ENERCA Recommendations for Centres of Expertise on Rare Anaemias

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ENERCA CONSENSUS RECOMMENDATIONS FOR EXPERT CENTRES IN RARE ANAEMIAS: A WHITE BOOK

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This White Book is dedicated to all the patients with a Rare Diseases

INTRODUCTION

- Rare diseases (RDs) is probably the area in public health in which join efforts within European Member States (MS) is most justified and crucial. A European common approach appears much more rational, efficient and effective than 27 national ones.
- In this sense, the establishment of a European Reference Network (ERN) of Centres of Expertise (CEs) in RD is a key strategy in order to improve the clinical management of these patients and to move forward the reducing of inequalities across Europe.
- This White Book (WB) constitutes a tool in that direction, which has been developed as the deliverable 5 of the ENERCA 3 Project (European NEtwork for Rare and Congenital Anaemias).
- ENERCA 3 is a project funded by the European Commission (EC) through its Executive Agency for Health and Consumers (EAHC). As a matter of fact, the aims of ENERCA are closely linked to the strategic objectives of the EU Health Programme 2008-2013, which particularly focus on reducing health inequalities across the European Union (EU) and tackling RD through a Europe-wide network. ENERCA also relates to one of the main objectives of the Commission's Work Plan for 2008, "putting citizens first", which includes improving patient safety and the quality of health services.
- Within that general framework, the WB aims to be a contribution in the specific area of rare anaemias (RAs). Accordingly, its specific objectives are: 1) To establish consensus recommendations on the services to be provided by Centres of expertise (CEs) in RA and 2) To establish consensus recommendations for the creation of a European Reference Network (ERN) of Centres of Expertise (CEs) in Rare Anaemias (RAs). As an added value, the WB will be a useful tool as a pilot experience for the development of other RD networking and it is also itself a tool of self assessment of the services provided by the centres.
- As established in the Directive 2011/24/EU of 9 March 2011 on the application of patients' rights in cross-border healthcare, the Networks in Rare Diseases are a very important tool that should be promoted. Some concrete benefits of an ERN of CEs in RAs can be identified as the following:

  - Access to experts and expertise throughout the European MS for both patients and health professionals, independently of the country of origin or practice, reduce inequalities and maximize the cost-effective use of resources.

- Epidemiological surveillance throughout EU by gathering comparable data on patients affected by RAs and allowing the implementation of preventive programmes for tackling RAs
- Fostering of best practices for prevention, diagnosis and clinical management.
- Promotion of knowledge dissemination of share of expertise and support of research, and increase awareness about RAs.
- To facilitate the transposition of the Directive 2011/24/EU of 9 March 2011 on the application of patients' rights in cross-border healthcare. The ERN between healthcare providers and CEs are a main interest point of the directive, especially for RD. The networks will be a tool to "improve the access to diagnosis and the provision of high-quality healthcare to all patients who have conditions requiring a particular concentration of resources or expertise, and could also be focal points for medical training and research, information dissemination and evaluation, especially for rare diseases" (recital 54 and article 12 of the Directive). The networks would also be useful concerning the establishment of national contact points (article 6 of the Directive).

- Accordingly, The WB is addressed to the agents in charge of the recognition of CEs and the creation of the ERNs; European and national authorities, health centres and health professionals. It is also addressed to the patients as in the WB is reflected the need to empower the patient's community in that process.
- The reader will find an up-to-date RA description and epidemiological information with the EU background policies for defining CEs and ERN. The aim is to provide stakeholders with a practical material and specific methodology used for the establishment of the ENERCA consensus recommendations. Therefore, methodology is presented in detail in a separate chapter. It is explained integrating the interdisciplinary approaches: legal and ethical, clinical and laboratory, and patient's expectations. For this, a working group was initially established integrating different profiles, namely the European Working group on Rare Anaemias (EGRA). The materials used such as the questionnaires for surveys and the evaluation of results can be found in the annexes. Consensus recommendations are presented in a friendly user document easy to interpret. Obviously, the WB content is not immutable and it is expected to be a dynamic working tool, modifiable, if necessary, in the time, as mentioned in the last chapter.
- One of the particular characteristics of ENERCA is the integration of the ethical and legal perspective in the networking design. A panel of jurists, experts in Medical Law, has been working together with other medical partners, in order to analyze the ethical and legal issues involved. Implementing mechanisms that guarantee the respect of the rights and interest of patients has been an important ENERCA concern. The outcomes of this important task can also be used as an example for other initiatives concerning RD, in general.
- Finally it must be stressed that the WB is the result of a co-ordinated and integrated consensus task developed by the EGRA and is presented according to the consecutive steps followed for moving towards the establishment of the consensus recommendations for the recognition of CEs and the creation of the ERN of CEs in RA.
- In this task we acknowledge all those persons and institutions that have contributed to make this book, a useful and realistic tool to improve the tackle of RA across Europe:

1. The European Commission, by means of its Executive Agency for Health and Consumers (EAHC); the external advisers that have actively been involved in the

agreement of the ENERCA consensus recommendations

2. The institutions that support the EGRA members work in this project;

3. All the ENERCA partners and centres that have participated in the different surveys performed across Europe; and

4. Each patient who has contributed to improve the contents of the book by answering the patients' expectations survey questionnaire.

THE ENERCA PROJECT

• As it has been said, this WB has been developed as the deliverable 5 of ENERCA 3. This project is described in brief in the following paragraphs

• The ultimate goal of ENERCA is **the prevention of rare anaemias and the promotion of policies** that lead to a healthier way of life for the well-being of European Union (EU) citizens. Accordingly, two pivotal aspects, a specific framework for cross-border healthcare and a European cooperation on health services are promoted.

• In this context, as in many other RDs, a large-scale network of experts and specialists working in the field of rare anaemia (RA), did not exist. That created a serious lack of information and knowledge-related troubles for both patients and health professionals. For this reason, ENERCA started back in the earlier 2002 with the purpose of **offering an improved public health service in every aspect of RA.** The ENERCA website, www.enerca.org, definitely provides a meeting place for such a service.

• Since 2002, ENERCA has taken an active role in the cooperation within the EU in the framework of helping health professionals and improving patients care. This allows to present, actually, a definitive European added value, as stated in the **EU Health Programme 2008-2013** *("common principles in all EU health systems aiming to ensure clarity and confidence with regard to authorities setting and monitoring healthcare standards, have to be implemented").*

• The three developing phases of ENERCA have been the following:

First phase (2002-2004) for setting up of a topic-focussed website, www.enerca.org, addressed to both health professionals and patients, with the aims of:

- Increasing the knowledge and awareness of RA among patients, their relatives, and care providers by the provision of clear and concise information, in their own language, about RA.
- Providing health professionals with a diagnosis flowchart for the identification of RA.

Second phase (2005-2008) covered both hereditary and acquired RA. The main outcomes were as follows:

- The development of a pilot study to initiate the mapping of existing clinical centres in RA in Europe.
- The creation of a database collection of haemoglobinopathies as a pilot experience for the future development of the European registry of RA.
- The promotion of prevention programmes, such as the neonatal screening of haemoglobinopathies in those European countries without existing epidemiological information. This has led to the knowledge of a prevalence of haemoglobinopathies in Catalonia and Latvia.
- The performance of a quality assessment pilot study for red blood cell (RBC) morphology and quantification of haemoglobins A_2 and F.
- The dissemination of ENERCA knowledge across Europe by providing an online forum, via the website and by approaching the national and European scientific societies, and finally, the organisation of the first European symposium on RA.

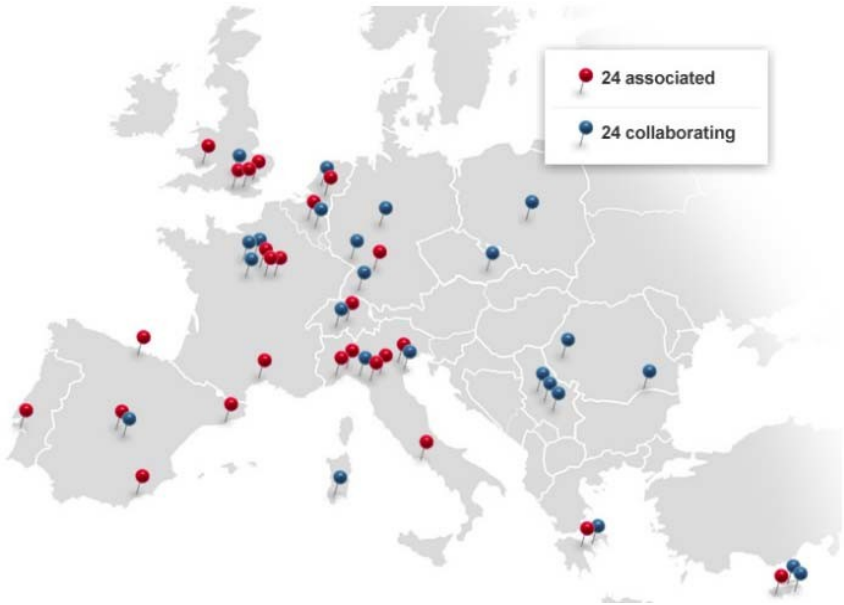
Third phase (2009-2012) for the creation of the ERN of CEs in RA, that not only will be a Network linking the existing European CEs, but will also become a platform for providing both information and services to every stakeholder of RA.

The **specific objectives** of ENERCA 3 include:

- The creation of an **ERN of CEs in RA.**
- The promotion of the **external quality assessment** (EQA) of procedures used for the diagnosis of RA.
- The publication of **ENERCA recommendations** for prevention, diagnosis and clinical management of RA.
- The improvement of health professionals' knowledge on RA by the organization of **topic focussed training courses** at the European and national levels and by the organisation of the 2nd and 3rd **European symposiums** on RA with the involvement of the patients' community.
- Increase social awareness of RA by means of developing **educational material** in the most widespread European languages.
- The **promotion of research and cooperation** among experts in RA

• ENERCA involves 48 partners (24 associated and 24 collaborating) distributed in 15 different European countries. Most of the partners have been working together since 2002 and all of them are well known and recognized experts in their respective field. Contact person and institutions details are listed in Annex 1

Figure 1 – ENERCA Consortium - Picture of ENERCA associated and collaborating partners



Partners are distributed in different working groups on the basis of six Work Packages (WP); three transversal: **WP 1 "Networking of expert centres"** (Leader: Interuniversity Chair in Law and the Human Genome, University of Deusto), **WP2 "Quality of patient care"** (Leader: UK National External Quality Assessment Service) and **WP3 "Education and training"** (Leader: Centre Hospitalier Universitaire de Montpellier) and three focused on public health issues and management of patients with Rare Anaemias (RA) classified into three main categories: **WP4 "Sickle Cell Disorders"** (Leader: Erasme Hospital-Université Libre de Bruxelles); **WP5: "Thalassaemia"** (Leader: Thalassaemia International Federation) and **WP6 "Very rare Anaemias"** (Leader: Universität of Ulm). **"Evaluation"**, **"Dissemination"** and **"Coordination"** of the project as a whole (**WP7, WP8** and **WP9** respectively) is led by the main partner (Hospital Clínic of Barcelona – University of Barcelona).

1. THE EXPERTISE IN RARE ANAEMIAS: AN OVERVIEW

1.1. Rare Anaemias. The concept

Anaemia, defined as the decrease of haemoglobin (Hb) concentration in blood, is a very common clinical manifestation in human pathology and may result from a wide variety of causes, either congenital or acquired. Anaemia is always a symptom or the clinical manifestation of an underlying disease, and never a disease itself. In general, there are three primary causes of anemia: (1) Bone marrow erythropoietic defects or reduced haemoglobin synthesis. (2) Hemolysis or excessive destruction of mature red blood cells, and (3) blood loss due to bleeding. Anaemia can be the consequence of a single disease (e.g, haemoglobinopathy, enzyme deficiency, etc), but it can be also the expression of external factors such as nutritional deficiencies, parasitic or viral infection, and other. Hb concentration is the most reliable indicator of anaemia, but since its normal distribution at population level varies with age, sex, and physiological status, the Word Health Organization (WHO) has defined the existence of anaemia when its concentration is less that 110 g/L in children and pregnant women, 120 g/L in non pregnant women and 130g/L in men. Moreover, measuring of Hb concentration is relatively easy and inexpensive, and currently all automated and semi-automated haematology analyzers measure Hb concentration with a great precision and accuracy. It is well known that iron deficiency in children and women and chronic diseases in adults and elderly, is the most frequent causes of mild to moderate anemia in Europe. However, there a group of anaemias that is considered rare because their frequency in our population is less than 5 cases for 10,000 individuals. These are the so-called “rare anaemias”, either of hereditary or acquired origin.

Diagnostic approach

More than 80% of rare anaemias (RA) are hereditary and in its dominantly inherited pattern, the allele responsible for the disease can be passed on from parents to their children with a probability of 50%. In recessive hereditary pattern, parents or other relatives can be healthy, because only the coexistence of two mutated alleles causes the disease, which can occur with a probability of 25% in each pregnancy.

Among hereditary anaemias, haemoglobinopathies are the commonest genetic defect worldwide with an estimated 269 million carriers. They are the consequence of mutations in the globin genes, which are responsible

for the synthesis of haemoglobin, the main component of red blood cells. These mutations are leading to abnormal proteins (haemoglobin variants) or to a decreased synthesis of globin chains (thalassaemias). In Europe, certain populations are particularly at risk of having a haemoglobinopathy. In Southern countries, their prevalence is higher than in central or northern Europe, but in all cases the prevalence is less than 1 per 2000 individuals. For this reason, in Europe, haemoglobinopathies and thalassaemias are still considered a particular group of rare diseases (RD) or rare anaemias (RA). Whereas thalassaemia syndromes are inherent in the autoctonous European at risk groups (Mediterranean anaemia), other haemoglobinopathies have been imported by immigration (Sickle-cell anaemia). As in any anaemia, the diagnosis on a RA is often prompted by pallor, noticed by the patient, the family, and/or the general practicioner (GP). Severity of clinical manifestations is directly proportional to the acuteness of onset, and many patients do not notice any symptoms when anaemia occurs insidiously. At the laboratory level, the diagnosis of anaemia includes two main steps:

1. General diagnostic tests: Complete Blood Count (CBC), reticulocyte count and morphology examination of the red blood cells (RBC)

2. Cause-oriented special diagnostic tests: Haemolysis tests and specific diagnostic tests (including genetic testing)

1. General diagnostic tests : CBC includes four parameters: a) haemoglobin concentration (Hb), the key of anaemia diagnosis, b) RBC count or concentration of RBCs, given as number of cells per liter of blood c) haematocrit or packed cell volume (PCV), given as the percentage of blood by volume that is occupied by the RBCs and d) RBC indices or calculations derived from a, b, and c), of great help for the diagnosis and classification of anemias. These indices are automaticaly measured by modern haematology analyzers and are mainly three 1. The mean corpuscular volume (MCV) or average size of the RBCs expressed in femtoliters. 2. The mean corpuscular hemoglobin (MCH) or average amount of hemoglobin inside a single RBC expressed in picograms (pg) and 3. The mean corpuscular haemoglobin concentration (MCHC) or average concentration of haemoglobin in the RBCs expressed as a percent. Sometimes the RBC distribution width (RDW), a measure of the variation of RBC width, can be also used for anaemia classification. Usually RBCs have a standard size of about 6–8 μm, but in certain disorders, a significant variation in RBC size can be present. Here the RDW value is a relatively good indicator of RBC size heterogeneity .RDW is specialy useful for differentiate iron deficiency

(increased value) from thalassemia (normal value) Reticulocyte count or number of circulating young RBCs (reticulocytes) is an important complementary test which indicates the bone marrow capacity to overcome the severity of anaemia. Accordingly, anaemias due to RBC destruction (haemolysis) are characterized by increased reticulocyte count (*regenerative* anaemias), whereas anaemias due to erythropoietic insufficiency (aplasia or ineffective erythropoiesis) are characterized by a lower reticulocyte count than to be expected from the severetiy of anemia (hypo-or *aregenerative* anaemias) In thalassaemias and in congenital dysertythropoetic anemias, where erythropoietic insufficiency coexists with some degree of haemolysis, the reticulocyte count may be variable

2-The reticulocyte count and MCV are, up to now, the most useful criteria for anaemia classification. According to MCV, anaemias are classified into microcytic (low MCV), macrocytic (high MCV) and normocytic (normal MCV). The two main causes of microcytic anaemias are iron deficiency and thalassaemia and the two main causes of macrocytic anaemias are cobalamin (vitamin B12) and folic acid deficiencies. Normocytic anaemias can be due to several different causes, not related with nutritional defects or thalassemia, being the most frequent haemolysis and erythropoietic failure. Here, the reticulocyte count is the most useful test for differentiate between these two conditions. In clinical practice, the most frequent cause of anaemia is iron deficiency (ID), characterized by a low MCV (microcytic anaemia). In southern Europe countries with higher “at risk” thalassemia population (mediterranean bassin) , this hereditary disorder can be easily taken by iron deficiency anaemia (IDA) because of the low MCV (< 82 fL) or microcytosis. Accordingly, in a patient with microcytosis the first step is always to exclude ID. If present, iron supplementation has to be given until the MCV recover its normal value. Howwever, if after treatment, MCV remain low, the coexistence of a thalassemic gene has to be investigated. It should be mentioned that there are a number of conditions where the MCV can be falsely rose masking the main clue of thalassaemia diagnosis. This is the case of patients with thalassemia and sickle cell anaemia (HbSS) co-inheritance or with other cause of haemolytic anaemia and increased reticulocyte count. This can falsely increase the value of MCV and masking the diagnosis of thalassemia if only the MCV is used for initial screening.

As part of the CBC, the blood film examination is sometimes very useful because it may provide a clue to the diagnosis of a particular RBC defect..

As indicated by Barbara Bain (Diagnosis from the Blood Smear. N Engl J Med 2005; 353:498-507), despite the advances in automated blood cell counting, the blood film retains a crucial role in the diagnosis of RBC disorders. This is particularly important in haemolytic anaemias, in congenital dyserythropoietic anemias and in the differential diagnosis of macrocytic anaemias. RBC morphology examination provides in some cases (i.e.: hereditary spherocytosis,, sickle-cell anaemia, hereditary siderobablastic anemia and thrombotic thrombocytopenic purpura) a definitive diagnosis, but, more often, it suggests a differential diagnosis that indicates further study. Morphological changes such as stippling and target cells in the blood film are not definitively associated with a haemoglobinopathy, but would be helpful findings in patients with moderate or severe anaemia associated with low MCV (Thalassaemia Intermedia, or major). Finally, RBC morphology examination has also the advantage of speed that may be important in severe anaemias such as those mentioned before.

2. Cause-oriented special diagnostic tests are the next step is the identification of the cause of the anaemia or its molecular mechanism. These tests include a group of laboratory procedures depending of clinical or laboratory diagnostic orientation of the anaemia (Dacie and Lewis. Practical Haematology, Elsevier, 11th Edition, 2012). In order to provide a first approach to the cause of the anaemia, several diagnosis oriented flowcharts can be found in the literature, mainly based on the morphological classification of the anaemia (microcytic, macrocytic and normocytic). In ENERCA web page (www.enerca.org) several flowcharts have been included for anaemia diagnostic orientation. For this, three patient's data have to be provided: sex, Hb and MCV. If anaemia is detected, one of the three available flowcharts will appear, depending on the MCV value: low (microcytic anaemia), high (macrocytic anaemia) and normal (normocytic anaemia). These flowcharts are not exhaustive and always require the advice of the haematologist, but they provide the basic information of how the investigation of anaemia causes can be undertaken in routine clinical practice. Using these flowcharts the most frequent rare anaemias (haemoglobinopathies, thalassaemias and haemolytic anaemias) can be easily recognized. Depending on the results of the recommended basic tests more specific tests (including molecular biology) can be performed. Some of these specific tests can be performed in general

haematology laboratories but other tests require to be undertaken in specialized laboratories. In all the cases External Quality Assessment Schemes (EQAS) are necessary for assessing the quality of practice or for obtaining a technical qualification Since the most specific tests are performed in few specialized laboratories, local (national or regional) EQAS organizations cannot establish a specific EQAS for these procedures due to its high cost. Accordingly, the EQAS for these procedures has to be established at European level as ENERCA 3 has done with some rare diagnostic tests for RAs.

Classification

RAs can be classified, according to its pathophysiology, into two main grops: 1- Erythropoietic/Bone Marrow defects and 2- Red Cell/Peripheral Blood defects. This means that all RAs are the consequence of intrinsic defects of the haematopoietic system leading to low RBC production (erythropoietic defects) or of the RBC leading to haemolysis (RBC defects). More than 80% of these disorders are hereditary and, therefore, without treatment, exception made of palliative therapies such as blood transfusions or erythropoietic stimulating drugs (Erythropoietin). In clinical practice there may be some confusion between RAs and the anaemias that appear in the course of nonhaematological or systemic diseases (secondary anaemias). This confusion is due to the fact that anaemia is not a disease but a clinical manifestation, and some rare diseases are associated with anaemia of variable degree. One example of this is the Rendu-Osler disease (hereditary telangiectasia), a relatively well known rare disease where anaemia, due to iron deficiency, is very common, and sometimes the first clinical manifestation of the disease. Furthermore, the anaemias due to chronic inflammatory diseases, vitamin deficiencies, immune diseases, malignancy or other rare disorders may probably be also considered RAs, despite have not been included here.

ENERCA is actively contributing to the WHO Update Platform ICD-10 of blood and blood forming organs. Here anaemias, in general, are classified into three main gropus: D50-D53 (nutritional anaemias), D54-D59 (haemolytic anaemias), and D60-D64 (aplastic and other anaemias). This ICD classifrication includes all kind of anaemias, hereditary, acquired, common and rare and ENERCA has extracted the RAs that have been individualy listed in ENERCA Web page. For practical purposes according to their mechanism, prevalence and/or relevant clinical and/or social impact in the European population these RAs have been classified into nine groups:

Group 1: Haemoglobin disorders: Haemoglobinopathies and Thalassemias Group 2. Hereditary Haemolytic Anaemias: Red blood cell enzymopathies and membrane defects Group 3. Hereditary erythropoietic failure or aplasia: Diamond Blackfan anaemia (DBA) and Fanconi Anaemia (FA), Group 4: Congenital dyserythropoietic anaemias (CDA), Group 5: Hereditary sideroblastic anaemias, Group 6: Hereditary disorders of iron metabolism defects, Group 7: Hereditary disorders of folic acid and cobalamin defects, Group 8: Paroxysmal nocturnal haemoglobinuria (PNH), Group 9: Anaemias due to rare complex mechanisms and Group 10: Anaemias of unknown underlying cause (AUC). The underlying cause of rare anaemias remains still unexplained in about 30% of patients, almost one third of which might be accounted for myelodysplastic syndromes.

AUC can be also due to complex clinical situations and multifactorial mechanisms, in general associated with systemic, non haematological, hereditary or acquired diseases. Their existence is a very important tackling exercise for clinical and biological research.

