of spherocytosis, splenomegaly and jaundice, the current diagnosis of HS requires the inclusion of a triparametric algorithm: 1. The mean corpuscular haemoglobin concentration (MCHC), provided by almost all hematology analyzers that is increased in the presence of reticulocytosis . 2. The EMA-binding test ,based on the measurement of the fluorescence intensity in RBCs after incubation with a fluorochrome, eosin-5- maleimide (EMA). EMA specifically binds to the Band 3 (anion carrier) which N-terminal cytoplasmic domain interacts with other proteins such as ankyrin and protein 4.2 , and decreases when Band 3 decreases (18) and, 3. RBC deformability measured by osmotic gradient ektacytometry (OGE) using the new generation LoRRca Osmoscan from Mechatronics (Figure 4). The curve profiles obtained with this instrument are the most accurate and sensitive diagnostic tool for a clear distinction between HS and the other two membranopathies (Figure 5), because in addition to the value of RBC deformability at different osmotic values, it allow to measure the decrease of the classical RBC osmotic fragility and the degree of cell hydration (19,20).

**Hereditary elliptocytosis** (HE; OMIM 109270, 130600, 179650,225450, 611804), is a clinically



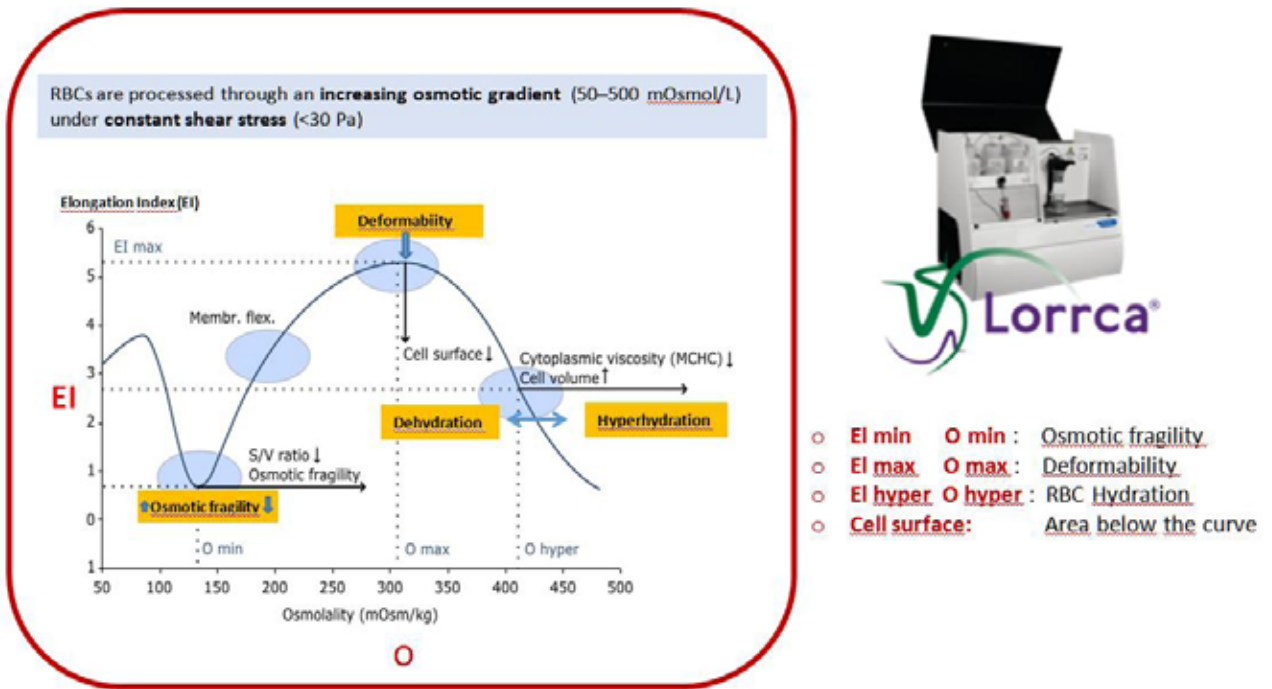


Figure 4. Osmotic Gradient Ektactometry (OGE) profile (Osmoscan curve) measured with LoRRca MaxSis