Further reading

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1.2. Rare Anaemias. Description

- It is not the objective of this chapter to provide extensive scientific information on every RA but, rather, to provide a brief overview to health professionals, experts on other RD, policy makers, patients and other stakeholders that may be interested of the main categories of RA, the heterogeneity of these disorders and the most common clinical features and complications that affect the patient.
- A list up to 62 different RA has been defined in ENERCA website (www.enerca.org). Specific disease's cards have been elaborated and translated into different languages. For all of them, the ORPHANET, MIM and ICD codes, definition, treatment and hereditary pattern, as well as health professionals leading with these disorders and patients' associations have been considered.
- RA's categories covered within the development of this WB are briefly described herein.

SICKLE CELL DISEASE

- ORPHANET code: 232
- OMIM code: 603903
- ICD-10 code: D57.0 / D57.2

Definition

- Haemoglobin (Hb) is a molecule that carries oxygen; it comprises four globin chains and is found in the red blood cells. The normal major Hb form found in the blood of newborns is called foetal Hb (HbF). A few months after birth, it is replaced by adult haemoglobin (HbA). When a specific mutation occurs in the β-globin gene, HbA is no more produced but well a structurally abnormal Hb called sickle haemoglobin (HbS). HbS is of low oxygen affinity and has a tendency to polymerise in certain circumstances such as hypoxia. Sickle cell disease (SCD) includes a group of conditions in which HbS is the major abnormal protein involved in the clinical disease. The homozygous state "HbSS" or sickle cell anaemia (SCA) is the most common and severe form of the disease. The other forms are compound heterozygote states and notably, HbSC, $HbSD_{Punjab}$, $HbSO_{Arab}$, HbSβ°thalassaemia, HbSβ^{+}thalassaemia.

Pathophysiology

- The tendency of HbS to polymerise when deoxygenated is the basic pathophysiological mechanism in sickle cell disease. Other mechanisms are involved such as abnormal interactions between sickle red blood cell and vascular endothelium leading to vascular damage, red blood cells dehydration, and chronic inflammation with activation of cells present in the vessels, and abnormalities of the vascular tone. Polymerisation leads to the loss of red blood cell deformability and their premature destruction i.e. haemolysis, and also to occlusion in the micro vascular circulation.
- However, SCD is a complex, multifactorial pathology. Genetic modulators such as foetal haemoglobin levels and the presence of α-thalassaemia, also influence the clinical course of the patients. The severity and course of the disease are also modulated by psychological or environmental factors such as malarial infection.

Mode of inheritance

- SCD has an autosomal recessive mode of inheritance. In SCD, the gene is situated on chromosome 11 and a mutation on one chromosome will result in

the carrier state. When two carriers (heterozygote) individuals mate, there is a 25% risk of having a homozygote or compound heterozygote offspring in each pregnancy. There is also a 50% risk of having a carrier child (HbAS) and another 25% of having a homozygote normal child (HbAA).

Diagnosis

- A full blood cell count, a separation of the haemoglobin fractions and quantification of HbS, Hb A_2 and Hb F are the key parameters in screening for haemoglobinopathies. In the case of neonatal screening for sickle cell disorders, only separation of the haemoglobin fractions is realised.
- Antenatal and neonatal screening programmes are the best tools for delivering prevention interventions and early, suitable care for families and patients. Antenatal screening with a view to identify at-risk pregnancies is feasible; a national antenatal screening programme is available in England.
- The diagnosis can also be established at birth, before any complication or painful crisis occurs. Five neonatal screening programmes financed by the local or national authorities in public Health are implemented in five countries of the EU: England, France, Belgium (Brussels, Liège), Spain (Madrid, Extremadura, Comunidad Valenciana and Pais Vasco), and The Netherlands. These programmes essentially aim to begin treatment to prevent the early mortality seen in infants secondary to pneumococcal septicaemia. They also allow early entry to other preventative programmes aiming to detect early organ complications of Sickle cell disease, the most established of which is the use of transcranial Doppler technology to assess stroke risk.
- Another opportunity for diagnosis is the case of an individual who presents with a clinical event whose aetiology may be SCD.

Management

- A few months after birth, when HbS level rises, the first symptoms may be expected. While in less severe sickle cell disorders, clinical problems may develop later in life.
- SCD is a chronic disease characterised by anaemia and damage of a number of organs i.e. spleen, lungs, central nervous system, liver, skin, eyes, kidneys … but punctuated by acute painful episodes. These random crises are of variable severity and triggered by different factors such as cold weather, infection, or dehydration.
- Chronic organ damages as well as acute, random painful crises can be life threatening. They also can have a profound effect on all aspects of life; as a consequence, psychological and social problems are very common in these patients and their families.
- These disorders are complex and of very variable prevalence. That means that the management of patients with sickle cell disease has to be offered by a multidisciplinary team of health and social workers. Specialist and local centres working in networks offer the best chance of providing a full range of services, specialist access and supervision when required but the majority of care can be delivered close to the patient's home by a local team.
- Management of SCD patients must include prevention programmes, curative, symptomatic and psychosocial interventions.
- The main principles of management from childhood to adulthood are:

- (The milestone) Education, information and advice regarding sickle cell disease (given to health workers, parents and/or patients)
- Prevention of infections i.e. extended vaccinations, penicillin prophylaxis and pneumococcal vaccination
- Follow-up of patients in view to identify those at risk for certain adverse outcomes such as:
    - For stroke by monitoring them by transcranial Dopplerscanning
    - For a severe disease by monitoring the number of painful events per year
- Prevention of acute events related to a surgical procedure or pregnancy
- Treatment of acute events i.e. blood transfusion for acute stroke or acute chest syndrome, antibiotics for infection, tailored analgesia for painful crisis …
- Prevention of acute or chronic events i.e. treatment by chronic blood transfusion or hydroxycarbamide
- Treatment of some chronic complications, by chronic blood transfusion (Not for chronic anaemia) or hydroxycarbamide
- Monitoring and treatment of iron overload
- If applicable i.e. severe disease, curative therapy by haematopoietic stem cells transplantation

• It is unlikely that all these and other services will be available within one centre in many places hence the emphasis placed on provision of a full range of services within a network arrangement. This allows for local expertise and is flexible, the key being that all professionals within such an arrangement have a clear understanding of their roles and responsibilities within such an organisation

Key messages

- Sickle cell disorders might be very rare or rare conditions in the different countries of the Europe Union. They have been introduced both from the south of Europe but also from Sub-Saharan Africa and Asia**.**
- Each sickle cell disorder is a multi-organ disease requiring life-long specialised care by a multi-disciplinary team
- Care is best provided in Expert Centres and networks with local centres
- Prevention of new affected births is possible and should be adopted where the frequency is increasing
- Prevention of several adverse events is possible if a neonatal screening programme is implemented

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BETA-THALASSAEMIA

- ORPHANET code: 848
- OMIM code: 141900, 604131
- ICD-10 code: D56.1

Definition

- The thalassaemias (Thal) are a group of hereditary disorders in which there is quantitative reduction of either of the two globin chains which make up the haemoglobin molecule. The reduction is due to mutations on the relevant globin genes on chromosomes 11 and 16. In beta thalassaemia (beta-Thal) there is reduced production of beta globin chains due to mutations on the beta globin gene on chromosome 11. The result is a reduced production of the haemoglobin molecule and consequently anaemia. Beta thalassaemia is the most significant clinically although there is a variation in the severity of the clinical consequences.

Pathophysiology

- The normal adult haemoglobin molecule (HbA) has a balanced amount of alpha and beta globin chains. Any reduced production of one will lead to an imbalanced α/β globin ratio and an excess of the chain which is normally produced. Thus in beta thalassaemia there is an excess of alpha globin chains. It is these unbound alpha globin chains which are responsible for the pathophysiology since they cause damage to the cell membranes of red cell precursors in the haemopoietic tissue leading to massive destruction of these cells and hence to ineffective haemopoiesis.
- The natural history of beta thalassaemia is further affected by increased iron absorption from the gut and/or blood transfusions, which are used to treat the anaemia. The result is iron overload as the iron storage proteins become saturated and unbound iron is released. The non-transferrin bound iron then causes oxidative damage to cells in vital tissues such as the heart, the liver and the endocrine glands leading to a multi-organ pathology.

Mode of inheritance and molecular defects

- Beta thalassaemia has an autosomal recessive mode of inheritance. The gene is located on chromosome 11 and a non-functional gene on one chromosome will result in the carrier or heterozygote state. When two carrier individuals mate, there is a 25% risk of having an affected offspring in each pregnancy. There is also a 50% chance of having a carrier child and another 25% of having a normal child.
- Different mutations can result in varying degrees of decreased production ranging from virtually no beta globin chains being produced to only a slight reduction. This will result in a variable clinical picture of the homozygote state, ranging from thalassaemia major to Intermedia. The clinical picture will also be modified by the co-inheritance of mutations which limit the chain imbalance such as those reducing alpha chain production (alpha-thalassaemia) or those increasing HbF production (e.g. hereditary persistence of fetal haemoglobin -

HPFH). In addition a frequently occurring Hb variant (HbE), widely distributed in Asia, results in reduced amounts of beta globin mRNA and may result in moderate to severe thalassaemia if co-inherited with a beta thalassaemia mutation.

Diagnosis

• The carriers of beta-thalassaemia have no clinical manifestations. In the homozygous state there is pallor from infancy, mild jaundice and later growth retardation, enlargement of spleen and liver and bone changes which are responsible for the characteristic facial features.Untreated iron overload will also result in a dark pigmentation of the skin. Most of these features are modified by early treatment.

• Both the heterozygous and the homozygous conditions in the beta-thalassaemia syndromes are diagnosed by the same spectrum of laboratory techniques:

- A full blood count including red cell morphology and red cell indices (MCV, MCH etc)
- The separation and quantification of the fractions of the Hb molecule,HbA, HbA2, HbF and possible variants by electrophoresis or HPLC
- Molecular diagnosis is used where the findings of the above techniques Are not diagnostic or if further characterisation of the homozygote state is needed, or if prenatal diagnosis is requested.

• In the clinical follow up of patients a series of specialised tests are used to monitor all aspects of treatment and to identify organ involvement. Such tests include regular measurements of serum ferritin, specialised magnetic resonance examinations (such as the T2* cardiac MRI and liver iron concentration), endocrinological tests, bone density, serological and molecular viral tests for the diagnosis of hepatitis viruses and HIV which may be acquired through blood transfusion among others. In a centre of expertise all the necessary tests for diagnosis and patient monitoring must be available and there should be readiness to introduce new tests as their clinical usefulness is recognised. One example of emerging technology is the measurement of labile plasma iron (LPI) currently being introduced for the monitoring of iron chelation in order tailor the treatment according to individual patient needs.

Prevention

• Reduction of affected births is achieved by a comprehensive programme of health education, screening to identify carriers (which may be premarital or ante-natal), genetic counselling and the choices of prenatal diagnosis and pre-implantation genetic diagnosis.

Management

• The carrier state of the thalassaemias needs no treatment, but genetic counselling is recommended. In the homozygous state the anaemia is more severe and is divided into two categories for decisions concerning management: thalassaemia major which is transfusion dependent, and thalassaemia intermedia or non –transfusion dependent thalassaemia, which includes beta thalassaemia ameliorated by genetic modifiers, and non-severe forms of HbE/β-thalassaemia.

• Transfusion dependent thalassaemia is managed by regular blood transfusions and iron chelating agents to remove toxic non-transferrin bound iron. In addition however there is need for regular monitoring to establish whether iron has accumulated and whether it has affected organ function. Any detection of organic involvement may necessitate the assistance of specialists such as cardiologists, endocrinologists and hepatologists. The need for

additional psychosocial support in these chronic syndromes, also makes the need for multi-disciplinary care a necessity. The complexity of management and the chronicity of the condition is also the basis of the proposal for Expert centres of care. Guidelines for the clinical management of thalassaemia have been published. Haemopoietic stem cell transplantation may be curative and is offered to patients when a compatible donor is available, particularly if the donor os brother or sister, thus reducing the danger of adverse reactions.

- Non-transfusion dependent thalassaemia syndromes have a wide spectrum of severity that have in common, the fact that there is no transfusion dependency in early life. Over the years complications arise such as hypersplenism, iron overload from increased iron absorption from the gut, bone deformities, growth failure, heart failure (either due to the anaemia or iron overload) and endocrine complications. Careful and timely monitoring of these complications is necessary from early life so that therapeutic interventions may be initiated, in order to limit complications and maintain quality of life. Decisive criteria for establishing the necessity to intervene by measures such as regular blood transfusions or iron chelation, have not yet been established and currently individual doctors make decisions not based on scientific evidence or based on criteria that were established for thalassaemia major.

- **Key messages**

  - Beta thalassaemia is a rare condition in continental Europe but common in the Mediterranean coast. In areas of low prevalence thalassaemia has now been introduced both from the south but also from the Middle East and Asia. Where thalassaemia is rare problems in patient care may arise.
  - Thalassaemia is a multi-organ disease requiring life-long specialised care by a multi-disciplinary team
  - Blood transfusion and Iron chelating therapy are the standard treatment modalities but haemopoietic stem cell transplant transplant is offered in suitable cases
  - Care is best provided in Expert Centres
  - Prevention of new affected births is possible and should be adopted where the frequency is increasing

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ALPHA THALASSAEMIA

- ORPHANET code: 846
- OMIM code: 141800, 604131
- ICD-10 code: D56.0 / D56.3

Definition

- The thalassaemias (Thal) are a group of hereditary disorders in which there is quantitative reduction of the globin chains, which constitute the haemoglobin molecule, due to mutations on the relevant globin genes on chromosomes 11 and 16. In alpha thalassaemia there is reduced production of alpha globin chains due to mutations on the alpha globin genes on chromosome 16 and the result is a reduced production of the haemoglobin molecule and anaemia.

Pathophysiology

- The normal adult haemoglobin molecule (HbA) consists of equal amounts of alpha and beta globin chains, which form pairs. Any genetically reduced production of one will lead to an imbalanced α/β globin ratio and an excess amount of the chain, which is normally produced. In the case of alpha thalassaemia the excess of beta globin chains will form a new molecule of beta chains (β4), which is unstable and also results in the early death of red cells causing haemolysis. This molecule is known as HbH and is found as a precipitate in young red cells in all forms of alpha thalassaemia but in greater abundance when three of the four alpha globin genes are mutated in HbH disease. In the foetus zero production of alpha globin chains will result excess gamma chains with the formation of a tetramer of these chains (γ4) known as Hb Bart's. This molecule cannot deliver oxygen to the tissues and causes massive death of red cells. The resultant severe anemia will cause heart failure in the fetus with swelling of tissues including the placenta, and problems of toxemia and delivery to the mother. This is known as Hydrops fetalis and fetal death is the usual outcome. If not recognized early there is also danger to the life of the mother.In a few cases intrauterine transfusion has allowed the baby to born alive even though transfusion dependent and requiring life long treatment. It is a choice that may be offered to parents after full explanation of the benefits and dangers.

Mode of inheritance

- Four genes (two on each chromosome 16) control the production of alpha globin chains. Mutations may occur on any of these genes and result in the following conditions:
  - α^+ carriers in whom only one of the four genes is inactive and this causes minimal haematological changes and is known as silent alpha thalassaemia
  - α^0 carriers in whom two genes are inactive in different chromosomes (in trans). These carriers can have a child with HbH disease only if coupled with an α^0 carrier with defective genes on the same chromosome (in cis).
  - α^0 carriers in cis are those who have two inactive genes on the same chromosome. They can have a child with HbH disease if they mate with a partner who is either an α^+ carrier, or an α0 carriers in trans. If both parents are α^0 carriers in cis, they can produce an offspring with no alpha chain production that will usually die in-utero since complete

absence of alpha chains will cause severe anaemia and heart failure in the foetus.

Diagnosis

• Both the heterozygote and the homozygote state in the thalassaemia syndromes are diagnosed by the same spectrum of laboratory techniques:

- A full blood count including red cell morphology and red cell indices
- The separation and quantification of the fractions of the Hb molecule, HbA, HbH and possible variants of the alpha globin chains
- Molecular diagnosis is used where the findings of the above techniques are not diagnostic of alpha thalassaemia, especially if the haematological findings suggest $\alpha^{0.}$ .DNA analysis is most often performed when there is a risk having an affected child (HbH disease or hydrops fetalis) and if prenatal diagnosis is requested.

Prevention

• Most alpha thalassaemia syndromes are not severe and are compatible with normal life duration and so prevention of new births is not normally practiced. The exception to this is when an at-risk couple has the possibility of having a pregnancy with homozygous alpha zero thalassaemia –Hydrops fetalis- and the health and life of the mother are threatened. In such a case the possibility of early detection by ultrasound examination of the pregnancy and prenatal diagnosis can be offered.

Management

• Alpha thalassaemia carriers: no treatment, except genetic counselling in the case of alpha zero thal carriers.

• HbH disease is a **mild** to moderate haemolytic anaemia; it is due the inheritance of three inactive globin genes. In most cases there is no need for blood transfusions except as a supportive measure in serious infections or other acute events, although there are exceptional cases of non-deletional mutations in which the clinical phenotype is more severe.

Key messages

- Alpha thalassaemia is rare in continental Europe although quite common in the Mediterranean coast
- There is need for expertise including molecular methods for the definitive diagnosis of alpha thalassaemia- trait, HbH disease
- Hydrops fetalis has other causes and is not always recognised as being related to alpha thalassaemia
- Association of alpha thalassaemia to other Hb disorders modifies the severity (e.g: SCD)

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HEREDITARY RED BLOOD CELL ENZYME DEFECTS

- ORPHANET code: 57, 362, 371, 712, 713, 766, 868, 33574, 35120, 86817, 90030, 90031
- OMIM code: 102730, 138300, 190450, 230450, 231900, 235700, 266120, 266200, 300653, 305900, 610681, 611881, 612631, 613470
- ICD-10 code:D55.0, D55.1, D55.2, D55.3, E74.0

Definition

- Erythrocyte enzyme deficiencies are inherited disorders that disturb red blood cell metabolism. They ultimately may lead to a decreased red cell life

span, causing hemolytic anemia. Some enzyme deficiencies lead to hemolysis only during periods of stress imposed by infection or administration of "oxidative" drugs, and in some individuals upon ingestion of fava beans (favism). Other enzyme deficiencies are associated with chronic hemolysis, a disorder designated hereditary nonspherocytic hemolytic anemia (HNSHA). Expression of the defective enzyme may not be confined to the red cells but may also be expressed in other tissues. In these cases non-haematological symptoms, such as myopathy and neuromuscular impairment, may (also) occur and be a prominent part of the clinical syndrome.

Pathophysiology

- Red blood cell metabolism enables the erythrocyte to maintain a number of vital cellular functions. The red cell's main source of energy is glucose, which is metabolized through the glycolytic pathway and through the hexose monophosphate shunt. Together, these pathways provide the cell with metabolic energy in the form of adenosine triphosphate (ATP), and reductive energy in the form of nicotinamide adenine dinucleotide phosphate (NADPH). In a bypass of glycolysis (the Rapoport-Luebering shunt) 2,3-bisphosphoglycerate is generated which is an important regulator of the oxygen affinity of hemoglobin. Furthermore, the red cell contains high concentrations of reduced glutathione (GSH) which serves to protect the red cell from oxidative damage. Finally, the nucleotide salvage pathway ensures maintenance of the red blood cell's adenine pool.
- Many red cell enzymes are involved in these pathways. Inherited disorders of a number of them may disturb the red blood cell's integrity and, ultimately, shorten its survival (Table 1). The exact mechanism by which this occurs is, at present, still unknown but ultimately involves premature removal of the metabolically deprived red blood cell from the circulation by the spleen and liver (extravascular hemolysis).
- The most common red blood cell enzyme defect is a deficiency of glucose-6-phosphate dehydrogenase (G6PD). The common polymorphic forms of G6PD lead to hemolysis only during periods of stress imposed by infection or administration of "oxidative" drugs, and in some individuals upon ingestion of fava beans (favism). Hereditary nonspherocytic hemolytic anemia also occurs as a consequence of other enzyme deficiencies, the most common of which is pyruvate kinase (PK) deficiency. Deficiencies of glucosephosphate isomerase (GPI), triosephosphate isomerase (TPI), and pyrimidine 5′-nucleotidase (P5N) are included among the relatively rare causes of HNSHA. In the case of some deficiencies, in particular those of glutathione synthetase, TPI, phosphoglycerate kinase (PGK) and phosphofructokinase (PFK) the defect are expressed throughout the body. Neurologic symptoms, myopathy and other symptoms constitute a prominent part of the clinical syndrome.

Mode of inheritance

• By far the majority of red blood cell enzyme disorders are hereditary in nature. Most of the defects are transmitted as autosomal recessive disorders (Table 1), while deficiencies of G6PD and PGK are X-linked. Adenosine deaminase (ADA) hyperactivity is a very rare disorder which is inherited in a dominant manner.

Clinical Picture and Diagnosis

• Red cell morphology in red cell enzyme deficiencies is, in general, unremarkable except for P5N deficiency, which is characterized by prominent basophilic stippling. The diagnosis HNSHA is essentially one that is established on basis of exclusion, i.e. a non-immune-mediated type of hereditary hemolytic anemia that is not a hereditary spherocytosis, or any other major alteration of red blood cell morphology. Hence, HNSHA is extremely heterogeneous both in etiology and in clinical manifestations. Diagnosis of the causative enzyme disorder underlying HNSHA is best achieved by determining red cell enzyme activity with a quantitative assay or a screening test. Molecular characterization of the defect confirms the diagnosis and is necessary for genetic counseling. It may also be helpful in recommendations for treatment, since patients with some enzyme deficiencies tend to respond more favorably to splenectomy than others.

• Individuals who inherit the common (polymorphic) forms of G6PD deficiency and defects of glutathione metabolism usually have no clinical manifestations. The major clinical consequence is acute hemolytic anemia in adults and neonatal icterus in infants. Usually the anemia is episodic and associated with stress, most notably drug administration, infection, and, in certain individuals, exposure to fava beans.

• In HNSHA the main clinical symptom is anemia of variable degree, ranging from severe transfusion-dependent hemolytic anemia to compensated hemolysis with a normal steady-state hemoglobin concentration. Chronic jaundice is a common finding, and splenomegaly is often present. Gallstones are common and ankle ulcers may be present. Pregnancy may precipitate hemolysis in patients with PK deficiency. In PK deficiency the increased 2,3-BPG levels ameliorate the anemia by lowering the oxygen-affinity of hemoglobin. Some PK-deficient patients present with hydrops fetalis.

• Some enzyme defects display characteristic nonhematologic systemic manifestations. In fact, these may be the only sign of the enzyme deficiency. For example, in some patients with phosphofructokinase (PFK) deficiency hemolysis is present without muscle manifestations, whereas in others both muscle abnormalities and hemolysis occur. Glutathione synthetase deficiency may be associated with 5-oxoprolinuria and neuromuscular disturbances, and such abnormalities may occur either with or without hematologic abnormalities. Some patients with glutathione synthetase deficiency manifest only the hematologic abnormalities. Patients with TPI deficiency nearly always manifest serious neuromuscular disease and susceptibility to infections; most of the patients die in the first decade of life. Neurologic symptoms have also been noted in patients with glucosephosphate isomerase deficiency.

Management

• G6PD-deficient patients should avoid drugs that might induce hemolytic episodes. An updated list can be found on http://www.g6pd.org. If hemolysis does occur as a result of drug ingestion or infection, red blood cell transfusion may be useful although generally not required. Good urine flow should be maintained in patients with hemoglobinuria to avert renal damage. Infants with neonatal jaundice resulting from G6PD deficiency may require phototherapy or

exchange transfusion

• Most patients with chronic HNSHA do not require therapy, other than blood transfusion during hemolytic periods. A small group of patients, however, may need to be transfused continuously. Chronic transfusion therapy usually requires iron chelation in case of iron overload.

• Patients with HNSHA may require splenectomy. Considering the heterogeneous etiology it is not surprising that the response may be difficult to predict. In general, splenectomy is beneficial in most patients suffering from deficiencies of PK, hexokinase, GPI, and PGK. Splenectomy in G6PD and P5N deficiency is often ineffective.

• In rare cases, PK deficiency has been treated successfully by stem cell transplantation.

Key messages

- Red blood cell enzyme disorders is a very heterogeneous group of disorders, both in etiology and clinical presentation
- The associated type of anemia is designated hereditary nonspherocytic hemolytic anemia
- Hemolysis may be either chronic or limited to periods of increased oxidative stress (specific drugs, infection, ingestion of fava beans)
- Some enzyme disorders are characterized by additional prominent non-hematological symptoms
- Diagnosis requires specific diagnostic tools available only in a limited number of centres in the EU
- No treatment other than supportive treatment is currently available
- Creation of European EC networks are required to increase knowledge of these rare disorders.

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ENERCA Recommendations for Centres of Expertise on Rare anaemias: a White Book

Table 1. Red blood cell enzyme disorders associated with haemolytic anaemia

Enzyme	Metabolic process	Hemolysis	Inheritance
Hexokinase (HK) deficiency	Glycolysis	Chronic	AR
Glucosephosphate isomerase (GPI) deficiency	Glycolysis	Chronic	AR
Phosphophructokinase (PFK) deficiency	Glycolysis	Chronic	AR
Aldolase deficiency	Glycolysis	Chronic	AR
Triosephosphate isomerase (TPI) deficiency	Glycolysis	Chronic	AR
Phosphoglycerate kinase (PGK) deficiency	Glycolysis	Chronic	XL
Pyruvate kinase (PK) deficiency	Glycolysis	Chronic	AR
Glucose-6-phosphate dehydrogenase (G6PD) Deficiency	Hexose monophosphate shunt	Acute, some chronic	XL

HEREDITARY RED BLOOD CELL MEMBRANE DEFECTS

Definition

- Red cell membrane disorders are inherited diseases due to defects of membrane or cytoskeletal proteins or altered membrane permeability, resulting in decreased red cell deformability, premature removal from circulation, and haemolytic anaemia of variable degree.
- Hereditary red cell membrane disorders include:

Hereditary spherocytosis (HS)

- ORPHANET code:822
- OMIM code:182870, 182900, 270970, 612653, 612690
- ICD-10 code:D58.0

Hereditary elliptocytosis (HE) and its most severe expression **Hereditary pyropoikilocytosis (HPP)**

- ORPHANET code:98864, 98865, 286,
- OMIM code:109270, 130600, 141700, 166900, 166910, 179650, 225450, 235370, 266140, 611804
- ICD-10 code:D58.1

South East Asian Ovalocytosis (SAO)

- ORPHANET code : 98868
- OMIM code: 109270.0002
- ICD-10 code: D58.1

Hereditary stomatocytosis (HSt)

- ORPHANET code:3202, 3203
- OMIM code:194380, 603528, 185000
- ICD-10 code:D58.8

Pathophysiology

- The red cell cytoskeleton is a very complex system consisting of multiple integrated proteins that provides the erythrocyte with its shape and deformability. As a consequence, a defect of a single protein may impair the structural and functional integrity of the whole system and result in an alteration of red cell shape. In general, abnormalities of spectrin, ankyrin, protein 4.2 and protein band 3, weaken the cohesion between cytoskeleton and the lipid bilayer, leading to the release of microvesicles and progressive transformation of the discocyte into a spherocyte. This typically occurs in hereditary spherocytosis.
- On the other hand, abnormalities due either to defective spectrin dimer-dimer interaction or defective spectrin-actin-protein 4.1 complex, results in hereditary elliptocytosis. Mutations responsible for HS are mainly localized in the genes coding for RBC membrane proteins (spectrin, ankyrin, band3 and protein 4.2), whereas mutations in α and β spectrin, protein 4.1 and the glycophorin C gene are responsible for HE. If cytoskeleton weakening is

excessive, red blood cells can undergo severe deformations, mimicking red cell fragmentation due to exposure to heat (HPP).

- Hereditary stomatocytosis (HSt) comprises a group of haemolytic anaemias mainly due to abnormality of red cell membrane permeability to monovalent cations. Overhydrated hereditary stomatocytosis (OHSt) is characterized by the presence of large numbers of stomatocytes on blood smears in association with moderate to severe anaemia, macrocytosis, and abnormal intra-erythrocytic sodium and potassium concentration (↑ $[Na^+]$, ↓ $[K^+]$, ↑$[Na^+ + K^+]$). The excess of cations increases red cell water content, producing large, osmotically fragile cells with a low MCHC. Mutations in Rh-associated glycoprotein (RhAG) have been reported in OHSt.

Dehydrated hereditary stomatocytosis (DHSt or hereditary xerocytosis) is characterized by decreased intracellular potassium content and loss of cell water, increased cytoplasmic viscosity and typically increased MCHC and MCV. Cell dehydration has only a marginal effect on survival of erythrocytes in DHSt, which is characterized by well-compensated anaemia. Linkage analysis suggested a segregation of the disease with a locus on chromosome 16q24.2-16qter; recently mutations in the PIEZO1 gene have been described in two unrelated families.

Other rarer and heterogenous forms of hereditary stomatocytosis (i.e. cryohydrocytosis, familial pseudohyperkalemia) have been described, some of them due to mutations in the transmembrane domain of band3 (SLC4A1 gene) and in glucose transporter 1 (SLC2A1 gene). Rare cases of cryohydrocytosis may present neurological abnormalities.

Mode of inheritance

- HS is the most common cause of congenital haemolytic anaemia in individuals of European origin. The transmission is autosomal dominant in 75% of cases, non-dominant in the remaining cases (including recessive forms and de novo mutations). Transmission of HE is predominantly autosomal dominant; homozygosity or double heterozygosity causes severe forms of haemolytic anaemia. Finally, most of hereditary stomatocytosis share a dominant pattern of inheritance.

Diagnosis

- The diagnosis of RBC membrane disorders is the final step of a diagnostic workout based not only on laboratory tests but also on clinical examination, personal family history, and the exclusion of possible causes of secondary spherocytosis. However, given the rarity and the wide clinical heterogeneity, the diagnosis of these defects can be difficult, in particular in mild and atypical forms. In these cases the diagnosis should be performed in Expert Centres.

- Laboratory hallmarks are: Presence of specific red cell abnormalities at blood smear examination such as spherocytes, ovalo/elliptocytes and stomatocytes. Blood film morphology should be performed in all individuals suspected to have anaemia with abnormal markers of haemolysis (bilirubin, reticulocytes, LDH, haptoglobin) and negative direct antiglobulin test.
- Demonstration of increased red cell fragility as assesses by NaCl osmotic fragility, acidified glycerol lysis test, cryohemolysis and pink test) or decreased fluorescence intensity of RBc labelled with Eosin-5-maleimide (flow-cytometry EMA-binding test). Increased red cell fragility and positivity to EMA-binding test are almost constant findings in hereditary spherocytosis, whereas are not informative in HE or HSt.

In severe/atypical HS and HE cases, or when HSt is suspected, the diagnostic workout is more complex requiring specific diagnostic tools, in particular:

- Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) of red cell membrane proteins that leads to the identification of the membrane biochemical defect.
- Spectrin functional analysis or tryptic digestion spectrin map performed in cases of suspected HE.
- Osmotic gradient ektacytometry or Laser-assisted Optical Rotational Cell Analyzer (LoRRca) which are considered the gold standard for the diagnosis of HSt.
- The use of molecular testing in RBC membrane disorders is indicated only in very severe HS forms or in genetic counselling since, in particular for HS, most of abnormalities are private mutations.

This "second level" diagnostic step is usually performed only in a few Expert Centres (or in EC networks) where these latter tools are available.

Management

- The main features of red cell membrane disorders are haemolytic anaemia, which varies from compensated haemolysis to severe haemolytic anaemia sometimes requiring exchange transfusion, repeated blood transfusions, and variable grades of jaundice, splenomegaly and cholelythiasis. Although the diagnosis is often made in childhood or adolescence, some cases are identified in adult age and also in elderly. Mild forms may be difficult to diagnose being associated with normal haemoglobin levels and bilirubin concentration. In some cases anaemia becomes evident only in concomitance of infection diseases or during pregnancy.
- Gallstones are common; iron overload may occasionally develop even in the absence of transfusions.
- The treatment of red cell membrane defects is based on supportive measures: folate therapy is recommended in severe and moderate forms of haemolytic anaemia, red cell transfusions may be required in severely anaemic cases, particularly in the first years of life, during aplastic crisis, infections, and pregnancy.
- Splenectomy is highly beneficial in the management of HS and HE but is contraindicated in OHSt because of an increased risk of thromboembolic complications. For this reason it is of utmost importance to ascertain the diagnosis before any splenectomy.
- Although iron overload is a rare complication in RBC membrane defects, iron ferritin levels should be monitored particularly in the presence of co-inherited HFE gene mutations associated to hereditary hemocromatosis. Gallstones

formation occurs frequently in RBC membrane defects and requires periodical abdominal ultrasound monitoring.

- Clinical patient's follow-up is usually performed in local Centres. However, because of the rarity and the wide heterogeneity of these disorders, a strict collaboration between EC and local centres is needed to offer the best chance of providing an appropriate diagnosis and a full range of services in particular in case of indication for splenectomy, management of acute/chronic complications, specific treatments (chelation therapy) and pregnancy. When required, genetic counselling is performed in EC.
- Creation of European EC networks (i.e. ENERCA) are also required to provide information for physicians and patients, and to allow the diagnosis of atypical cases and very rare disorders.

Key messages

- RBC membrane disorders are a group of rare/very rare diseases.
- The genetic heterogeneity is reflected in a wide variation of the clinical phenotype ranging from very severe to compensate haemolytic anaemia.
- Diagnosis of severe/atypical forms requires specific diagnostic tools available in CEs.
- An appropriate diagnosis becomes mandatory particularly when splenectomy is required.
- Regular follow-up for monitoring gallstones and iron overload should be always considered.
- Creation of European CEs networks are required to increase knowledge of these rare disorders.

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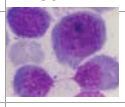
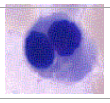
CONGENITAL DYSERYTHROPOIETIC ANAEMIAS

Definition

- DefinitionCongenital dyserythropoetic anaemia (CDA) is a disease category consisting of a group of hereditary anaemia's resulting from mutations of different genes. Although related by some common features, clinical appearance and hence methods of diagnosis as well as specific therapeutic measures are different for the different subtypes. An overview of this heterogeneity is shown in table 1

Table 1 –Congenital dyserythropoietic anemias

CDA type	I	II HEMPAS	III familial	III sporadic	Variants
Inheritance	Autosomal-recessive	Autosomal-recessive	Autosomal-dominant	Variable	Autosomal-recessive or x-linked
Cases reported	~150	> 500	3 families	< 20	~70
Morpho-logy					
Gene Locus	*CDAN1* 15q (15.1.3)	*SEC23B* 20p11.23-20p12.1	Unknown 15q (21-25)	Unknown Unknown	Unknown Unknown
Dysmorpho-logies	Skeleton, Others	Variable, rare	B-Cells Retina	Variable	CNS Others

Pathophysiology and clinical features

- The common features shared by the different subtypes are
  - Evidence of a congenital and/or hereditary disorder
  - Evidence of ineffective erythropoiesis – complete blood count, bilirubin, haptoglobin, serum transferrin receptor, reticulocytes, bone marrow examination
  - Characteristic morphological abnormalities of erythrocytes and erythroblasts
  - Exclusion of haemolytic and megaloblastic anaemia's, disorders of haemoglobin synthesis

- Even though symptoms are present in infancy or newborns, the diagnosis is often made in older children, adolescents or adults. As in other disorders with autosomal recessive pattern of heredity, siblings may be affected, but family history may be negative. With the exception of the familial type III, parents and offsprings are healthy, although by molecular genetics the trait can be detected.

- Diagnosis

The common clinical features allow suspecting the diagnosis of CDA, to be followed of special tests specific for the subtypes described below. It is recommended to do and to interpret these tests in the few expert centres for CDA in Milano IT, Naples IT, Paris FR or Ulm DE. The key feature to CDA as distinct from haemolytic anemias is the absence of adequate increase of reticulocytes in spite of anaemia. However, this may be also true in young children or in aplastic crises in patients with haemolytic anaemia.

CDA Type I

- ORPHANET code: 98869
- OMIM code: 224120
- ICD-10 code: D64.4

- The anaemia is normocytic or macrocytic. Red cells show distinct aniso-poikilocytosis and basophilic stippling. The typical changes of the erythroblasts in the bone marrow (erythropoietic hyperplasia, abnormalities of the chromatin structure, and chromatin bridges between cells) are highly sensitive. Similar changes may be seen in a few cells in other anaemias with erythropoietic hyperplasia. Their specificity for the diagnosis of CDA I (as well as in the other types) relies on the mosaic of morphological aberrations and the frequency of their occurrence. Rare cases of myelodysplastic syndromes (MDS) show similar changes as seen in CDA, and MDS is the most frequent erroneous diagnosis.

- Final proof of the diagnosis is based on presence of mutations of the codanin (CDAN1)-gene. They may be found in one of 28 exons, and therefore sequencing or the gene is needed to ultimately proof the diagnosis

CDA type II

- ORPHANET code: 98873
- OMIM code: 224100
- ICD-10 code: D64.4

• Anaemia is normocytic or moderately microcytic. Red cells show distinct aniso-poikilocytosis, basophilic stippling and a few nucleated cells. The typical changes of the erythroblasts in the bone marrow (erythropoietic hyperplasia, bi –or polynucleated late erythroblasts, karyorrhexis) are highly sensitive. Most cases show Pseudo-Gaucher macrophages with birefringent needles seen in the polarisation microscope. Similar changes may be seen in a few cells in other chronic anaemias with erythropoietic hyperplasia. Their specificity relies on the mosaic of morphological aberrations and the frequency of their occurrence.

• Red cells undergo lysis when incubated with serum acidified to ph 6.7 from 40% to 60% healthy individuals, but (in contrast to PNH-cells) never with autologous serum. This observation led to the term HEMPAS (Hereditary multinuclarity with a Positive Acidified Serum test) that is still used as a synonym.
Final proof of the diagnosis is now based on

- The analysis of transmembrane proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the most sensitive and specific well standardized biochemical test for the diagnosis of CDA II.
- Mutations of the SEC23 B-gene. They may be found in one of 20 exons, and therefore sequencing or the gene is needed to ultimately proof the diagnosis.

• At present, an analysis of these features is attempted at the expert centres for CDA in the framework of ENERCA to show whether the less time consuming SDS PAGE is of sufficcient specificity and sensitivity to dispense the mutation analysis.

CDA III

• ORPHANET code: 98870

• OMIM code: 105600

• ICD-10 code: D64.4

• First described in 1962 under the name of Hereditary Benign Erythroreticulosis it is observed in members of a large family living in Northern Sweden. At present, the fifth generation of this family is being investigated. Sporadic cases were described, but their final definition awaits identification of the mutating gene(s). Anaemia is less severe than in Type I and II patients and transfusions are not required. In contrast to other types, there is no clinically relevant iron overload. The significant anomaly is giant multinucleated erythroblasts resembling the erythroblasts seen in the transient erythroid

aplasia initiated by parvovirus B19. They are occasionally seen in acquired haematopoietic neoplasias such MDS or malignant myeloma.

CDA –variants

- ORPHANET code: 85,98869,98873,98870
- OMIM code: 224120, 224100, 105600
- ICD-10 code: D64.4

- This heterogeneous group fulfils the general criteria of the CDAs, but cannot be attributed to one of the three above described groups. The bone marrow may show bizarre erythroblasts, and similar changes are often seen in only one or few families. One entity with thrombocytopenia is due to a GATA-I mutation. Molecular genetics of the others are unknown.

Management

- Data beyond single case reports are available for types I and II only. Patients with CDA I respond to treatment by Interferon –alpha, to be continued throughout live. Nearly normal haemoglobin concentration is achieved. Pegulated preparations with very low doses of about 50 µg once a week are sufficient for maintenance. When interferon therapy is stopped, haemoglobin levels will return to previous values. Increase of iron enteric iron uptake is abrogated, but if iron overload is present before interferon treatment, iron depletion may be considered to speed the normalisation of storage iron.

- Patients with CDA II, which often show a more intensive peripheral haemolysis than those with other types, benefit from splenectomy. In contrast to Hereditary Spherocytosis (HS), the red blood count does not become normal, but the rise of haemoglobin is usually sufficient to abrogate the need of transfusions in severe cases and to improve physical ability. Considering the life long risks of asplenism, splenectomy is an elective measure dependent on the severity of the anaemia, symptoms, age and confounding risk factors. Splenectomy does not correct the up regulated iron uptake and development of iron overload. Consultation with an expert centre is strongly recommended.
Interventional and palliative therapy in both types is based on the sequalae shown in the Table 2

Table 2 :

CDA: consequences and complications

• Severe anemia, regular transfusions	~ 10 %
• Skeleton abnormalities by marrow expansion	~10 %
• Skeleton malformations	~10 %
• Splenomegaly:	> 90%
• Gall stones:	>70%
• Aplastic crisis:	< 10%
• Leg ulcers:	< 10%
• Bulky extramedullary erythropoiesis	~ 5%
• Iron overload, if not timely treated:	> 80 %

• Regular transfusions, albeit contributing to iron overload, have to be given in severe cases beginning in early childhood to guarantee normal growth and development and to avoid relevant bone changes by marrow expansion. Experiences of more frequent disorder such as thalassemia intermedia are used for the transfusion program. In adulthood, transfusions can often stop according to alleviation of anaemia symptoms. Occasional transfusions may be needed in aplastic crisis, intercurrent infections or pregnancy. In pregnancy, an Hb-level of less than 8 g/dl should be avoided to ensure the integrity of the foetus.

• CDAs are iron loading anaemias. Up regulation of enteric iron uptake is the consequence of ineffective erythropoiesis mediated by GDF 15. Increase of storage iron may become evident at any age. There is no close correlation to clinical severity as ascertained by haemoglobin levels. Control of iron burden by regular measurement of serum ferritin, endocrinological function and non-invasive checks of liver iron by MRI should follow the procedures used in thalassemia intermedia. The same is true for the indication and procedure of iron depletion including regular phlebotomies if compatible with physical ability and the patient's preferences.
Cure by transplantation of allogenic hematopoetic stem cells was successful in a small number of severe cases.

• In conclusion, management of patients with CDA has to be based on exact diagnosis of the type of CDA, grading of severity and timely recognition of risks by life long follow up. Information's for physicians and for patients on these very rare disorders are available from ENERCA and/or Expert centres. Assurance of physician's expertise and respectful consideration of the patient's perceptions are mandatory for the patient's compliance, the cornerstone to maintain quality of live and improve life expectancy.

Key messages

- Congenital dyserythropoetic anaemia (CDA) consist of a group of hereditary anaemia's resulting from mutations of different genes
- Clinical appearance, methods of diagnosis and specific therapeutic measures are different for the subtypes
- Cure is only possible by allogenic hematopoetic stem cell transplantation, but a normal life expectancy and quality of live can be achieved by specific and palliative therapy
- Assurance of physician's expertise and cooperation with experts centres are mandatory for the patient's compliance, the cornerstone to maintain quality of live and improve life expectancy

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BLACKFAN DIAMOND ANEMIA

- ORPHANET code: 124
- OMIM code: 105650, 606129, 606164, 610629, 612527, 612528, 612561, 612562, 612563, 613308, 613309
- ICD-10 code: D61.0

Definition

- Diamond Blackfan anemia (DBA) is a rare bone marrow failure syndrome characterized by severe normochromic macrocytic anemia and reticulocytopenia, typically presenting in the first year of life. Patients generally show a decreased number of erythroid progenitors in their bone marrow. Neutropenia and thrombocytopenia are rarely present. Erythrocytes in DBA patients frequently express fetal hemoglobin (HbF) and show increased adenosine deaminase (eADA) activity (Vlachos 2008). DBA is associated with an increased risk of malignancies, especially hematopoietic neoplasms and osteogenic sarcomas (Vlachos 2012). In 30 to 47% of cases patients show physical malformations involving head, thumb, heart, and urogenital system. Growth retardation is also frequent.

Pathophysiology

- The first DBA gene, ribosomal protein (RP) S19, was identified in 1999 and is mutated in about 25% of patients. Mutations in an increasing number of other genes encoding RPs of the small (RPS24, RPS17, RPS7, RPS10, RPS26) and large (RPL35A, RPL5, RPL11, RPL26) ribosomal subunits have been described in DBA patients. All mutations are present on a single allele and no genotype/phenotype correlation has been observed regarding the hematological parameters, but certain genes are more frequently associated with specific physical malformations. DBA is considered a ribosomopathy, a term initially proposed for dyskeratosis congenita. In eukaryotes, the ribosome is composed of four different ribosomal RNAs (rRNAs) and 79 ribosomal proteins. Although 5S rRNA is transcribed by RNA polymerase III, 28S, 5.8S, and 18S rRNAs are processed from a 45S precursor transcribed by RNA polymerase I. The maturation of pre-rRNA occurs in the nucleolus through a complex pathway involving both endo- and exonucleases that remove external and internal transcribed sequences (ETS and ITS). During these steps, the 45S pre-RNA associates with ribosomal proteins, ribonucleases, RNA helicases, small nucleolar RNPs (snoRNPs) and other accessory factors, to form 90S pre-ribosomes. During the maturation process, the 90S preribosome is separated into pre-40S and pre-60S subunits that are exported to the cytoplasm where their maturation is completed. Mature 40S subunits include 18S rRNA and 33 ribosomal proteins, whereas mature 60S subunits contain 28S, 5.8S, 5S rRNAs and 46 ribosomal proteins. Molecular mechanisms underlying the causal effect between RP haploinsufficiency and anemia have not been completely elucidated. A generally recognized pathogenetic hypothesis implies defective

ribosome biogenesis leading to apoptosis in erythroid progenitors. This mechanism has been named ‘‘ribosomal stress,’’ and there are indications that it may be signalled through p53. Mutations in DBA genes, along with their functional consequences and phenotype association, have been catalogued in the DBA Mutation Database, created by the ENERCA group from Novara/ IT in 2008 and available via www.dbagenes.unito.it. It includes information on molecular mechanisms involved in RP mutagenesis and more detailed information about inheritance. Its update arises from the collaboration of Czech, French, German, Swedish, American, and Italian DBA clinical and research groups and was recently supported also by ENERCA.