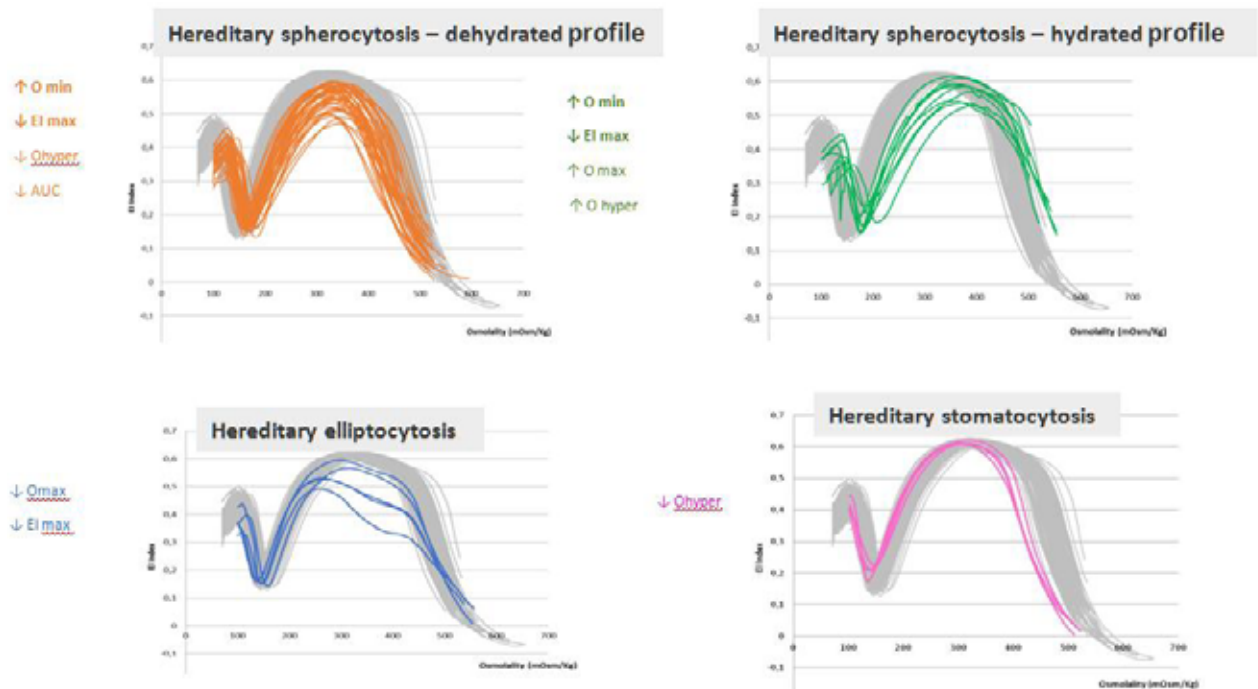
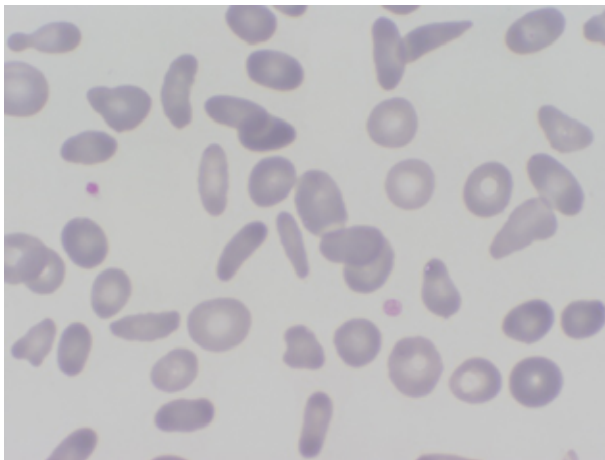
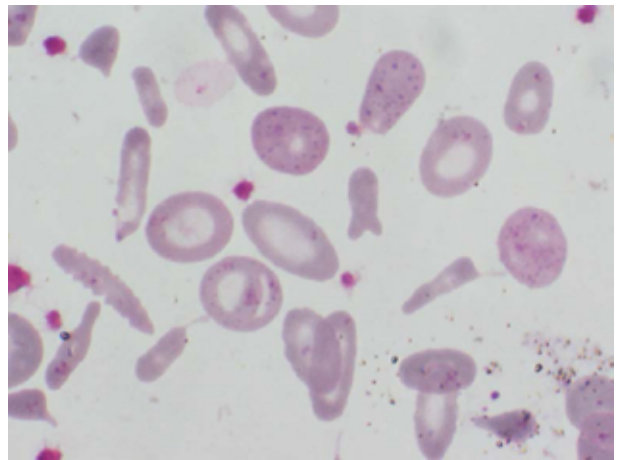