Mode of inheritance

• DBA is inherited with an autosomal dominant transmission with an incomplete penetrance. Most cases are sporadic. Recently, some cases of mosaicism have been reported.

Diagnosis

• Expressivity is widely variable, also among carriers of the same mutation within the same families. The diagnosis may be difficult and is made after the exclusion of other primary and secondary causes of erythroid aplasia. The molecular analysis is important to confirm diagnosis and should include also techniques able to detect large deletions. The diagnostic criteria have been revised in a paper resulting after an international consensus conference, to which we refer for a more detailed discussion in Table 3

Table 3 -

Diagnostic criteria – Age less than 1 year – Macrocytic anaemia with no other significant cytopenias – Reticulocytopenia – Normal marrow cellularity with a paucity of erythroid precursors
Supporting criteria o Major – Gene mutation described in ‘‘classical’’ DBA – Positive family history o Minor – Elevated erythrocyte adenosine deaminase activity – Congenital anomalies described in ‘‘classical’’ DBA – Elevated HbF – No evidence of another inherited bone marrow failure syndrome

• A diagnosis of ‘‘classical’’ DBA is made if all the diagnostic criteria are met. When there is a positive family history, an otherwise normal individual should be considered as having ‘‘non-classical’’ DBA if a mutation shared by affected

family members is present. Anyone suspected of having DBA, but with insufficient diagnostic criteria, should be considered as having sporadic, non-classical DBA if a reported mutation is present. A patient can be assigned as having a ''probable'' diagnosis, if three diagnostic criteria are present along with a positive family history.

- Of note, macrocytosis may be masked by iron deficiency or thalassemia. The erythrocyte adenosine deaminase (eADA) activity, not influenced by prior transfusions, is elevated (‡3 SD) in 80–85% of patients classified as having DBA. In contrast, 90% of patients classified as having Transient Erythroblastopenia of Childhood (TEC) have normal eADA activity. Elevated eADA activity, increased fetal hemoglobin (HbF) and mean corpuscular volume (MCV) are not very strong independent criteria, however, these factors should be seriously considered when evaluating a sibling as a stem cell transplant donor. If the first three diagnostic criteria are present, but there is no paucity of red cell precursors in the bone marrow and no supporting criteria, the diagnosis of DBA cannot be made. A bone marrow evaluation should be repeated at a later date as red cell marrow hypoplasia may develop after anaemia and reticulocytopenia. Furthermore, thrombocytopenia and neutropenia are not uncommon findings.

- An evaluation of the family of a proband is necessary. All immediate family members should be evaluated with a thorough relevant history (anaemia, cancer, birth defects, etc.), complete blood count including red cell indices, eADA activity and HbF. If the proband has a mutation, then the parents and siblings need to have appropriate mutation analysis. The nature of any other positive findings will dictate the extent of the family evaluation.
- Molecular and e-ADA analyses are usually performed only in a few Expert Centres (or in EC networks).

Management

- First-line therapy in DBA patients is steroid treatment. Although 80% of patients have an initial steroid response, less than half the patients can be maintained on a safe and effective dose. Thus, many of these initial responders may experience temporary or definitive steroid resistance of dose-limiting toxicity. Patients who do not respond to steroids undergo chronic blood transfusions and need iron chelation to avoid secondary hemochromatosis. Preliminary data suggest that patients with DBA are more likely to develop iron overload than patients with thalassemia, another disease treated with chronic transfusions. Twenty percent of patients inexplicably achieve remission. DBA can be treated successfully by allogeneic stem cell transplantation.
- Clinical patient's follow-up is usually performed in local Centres. However, because of the rarity and the wide heterogeneity of these disorders, a strict collaboration between EC and local centres is needed to offer the best chance of providing an appropriate diagnosis. When it is required, genetic counselling is performed in EC.
- Creation of European EC networks, such as ENERCA, are also required to provide information for physicians and for patients on this rare disorder.

Key messages

- DBA is a rare inherited pure erythroid aplasia with a wide clinical and molecular heterogeneity.
- There is not a clear genotype/phenotype correlation
- Diagnosis requires specific diagnostic tools available in EC.
- Regular follow-up for monitoring iron overload is mandatory
- Creation of European EC networks are required to increase knowledge of these rare disorders.
- Prevention of several adverse events is possible if a neonatal screening programme is implemented

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FANCONI ANAEMIA

- ORPHANET code: 84
- OMIM codes: 227650; 227646; 227645; 300514; 605724; 608111; 609054; 610832; 600901;614083; 613390; 614082; 614087; 613951; 603467; 609053; 602956
- ICD-10 code: D61.0

Definition

Fanconi anemia (FA) is a rare inherited syndrome characterized by bone marrow failure (BMF), congenital abnormalities (upper limb abnormalities, café au lait skin spots, short stature, microcephaly, etc..) and cancer predisposition, principally acute myeloid leukaemia (AML), myelodysplastic syndrome, and squamous cell carcinomas (SCC) of the head and neck and anno-genital region. Malignant transformation typically arises during the second or third decade of life. The age of onset of the haematological disease is usually during the first decade and, remarkably, 80% of FA patients will develop BMF before the age of 15, being the actual risk of the BMF above 90% by age 40. To date, 15 complementation groups have been reported (FA-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O and P) associated with mutations in the corresponding FANC genes. Among all the FA complementation groups, FA-A is the most frequent (average 65%). FA-G and FA-C account for approximately 10-15% of FA patients each while the other genetic subtypes are very rare. In Mediterranean countries, FA-A is even more prevalent and FA-C is rare.

Pathophysiology

- FA proteins participate in the FA/BRCA genome stability pathway which is responsible of sensing and processing stalled DNA replication forks.
- In response to stalled replication forks, 8 FA proteins, including FANCA and the ubiquitin ligase FANCL, assemble in a FA core complex that is required for the activation by monoubiquitination of a second complex formed by FANCD2 and FANCI (the ID complex). The active ID complex then binds to histone γH2AX at damaged chromatin, and coordinates further homologous recombinational repair by downstream FA proteins including FANCJ/BRIP1, FANCN/PALB2, FANCD1/BRCA2, and FANCO/Rad51C, four breast cancer susceptibility genes in monoallelic mutation carriers.
- In the absence of a functional FA pathway, breaks at stalled replication forks accumulate or misrepair leading to chromatid-type chromosome fragility and exchanges (radial figures) which subsequently lead to cancer or cell death of hematopoietic progenitors (anemia) or during development (malphormations).
- Disruption of the FA pathway also leads to hypersensitivity to cross-linking agents and oxygen, cell cycle arrest, overproduction of TNFα and other pro-inflammatory cytokines, increased apoptosis, telomere shortening, defective p53 induction and intrinsic stem cell defects
- The metabolism of aldehydes and alcohol has recently been implicated in the pathophysiology of FA

Mode of inheritance

- There are at least 15 independent FA complementation groups (FANC-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O and P) each connected with a distinct disease gene. With the exception of FANCB (which is X-linked and mutated in less than 1% of patients), all FA genes are autosomal recessive. The great majority of patients (>90% is most countries) have mutations in FANCA, FANCC and FANCG. Approximately, 2/3 (EEUU) to 3/4 (South European countries such as Spain) of patients belong to the FANCA subtype.

Diagnosis

- The diagnosis can be established at birth or even prenatally due to FA-related malformations or more usually during infancy at the age of onset of the haematological disease, typically during the first decade of life.
- FA cells are hypersenstitive to DNA-crosslinking cytostatic drugs such as

mitomycin C (MMC) or diepoxybuthane (DEB), and the final diagnostic confirmation of FA fully relies on an excess of chromosome fragility after in vitro treating the patient's cells with one of these agents.

- Chromosome fragility tests are usually performed by conventional cytogenetics in peripheral blood T-cells but the analysis of skin fibroblasts is sometimes necessary especially in mosaic patients with a subpopulation of healthy cells in blood due to in vivo reverting mutations. 15 to 20% of FA patients are mosaic and in some of them the hemathology improves due to clonal expansion of the genetically reverted cells.
- Complementary diagnostic assays are the analysis of an excess of cell death or cell cycle arrest at the G2 phase of the cell cycle upon treating the cells with MMC. In the great majority of patients (including FANCA, FANCC and FANCG) the analysis of the lack of FANCD2 monoubiquitination by western blot can also be used to re-confirm the diagnosis.
- Since there are at least 15 independent FA complementation groups (FANC-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O and P), the genetic subtyping to find the disease gene in every FA case is usually done by retroviral genetic complementation, western blot or direct mutational analysis.
- Mutational analysis is performed with conventional DNA sequencing techniques and must include the detection of large intragenic deletions involving one or several exons by multiple ligation probe amplifications or related techniques, especially frequent (approximately 20%) in FANCA patients.
- A chromosome fragility test can also be done prenatally in ammiocytes when required although a molecular diagnosis test is the preferred option when the patient's mutations are available. It is also possible to perform preimplantational genetic diagnostic with selection of an HLA-compatible embryo to be used as donor in future hematopoietic stem cell transplantation (HSCT) of the sibling.

Management

- Many patients will require surgery of skeletal (limb) and other malformations since birth.
- Regarding BMF, 75% of patients may initially respond to androgens, classically oxymetholone. However, side effects include masculinisation, final short stature, hepatic peliosis, liver adenomas and hepatocellular carcinomas. Recent reports suggest that danazol is a well tolerated therapeutic option to prevent/delay BMF in FA patients.
- Blood transfusions are eventually required to overcome BMF.
- HSCT is the only life-saving procedure for FA patients with available donor using clinical protocols specifically designed for FA patients. The source of hematopoietic progenitors can be umbilical cord blood, bone marrow or mobilized blood.
- Pre-HSCT conditioning with chemotherapy or chemo-radiotherapy is required, predisposing the recipient to bacterial, fungal, and viral infections, which constitute a significant source of morbidity and mortality after transplant.
- SCC is the most significant long-term complication after HSCT of FA patients, being the acute and chronic graft-versus-host disease (GVHD) a significant SCC risk factor.
- Most of these complications could, in principle, be avoided after transplantation with autologous gene corrected hematopoietic stem cells. Gene therapy clinical trials are currently underway.
- Management of FA patients must also include a long term cancer risk follow up for the prevention and early detection of leukaemia and solid tumours.

Key messages

- FA is a genetically (15 genes involved) and clinically heterogeneous chromosome instability syndrome characterized by bone marrow failure, malformations and cancer predisposition
- The final diagnostic confirmation of FA fully relies on an excess of chromosome fragility after in vitro treating the patient's blood cells with DNA interstrand cross-linking agents such as MMC or DEB
- Prevention of new affected births is possible by prenatal testing by chromosome fragility or mutational analysis in amniocytes or CVS
- While androgens can prevent/delay BMF, the only curative treatment for FA patients is HSCT using protocols specifically designed for FA patients.

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HEREDITARY OR CONGENITAL SIDEROBLASTIC ANAEMIA

ORPHA code: 480, 699, 1047, 2598, 2802, 3463, 75563, 98362

MIM code: 222300, 301300, 301310, 530000, 557000, 598500, 600462, 604928, 610819, 613561

ICD code: D64.0, D64.3, E10.7, H48.0, H49.8, G71.3

Definition

- Hereditary (or congenital) sideroblastic anaemia (HSA or CSA) is a group of disorders sharing a single feature: the presence of ring sideroblasts on microscopic examination of bone marrow smears stained for iron. These are formed by the perinuclear arrangement of erythroblast mitochondria containing

pathological deposits of iron. All patterns of inheritance are observed, and both sexes can be affected. Severity varies from mild to severe, some are syndromic and may be multisystem, and age of onset varies from birth to the 9th decade.

Pathophysiology

- The pathophysiology varies between and within subtypes. In defined, non-syndromic types, nuclear genes are involved encoding mitochondrial proteins required for haem synthesis (*SLC25A38, ALAS2*) or Fe-S cluster biogenesis (*GLRX5*). SLC25A38, a putative mitochondrial glycine importer, is essential for erythroid haem synthesis prior to production of ALA. ALAS2 is the erythroid-specific rate-limiting haem synthesis enzyme delta-amino-levulinate-synthase, and GLRX5 is required to maintain cytoplasm Fe-S and decrease IRP1-inhibition of ALAS2 synthesis in erythroid cells. The cause of the anaemia in these non-syndromic types is thought to be one of decreased erythroblast haem. Iron continues to enter the mitochondrion and precipitates affecting mainly intermediate and late erythroblasts. Compensatory expanded but ineffective erythropoiesis causes increased iron absorption and a risk of iron overload. The clinical presentation can be symptoms of anaemia or of iron overload.
- In defined, syndromic types the other tissue(s) involved may determine the presenting features and the anaemia noticed secondarily during investigation. On the other hand severe anaemia may be the presenting feature with non-erythroid tissue involvement emerging later. Non-erythroid haemopoietic lineages may be affected and pancytopenia, neutropenia or thrombocytopenia can also occur. The genes involved may be nuclear or mitochondrial. Nuclear genes include *SLC19A2, ABCB7, PUS1* & *YARS2*, encoding respectively the plasma membrane high-affinity thiamine transporter THTR1, the inner mitochondrial membrane transporter ABCB7 required for cytoplasm and nuclear Fe-S cluster protein assembly and two enzymes (pseudo-uridine synthase1 and tyrosyl-tRNA synthetase) involved in mitochondrial protein translation. Additional tissues affected are inner ear & endocrine pancreas (SLC19A2), cerebellum (ABCB7), muscle (PUS1 & YARS2).
- Heteroplasmic inheritance of large deletions of mtDNA including, overlapping or adjacent to the common 4977bp one, is also implicated in the Pearson's-marrow-pancreas syndrome (PMPS). The proportion of deleted mtDNA determines the degree to which a susceptible tissue is affected and varies between tissues and amongst people. In PMPS a high proportion in erythroid cells leads to a very severe anaemia. If the proportion decreases in the bone marrow the anaemia may remit and some patients survive to develop the Kearns-Sayre encephalomyopathy. Most cases of syndromic HSA present in the 1st and 2nd decade of life.

Mode of inheritance

- All patterns of inheritance occur: X-linked (ALAS2, ABCB7), autosomal recessive, autosomal dominant and mitochondrial. In autosomal recessive types, parents and heterozygous siblings are unaffected; their carrier status can only be determined by DNA analysis. On the other hand, non-anaemic female carriers of the X-linked types usually have two populations of red blood cells in their peripheral blood (one microcytic & hypochromic and one normocytic & normochromic) and are clinically unaffected. If there is skewed X chromosome inactivation in favour of the sideroblastic anaemia allele, female carriers of XLSA may be clinically affected by anaemia and by iron overload to an extent depending on the degree of skewed X chromosome inactivation as well as the severity of the genetic defect. A family history is more likely to occur for X-linked and autosomal dominant types of sideroblastic anaemia than for the autosomal recessive types. Despite maternal inheritance of mtDNA deletions

the resulting syndromic type of SA appears to occur sporadically.

- Cases not yet diagnosed (40%) are both syndromic and non-syndromic and exhibit most types of inheritance (autosomal dominant, presumed autosomal recessive and probable X-linked).

Features suggestive of hereditary sideroblastic anaemia

- With such a diverse group of disorders, HSA should be considered a cause in any hypo-regenerative anaemia unexplained by common causes. This should be considered in particular if (1) any degree of red cell hypochromia is observed on blood film examination, or (2) there is evidence of increased ineffective erythropoiesis such as increased bilirubin, increased serum transferrin receptor, unexplained iron-overload or unexplained non-specific dyserythropoiesis observed on bone marrow smear examination, or (3) Pappenheimer bodies, excess elliptocytes, a dimorphic appearance of the red blood cells and basophilic stippling are seen on Romanowsky-stained blood films.
- A chronic or acute hypo-regenerative microcytic, hypochromic anaemia with a broad distribution of cell size (raised RDW) in the absence of iron deficiency and unexplained by thalassaemia is suggestive either of one of the non-syndromic types caused by defects in *ALAS2, SLC25A38* or *GLRX5* or of the syndromic XLSA with ataxia caused by *ABCB7* defect.
- Vacuolated developing erythroid and/or myeloid bone marrow cells accompanying a severe macrocytic anaemia in a child could indicate the Pearson's marrow-pancreas syndrome.
- Isolated chronic, hypo-regenerative macrocytic anaemia in a woman with no obvious cause could indicate heterozygosity for a severe variant of X-linked sideroblastic anaemia (*ALAS2 or ABCB7*) with skewed X-chromosome inactivation.
- Refractory normocytic or macrocytic anaemia associated with symptoms suggestive of a mitochondrial disorder such as exocrine pancreas deficiency, malabsorption, hypotonia, poor exercise tolerance, lactic acidosis, respiratory acidosis, renal tubulopathy could indicate Pearson's marrow-pancreas syndrome (mtDNA deletion) or the MLASA syndrome (*PUS1, YARS2*). Macrocytic anaemia is also a feature of the thiamine responsive megaloblastic anaemia caused by *SLC19A2* defect additionally associated with deafness and diabetes.

Diagnosis

- Staining blood or bone marrow smears for iron (Perls' stain) is required to detect ring sideroblasts. Electron microscopy may be required to confirm the location of the deposited iron. Secondary acquired SA caused by toxins, drugs, hypothermia or certain nutritional deficiencies, the clonal primary acquired SA (RARS) that is usually associated with SF3B1 gene variations, and SA secondary to certain types of beta thalassaemia are more common and should be excluded first.

- Different genes are involved. Most causative variations are private requiring full sequence analysis of the relevant parts of the gene. Which gene to test requires taking into consideration red cell size, clinical presentation, age, sex and the response of the anaemia to pyridoxine (vit B6) (if microcytic) or thiamine (Vit B1) (if macrocytic). Full physical examination is essential and also where indicated full neurological, endocrine, cardiac or immunological assessments.

Certain management aspects

• All HSA patients require a multidisciplinary approach and regular review at an expert centre. For the non-syndromic HSA types, treatment and management of anaemia and iron overload are required with regular monitoring for complications of either to provide early intervention and treatment. Patients with severe refractory anaemia (e.g. *SLC25A38*) may require regular blood transfusions for survival and warrant consideration for bone marrow transplantation. Most patients with XLSA caused by missense *ALAS2* defects respond to pyridoxine and require supplements for life.

• Syndromic HSA types require the additional input of specialists for non-haematological aspects (neurological, auditory, cardiac, renal, hepatic, ophthalmological, metabolic, endocrine, immunological etc.). Some require referral to a centre specialising in mitochondrial disorders for assessment and regular review. Different symptoms arise at different times in different patients and health monitoring must take that into account. Treatment is usually supportive, standard for the complications that occur, and may include anti-oxidant vitamins and other supplements to try to preserve mitochondrial function and protect against free radical damage. TRMA (*SLC19A2*) patients respond haematologically to thiamine supplements. General body iron overload should be avoided and treated where possible. Tissue iron distribution may be unusual with iron overload evaluation requiring quantitative MRI. Emergency treatment for metabolic crises, overwhelming infection and other crises may be required. Implementation of interventions that address special needs as they arise such as speech therapy, physical therapy, mechanical aids of various types, extra tuition, development of computer skills bring large benefits for all involved.

Key messages

- Hereditary sideroblastic anaemia is a heterogeneous group of rare, non syndromic and syndromic conditions that occur within all populations for whom access to appropriate treatment often requires specific diagnosis.
- The prevalence in Europe is likely to be about 1 in 400,000 but figures are still being collected for ENERCA3.
- Facilities for quality diagnosis and clinical care are available in Europe and networking these centres should improve information sharing, rates of diagnosis and development of standards essential for equity in service provision.

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HEREDITARY NON-SIDEROBLASTIC ANAEMIA WITH HYPOCHROMIA

Definition

- Hereditary non-sideroblastic anaemia is a group of disorders sharing the following features: 1) the presence of a mild to severe hypochromic microcytic 2) the absence of >5% of ring sideroblasts on microscopic examination of bone marrow smears stained for iron. (For definition of ring sideroblasts please see section HEREDITARY OR CONGENITAL SIDEROBLASTIC ANAEMIA). Inside this group one can find the following diseases: Aceruloplasminemia, Atransferrinemia, DMT1-deficiency Anaemia and Iron-refractory Iron-deficiency Anaemia (IRIDA). Each of this disease presents specific particularities, but all of them are inheritance as autosomal recessive genetic diseases.

Aceruloplasminemia
- ORPHANET Code: 48818
- OMIM Code: 604290

Atransferrinemia
- ORPHANET Code: 1195
- OMIM Code: 209300

DMT1-deficiency Anaemia
- ORPHA83642
- OMIM Code: 206100

IRIDA
- ORPHANET Code:
- OMIM Code: 206200

Pathophysiology

- The pathophysiology varies between subtypes.
- Aceruloplasminemia is a rare genetic disease linked to mutations of the ceruloplasmin (*CP*) gene, encoding ceruloplasmin, the principal copper transport protein in plasma also involved in iron release from macrophages and other cells. Mutations in *CP* lead to a total or reduced amount of the protein or to a reduced activity. Deficiency of *CP* causes moderate anaemia with iron accumulation in liver, pancreas and basal ganglia. Laboratory and clinical expression of aceruloplasminemia includes low or absence serum ceruloplasmin, low serum copper levels, mild-moderate microcytic anaemia with low serum iron and high serum ferritin, diabetes mellitus, and late-onset neurological symptoms, including retinal degeneration, ataxia, involuntary movements and dementia.
- Atransferrinemia is extremely rare genetic disease caused by mutation in the transferrin *(TF)* gene that leads to a strong reduction of the transferrin protein,

the protein that transports iron in the blood circulation. Affected subjects show severe microcytic-hypochromic anaemia since birth with the development of iron overload of liver and other organs. External signs are pallor and fatigue. Atransferrinemia appears early in life being its age of onset in neonatal or infancy period. Laboratory values of transferrin are half-normal in carriers and very low in affected patients.

- DMT1-deficiency anaemia, also known as familial microcytic hypochromic anaemia with hepatic iron overload due to defects in DMT1, is a very rare genetic disease caused by mutations in the *SLC11A2* gene, encoding the DMT1 iron transporter. DMT1 is a key mediator of iron absorption and iron transfer from endosomes into the cytosol of developing erythroid cells. DMT1 deficiency leads to severe microcytic hypochromic anaemia present from birth and the development of iron overload in the liver. Only a few families are known with this disorder.
- Iron-Refractory Iron-Deficiency Anaemia (IRIDA) is a rare genetic disease, linked to mutations of the *TMPRSS6* gene, encoding the serine protease matriptase-2. The mutations lead to a reduced activity of matriptase-2 in hepatocytes and thus to an increased amount of the hormone hepcidin which inhibits intestinal iron absorption. *TMPRSS6* deficiency leads to microcytic hypochromic anaemia of moderate degree from the 3rd or 4th month of life due to defective iron absorption because of inappropriately high production of the iron hormone hepcidin. The disorder is sometimes diagnosed later in life, in adolescents or young adults.

Mode of inheritance

All these 4 types of hereditary non-syderoblastic Anaemias are inherited as autosomal recessive genetic diseases. Therefore, affected patients inherited two genetic defects in the particular gene (i.e. *CP, TF, SLC11A2 or TMPRSS6*). Parents and heterozygous siblings are unaffected and carried only one genetic defect; their carrier status can only be determined by DNA analysis.

For each of these subtypes, when two carriers (heterozygote) individuals mate, there is a 25% risk of having a homozygous or compound heterozygous offspring in each pregnancy. There is also a 50% risk of having a carrier child and another 25% of having a homozygous normal child.

Features suggestive of hereditary non-sideroblastic anaemia

- Hereditary non-sideroblastic anaemia should be considered a cause in any hypo or mildly -regenerative microcytic, hypochromic anaemia unexplained by common causes. Especially, common genetic causes such as thalassaemia, and Wilson disease in the case of low values for ceruloplasmin, should be ruled out. Common non-genetic causes of microcytic and hypochromic anaemia such as gastric bleeding, coeliac disease or other autoimmune gastritis, blood loss, infection by helicobacter pylori and anaemia of chronic disease should be also excluded.

Diagnosis

• Different genes are involved and particular diagnosis should be considered on the basis of biochemical findings (serum iron, serum ferritin, transferrin saturation, ceruloplasmin and serum transferrin and hepcidin levels), clinical presentation and age of onset. Once excluded common causes, very low values of transferrin or ceruloplasmin together with low serum iron and high serum ferritin suggest atransferrinemia or aceruloplasminemia, respectively. Aceruloplasminemia patients present late disease onset with iron-overload not only in the liver but also in the brain, which causes neurological symptoms. IRIDA patients present unexpectedly normal/high hepcidin levels for their low serum iron values. DMT1 patients are severely anaemic since birth and present high levels of serum iron and transferrin saturation; all but one reported patient present liver iron-overload. Most causative genetic mutations are private, requiring full sequence analysis of the relevant gene; what requires contacting a genetic diagnostic expert centre (see GeneTest or ENERCA for accredited laboratories).

Certain management aspects

• All patients require regular review at an expert centre. Treatment and management of anaemia and of iron overload or iron deficiency depending on the defect) are required with regular monitoring for complications of either to provide early intervention and treatment.

• For aceruloplasminemia patients there is no established treatment for neurological symptoms. Liver iron overload can be reduced by phlebotomy therapy, although the volume of blood removed at each session and the timing of repeated phlebotomies must be carefully controlled. In patients with very low haemoglobin levels or those intolerant to phlebotomies iron chelation therapy (such as deferoxamine, deferiprone or deferasirox chelation) should be done.

• Patients with atransferrinemia are treated with periodic infusions of normal plasma (which contains transferrin) or purified apotransferrin. Treatments with chelating agents such as deferoxamine or deferasirox, phlebotomy of both are required to diminish tissue iron overload.

• Severe anaemia in patients with DMT1 defects may require erythrocyte transfusions, although not as continued as for beta-thalassemia patients. Erythrocyte transfusions or continuous oral iron supplementation contribute to iron overload and should be carefully monitored. Iron chelation treatment to reduce liver iron accumulation has been proven to be inefficient in one patient. Since the anemia is poorly responsive to iron treatment and the patient may develop severe liver iron overload, these patients should be very carefully treated with iron supplementation or erythrocyte transfusions. In contract, Erythropoietin (EPO) treatment can allow transfusion independency and improves the anaemia.

• Patients with IRIDA are unresponsive to oral iron and partially respond to parenteral (I.V.) iron that should be administered especially during growth.

Key messages

- Hereditary non-sideroblastic anaemia is a heterogeneous group of rare and non syndromic conditions that occur within all populations for whom access to appropriate treatment often requires specific diagnosis.
- The prevalence of aceruloplasminemia, atransferrinemia and DMT1-deficient anaemia is very low, estimated to be <1-9/ 100,000. IRIDA is the most common cause of genetic non-sideroblastic microcytic hypochromic anaemia. Precise figures are still being collected for ENERCA3.
- Facilities for quality diagnosis and clinical care are available in Europe and networking these centres should improve information sharing, rates of diagnosis and development of standards essential for equity in service provision.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

1.3. Rare anaemias. Possibility of cure

Most therapeutic approaches for the management of rare anaemias are able to provide patients with symptomatic relief, the possibility to prolong life and improve quality of life. There are two main approaches which provide the possibility of cure, in the sense that the beneficiary may not require any further treatment and go on with life in good health. These approaches are haemopoietic stem cell transplantation and gene therapy.

Haemopoietic stem cell transplantation (HSCT)

- HSCT has the possibility to 'cure' several of the rare anaemias including, thalassaemia, sickle cell disease, severe aplastic anaemia, Fanconi anaemia, Diamond –Blackfan anemia and Paroxysmal Nocturnal Haemoglobinuria. In each case there are indications and contra-indications and adverse effects that should be considered and so it cannot be regarded as a solution for all patients in any given diagnostic category.
- This procedure requires that the patient has a family HLA matched donor. For chronic anaemias unrelated matched donors increase the risk of serious reactions. This means that few patients have suitable donors. However, since the first curative HSCT to a thalassemia patient from an human leukocyte antigen (HLA) identical sibling donor in1982 more then 3000 successful transplantations have been reported. The patients' age also plays an important role in the outcome of the procedure since younger patients have fewer complications and can withstand the rigours of the myelosupression and the side effects of immunosuppressive medication. In patients younger than 30

years cure rates are 70-90% if there is a sibling donor, whereas transplant related mortality reaches 50% in patients over 40 years old. The overall event-free survival has been recently reported as high as 89%-97% for thalassemia patients with no advanced disease and of 80%-87% for patients with advanced disease. In the French group of SCD patients, cure rates of 95% have been reported since 2000, in patients without cerebrovasculopathy and using cord blood transplant.

- The potential benefits of cord blood transplant instead of, or in association with HSCT, are the low risk of viral contamination from a graft, the decreased incidence of graft rejection, and easier accessibility.
- Decisions concerning the suitability of each case for transplantation, is the responsibility of the centre of expertise in collaboration with the transplant centre. The follow up is also a combined responsibility of the multidisciplinary team and the transplant centre.

Gene therapy

- This is an attractive treatment for the rare anaemias since it eliminates the need for a matched donor and can provide a radical cure. The practical application of gene therapy is still experimental but has reached Phase I/II clinical trials in thalassaemia. The beta globin gene of the haemoglobin molecule has been one the first genes studied at and is one of the most likely to benefit from gene therapy. It is for this reason that thalassaemia and sickle cell disease are indeed ahead in the clinical field although gene therapy in the laboratory is being investigated for other rare anaemias such Diamond-Blackfan and Fanconi Anaemias.
- The clinical trial in thalassaemia has been initiated in France and one patient with severe HbE/ β^0 –thalassaemia is now transfusion independent for over 2 years. The technique involves the introduction a functional beta globin gene to the patient's haemopoietic stem; several parts of the process are quite close to a stem cell transplantation procedure. There are many possible hazards inherent in this undertaking and the need for careful pre-clinical and clinical studies is imperative before introduction to clinical practice. The one successful case has increased hope and expectations in both clinicians and patients.

1.4. Rare Anaemias. Epidemiology

Introduction

- Clinical epidemiology, originally confined to the global problems of infectious diseases, became, in the last 50 years, the fundament of today's evidence based medicine. No medical stakeholder is able to oversee the abundance of data describing prevalence, incidence, clinical patterns and health risks from all regions in the world. The majority of studies are concerned with common diseases, but there is still a paucity of data for rare diseases, which came in the scope of medical stakeholders in the last decade. ENERCA is concerned with the special problems of rare anaemias, and aims to obtain valid epidemiological evidence of their prevalence, life expectancy and effects of therapy.
- In rare anaemias, as in other rare and very rare disorders, supranational networks only can provide valid data. ENERCA has concentrated on data from the member states (MS) of the European Union (EU) (nevertheless including patients from Switzerland and other Non-EU European countries) and also on a subset on Rare Anemias. However, the methodological experience gained by ENERCA can (and has been) be used for other rare blood diseases. In the context of this section of the White Book, methodological experience denotes primarily the advances and procedures of networking, but also the problems to bundle

results of different general and medical cultures in the different countries. For example, it is often overlooked that social-economic conditions and medical practices are different in the different MS, resulting in different recognition of the epidemiological data studied.

Methods and definitions

- Frequency is a general term to compare differences between the occurrence of a given disease, in the case of rare anaemias between populations defined by geographic regions or populations of different origin (formerly called races) in a given region. The latter became of paramount importance with the ongoing immigration of people the mediterranian basin to North- and Middle Europe and from Asia and Africa to all MS, as mentioned above. Frequency is useful in any first attempt and useful to decide on the methodology to estimate more exact parameters. No one would challenge the fact, that thalassemia is much more frequent in the Mediterranean or in immigrants to the North European countries. However, mathematically defined parameters are needed for research and health care planning. **Prevalence** or more exact prevalence

proportion is the main indicator used for any epidemiological studies in congenital diseases, representing the vast majority of conditions considered in disease category. PNH is an exception, with incidence being of major significance alike for the situation of cancers. Prevalence is the probability that an individual is recognized as a case at time, with the total population considered as denominator. However, for very rare anaemias included in ENERCA, usually **period prevalence** is measured, using a defined number of observation years rather than an index day as used for the so called **point prevalence** in common diseases. Another useful measure is the number of affected children of all live births in a given observation period; in this case, the period of definition of live birth (e. g. days after birth) should be indicated, to avoid bias due to early mortality. An effective instrument to measure true prevalence proportions isare obtained with pre- and post-natal screening programs, as described for SCD.

- Ideally, all cases of a given disease or disease category in a population at time should represent the “true” prevalence. However, in the case of rare anaemias, the detection rate (number of detected / number of existing cases) is often less than 1. As shown by the work of ENERCA, there are strong indicators that the detection rates in the MS vary considerably dependent on socio-economic conditions, and the same is true for the proportion of misclassifications. These data are of importance for ENERCAs attempts to equalize the diagnosis of rare anemias in the European countries.

Sources of data

- Registries are among the main sources for epidemiological data in rare anemias. They are a structured collation of cases of one of the disease categories considered by ENERCA III, in the rule in a digital data bank. Harmonisation of the basic structures and basic data fields were attempted in some disease categories. Originally, a joint ENERCA epidemiological registry including all disease categories was discussed. Differences of national regulations for data protection as well as limitations of time and resources prevented to realize this target. However the work done by several ENERCA working parties, and intensive discussions in the Executive Committee meetings and symposia yielded very useful experiences on the suited strategies, methodological problems, pitfalls and risk of biases in supranational projects of epidemiology of rare disease, a shown paradigmatically for the rare anemias.
- An inherent, unsolved problem of all registries is sustainability. Time trends, so important considering the influence of scientific as well as population

changes due to both demographic evolution and migration can only be ascertained if sustainability can be guaranteed by recourses of much longer duration of sponsorship than available to day.

• **Clinical trials**, even though primarily directed to progress of therapeutic measures and often dependent on industrial interests are another source of epidemiological data. Only few such trials are performed in the group of very rare anemias and are not supported by ENERCA. However, data from such trials are available for some of the disease categories considered by ENERCA.

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Epidemiology of Rare Anaemia categories

• According to their frequency, the rare anaemias can be classified into two main groups

1. Hemoglobinopathies (Sickle cell disease and Thalassemia syndromes). It was a priory known that these disorders are endemic in the MS surrounding the Mediterranean, due to the holoendemic malaria still present up to the 20th century. Prevalence in the MS of middle and Northern Europe MS, are largely the result of history of migration and the influence of the Turkish dominance of the Balkan and today's Hungary and Austria. Considering the new migration starting after Word War 2, and the ongoing immigration from South to North and from East to West, data from the middle and North European MS are of particular interest for health care planning in the EU.
2. Very rare anaemias (VRAs) such as enzyme disorders of the red blood cell, red blood cell membrane disorders, Congenital Dyserythropoetic Anemias (CDA), Diamond–Blackfan anaemia (DBA) and Fanconi Anaemia, Primary iron metabolism disorders, including Hereditary Sideroblastic Anaemia (HSA) and other VRAs , Congenital defects of vitamins B12 and folic acid and Paroxysmal Nocturnal Haemoglobinuria (PNH). Here, only few data on regional distribution were available, ENERCA has added data supporting the hypothesis that in contrast to the diseases mentioned above, prevalence is not dependent on environmental factors but rather on rate of new mutations, consanguinity and detection rates at least in disorders of autosomal recessive heredity.

SICKLE CELL DISEASE

Table Neonatal/newborn screening for sickle cell disease financed by national authorities within the European Union

	Implementation	Type	SCD prevalence	Reference
England	**1985**	**Universal**	**±1:2000**	**Streetlly, 2010**
France	**1992**	**Targeted**	**±1:700**	**Bardakdjian, 2009**
Brussels (Belgium)	**1994**	**Universal**	**±1:1600**	**Gulbis, 2009**
The Netherlands	**2007**	**Universal**	**±1:4200**	**Bouva, 2010**
Madrid	**2003**	**Universal**	**±1:6250**	**Cela de Julian, 2007**

- Sickle cell disease in Europe is predominantly a disorder seen in immigrant communities. The gene in its carrier state form arose originally as it offered some degree of protection against malaria hence it is most commonly seen in people originating from malarial areas, most usually people of African origin. The gene however is seen in many other communities, it is present in many groups of Middle Eastern origin; some Indian groups also have a high prevalence. Intermarriage is spreading the gene into communities with historically low prevalence. The communities involved are largely immigrants

within Europe although there is low background prevalence in the Caucasian community and some indigenous Southern European people also carry the gene. Many of those affected by Sickle cell disease are relatively recent arrivals in Europe; they have tended to concentrate initially in large urban centres with already established communities whilst looking to establish them. This results in a very uneven distribution of Sickle cell disease throughout Europe, the disorder being a major health issue in some large urban centres whilst remaining a rarity in some rural areas. The affected individuals may not have the language of their country of residence; they may be asylum seekers or be towards the lower end of socio economic spectrum, all these issues are a challenge in proving suitable services to manage adults and children with these conditions.

- Prevalences come from five neonatal screening programmes financed by the local or national authorities in public Health and implemented in five countries of the EU: England, France, Belgium (Brussels, Liège), Spain (Madrid, Extremadura, Comunidad Valenciana and Pais Vasco), and The Netherlands.(2–6)
- When a systematic neonatal or newborn screening programme was implemented (All countries except France), the prevalence of sickle cell disease ranged from 1:2000 to 1:6250 live births.

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Thalassemia syndromes in Europe

Data on both these conditions is limited since proper surveys are few and mostly depend on neonatal screening. Many countries have no data (Table 5) .

Table 5. Alpha thalassaemia and sickle cell disease in Europe

Country	α^0	α^+	α overall	S carriers	SCD patient s		References
Albania				1.4%	174		Boletini E et al Hum Genet 1994 Manika Face-Kreka report (unpublished)
Belgium				0.065%	358		Lè PQ et al Med TRP.(Mars) 2010 Gulbis B et al Hemoglobin 2008 Gulbis B et al J Clin Path 2009
Bulgaria	0.5 %	1.6%		0	0		Petkov GH, Efremov GD. Hemoglobin 2007
Cyprus	1%		10%	0.2%	40		Kyrri AR ey al Hemoglobin 2009 Kyriakou K et al Hemoglobin 2000
Denmark			0.16%	0.11%			Derived from reports by Birgens et al
France				0.62	3000 May be 7000		Thuret I et al J Clin Path 2010 Bardakdjian-Michau et al. J Clin Path 2009 Berthet S et al Arch Pediatr 2010
Germany			0.01%		1500		Dickerhoff R et al J Clin Path 2009
Greece			8.4%	0.53%	1080		Loukopoulos D Semin Hematol 1996 Kanavakis E et al Am J Hematol 1986 Voskaridou E. Ann Hematol 2012
Italy			4.1%	2%	829		Fichera M et al Hum Genet 1997 Velati C et al Br J Haem 1986
Netherlands			3.6%	0.03%	616		Giordano PC et al. Hemoglobin 2004 Peters M et al Arch Dis Child 2010 Giordano P Prenat Diagn 2006
Norway					15		Graesdal JS et al 2001

Turkey				0.44%	1050		Guler E et al Ped Hematol Oncol 2010
Portugal			5-10%		500		Peres MJ et al Hemoglobin 1995 Peres MJ et al Acta Med Port 1996
Spain				0.016% (0.001-0.03)	350		Manu-Pereira M, Corrons JL. J Clin Path 2009 Cela de Julian E etal An Pediatr (Barc) 2007
England			2.5%	0.054%	12000		Streetly A et al J Clin Path 2010 Sorour Y et al J Med Screen 2007

• The thalassemia genes are indigenous in many parts of the world, from the countries of the Mediterranean basin, the Middle East, Asia including the Indian subcontinent, southern China and South East Asia. Migrations have carried the mutated genes to non-endemic areas so the presence of these disorders is now almost universal. From the known carrier frequencies it is calculated that around 60,000 new affected births of major and intermedia thalassemia occur every year, although in some high prevalence areas prevention programmes limit these births. Most births (around 90%) are in Asia where poor healthcare development results in early death of many affected individuals. Global epidemiology for beta-thalassemia is shown in Table 6

Table 6. Global Epidemiology of Beta – thalassemia

WHO Region	**Carrier Range**	**Annual Affected Births**
Europe	0.1%-15%	1636
East Mediterranean Region	1.5%- 6%	8128
South Asia	2.2% - 16% (up to 30% HbE)	41366
Asia Pacific Region	0.4% - 6.8% (up to 30% HbE)	5945
Americas	0.4% - 1.3%	614
Africa (Algeria only)	3%	123
Total		**57812**

Figures from the TIF database, derived from published carrier information, and other databases, including the APoGI and March of Dimes databases.

• Europe is a continent where beta thalassemia has a very variable prevalence since in the southern Mediterranean coastal area the thalassemia genes are prevalent while in the northern countries they are rare in the indigenous populations. However migrations have over the last few decades introduced the disease in most of the northern areas. In most European countries migrants now have reached around 10-12% of the population [1]. These migrants originate not only from the southern states of Europe but also from Asia, the Middle East and Africa. In each country the migration patterns are different, often related to the past or present relationships of host countries to the countries of origin and also to economic factors. Most migrations have been south to north and so from high prevalence areas to low prevalence areas. This has created a new public health problem in Europe as chronic, hereditary diseases which also require expensive and demanding treatment have increased and made new demands on health services.

Table 7. Beta thalassemia in Europe – based on the carrier rates of immigrant groups as well as the indigenous population (data from the TIF database, with migration data derived from the MPI database)

Country	Percentage carriers	Affected births/1000 live births
Albania	5	0.625
Azerbaijan	8	1.6
Austria	0.2	0.001
Belgium	0.2	0.001
Bulgaria	2.5	0.16
Cyprus	15	5.2
Denmark	0.26	0.0017
France	0.7	0.012
FYROM	2.6	0.17
Germany	0.28	0.002
Georgia	3	0.225
Greece	8.1	1.6
Italy	4.1	0.4
Malta	3	0.225
Netherlands	0.4	0.004
Portugal	1.4	0.045
Romania	1	0.02
Serbia	1.2	0.036
Spain	1.52	0.06
Sweden	0.17	0.0007
Switzerland	0.4	0.004
UK	0.44	0.005

Further reading

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- Christianson A, Howson CP, Modell B. March of Dimes Global Report on Birth Defects. March of Dimes Birth Defects Foundation, White Plains, New York 2006

RED BLOOD CELL ENZYMOPATHIES

- There are no exact and verified figures regarding the frequency of red blood cell enzyme disorders. Basically, this is due to the lack of a certified EU registry. Also, like in disorders of the red cell membrane, some enzyme disorders will be difficult to identify because they are either very rare or clinically mild. This latter fact may, for instance, explain the discrepancy between the estimated number of cases affected by pyruvate kinase deficiency (*i.e.* 1:20,000 in the general white population) and the true number of identified cases. ENERCA enabled a comparison of these numbers. It was concluded that both in The Netherlands and Italy, 2 countries with a large and well-characterized database of patients with PK deficiency the true frequency was, in fact, about 10 times lower than predicted. As stated, this may either be due to a high number of patients showing a mild to very mild clinical picture or to a lack of awareness, or both. A similar situation might be applicable to haemolytic anaemia due to pyrimidine-5'-nucleotidase deficiency.
- The recently conducted survey by ENERCA3 partners of WP6 also brought to light that another important issue contributes to the limited knowledge on epidemiology of red cell enzyme disorders. This concerns the fact there are currently only a limited number of laboratories in the EU capable of performing all the necessary tests, either on the biochemical level or on the genetic level, required to diagnose red cell enzyme disorders. Whereas a considerable number of laboratories are performing diagnostic tests for detection of the two mostfrequently occurring red cell enzyme disorders, *i.e.* deficiencies of glucose-6-phosphate dehydrogenase and pyruvate kinase, only few laboratories offer the complete panel of tests for detection of the other 12 rare enzyme disorders of the red blood cell. This fact probably contributes to the relatively high amount of patients with hereditary haemolytic anaemia that remain undiagnosed. In addition, it affirms the belief among ENERCA partners that the true worldwide population frequency of the rare and very rare enzyme disorders of the red blood cell may, in fact, be significantly higher than that reported in the literature. This may for instance be true for a deficiency of glutathione synthetase.
- The currently available data regarding the worldwide number of families/cases diagnosed with anaemia due to very rare enzyme disorders are (table 8) :

Table 8. RBC Enzymopathies

Disease	**Cases**
Pyruvate kinase deficiency	>500 families
Pyrimidine-5'-nucleotidase deficiency	>60 families
Triosephophate isomerase deficiency	50 – 100 cases
Phosphofructokinase deficiency	50 – 100 cases
Phosphoglycerate kinase deficiency	40 families
Class I glucose-6-phosphate dehydrogenase deficiency	>50 families
Glucose-6-phosphate isomerise deficiency	>50 families
Glutathione synthetase deficiency	>50 families
Hexokinase deficiency	20 cases
Adenylate kinase deficiency	12 families
Glutamate cysteine ligase deficiency	12 families
Aldolase deficiency	6 cases
Adenosine hyperactivity	3 families
Glutathione reductase deficiency	2 families

RED BLOOD CELL MEMBRANOPATHIES

No definite information is available regarding the epidemiology of red blood cell membrane defects in the EU. This is because official EU registries for these pathologies do not exist. Moreover, some forms are difficult to identify because they are either very rare or phenotypically mild.