Figure 5. Osmoscan curve profiles of RBC membranopathies



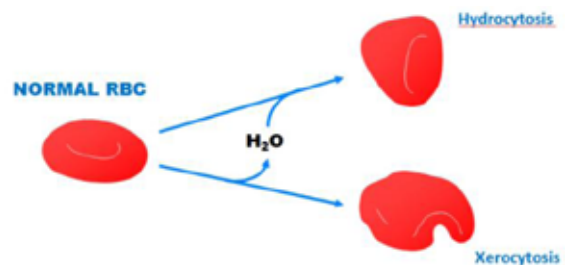
**Figure 6.** Peripheral blood smear stained with MGG from a patient with hereditary elliptocytosis



**Figure 7.** Peripheral blood smear stained with MGG from a patient with hereditary pyropoikilocytosis (HPP)

milder disorder than HS characterised by the presence of elongated RBCs or elliptocytes in peripheral blood (Figure 6). Like HS, HE is due to a defect in skeletal proteins (mainly Band 4.1) that alter the elasticity of the membrane, preventing its recovery after elongation. Since there is not a loss of membrane RBC, osmotic fragility and MCHC are normal. The OGE is also very useful for the differential diagnosis between HS and HE (Figure 5). In the severe clinical form of HE known as hereditary pyropoikilocytosis (HPP), the alpha-spectrin (SPTA-1) gene mutation in heterozygous state is associated “in trans” with a silent mutation known as alpha “Lely” (Low expression Lyon) leading to severe HHA with markedly abnormal RBC morphology and decreased heat stability (Figure 7)

**Hereditary stomatocytosis** (HSt; OMIM 194380, 185000) arise from genetic defects of RBC transport proteins, which leads to abnormal cation permeability and the consequent changes in RBC hydration. Accordingly, HSt can be classified into dehydrated HSt (hereditary xerocytosis) and overhydrated HSt (hereditary hydrocytosis). The most frequent form is the dehydrated hereditary stomatocytosis (DHS) or Hereditary Xerocytosis (HX), where RBCs are dehydrated due to a cation leak, primarily of potassium, that leads to a decreased cellular potassium concentration and water. The difference between overhydrated (hydrocytosis) and dehydrated (xerocytosis) HSt is shown in Figure 8. HX is characterised by slight or



**Figure 8.** RBC dehydration and overhydration. The dehydrated RBC is called xerocyte and is observed in hereditary xerocytosis. The overhydrated RBC is called stomatocyte and it is observed in hereditary hydrocytosis

moderate anemia associated with markedly increased number of reticulocytes and increased MCHC (340–370 g/L). OGE reflects a characteristic pattern of mixed reduced deformability index and dehydration given by a leftward shift of the minimal osmolality point (Figure 5). HX is an autosomal dominant disease that is caused by gain-of-function mutations in *PIEZO1* and in *KCNN4*, also known as ‘Gardos channelopathy’. The role of Gardos channel in normal erythrocytes has not yet been defined, but in HX-associated mutant *KCNN4* channels demonstrate alterations in channel kinetics and trafficking (21)

The treatment of HHA due to RBC membrane defects are always palliative, depending on the severity of anaemia. In general it is based on blood transfusion and concomitant administration of folic acid. When

the anemia is severe, removal of the spleen (splenectomy) is the therapeutic option. In HS, splenectomy allows a complete recover of haemoglobin concentration, whereas in the HE this recovery is only partial. Due to the important defensive effect of the spleen against infections, the splenectomy is not recommended under the 5 years of age in order to avoid the possibility of a fulminant pneumococcal sepsis. In all cases, however, before splenectomy it is recommended to administrate a polyvalent pneumococcal vaccine. In HSt, splenectomy is not recommended because it facilitates thrombotic events due to an increase of thrombophilia (22)

### Erythroenzymopathies

Erythroenzymopathies are hereditary diseases due to defects of RBC metabolism, in general, an enzyme deficiency associated with acute or chronic haemolytic anaemia. An association of enzymatic defects with HHA has been described in 14 of the 38 enzymes that make up the erythrocyte metabolism (Table 1)

**glucose-6-phosphate dehydrogenase deficiency** (*G6PD*; OMIM 300908, 134,700), is the most common human enzyme deficiency, affects the antioxidant system, and shows an hereditary transmission linked to sex. It is especially frequent in Africa, Asia and in the Mediterranean region (23).

Clinically, the G6PD deficiency occurs with haemolytic crisis sometimes associated with severe anaemia, in general triggered by the intake of oxidant substances, like fava beans (favism), or certain drugs. Due to this, the carriers of a G6PD deficiency can remain asymptomatic for many years, until a contact occurs with the substances that may trigger the haemolytic crisis. Among the drugs that can induce haemolysis in G6PD deficiency, can be mentioned certain analgesics, sulphonamides, antimalarials, and antibiotics. Favism, a severe haemolytic anaemia induced by fava beans ingestion or exposure to pollen from the plant, is the most frequent clinical manifestation in caucasians bearing the deficient G6PD Mediterranean variant (24). There are also ultrarare forms of G6PD deficiency that do not obey to polymorphic variants but to sporadic variants that present with a chronic

non-spherocytic haemolytic anemia (CNSHA). Other factors that can induce haemolysis in the G6PD deficiency are viral infections, especially influenza and hepatitis, diabetic ketoacidosis, and other different metabolic situations

The diagnosis of G6PD deficiency is based on the clinical history, and the exclusion of the autoimmune mechanism through the negativity of the direct anti-globulin test (DAGT) or Coombs test. During the haemolytic crisis, the observation of the smear shows the presence of eccentrocytes or RBCs subjected to oxidative stress where haemoglobin is pushed off to one part of the cytoplasm (Figure 9). For screening purposes, the fluorescent spot test is used and based on demonstrating the formation of NADPH (fluorescent) from NADP (non-fluorescent) in a drop of blood (Beutler's G6PD fluorescence spot test) or the reduction of methaemoglobin in the presence of methylene blue (23)

**Pyruvate kinase deficiency** (PK; OMIM 266200) is the second erythroenzymopathy in frequency after G6PD deficiency. PK is an enzyme of the anaerobic glycolysis (Embden-Meyerhof pathway) and since mature RBCs rely exclusively on glycolysis for their energy production PK deficiency (PKD) as happens with almost all the glycolytic enzymes, results in chronic hemolytic anemia (25). PKD is an autosomal recessive disease and generally the cause of CNSHA. Up to date more than 250 mutations in the PKD gene (*PKLR*) that encodes the liver and RBC pyruvate kinase isoforms are known to be causative of this condition. PKD is characterized by a highly heterogeneous clinical manifestations with frequent double heterozygous of non-missense mutations (26). Very recently, a concise guide to PK deficiency for primary care providers, but also for haematologists, healthcare providers and medical students has been published. This guide is useful for the better understanding of PKD deficiency during childhood and adolescence (27)