- **Hereditary spherocytosis** (HS) is a relatively common inherited haemolytic anaemia that occurs in all racial groups and is particularly common in individuals of northern European ancestry. Its prevalence, considered in the past to be 1:5000 is now, more realistic, estimated to be 1:2000 based on studies of decreased erythrocyte osmotic fragility in blood donors. Notably, mild/asymptomatic forms can easily be missed and the wide heterogeneity of the molecular defect makes the diagnosis difficult.
- **Hereditary elliptocytosis (HE) and Hereditary pyropoikilocytosis (HPP)** are two variant forms of the same entity and differ in their severity and frequency. HE has an estimated frequency ranging from 1:1000-1:4000 and is ubiquitously distributed, although it is more common in African Blacks and patients from Mediterranean descendant. The resistance to malarial infections by the elliptocytic cells may explain the high frequency of hereditary elliptocytosis found in malaria-endemic areas, in particular in some parts of West Africa. Here, HE reaches a prevalence of 2%. HPP is a very rare condition and only a limited number of families have been reported in Europe.
- **Southeast Asian Ovalocytosis** is very common (prevalence 5-25%) in malarial-endemic areas in Melanesia, Malaysia, Philippines, Indonesia and Southern Thailand. In Europe it is very rare.
- **Hereditary stomatocytosis** is a very rare and highly heterogeneous disorder. The 2 most common of these are dehydrated stomatocytosis (hereditary xerocytosis) with an estimated prevalence of 1: 50.000, and overhydrated stomatocytosis (1-9:1.000.000). The few reported patients are mostly of European origin. Hereditary stomatocytosis is easily misdiagnosed as HS.
- A recent ENERCA3 survey on diagnosis and management of rare/very rare anaemias indicates that the number of RBC membrane disorders is about 4-5 times higher than the number of registered cases of erythroenzymopathies, and 10-15 times higher than cases with congenital dyserythropoetic anaemia type II.

CONGENITAL DYSERYTHROPOIETIC ANAEMIAS (CDA)

- No data on global distribution and frequency were available before the work done in the frame of ENERCA 3. In the task group of WP 6, worldwide reports were collated in the German Registry on CDA data bank. Within ENERCAs network, the code initially used for the German Registry was adopted to unequivocally identify all cases of CDA based on case reports from the literature, correspondence with caregivers of CDA patients (always including consultation to confirm the diagnosis and management) and centres in Naples/ IT (A. Iolascon), Milano /IT (A. Zanella, P. Bianchi), London (S. Wickramasinge) and Oxford (R.Renella) UK, Paris/FR (J.Delaunay) and Bukarest/ RO (A. Colita). All cases from the German speaking countries, Poland and the Czech Republic were primarily included in the German Registry's data bank.
- CDA has been observed in many regions of the world, with most cases reported from Europe and North Africa, but also from Asia, Australia/ New Zealand and the Americas. Prevalence data can only be estimated from Europe. There is no evidence that environmental factors, such Malaria exposure, play a role.
- An attempt to estimate prevalence was made in Europe, covering all EU-MS and Switzerland We used the period prevalence of 50 years, including 1968 (first reports on CDA) up to 2008, and limited the data on CDA I and II. They were shown in a European geocode. Briefly, the results were:

Prevalence of CDA II is about two three times higher as compared to CDA I

1. Prevalence's show large differences in the various countries
2. Prevalence depends on the presence of Registries collecting all CDAs or Registries devoted to one type (CDA I in the UK, CDA II in Italy).
3. 40 years period prevalences for CDA I vary between 0 and 0.59 /per million inhabitants with an average of 0.24
4. 40 years period prevalences for CDA II vary between 0.04 and 2.46 /per million inhabitants with an average of 0.71

- Different awareness of the diagnosis CDA, publication bias and consanguinity rates are most probably the cause of the geographic differences estimated.
- Point prevalence and number of affected individuals of all live births are close to this figure, with a median life expectancy more the 50 years (assessed for the German speaking countries only).True prevalence is probably higher than shown by the difference of period prevalence estimated, based on the observation that many cases notified to the German Registry are adolescents or adults.

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BLACKFAN DIAMOND ANEMIA (BDA) to be reviewed by Irma Dianzani - pending

- Clinically, DBA is a very heterogeneous disease that is inherited in an autosomal dominant fashion (Vlachos 2008). Classical DBA affects about seven per million live births and presents during the first year of life. However, the identification of 11 genes that are mutated in patients with DBA and extended investigation within the families of the affected patients allowed the discovery of non-classical cases with less distinct phenotypes. These phenotypes include mild increase of erythrocyte volume and/or increased erythrocyte ADA activity in adults as well as children with otherwise normal haematology. It is therefore expected that many patients with mild DBA forms are under-diagnosed. Another level of heterogeneity could be due to mosaicism that has been revealed in several cases. Extended genomic studies are expected to reveal modifier genes that could also explain the phenotypic heterogeneity.
- The implementation of accurate patient registries and regular update of the locus specific DBA Mutation Database (www.dbagenes.unito.it) will certainly allow to better define the genotype/phenotype correlations in DBA. In Europe DBA patient Registries have been started in France (Faivre 2006), Germany (Faivre 2006), Italy (Boria 2010), Czeck Republic (Pospisilova 2012), UK (Orfali 2004). An attempt to produce a European Registry is ongoing with ENERCA's support.

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FANCONI ANAEMIA

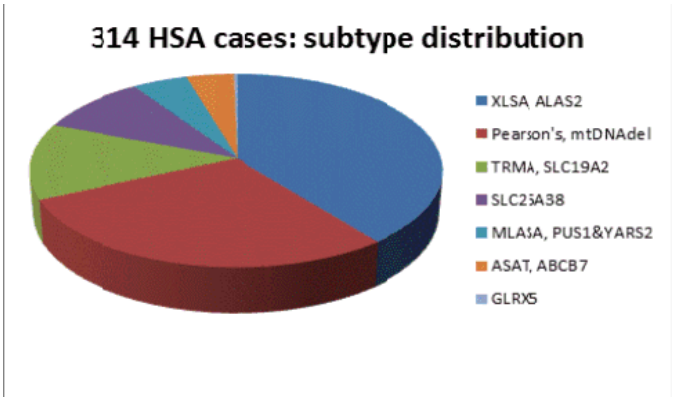
All the available epidemiological sources indicate that the prevalence of FA for all Europe is 0.03/10,000 inhabitants. The frequency of mutation carriers ranges from 1:65 in some consanguineous ethic groups such us the Spanish Gypsies to 1:209 in the overall Caucasian population. Other ethnical groups with higher incidence of FA are Ashkenazi Jewish and white Afrikaners from South Africa due to founder mutations in FANCC and FANCA respectively.

HERDITARY CONGENITAL SIDEROBLASTIC ANAEMIA

- Hereditary or congenital sideroblastic anaemia of a defined type occurs rarely. There are at least 310-330 case reports from about 255 families in the literature. It is a group of heterogeneous disorders, some of which appear to occur more frequently than others. The figures obtained so far are those collected from the literature by a single individual, or by personal communication from other diagnostic laboratories, and are shown in table XX. A consensus view from ENERCA colleagues has yet to be arrived at and the database added to by others in the ENERCA network. It is hoped that a more accurate estimation of the number of patients in Europe will soon be obtained in this way despite the absence of specific registry.

Table 9

	Reported cases	**Families**
All	310-330	255
Non- syndromic X- linked sideroblastic anaaemias (*ALAS2*)	124	92
Pearson's Syndrome	90-100	90-100
Thiamineresponsivemegaloblastic anaemia (*SLC19A2*)	40	30
Non-syndromic, autosomal recessive, pyridoxine-refractory SA caused by *SLC25A38* variations	31	28
MLASA syndrome types of HSA	15	8-9
X-linked sideroblastic anaemia with ataxia (ASAT) caused by *ABCB7* variations	13	5
Non-syndromic, autosomal recessive, pyridoxine-refractory SA caused by *GLRX5* variations	1	1



- In one diagnostic laboratory which does not accept referrals from patients requiring investigation for Pearson's syndrome (*mt DNA deletion*) or for TRMA (*SLC19A2*) 39% referrals remain undiagnosed . If this applies elsewhere, the number of cases would increase to a total of about 430-450. This represents fewer than 1 in 10,000,000 cases worldwide. This will be an underestimation however. A local regional estimation of the most common subtype (XLSA due to ALAS2 defect) is 1 in 1,000,000. From the data collected above, this would give

an approximate predicted prevalence of about 1 in 400,000 for any type of HSA.

- For other forms of hereditary iron disorders there are no prevalence data yet, but single case reports and reviews were reported by ENERCA partners; their contributions allow a preliminary classification of these very rare disorders as a prerequisite for future epidemiological studies.

Inherited Iron disorders: hereditary forms of non-sideroblastic anaemia

Hereditary or congenital non-sideroblastic anaemias are rare (IRIDA, aceruloplasminemia) or very rare (atransferrinemia and DMT1-deficient anaemia) disorders depending on the subtype. Prevalences of these diseases are very low and so far are only estimates; a more accurate estimation of the number of patients in Europe will be obtained by ENERCA expert consensus in the near future.

About 60 patients with **aceruloplasminemia** from several countries including Japan, China, Ireland, Belgium, France, Italy and USA have been described. Most patients are of Japanese origin. The frequency of homozygosity for deleterious *CP* mutations in non-consanguineous couples in Japan was estimated to be 1 per 2,000,000.

Atransferrinemia is an extremely rare genetic disorder with only 12 families and 14 affected individuals reported world-wide (personal literature collection of cases). The transferrin defects have been characterized at the molecular level in only 5 of these families.

DMT1-deficient Anaemia or Familial microcytic hypochromic anaemia with hepatic iron overload due to defects in DMT1 has been described in only 5 patients so far. The exact prevalence of this disease it is not known but it is estimated to be <1 / 1, 000, 000.

For Iron-refractory Iron deficiency Anaemia (**IRIDA**) at least 43 patients from 27 families distributed world-wide have been described. The estimated prevalence of the disease is also <1 / 1, 000, 000.

Other new entities such as IRIDA-like patients without mutations in the *TMPRSS6* gene may exist and are waiting for further clinical and genetic characterization.

2. CENTRES OF EXPERTISE ON RARE ANAEMIAS: NETWORKING POLICIES

The EU Perspective – Background

- The EU considers diseases to be rare when they affect not more than one in 2,000 persons in the EU and this definition has been adopted today in all European countries, with few exceptions (e.g. UK, Russian Federation, where the diseases affecting less than one in 100,000 persons are considered rare). The definition first appeared in EU legislation in Regulation (EC) N° 141/2000 in December 1999 on Orphan Medicinal Products and in the Community Action on RDs in the field of public health (1999 – 2003)
- It is estimated that 5,000 – 8,000 distinct rare diseases (RDs) exist, affecting 6% to 8% of the European population in the course of their lives. All together the diseases characterized as rare, affect in Europe, 27 to 36 million people. Within rare, there are "very rare" and "ultra rare" diseases. In fact, most of them affect 1 in 100,000 people or less and some of these, the ultra rare, have a prevalence of 1:2,000,000.
- The specificities of RDs, including a limited number of patients and scarcity of relevant knowledge and experience, certainly single them out as a very special domain of very high European added-value. In addition, the lack of specific health policies for RDs and their scarce and scattered research performed in highly specialized labs and Centres throughout the EU, translate into a difficult of access to clinical care and consequently a delayed diagnosis. Therefore, a call for European and international cooperation, to ensure sharing of this knowledge and benefiting of combined resources is extremely necessary.

Rare Anaemias – Background

- One important, and relatively homogeneous, group of RDs is that in which “anaemia” is the first sign and the most relevant clinical manifestation of the disease. This group of RD has been defined as “rare anaemias” (RAs), and the EC recognized, for first time, its importance by approving for financing a DG-SANCO Project entitled “European Network for rare congenital diseases” (ENERCA). It is noteworthy that this Project started in 2003, one year before the creation of the High Level Group (HLG) in July 2004 that brought together experts from all the Member States in several areas of expertise. For RAs this was a great advantage because it facilitated the progressive development of ENERCA tasks in parallel to

the development of the different HLG areas of action: a) Cross-border healthcare purchasing and provision, b) European workforce for health professionals, c) European reference networks, d) Health technology assessment, e) Information and e-health, f) Health impact assessment and health systems and g) Patient safety and quality of care.

- Since the existence of ENERCA, many people in Europe, including some health professionals, didn't know the existence of RAs because, in many cases, the cause is unknown or extremely infrequent, and/or have no causal treatment, exception made of some special types of RA. Moreover, as mentioned previously, RAs have been systematically underestimated by public health officials, because, for a long time, anaemia, in general, has been confused with iron deficiency. The first ENERCA Project (ENERCA 1) started in the early 2004 and was devoted to congenital rare anaemias only (*European Network for Rare Congenital Anaemias*). ENERCA 1 allowed the establishment of the necessary background for a sustainable coordination in the area of health information, collection of epidemiological data, comparability issues, exchange of data and information within and between Member States At this time, it has also facilitated a rapid reaction to RA diagnosis and treatment. In 2005, a second ENERCA Project (ENERCA 2) was approved for financing by the EC and covered, in addition to congenital anaemias, all rare causes of anaemia, either hereditary or acquired (*European Network for Rare and Congenital Anaemias*) . Besides this very important issue, ENERCA 2 dedicated many and important activities to the field of Health Information, patient's data collection, education and training and standardization/quality assessment for special RA diagnostic procedures. This has provided a first and unique approach for prevention, diagnosis and treatment of rare congenital anaemias.

2.1. Centres of expertise and chronic diseases: the WHO recommendations.

- Since the year 2000, non communicable diseases (NCDs) and especially chronic conditions (chronic diseases) have been set a priority in health prevention by the World Health Organization (WHO). Targeted disorders include cardiovascular diseases, diabetes, cancers and chronic respiratory diseases. Genetic disorders are also included in the broader context of Non Communicable Diseases prevention programmes that have been proposed by WHO at a global level.

- The 2008-2013 action plan for the global strategy for the prevention and control of NCDs (http://www.who.int/nmh/publications/9789241597418/en/) has defined six main objectives, which propose actions to be set at WHO and at the international levels, as well as by Members states. Although genetic diseases do not share the risk factors common to the four targeted NCDs and in fact require different, specific interventions for their control, genetic disorders will greatly benefit from the development of services for NCDs. Objective four of the action plan specifically focuses on the need to establish national reference centres and networks and their role in research programmes for the prevention and control of NCDs.

- As already stressed, Haemoglobin (Hb) disorders, especially sickle cell disease (SCD) and thalassaemia (Thal), are among the most prevalent inherited diseases at the global level. We have also mentioned that the world distribution of Hb disorders has changed during the past years: initially restricted to areas of historically high frequency, e.g., Africa, Asia and the Mediterranean basin, it has now reached important levels in high income countries in Europe and elsewhere due to globalization. As a result of the information, data,

knowledge and experiences collected through the implementation of effective prevention and management, programmes for thalassaemia in, albeit, few countries, and of the considerable research in more recent years, there is ample evidence today, of effective control strategies. For examples, in some countries, like Cyprus or Sardinia, a dramatic decrease in the number of affected patients has occurred and effective measures of management has led to high survival rates and good quality of patients' life. However, in most countries such programmes do not exist or function sub optimally and the burden of the disease remains very high.

- In 2006 further development was made in the preparation and the adoption by the WHO Executive Board and the World Health Assembly of resolutions on both, sickle cell disease (EB117.R3 and WHA59.20) and thalassaemia and other haemoglobinopathies (EB118.R1).
- Resolutions on Haemoglobinopathies urge Member States to: i) Implement and reinforce national programmes on Hb disorders; ii)

Evaluate the impact of national programmes; iii) Intensify the training of all health professionals; iv) Promote community education; v) Promote international cooperation; vi) Develop and strengthen medical genetic services; vii) Support basic and applied research. They also request the Director-General to: i) Provide technical support and advice to national programmes; ii) Expand the training and expertise of personnel; iii) Support the further transfer of affordable technologies; iv) Draft guidelines on prevention and management; v) Foster the establishment of regional groups of experts; vi) Support needed research.

- In addition to these specific resolutions, the World Health Assembly recently adopted, in May 2010, a more general resolution on birth defects, supported by both, the Child and Adolescent Health and Human Genetics units of WHO. This resolution deals with congenital disorders (as a synonymous of birth defects) but also involves inherited diseases such as SCD & Thal. The report of the WHO Secretariat accompanying the resolution insists on the health care services that should be available for the prevention and care of birth defects. These include:

- core network of appropriate specialist clinical and laboratory services that can be expanded in response to demand;

- integration of approaches to the prevention and care of birth defects into primary health care, with an emphasis on maternal and child health;

- education and training for health-care providers, particularly those in primary health care;

- establishment of effective mechanisms to foster development of patient–parent support organizations, and collaboration with them in caring for people with birth defects and their families;

- Moreover the WHO Secretariat recommends the establishment or

strengthening of national programmes for the control of birth defects and stresses the usefulness of technical tools such as the revised international disease classification (IDC-10) and the definition of effective community services. It also encourages the identification of useful models that can be applied to low and middle income countries.

- WHO resolutions have a global diffusion to all member states em implementation. The implementation of these resolutions relies on the support of all committed parties, including networks such as ENERCA and NGOs in official relations with WHO such as TIF, which is also an ENERCA 3 partner.
- ENERCA has collaborated with WHO during the second and third phases of the project (ENERCA2 and 3). During ENERCA 2, the project was represented at a WHO-TIF meeting, on the "*Management of Haemoglobin Disorders*", held

in Cyprus (WHO 2007). The former WHO Human Genetics programme responsible officer, Dr V Boulyjenkov, has been a collaborating partner of ENERCA3 workpackage on education and training. Leaders of WHO Collaborating Centres have been involved in other workpackages of ENERCA 2 and 3. ENERCA 3 included a communication on WHO resolutions on haemoglobin disorders during the Madrid 2010 ENERCA symposium (communication by Dr Patricia Aguilar Martinez and press release). This cooperation and partnership greatly contributed to the promotion of the WHO relevant resolutions.

- ENERCA has been also involved in the International Disease Classification (IDC) revision and partners have actively participated in a proposal of modifications and improvements of the classification of rare red cell disorders, including rare anaemias and iron related diseases.
- Finally the ENERCA3 project itself is a vector for the implementation of both resolutions on haemoglobinopathies and of the resolution on Birth defects, in all their aspects, including the epidemiological assessment of the burden of the disorders in Europe, the establishment of recommendations for the clinical an laboratory practices, medical education and information of patient and the public, and the provision of information and guidance to national health policy makers through the redaction of the present White book. The comprehensive work done by the ENERCA network in Europe could serve as a model for other WHO regions, including those from low and middle income countries and for other RD.

- **Further reading**

1. WHO, 2008, 2008-2013 Action plan for the global stratetgy for the prevention and control of Noncommunicale Diseases. WHO, Geneva, Switzerland
2. Angastiniotis MA, Hadjiminas MG. Prevention of thalassaemia in Cyprus Lancet. 1981;1(8216):369-71.
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4. WHO, 2006, *Executive Board Resolution on Thalassaemia and Other Haemoglobinopathies.* EB118.R1. WHO, Geneva, Switzerland
5. WHO, 2007, Report of Joint WHO/TIF Meeting. *Management of Haemoglobin Disorders*, Nicosia, Cyprus, 16-18 November 2007
6. WHO, 2010, *World Health Assembly Resolution on Birth Defects.* WHA63.17. WHO, Geneva, Switzerland
7. WHO, 2011. Report of a WHO consultation on community genetic services in Low and middle income countries. Geneva, Switzerland, 13-14 September 2010

phasizing that the WHO Director General is committed to their

2.2. Centres of Expertise - The European Union Committee of Experts in Rare Diseases (EUCERD)

Political framework on RDs at the country and European level

• Since the 1990s, at both the EU and Member State (MS) level, the following several initiatives and political issues in the field of RDs were undertaken:

1. At MS level
    a. Sweden established the first Centre of Expertise in 1990 and an information centre in 1999
    b. Denmark established an information centre in 1990 and Centre of Expertise in 2001
    c. Italy adopted a degree on RDs in 2001
    d. France developed the Orphanet web-portal in 1997 with the support of the French Ministry of Health, followed by the first national plan/strategy in 2004
2. At EU level
    2.1 Key Policy documents work programmes and research
        a. Orphan Medicinal Products Regulation (EC N^{o} 141/2000) in December 1999
        b. Commission Communication on RDs: Europe's Challenges, adopted in 2008
        c. This set out the overall Community strategies to support MS and paved the way for the Council Recommendation for action in the field of RDs.
        d. The Council Recommendation adopted in June 2009 engaging the responsibilities of MS and concentrates on supporting and strengthening the adoption of national plans for RDs before the end of 2013.
    2.2 Work programmes at the EU level
        a. A Community Action programme on RDs (1999 – 2003), as a first EU effort aiming to ensure a high level of health protection in relation to RDs.
        b. RDs became a priority in the Second EU Health Programme (2008 – 2013).
    2.3 In addition research on RDs has been addressed both in EU Framework Programmes for Research and Technological Development since the early 1990s and more specifically through FP6 (2002 – 2007) and FP7 (2007 – 2013) under the theme of 'Cooperation'.

• One of the seven themes in the key EU political frame mentioned above, the Council Recommendations [1], ref? is the creation of Centres of Expertise (CsE) and European Reference Networks (ERNs) for RDs which constitute the focus of this book. ?.

• In the context of Centres of Expertise (CsE) and of European Reference Networks (ERNs), the work performed by RDTF on 'Standards of Care' produced between 2005 – 2008 was of particular value. This was supported by one of the working groups of the High Level Group (HLG) on Health Services and Medical Care, established in 2005 by the Directorate General for Health and Consumers (DG SANCO). The HLG had the mission to take forward the DG SANCO's recommendations made in the reflective process on patient mobility providing assurance about safety and quality of cross-border healthcare and fostering health system cooperation in improving healthcare for all. This work concluded with the publication of milestone reports and recommendations : "Overview of current Centres of Expertise on RDs in the EU (2005)", "State-of-the-Art Centres of Reference for RDs in Europe (2006)" and "Recommendations (2006)", and the RDTF Final Report: "State-of-the-Art and Future Directions" (2008). Based on the experience of countries with existing processes for Expert Services in place, the EC/ RDTF, described the following quality criteria for CsE

- Appropriate capacities to diagnosing, follow-up and managing patients with evidence of good outcomes, where applicable;
- Activity and capacity to provide relevant services at a sustained level of quality;
- Capacity to provide expert advice, diagnosis or confirmation of diagnosis, to produce and adhere to good practice guidelines;
- Demonstration of a multi-disciplinary approach;
- High level of expertise and experience as documented through publications, grants or honorary positions and training activities;
- Strong contribution to research
- Involvement in epidemiological surveillance such as registries;
- Close collaboration with other expert centres at national and international level and capacity to network;
- Close links and collaboration with patient organisations

• Should we mention the RDTF before the EUCERD? Later on in the chapter the activities of RDTF are mentioned however there is no data about its creation

• The European Union Committee of Experts on RDs (EUCERD) was established via the EC decision in 2009 (2009/872/EC) to continue and replace the work of the Rare Disease Task Force (RDTF), and has began since then to assume a pivotal role in the preparation and implementation of Community activities in the field.