Other enzymatic deficiencies associated with CNSHA are listed in Table 1. As can be observed, the clinical expression of the deficiency is not restricted to the erythrocyte, but extends to other tissues leading to a hepatopathy, myopathy or neurological disease, in addition to chronic anemia (28).

**Table 1.** RBC Enzymopathies associated with hereditary hemolytic anemia

Enzyme	Hemolysis	Other clinical manifestations	Inheritance	Number of cases described	Gene
Adenilatekinase deficiency	Chronic	Neuropathy	AR	12 families	<i>AK1</i>
Adenosine deaminase hyperactivity	Chronic	No	AD	3 families	<i>ADA</i>
Aldolase deficiency	Chronic	Neuropathy	AR	6 cases	<i>ALDOA</i>
Phosphofructokinase deficiency	Chronic	Myopathy Glucogenosis	AR	50-100 cases	<i>PFKM</i>
Phosphoglyceratekinase deficiency	Chronic	Neuropathy Mental retardation	XL	40 families	<i>PGK1</i>
Glucose phosphate isomerase deficiency	Chronic	No	AR	> 50 families	<i>GPI</i>
Glucose-6-phosphate dehydrogenase deficiency	Acute (*) Chronic (**)	No	XL	Chronic variants - > 50 families	<i>G6PD</i>
6-Phosphogluconate dehydrogenase deficiency	Chronic (***)	No	AR	6 families	<i>6PGD</i>
Gamma glutamil cysteine synthetase deficiency	Chronic	Neuropathy Metabolic acidosis	AR	12 families	<i>GGCS</i>
Glutathione reductase deficiency	Acute/Chronic	Cataracts	AR	3 families	<i>GSR</i>
Glutathione synthetase deficiency	Chronic	Neuropathy Metabolic acidosis	AR	> 50 families	<i>GSS</i>
Hexokinase deficiency	Chronic	No	AR	21 cases	<i>HK1</i>
OPyrimidine 5'-nucleotidase deficiency	Chronic	No	AR	> 60 families	<i>NT5C3A</i>
Pyruvatekinase deficiency	Chronic	No	AR	> 500 families	<i>PKLR</i>
Triose phosphate isomerase deficiency	Chronic	Neuropathy (severe)	AR	50-100 cases	<i>TPI1</i>

(\*) After drug or fava beans ingestion or infection

(\*\*) Ultra rare variants

(\*\*\*) With acute crisis after fava beans ingestion

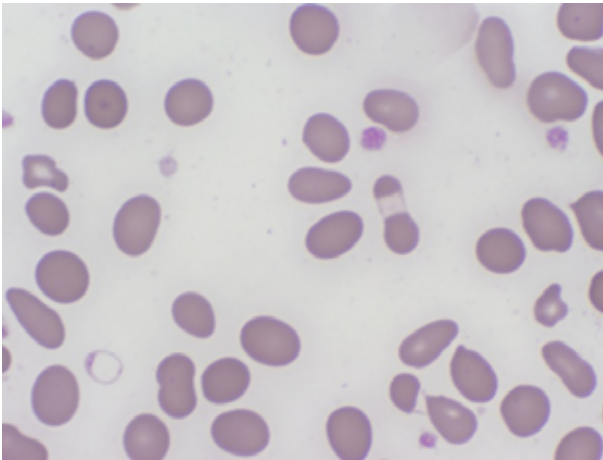
## Hemoglobinopathies

Haemoglobinopathies are the most frequent RBC defects in comparison with membranopathies and erythroenzymopathies. are the consequence of globin gene mutations that can alter the synthesis (thalassaemias) or the structure of haemoglobin (structural haemoglobinopathies). Its worldwide prevalence is around 269 million carriers, and in Europe there are risk populations, especially for thalassemia, which are located in the geographical regions surrounding the Mediterranean basin (Mediterranean Anaemia). During childhood and adolescence their clinical expression is always an HHA sometimes with severe