• EUCERD is responsible to assist the EC in cooperation and consultation with the specialized bodies in MS, the relevant European authorities in the field of research and public health actions and other relevant stakeholders including the patients. Moreover it will foster exchange of experiences, policies and practices between these parties. EUCERD activities have been supported by the Joint Action N^{o} 2008 119 (RDTF/EUCERD) and will be supported by a new Joint Action, to commence in March 2012. A valuable piece of work undertaken by EUCERD currently was the preparation of an informative and descriptive overview of activities on RDs at the EU and MS level, up to the end of 2010. The principal information sources included EC websites and documents, OrphaNews Europe, RDTF publications, EUCERD publications and meetings (*2010*/*2011*), reports on orphan drugs, EURORDIS websites of Patient Organisations and national alliances, EUROPLAN questionnaire and Conferences final reports and Orphanet web portal.

• The EC is responsible, with the support of the EUCERD, to review all this information and produce by the end of 2013 an implementation report on both the Council Recommendation and Commission Communication in order to

assess the extent to which the proposed measures are implemented and working effectively and very importantly to evaluate the needs for further action.

• The EUCERD undertook the commitment to take further this work and produce recommendations on quality criteria for national CsE on RDs based on the above and which have been adopted in October 2011 (3rd EUCERD meeting agenda item 2.1.2).

The recommendations directly derive from the following documents:

- Commission Communication, Rare Diseases Europe's Challenge

  http://ec.europa.eu/health/ph_threats/non_com/docs/rare_com_en.pdf

- Council Recommendation (2009/C 151/02) of 8 June on an action in the field of rare diseases

  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2009:151:0007:0010:EN:PDF

- Directive (EC 2011/24/EU) of the European Parliament and of the Council on the application of patients' rights in cross-border health care
  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:088:0045:0065:EN:PDF *EUCERD Recommendations on Quality Criteria for National Centres of Expertise for Rare Diseases (For adoption on 24 October 2011)* 4

- Work of the HLG on Health Services and Medical Care during 2005

  http://ec.europa.eu/health/archive/ph_overview/co_operation/mobility/docs/highlevel_2005_013_en.pdf

- RDTF Report: Overview of Current Centres of Reference on rare diseases in the EU (September 2005)

  http://www.eucerd.eu/upload/file/Publication/RDTFECR2005.pdf

- RDTF Report: Centres of Reference for Rare Diseases in Europe – State-of-the-art in 2006 and Recommendations of the Rare Diseases Task Force (September 2006)

  http://www.eucerd.eu/upload/file/Publication/RDTFECR2006.pdf

- RDTF Report: European Reference Networks in the field of Rare Diseases: State of the art and Future Directions (July 2008)

  http://www.eucerd.eu/upload/file/Publication/RDTFERN2008.pdf

- EUCERD Workshop Report: Centres of expertise and European Reference Networks for Rare Diseases (8-9/12/2010)

  http://www.eucerd.eu/upload/file/WorkshopReport/EUCERDWorkshopReportCECERN.pdf

- EUCERD Workshop Report: National centres of expertise for rare diseases and networking between centres of expertise for rare diseases (21-22/03/2011)

  http://www.eucerd.eu/upload/file/EUCERDReport220311.pdf

- EUCERD Report: Preliminary analysis of the experiences and outcomes of ERNs for rare diseases (May 2011)

  http://www.eucerd.eu/upload/file/Reports/2011ERNAnalysis.pdf

- EUROPLAN: Recommendations for the development of National Plans and Strategies for rare diseases

http://www.europlanproject.eu/public/contenuti/files/Guidance_Doc_EUROPLAN_20100601_final.pdf

European Centres of Expertise (CEs)

- Currently and through the work of EUCERD it is recognised that there is a great heterogeneity in the area of national CsE for RDs in Europe, both in terms of the initiatives already in place, designated criteria and the state of advancement of MS in the provision of Expert Centres for rare diseases.
- With regards to CsE in the different European countries, four different actions can be identified:

1. CsE officially designated and supported at the national level, usually by the Ministry of Health and referred to as official centres;
2. CsE at the Regional level designated and fully or partly supported by Regional or Central Health Authorities
3. Self-designated/non-designated CsE where experts or expertise may exist but in the absence of one designation processes and
4. Countries that are currently planning CsE designation

- With regards to CsE at MS Level the current situation is the following:

1 A few countries currently having officially designated National CsE, such Denmark, France, Norway, Spain and the UK[62]

2 A greater number of countries having self-declared/non-designated National CsE acknowledged by Nationa Health Authorities (NHAs) to varying degrees, such as Austria, Belgium, Czech Republic, Germany, Greece, Hungary, Ireland, the Netherlands, Slovakia, Sweden and Switzerland

3 An equally great number of countries planning to elaborate designation procedures in the future, mostly in the context of future national plans/strategies for RDs, such as Austria, Belgium, Czech Republic, Germany, Greece, Hungary, Ireland, the Netherlands, Portugal, Romania, Slovenia and Turkey

European Reference Networks (ERNs)

The philosophy and principles for the development of networks of expert centres was explained in Chapter 2, in the context of the EUCERD initiative. The need for centres of expertise for rare and chronic anaemias arises from the need to have a concentration of expertise based on disease specific criteria. A centre expertise may deal with some and sometimes all of the rare anaemias. Specialisation is usually a result of the local prevalence of an anaemia or the local academic interest which drove research and so concentrated of cases. In practice however the aim is for equity and access of patients to safe and good quality healthcare and the provision of such a service is complicated by the rarity and complexity of cases and the often poor local experience which makes both diagnosis and case management difficult. Diagnosis requires highly specialised tests and experience which is outside the scope and resources of individual centres. In order to satisfy patient equity and access and at the same time save resources, networking between centres becomes imperative.
The need for networking of centres of expertise arises from:

1. Epidemiology – the rarity of each individual or category of anaemia
2. The complexity and often expense of diagnostic procedures.
3. The complexity of case management, and poor knowledge of the natural history and the effects of treatment. This leads to the need for clinical support and exchange of expertise
4. The need for equitable access of patients to good quality healthcare.
5. The need to enhance research in rare anaemias, which the scarcity of cases in any one location makes for poverty of results.
6. The need to develop orphan drugs.

The epidemiology of rare anaemias has been described in chapter 1 (1.3 p64). One particular benefit of networking is the possibility of sharing data and registries, thereby developing a more accurate picture of incidence and prevalence.

Accuracy of diagnosis is a prerequisite both to correct treatment and to correct epidemiological surveillance. Networking allows a laboratory to apply the routine haematological tests to at least categorise the anaemia (see chapter 1.2) and then send samples to a laboratory of the network which specialises in the particular category for a definitive diagnosis. Within a network a diagnostic algorithm can be developed which will direct specimens in order to increase the diagnostic yield (Glembotsky AC et al 2012). This exchange of 'data, biological samples and other diagnostic materials' allows improved diagnosis without the need for patient travel. An example of such a network is the HMA-IRON consortium which deals with rare genetic anaemias of iron metabolism (http://www.e-rare.eu). Linking this specialised network to other networks for rare anaemias will undoubtedly increase diagnostic yield. The ENERCA project has from the onset promoted these collaborations between expert partners and through its website offered diagnostic algorithms.

Networking for clinical care is particularly needful if the requirement for good quality healthcare is to be met. The 62 rare anaemias described in the ENERCA website some treatment modalities, such as blood transfusions, splenectomy or stem cell transplantation, but the correct use in each individual case or diagnostic category requires expertise and adherence to already published evidence based guidelines where these exist. In addition the complexity of complications in each disease entity, make both monitoring for early detection and the therapeutic approach complex. Some of the complications are inherent in the natural history of the anaemia (as are the consequences of vaso-occlusion in sickle cell disease or the renal complications of PNH) and some are the result of treatment (such as iron overload due to transfusion dependency). Even where protocols and guidelines exist, experience is necessary in decision taking. This means that consultation with experts is important. The means for such consultations must be provided and should consider the EUCERD principle that 'expertise travels rather than the patient'. In order to satisfy this requirement electronic communication tools (including tele-medicine) are the preferred means for the exchange of expertise. In rare anaemias such tools will allow exchange of images, including morphological images (such as marrow preparations) as well as radiological (such as MRI T2* for iron deposition in vital organs). The need for support arises at a local level (centre of expertise to secondary centre), at national level or even between countries. Interstate, cross border clinical support is particularly relevant in the very rare anaemias.

• Another major component of EUCERDs work particularly in 2011 was the preparation of Recommendations on quality criteria for National Centres of Expertise for RDs based on the previous work of the High Level Group and RDTF as mentioned previously and the experience, information and data produced by projects funded by EU through various calls on this subject. The homogeneity of criteria as for CsE being a key concern in the context of ensuring quality of healthcare for RDs across the EU and the benefits gained through the implementation of the EU Cross-border Directive. Work was thus necessary to develop existing principals and elaborate and/or revise the existing work on ERNs for RDs and importantly on the criteria required for CEs to participate in these.

• The concept of ERNs is based on the mobility of expertise rather than the mobility of patients (which should be possible but only when really necessary and in a fair manner). They represent an innovative European coordinated dispersal of cooperation between MS health systems with high European added value, mutualising expertise and resources, sharing knowledge for highly specialized and complex medical conditions. MS have agreed to identify appropriate CsE throughout their national territory by the end of 2013, with the help and support of the conclusions derived from EUCERD and EU, funded projects such a the EUROPLAN, a three year project 2008-2011 created to give support to EUCERD activities and to provide NHAs with supporting tools for the development and implementation of national plans/strategieson RDs). A prerequisite in this effort is for EC to support MS to create and foster the participation of CEsin ERNs, respecting national competences and rules to their authorization or recognition. The critical role of ERNs is also explicitly mentioned in Article 12,13 of the European Cross-border Healthcare Directive (see below)

• EUCERD has thus focused work on analysing the outcomes and experiences in late 2010 through a workshop of the information compiled through a number of pilot ERNs for RDs projects which have been awarded 3-year funding (some receiving more than 13 years support) from the EC (in the context of the Community Action Programme on RDs, 1997 – 2007 and the Second Programme of Community Action in the field of health, 2002 – 2013 as mentioned previously. These included Networks on Dysmorphology, Cystic Fibrosis, Paediatric Hodgkin Lymphoma, rare paediatric neurological diseases, Langerhans Cell Histiocytosis, Genodermatoses, Alpha- 1 antitrypsin? Registry, Porphyria, rare bleeding disorders, Duchenne Muscular Dystrophy , and Rare Anaemias (ENERCA).

• Despite the wide heterogeneity noted in the activities and geographical coverage of these ERNs, it was possible to recognize various benefits, but also weaknesses and needs for further action and co-ordination both at the EU level, Directorate General for Research and Innovation (DG RTD) and DG SANCO, but also at the MS level. Valuable resources developed and worth mentioning, however include:

- Shared database/registries
- Guidelines and information
- Training tools and training sessions
- Shared tools for tele-experience

• A major concern deriving and worth noting was the need for such types of infrastructures to have long-term funding and support for the sustainability and appropriate functioning.

A second workshop held by EUCERD in March 2011 discussed in depth a number of priority topics identified in the 2010 workshop. In this workshop focus was given on the models of organisation of expertise at the national level since the consensus in 2010 was that the expertise should be identified at the national level before networking at the EU level and development of ERNs can take place. Following presentation of such work in different European countries, it was highlighted that:

- National CsE in terms of disease coverage is closely related to the organisation of health care in each country - The definition of a national centre of expertise differs from one country to another: some have specialized centres (by disease or group), and/or generally a list of centres (for all RDs) – reflecting differences in the size of the countries. Some CsE are focused on clinical management, others undertake research and yet others have a focus on technology and/or expert intervention or on expert advice/production of guidelines. Many CsE may do more than one of the above activities. Therefore, missions of National CsE and their financing are key themes.
- Lack of designated National Centres does not implicate a lack of expertise, but designation implies commitment on behalf of the state in terms of sustainability, financing, monitoring and evaluating.
- Outcome indicators and guarantees of quality, are key to ensuring the expertise of a National CsE. Work has already been done through the EUROPLAN PROJECT and implementation by MS needs now to be noted.
- Not all RDs can be covered at the national level by CsE and European networking provides the only solution
- High Level Group/RDTF criteria can be useful as the starting point for designation and through EU networking, sharing of tools, expertise,

  guidelines will be possible. Such discussions culminated in the consensus document 2.1.2. on the Recommendation on quality criteria for national CsE.

• Additional criteria for National CsE were further more suggested in the EUCERD workshop of 2012 of the needs on Cross Border Health Care Directive aiming to clarify better standards and indicators needed for the evaluation and auditing of national CsE and ERNs such as the below:

- These national centres must be open to European collaboration;
- National centres must accept to be evaluated 3 or 5 years after their designation;
- Centres are not obliged to fulfil all the criteria when they are designated, but they should have a strategy in place to attain quality criteria when not yet already achieved
- The population and geographical size of the country should be considered when organising expert care for rare diseases at national level, i.e. national centres of expertise should network with proximate centres, such as the French model of *'centres de compétence'*;

- Centres should be responsible for improving the delay to diagnosis and for building healthcare pathways from primary care;
- Centres should have a quality management system in place to assure quality of care which takes into account European norms;
- Centres should adhere to good practice guidelines where they exist;
- Centres should be responsible for establishing health care pathways which both aid diagnosis and aid the coordination of care between different medical specialities;
- Quality of care indicators and process indicators should be developed and monitored;
- Collaboration with other expert centres at European/national/regional level,if exist, is important;
- The designation/quality criteria should be adapted to the national situation/ to the specificities of the disease/disease group;
- Centres should have a continuity plan in place for sustainability in terms of personnel;
- Continuity of care between childhood and adult care should be assured by centres;
- Centres should make appropriate arrangements for patient referrals from other countries.

• At this point, it is essential for MS to identify, in time, individual expertise and Centres, otherwise patients will seek care unnecessarily abroad, introducing new and extremely challenging problems in the implementation of the Cross Border healthcare. In terms of ERNs the highlighted observations include:

- That there is a clear distinction between the missions of CsE and of ERNs;
- The current networks are mainly expert's networks not necessary officially recognized as national centres;
- Expert clinical care and clinical research go always together, and both activities have to be developed simultaneously by the same network;
- ERNs contribute to Research and Development (R&D)
- The establishment of ERNs is a process that requires long term effort
- It is waste of money to establish ERNs if renewal of funding is not feasible.

• It was also highlighted that there cannot be two parallel systems for designate the CsE : In order to avoid confusion and duplication of efforts, nationally designated CsE and ERNs CsE, should be the same entity.

Conclusion

• EUCERD has initiated the process of elaborating recommendations on ERN to serve in the elaboration of the criteria for ERN to be established by the Committee on Cross-Border healthcare. Draft recommendation will be discussed in June 2012 (in the context of EUCERD Join Action) and their adoption expected is by EUCERD in Nov 2012. So far a number of recommendations have a consensus including:

- ERNs are established between CsE designated at MS level. Accordingly, CsE need to be identified at national level before ERNs can be built

- Expertise and information (data) should travel rather than patients: clinical reports, biological samples, radiologic images and other diagnostic materials. The use of e-tools has to be reinforced.
- The purpose of ERNs are the following;
    - Establish healthcare pathways for people living with rare diseases
    - Store data, share knowledge and specimens, develope tools for tele-expertise
    - Prepare guidelines, updated information, training tools,
    - Provide training and expert-opinion

• In the meantime MS are urged to work on designation of CsE using 'EUCERD Recommendations on quality criteria for CsE as adopted in October 2011 before November 2012. This is essential to allow the establishment of ERNs which will be supported in the context of the 3rd Health Programme of the EU.

• MS are best placed to oversee the designation and sustainability of CsE and their involvement in ERNs as they have the primary responsibility of the organisation, financing and delivery of healthcare services.

• The Commission will support MS by cooperating in the development of diagnosis and treatment capacity, in particular to:

- Make health professionals aware of the tools available to them at Union level to assist them in the correct diagnosis or rare diseases, in particular the Orphanet database, and the European reference networks;
- Make patients, health professional and those bodies responsible for the funding of healthcare aware of the possibilities offered by Regulation (EC) NO 883/2004 for referral of patients with rare diseases to other Members States even the diagnosis and treatments which are not available in the Member State of affiliation.

ENERCA's contribution

Through the ENERCA projects (1 & 2) specific and comprehensive work as mentioned previously in this chapter, has been focused on the compilation of a set of recommendations for setting the recommendations for Centres of Expertise in Rare Anaemias. This has been achieved through surveys and very importantly with the contribution and consideration of the patients' perspective (see chapters on recommendations).

Further reading – Up to 7

2.3. The role of National Health Authorities in the recognition and sustainability of Centres of Expertise

A more practical approach of this chapter including future steps

Article 100 A(3) of the 1987 Single European Act required the Commission when taking harmonizing/measures to take as above for its proposals a high level of health protection. It was not, however, until The Treaty of Maastricht in 1993 that the EU had explicit competence in the field of public health through Articles 3(0) and 129, stipulating that the community should contribute to the attainment of a high level of health protection in two areas: disease prevention

and health protection. This stipulation was strengthened in the 1997 Amsterdam Treaty through article 152 EC. Through the latter, it is required from the EU to 'mainstream' health protection in all its policies and activities. The expanded Article 152 (2) sets outs the division of powers between MS and EU institutions in the field of public health. Under the Lisbon Treaty further 'mainstreaming' of public health was ensured with a new article 9 and by replacing Article 152 (5)with article 168 (7), the issue of the responsibilities of the MS to include the management of health services and medical care and the allocation of the resources assigned to them was further clarified.

Resources available for the Public Health programme 2008-2013 is €365.6 million with a significant increase anticipated in the upcoming programme after 2013. The objective of the programme covers:

- Citizens health and security
    - Health threats
    - Citizens safety
- Promotion of health
    - Fostering of healthy, active ageing on bridge inequalities and
    - Tackling health determinates

The EU health policy: contradiction and challenges

Health policy in the EU has a fundamental contradiction at its core, since MS health systems involve interactions with people (e.g. staff, patients), (e.g. pharmaceuticals and devices) and services (e.g. provided by healthcare funders and providers) all of which are granted freedom of movement across border by the same Treaty and in this context many national health activities are in fact subject to EU law and policy.

Overall, there is a gap or a 'constitutional asymmetry' in the EU approach to health policy, especially in relation to the delivery and funding of health care services. Health is and will continue to be an area within which the competence of the EU institutions is highly constrained as reconfirmed also by the Treaty of Lisbon article 168(7) on the functioning of the European Union.

The political framework on RDs at the country and European level

Since the 1990s at both the EU and Member State (MS) level, several initiatives and political concepts in the field of RDs began[1].
At member state level, there is a great heterogeneity in their health care delivery, infrastructure of their health system, resources, health priorities, capacities and thus great heterogeneity in the state of advancement of national policies, plans or strategies for RD.

In the context of National Centres of expertise as highlighted in the commissions communication 'Rare Diseases Europass Challenge' of Nov 208 and the Council Recommendation of Jan 2009 there is a strong agreement that National Centres of Expertise should be established in the framework of national policies for RDs.

MS with a designation process in place are expected to share their experience with other countries and should harmonise their approaches and designation quality recommendations, the responsibilities of MS for the organisation, financing and delivery of health care must be respected. Recommendations must also take into account the diversity of health care system and economies and the differences between large/medium/small size countries in respect of both geography and population.

Through a significant number of actions, programmes, projects, activities at the national and European level, and collection of updated reliable information it became clear by the end of 2010 which were the main areas requiring more in depth action.

The European Project for Rare Diseases National Plans Development (EUROPLAN) 2012-15 is built exactly to provide technical support for developing and implementing national plans/strategies (NP/NS) on rare Diseases (RD) in EU Member States, EFTA/EEA and non-EU countries. Integrated, comprehensive, long-term strategy will be proposed in the framework of principles and guidelines of the key policy documents

Its objectives include the provision of support for the establishment of national plans and strategies for rare Diseases at member state level[2].

In fact European Conferences in the context of EURPLAN have been organised in 2011 in Bulgaria, Denmark, France, Germany, Greece, Hungary, Ireland and in 2010, Italy, Netherlands, Poland, Romania, Spain Sweden the UK and Croatia.

Current stages of development of national plan/strategies for RD in EU MS (2011 EUCERD Report, p.17).

MS with past or updated or currently adopted national plans include: France, Portugal, Greece, Bulgaria, Spain, and the Czech Republic - albeit these plans/strategies vary in scope and/or financing. Issues that will ultimately influence the extent of their impact at national level. Some short reference is made below:

- France was the first country with a comprehensive plan in place (since 2004) and allocated funding - first plan 2004-2008 and second French National Plan was elaborated during 2008-2010 and launched on February 2011 with a budget of €180 million and focusing on three main axes: improve quality of heath care for 3,000,000 patients with RDs, develop research and increase European and International cooperation in the field.

2

1. *To establish an interactive rare diseases policy makers public health network: the network will provide technical assistance & capacity building skills to policy makers in charge of developing a NP/NS. Participants will be supported methodologically and technically. A website will be implemented for sharing and disseminate experiences and documents, and activities will be organised to share and exploit experiences, strengthen interactions, provide new skills in developing public health strategies and plans.*

2. *To produce a complete, coherent and feasible operational proposal for NP/NS: trough public health networking and sharing experiences, every participant will contribute to produce a operational proposal for NP/NS, according to specific country features (size, GNP, health care system), selecting EUROPLAN indicators for future data collection, and identifying strengths and critical aspects in developing public health strategies and plans.*

3. *To support EURORDIS National Conferences: EURORDIS identifies and organises National Conferences (NC) to support the process of elaboration of NP/NS and assess the transferability of EU policy documents in countries that did not organise a EUROPLAN NC and countries that did it but need to sustain the process.*

- Portugal more recently (2008) with a Ministerial decree adopted a national plan, expected to have been implemented within on initial timeframe from 2008 to 2010 followed by a consolidation period from 2010 to 2015. This is focused on similar axes in to the French ones and includes 30 intervention strategies, 9 education and training strategies and 8 strategies for data collection. The budget has not yet been finalised and only partially released with consequent delays in its schedule implementation.

- Greece – Upon the request of the patients' alliance (PESPA), a commission of governmental officials, health professionals and POs was formed in 2007. a national plan has been developed to be implemented between 2008 and 2012 focusing on eight important priorities. Although an initial estimate for the budget required was made, no funding has been officially allocated to the National Plan of Action for Rare Disorders, and none of the eight strategic priority actions have yet started. As of yet, is no legal framework for the Plan.

- Bulgaria. The national plan has been approved by the Bulgaria NHAs (2009-2013). It is currently active with an allocated budget and consists of 9 priorities. It is supported by a national consulting council including health professionals, MOH representatives and a representative of the patients National Alliance.