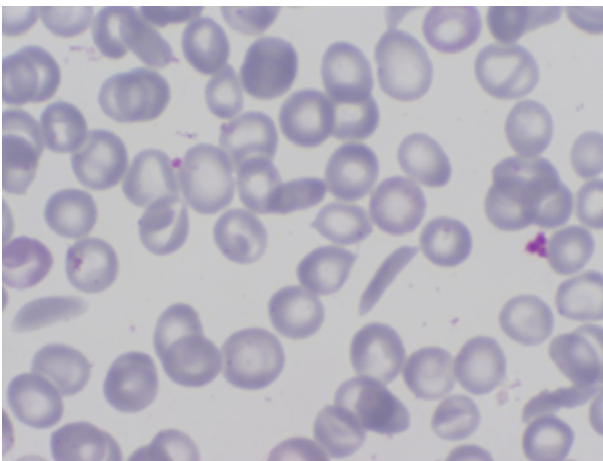
acute hemolytic anemia or with certain degree of dyserythropoiesis and decreased MCV (29).

**Sickle-Cell Disease (SCD).** This is the most frequent haemoglobinopathy due to HbS (OMIM 603903) that results of the substitution of valine for glutamic acid in the sixth position of the globin beta chain. HbS and in its homozygous form (HbSS) or combined with other haemoglobinopathies, under an hypoxic condition, decreases its solubility leading to a hemoglobin transformation that creates the characteristic RBC shape distortion (sickle cell) and cell rigidity that drastically decreases its deformability (Figure 10). This is the cause of the hemolytic crises, and the occlusion of small vessels (capillaries) in many organs that





**Figure 9.** Peripheral blood smear stained with MGG of a hemolytic crisis due G6PD deficiency. Presence of an eccentric nucleus where due to oxidative stress the haemoglobin is pushed to one part of the cytoplasm.



**Figure 10.** Peripheral blood smear stained with MGG of a patient with homozygous Sickle-Cell Disease (SCD). An evident sickle-cell can be observed in the centre of the image

lead to multiorgan microinfarcts and severe painful episodes (30). These recurrent hemolytic crises associated with painful episodes are called vaso-occlusive crises. These children have a high risk of infections and the disease is associated with a shortened life span. However, recent medical advances have significantly increased survival and quality of life for individuals with SCD. One of these has been the neonatal screening programs for HbS that allow an early diagnosis of the disease and its preventive treatment from the first

years of life. As a consequence, the frequency of complications have significantly reduced, and mortality during early childhood and adolescence has dramatically decreased (31). Despite these advances, however, adolescents with SCD continue to face many challenges of living with a chronic condition that requires lifelong medical management that creates a strong problem of scholarship due to the frequent transfusion requirement and the concomitant hospitalisation. These inconveniences decrease the patient's quality of life and in some cases may place them at risk of psychiatric symptoms and disorders. Several studies focusing on children and adolescents with SCD, suggest greater risks for anxiety, psychosocial difficulties and depression (32)

Over the last 30 years, it has been observed a significant increase of HbS in Europe due to the immigration impact of populations from other geographical areas, mainly from Sub-Saharan African regions but also from Asia and Central America, where this disease is very prevalent due to the protection that it has offered against malaria (6, 29)

**Thalassemia.** The Thalassemia syndromes are due to the decrease in the synthesis of a globin chain (alpha or beta), as the consequence of the absence, diminution or defective translation of specific messenger RNA (mRNA) caused by deletions or point mutations of the globin genes. While point mutations predominate in beta genes, large deletions are more frequent in alpha genes. According to the type of mutation and the intensity of the synthesis decrease, the severity of the clinical picture can be more or less intense (33).

In beta thalassemia, the milder forms consist of a slight or moderate hypochromic and microcytic anaemia (thalassemia trait) whereas the more severe clinical forms can be classified as "thalassemia major" or "thalassemia intermedia" depending on the severity of the anaemia and the periodic transfusion requirements, respectively. In alpha thalassemia, as the genetic cluster has two genes, the mutation of a single allele, is relatively common in Southern Europe and characterized by a moderate microcytosis (MCV around 80 fl) without anaemia (alpha thalassemia trait), whereas if more than one allele is affected more severe forms of alpha-thalassemia may occur. The mutation of three alleles gives rise to haemoglobin H (HbH), a clinical form very similar to intermediate

beta thalassemia but with the presence of HbH (beta-globin tetramers), a result of the excess or imbalance of beta chains due to the decrease of alpha chains. The complete loss of the four alleles (homozygous alpha-thalassemia) is incompatible with life (hydrops foetalis).

Thalassemia has been shown to be one of the most common genetic disorders worldwide, and in moderate clinical forms it arises during childhood or adolescence. The most frequent clinical forms of thalassemia are the beta-thalassemia intermedia and alpha thalassemia trait depending on the geographical area. In some European Countries like Greece, Italy, Cyprus and Turkey, thalassemia intermedia and major are relatively more frequent than in others like Spain, France and Portugal where predominate alpha and beta thalassemia trait (29).

The mutations associated with thalassemia now number over 1,530, and these range from single-nucleotide variations to large genome rearrangements (34).

Routine diagnosis of both forms of thalassemia is based on the data provided by the CBC and the study of haemoglobins by electrophoresis or high-performance liquid chromatography (HPLC). In the case of beta thalassemia trait an increase in the HbA2 fraction is always observed, whereas in alpha thalassemia trait the haemoglobin pattern is normal and a molecular study is mandatory. An accurate family study is also very important to prevent diagnostic errors and the application of unnecessary treatments. In addition, an appropriate identification of the carrier condition allows the identification of couples at risk in about a 95% of patients, while the remaining cases arise from point mutations

## Conclusion

Anemia constitute a serious health problem during the adolescence, due to its adverse effects on mental and physical development on this important stage of human life. Managing anemia in childhood depends on how severe the symptoms are, and if these symptoms are not critical, the adolescents do not refer to pediatricians and the diagnosis and treatment of the anemia may be delayed until the patients may appear with a more severe anemia picture. The most frequent cause of anemia during adolescence is iron deficiency more

evident in female gender due to chronic blood loss as consequence of menstruation. Moreover, serum ferritin levels of females are found significantly lower than that of males. These nutritional origin anemias, in general of low expression, are successfully treated with the administration of iron and other nutrients like vitamins (VitB<sub>12</sub> and folic acid). In adolescence, however, relatively common symptoms can hide underlying rare diseases leading to misdiagnosis and delaying treatment. This is the case of some rare anaemias that, due to their low prevalence, knowledge and medical expertise is scarce leading to inadequate health care offerings with denied diagnosis and treatment. Some rare anemias leading to a severe decrease of hemoglobin concentration typically disable, the quality of life of an adolescent that may be affected by the lack or loss of autonomy due to the chronic, progressive, degenerative, and frequently life-threatening aspects of the disease.

The fact that there are often no existing effective cures, adds anxiety to the high level of pain and suffering endured by patients and their families. A child with a rare anemia associated with severe clinical manifestations not only affect the person diagnosed but also impact families, friends, care takers and society as a whole. Hematologists and pediatricians have to be aware of this special situation and take the necessary care to establish the correct differential diagnosis of the cause of the anemia

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