- Spain. The interterritorial Council of the Spanish NHS in 2009 approached the National plan of RDs, set within the framework of the Quality Plan of the Spanish National Health system. Spain as it Italy, there is a decentralised health administration of the Autonomous Communities (regional government) and the strategy will act as a framework for the different regions which will in turn be in charge of implementation. The strategy focuses on seven strategic aspects and is structured into three parts: the first part, 'General aspects', includes the justification, the purposes of the Strategy (its mission, principles, the values it inspires), the definition of rare diseases and their situation in Spain. In addition it covers their historical development end epidemiological situation. Finally, it sets out the strategy development methodology. The second part, 'Development of strategic lines', sets out the objective and recommendations. The participants of the Strategy decided, by consensus, to establish the following strategic lines: information on rare diseases, Prevention and early detection, Healthcare, Therapies, Integrated health and social care, Research and Training.

- Czech Republic – A ten year strategy (2010-2020) was adopted in 2010 by the Czech Republic. This outlines existing efforts and proposed major targets and measures for improving the situation to be subsequently specified in more detail in the context of a 3 year National Action Plan that will define 'sub-tasks', instruments, responsibilities duties and indicators.

- Austria - In 2010, the working group elaborating the future national plan adopted the definition of the strategic priorities to be covered by the national plan. In Austria 3 public medical universities and one private address adequately medical needs of patients with RDs albeit there is recognition of the need for more improvement. Other unofficial centres of expertise are also in place. A coordinating centre of RD established in 2011 at the Austrian Health Institute (Gesundheit O Sterreich GmH) and

funded by MOH, was given the task to work on the National Plan for Rare Diseases.

- Belgium - In 2010, phase 1 of the recommendations and proposals were elaborated for the Belgian Plan for Rare Diseases, covering the following four central topics (1) diagnostics and treatment; (2) codification and inventory; (3) information, awareness and patient empowerment; and (4) access and cost.

- Cyprus - It was announced on 28 February 2009 that in order to coordinate the best possible existing services for treating rare diseases, and to develop research activities, the Ministry would establish a National Committee for Rare Diseases and apply a strategic plan for rare diseases. The National Steering Committee has since been established and a draft national plan for rare diseases has been elaborated.

- Germany - The Federal Ministry of Health in Germany initiated a national action league for people with rare diseases - Nationales Aktionsbündnis für Menschen mit Seltenen Erkrankungen (NAMSE) – in Berlin on 8 March 2010. NAMSE is a coordination and communication platform comprising all key bodies and organisations. This platform provides the basis for further concerted action, including the implementation of a National Action Plan on Rare Diseases. All partners, the major institutions and stakeholders of the German health care system, adopted a common declaration to improve the health situation for people with rare diseases in Germany.

- Italy – An agreement was signed between the Governement, the Regions and the special statue Provinces of Trento and Bolzano on the proposal of the Ministry of Labour, Health and Social Policy concerning guidelines for the correct use of bound resources by the special statute Regions and Provinces, as provided in art. 1,par. 34 and 34bis, Law dated 23 December 1996, n.662, in order to implement the primary and nationally important objectives for year 2010, including the allotment of €20 million for rare diseases. On 11-13 November 2010 the Italian Federation for Rare Diseases (UNIAMO F.I.M.R ONLUS) in collaboration with EURORDIS organised a national conference on rare diseases in Florence in the context of the EUROPLAN project.

- Romania – In 2010 Romanian National Plan for Rare Diseases was elaborated and transmitted to the Health Ministry, and was included in the national strategy for 2011. the next step will be the implementation of the plan.

- United Kingdom – A UK National Conference on Rare Diaseases, organised by RDUK and Eurordis in the context of the Euroopean conference, took place on 16 November 2010 in Manchester to examine proposals for a plan.

- Luxembourg constitutes an example of the challenges faced by patients with RDs in the absence of national plan for RD and CsE. 95% of patients with RD travelled to neighbouring countries (e.g. Germany) as per the EC Regulation 883/2004. Cross border healthcare amounted to 17.5% of health expenditure in 2009. Challenges were identified both on the side of patients, 28% feeling that they 'co-ordinate' themselves their health, 18% paying themselves for the medical care, 10% renounced psychosocial support and importantly 71% cited linguistic problems implicated in seeking health care cross-border.

Other MS which have not taken yet official first steps or are still at very early phases, include Denmark, Estonia, Hungary, Netherlands and Slovenia.

Further reading

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2. Collins PW, Baglin TP, Dang R, Evans G, Greaves M, Laffan M, Pasi KJ, Rose P, Stanworth S, Toh CH. Non-malignant haematology research in the UK: looking forward to new opportunities. Br J Haematol. 2010; 150(6): 732-6

3. http://www.fanconi.org.uk/Network

2.4 European policies for quality assessment

Quality assessment for laboratories

Laboratory quality is demonstrated through adherence to a quality management system that is audited against international standards. These standards differ from professional, best practice guidelines, which may deal with the selection, reporting and interpretation of diagnostic tests, in the case of laboratory practice, and are based on recommendations from experts. . Accreditation and certification are different: according to ISO/IEC Guide 2, accreditation is defined as the procedure by which 'a an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks', whereas certification is a procedure by which 'a third party gives written assurance that a product, process or service conforms to specified requirements'. For diagnostic laboratories, the following references provide the detail for the development of a quality management system (note that this list is not exhaustive):

- ISO 15189:2007 Medical laboratories – particular requirements for quality and competence. This ISO standard is the key accreditation standard for medical laboratories.
- ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories
- ISO 9001:2000 Quality management systems – Requirements
- ISO 9000:2005 Quality management systems – Fundamentals and vocabulary
- European Communities Confederation of Clinical Chemistry: Essential Criteria for Quality Systems of Medical Laboratories; *Eur J Clin Chem Clin Bioch* (1997); 35: 121-132
- European Communities Confederation of Clinical Chemistry: Additional Essential Criteria for Quality Systems of Medical Laboratories; *Eur J Clin Chem Clin Bioch* (1998); 36: 249-252

Laboratory accreditation is undertaken by a national accreditation body. It is possible for laboratory services to be inspected by government agencies or professional societies and in some countries this may be required in law. .. There is no uniform requirement for laboratory accreditation throughout Europe although it may be mandatory in some countries, either for all laboratories or those providing publicly funded services. End user organisations may also drive accreditation compliance from their service suppliers through the demands of their own quality management system. This same drive may not exist where the end user is an individual patient or clinician.

Accreditation is a process by which an authorised body or organisation gives formal recognition that a laboratory is competent to carry out specific tasks; it is a procedure that ensures the correct conditions for the provision of a quality service exist but does not necessarily measure the quality of the laboratory's output directly. It provides a measure of confidence for the service user and it should be strongly encouraged for expert centres as it provides a means for the harmonisation of laboratory practice. Laboratory accreditation covers all aspects of the service provision, including organisation and the quality management system; personnel; premises and environment; equipment, information systems and materials; the pre-examination, examination and post examination phases of the diagnostic testing process; evaluation and quality assurance. A key quality indicator is the laboratory's use and performance in external quality assessment and participation in EQA, where available, is required for accreditation.

When a network of expert centres is considered, providing diagnostic services across national borders, adherence to an internationally recognised accreditation standard such as ISO15189 becomes more significant, providing independent assurance of the level of service provided. Accreditation is of particular importance where there is a choice of service provider. Private funders of healthcare, such as healthcare insurers, may demand that diagnostic services are only purchased from an accredited provider. Accreditation differs from licensing, in that it is frequently voluntary with inspection by professional

assessors. Mandatory licensing against l standards set by government authorities is also a driver of improvement but there is a conflict of interest if the licensing authority is an agency of a government responsible for the provision of funding to maintain and improve the service.

The role of external quality assessment in the demonstration of competence

External quality assessment (EQA) was first established in the late 1960s as a means of improving the inter-laboratory performance in laboratory medicine. Since that time it has been widely adopted as a recognised part of laboratory quality management and participation in EQA is an essential requirement for laboratory accreditation. EQA complements but does not replace internal quality control (IQC). Whereas IQC will demonstrate that the results of one batch of investigations is comparable to the previous, EQA provides a long term, retrospective analysis of a laboratory's performance in comparison with that of its peers.

Within Europe, there are a number of EQA provider organisations; these may be public or commercial enterprises, operated on a national, regional or local basis. Some operate internationally, within Europe and beyond; by the same token, laboratories in European member states may also participate in EQA programmes from providers outside Europe. Determination of the accuracy and comparability of results between testing centres through the use of EQA is essential for the harmonisation of testing procedures and improved patient care, especially where the methodology is largely manual or only semi-automated. EQA is increasingly a means of assessment of the state of the art in diagnostic testing, providing evidence for the suitability of particular kits, reagents and methods.

EQA is of particular importance in the provision of testing for rare anaemias (RA), as it provides evidence of competence to users of the diagnostic service. This is essential for an expert centre in RA diagnostics, to which patients (or their samples) may be referred for investigation. Definitive testing for RAs often requires complex diagnostic testing, frequently using manual procedures and requiring the subjective interpretation of results. In these cases, EQA can give confidence in the laboratory's output and ensure that results are comparable wherever they are performed. This is of significance when patients may be referred across national boundaries. The best operated EQA programmes will have as their goal the improvement of laboratory performance through education, challenging participants at the levels of clinical decision making and sharing best practice, so that all the participant laboratories can learn from the best.

The selection of an EQA scheme is the responsibility of the laboratory. A well operated EQA scheme will adhere to the following principles:

- Frequent distributions, to ensure timely performance assessment
- Stable, homogeneous survey material that resembles patients' samples as closely as possible. This may be difficult since this often conflicts with the necessity for samples to retain their integrity during the period the survey is open.
- Reliable, valid target values.
- Rapid feedback following data analysis.
- Structured, informative and intelligilble reports.

Accreditation is available for external quality assessment programmes and laboratories should aim to use an accredited EQA scheme where possible; however, it is not possible to make this an absolute requirement until such time as EQA programme accreditation becomes universal. EQA schemes are accredited in a similar fashion to medical laboratories but the key standard in this case is ISO/IEC 17043: Conformity assessment – general requirements for proficiency testing. PrEN14136 (Use of external quality assessment Schemes in the assessment of the performance of in vitro diagnostic examination procedures), is also of importance as it specifies the requirements of an EQA scheme and in particular EQA survey material for the evaluation of in-vitro diagnostic devices.

Laboratories may find that there is no EQA available for some tests utilised in the field of RA. In this case, laboratory accreditation is still possible but the laboratory may need to demonstrate interlaboratory comparability in some other way, e.g. by the interchange of patients' material with other centres providing a similar service.

Within the ENERCA project, educational EQA exercises have been undertaken for specific investigations, for example, for the measurement of Hb A_2 for the diagnosis of beta thalassaemia carrier status and blood film morphology. The project has assessed the provision of EQA across Europe, with the objective of identifying the most significant gaps.

The value of good medical practice in the management of rare anemias; rules and policies across Europe and the medical world

In contrast to the precise regulations which control and assess the quality of Laboratory practices, the quality of Medical Services cannot be easily assessed and expressed in quantitative terms. Consequently, the evaluation procedures which have been adopted to ensure the accreditation of physicians, hospitals or other health-providing Agencies are not precisely defined.

Most of the available documentation related to good medical practice is of British origin. Formal guidelines or clinical indicators published by other European countries are rare, although this is not to say that there is a lack of interest on their behalf. Across the Atlantic, good medical practice is also of utmost importance for US hospitals and physicians, and has led to the publication of several guidelines, instructions and comments, as well as the development of various auditing systems.

A search of the literature does not yield a very clear picture. Information about the assessment of the quality of clinical services can be found under various titles. On one end of the spectrum are titles of direct relevance such as "clinical effectiveness", "good medical practice", and "clinical governance", defined as "*the systematic approach to maintaining and improving the quality of patient care within a health system"* or, according to the British National Health Service, as "*a framework through which NHS organizations are accountable for continuously improving the quality of their services and safeguarding high standards of care by creating an environment in which excellence in clinical care will flourish"* . On the other end of the spectrum are titles with less clear clinical relevance such as "Quality Assurance", "Good Clinical Practice" and "Clinical trials legislation", which refer more to the auditing of clinical trials or the evaluation of various medications and less to the assessment of the quality of patient care. However, it is clear that, here also, the guidelines concerning management of the patients cannot be different from those defined as "good medical practice" and must be rigorously observed because the trials and drug evaluations which are carried out involve not only the patients who are participating in a given trial but also the numerous patients who will be treated in the future following the conclusions of it.

Another factor, which has to be taken into account, is that good medical practice is closely associated with good education and thus creates some overlap, as a large amount of relevant publications consider both issues together.

The present chapter addresses "good medical practice" in reference to the concept of quality of care for the patient, either in general or in the case of specific diseases. Despite the already mentioned confusion regarding terminology and content, the proposed clinical criteria for the assessment of quality of care converge to the same principles and rules. For example, those issued by the British General Medical Council are summarized as follows: "*Patients must be able to trust doctors with their lives and health. To justify that trust, a registered doctor must show respect for human life and make the care of patients his primary concern. He/She must also provide a good standard of practice and care, treat the patients with dignity, work in partnership with the patients, listen to them and provide true and honest answers.*"

Caring for thalassemia and SCD is thus a great challenge for the treating physicians and demands provision of medical services of the best level possible. In terms of a qualitative characterization of these services, all "duties" of an active physician must be performed with utmost respect and care (as defined by the General Medical Council). This category of sensitive patients must be treated with politenesss, dignity, and confidentiality. Treating physicians should be fully updated on recent advances, and keen to collaborate with fellow specialists in order to achieve the maximum benefit for their patients. Patients should be given ample information, have the opportunity to discuss their problems, and to participate in decision making. Lastly, treating physicians should be honest and clear towards their patients, protect them against anything they consider as unjustified risk, and avoid any kind of discrimination.

The above is a good example of a qualitative characterization of good medical care. It must be, however, complemented by criteria for its quantitative assessment as well. For example, according to a seminal methodology paper (Mainz, 2003), quality of care can be defined as *"… the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with profession al knowledge.*" How is then this "degree" measured?

The quantitative expression of quality of care is difficult indeed and must not in any way be based on personal, subjective opinions or feelings. This has led to the development of various types of "indicators" (also, "screens" or "flags") that are used as guides to monitor, evaluate and improve the quality of patient care, clinical support services, and organizational functions that affect patient outcomes. They may serve to make comparisons between hospitals or measure potential improvements within a single hospital over time. Of course, indicators are based on standards of care previously set by various academic or hospital panels on the basis of the available evidence. They therefore reflect the prevailing conditions and may change accordingly.

There are various types of indicators, and may refer to undesirable events, patient options, for example the patient's choice for health providers, as well as other quantifiable characteristics of patient care such as the accountability, regulations and accreditation of the audited service. Indicators may be based on rates (e.g., number of actual events over total possible events within a given time period), or raw numbers (e.g., of sentinel (undesirable) events), or may be related to structure (material or human resources and organization of the environment in which various events take place), medical procedures (given care) and outcome (state of health or events that follow the provision of care, and that may be affected by it).

Another category of indicators are disease-specific indicators, which examine all of the above in relation to one disease or condition only. Such indicators are particularly relevant for the assessment of care of thalassemia, sickle cell disease (SCD), and other rare anemias, because such medical conditions cause a lot of misery and pain to the patients, are the source of great unhappiness to the patients' families, give rise to great demands for blood for transfusion and require expensive medications for continuing treatment. Most importantly, these conditions are mercilessly chronic and have no chance of being cured.

Of course, good medical practice does not apply to individual physicians only; hospitals, outpatient clinics, the laboratories and any Institutions providing health care are subject to Quality Assessment. "QA and Improvement" (QA/I) programs are the tool by which each organization may define the quality of its activities, measure the status of this quality through the appropriate indicators and initiate improvements in performance. Such programs provide the means for the quantitative characterization of the services which are provided to thalassemia, SCD and the rare anemias (and, indeed, all) patients.

Setting up QA/I programs requires a clearly defined process for monitoring, which should include the appropriate indicators, the methods by which the indicators will be measured and those by which the data will be collected and displayed, the individuals who will be responsible, the procedures that will be used to determine the actions which will allow the desired improvement to take place, and the overall expected benefits from employing the QA/I program. In fact, here also, carefully selected indicators are the most objective tool to evaluate the quality of patient care because they can (a) provide rates of specific actions or events (numerators) over the corresponding ideal practices recommended by national organizations or specialty societies (denominators), (b) guide the analysis of (usually unanticipated) events requiring individual reviews, (c) help evaluate statistical data and results in order to identify good or poor patterns and trends which cannot be revealed by individual case analysis, (d) feed the process for analyzing morbidity and mortality data, and (e) peer review specific cases. The final auditing of the aforementioned criteria lies with the quality and integrity of the peer reviewers; the more objective and undisputable are the criteria, the more true and useful are the conclusions and recommendations which will be extracted from their analysis. Obviously this is a prerequisite for fair comparisons, identification of weaknesses, and, of course, for suggesting corrective actions.

An issue often identified through QA/I programs concerns Continuing Medical Education (CME) which is a major pillar of good medical practice. This issue is repeatedly reviewed in the recent literature; it is also intensively promoted not only by several scientific societies and administrative authorities, but also by an ever-increasing number of individuals, i.e., patients through their associations and interested physicians through specific social programs. ENERCA is a typical example of this latter activity. Patients with rare anemias are not seen so often in everyday medicine; however, attending physicians are expected to be fully updated with regards to patients' condition and must perform their duties according to the best possible guidelines of clinical practice. A similarly high level of attention and care is expected from the hospitals and the respective social services. Treating physicians who are deeply involved in all these processes need a lot of up-to-date medical knowledge and their hospitals require substantial administrative preparation. Continuous

Training is therefore an essential prerequisite for their ability to provide good clinical practice and ENERCA supports this activity by organizing workshops and seminars, publishing instructions and other documents and setting up an *ad hoc* website that provides important information.
Of course, good medical practice ultimately depends on an additional factor which cannot be taught or imposed: the "culture" of the treating physician; his or her own way of approaching the patient with kindness and patience, positive attitude in life, moral beliefs and correct behavior. It is all these qualities which, along with good training, ensure the best clinical practice in the field of rare anemias.

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3. METHODOLOGY FOR THE RECOGNITION OF CENTRES OF EXPERTISE AND NETWORKING. THE EXPERIENCE IN RARE ANAEMIAS

3.1. The ENERCA Group on Rare Anaemias (EGRA)

• A working group within ENERCA partners was created integrating different professional profiles, namely the European Group on Rare Anaemias (EGRA). EGRA was composed of a) physicians, b) molecular biologists, c) legal –ethical experts and d) patients associations.

• The methodology followed in order to achieve the objectives of the EGRA has been characterized by three main notes:

- **Interdisciplinary**, that is to say, integrating the work of experts from different areas. The quality of a centre is based on clinical and laboratory requirements, but also on ethical and legal criteria, and patient's expectations must be also taken into consideration.

- **European coverage has been one of** the main concerns. Accordingly, the EGRA group is made up of experts from eight different European countries: Belgium, Cyprus, France, Germany, Italy, Spain, The Netherlands and United Kingdom. Involvement of other MS, especially New MS, has been assured by a widespread of the different surveys performed, the seeking of experts from different countries and the involvement of TIF, as international umbrella for patients' associations.

- **Evidence based.** In order to assure that the final results are as realistic as possible, the starting point was to approach the current situation, a kind of picture of the real practice.

• Working phases have been developed from three different perspectives, led by an expert in the concrete field but at the same time involving the other EGRA members:

- Legal and Ethical perspective
- Clinical and Laboratory perspective
- Patient's expectations perspective

- With this general approach, the concrete steps have been the following:
    - Analysis of the current situation for the identification of the main relevant issues that have to be addressed into recommendations for the recognition of centres of expertise and the creation of European reference networks.
    - Surveys based on this analysis, in order to check in practice how to translate these relevant issues into recommendations in a realistic way.
    - Analysis of the Surveys' results
    - Establishment of the ENERCA recommendations for Centres of Expertise (CEs) and for the European Reference Network (ERN) on Rare Anaemias (RA) with the cooperation of external advisers and a large number of centres, professionals and patients.

The consecutive working phases were performed as detailed in the following section.

3.2. Current situation analysis in Europe. Relevant issues

In order to identify the main relevant issues that have to be addressed into recommendations different mechanisms have been followed by each specific perspective. To investigate each aspect of the available and prposed services for rare anaemias, a series of questionnaires were prepared through the Enerca project. These questionnaires addressed the issues of legal and ethical practices in networking and exchanging samples and information, clinical and laboratory standards with external quality control. A literature review was conducted using a specific methodology. A separate questionnaire was prepared to explore the patients' view of the services and their expectations of an ideal centre of expertise.

The questionnaires:

a) **Legal and ethical:**

- A survey was conducted by means of a questionnaire sent by e-mail to the 48 ENERCA centres (ENERCA partners). The objectives were two: first one, to detect the way by which the centres manage in clinical practice the issues related to patient's rights: information and consent and rights concerning data and samples; and second one, to find out if there were some homogeneous procedures..
- The questionnaire included a list of items that were proposed by the legal experts and consensuated by the EGRA **(Annex 1)**; it was made to be answered in a short time.
- The content of the questionnaire was structured in seven groups of items that refer to the following matters:

    - Checking quality standards in the circulation of samples or data through Europe;
    - Organizational issues involved in data transfer and patient mobility;
    - Personal Data Issues;
    - Obtention, use, transfer, storage and destruction of biological samples;
    - Referring patients;
    - Genetic Counselling;
    - Knowledge/awareness about the applicable legislation

- Answers from 23 centres (48%) were received distributed in the following countries: Belgium (1), Cyprus (1), Denmark (1), France (6), Germany (1), Grece (1), Italy (4), Netherlands (1) Portugal (1), Romania (1), Serbia (2), Spain (1), UK (2). Among these centres were private and public ones, clinical departments, diagnostic laboratory, research laboratories and others (eg. blood transfusion service).
- The answers were analized in order to have a comparative idea of the criteria and procedures followed by different centres and to identify the items that should be studied from the legal and ethical perspective. The main conclusion was the confirmation about the general awareness of the respect to ethical / legal principles in the clinical practice, as well as the lack of homogeneous criteria of procedure in the practices (see **Annex 2).**
- Two representative examples were the following. First, the answers to the question concerning the period of storage of samples: 45 % answered "years", 27% didn't answered, and the other answers indicated different periods ranging

from “6 months” (5%) to “unlimited period” (4%), or “no time established” (9%). Second, the answers to the question concerning the requirement of the patients consent to send the samples to other centres for diagnosis purposes: 54% answered “yes,” 32 % answered “no”, and 14% didn’t answered.

- It was confirmed the need to make a legal analysis in order to explore the possibilitys of harmonisation, and to have some homogeneous criteria in a network, among which patients, data and samples are going to circulate.
- These conclusions have be sent to national legal experts of ten Member States, who may use them as a basis for a legal study that should have a similar outline.

b) Clinical and laboratory

- The methodology is here described for identifying the characteristics and the necessary components of CEs in RA, so that recommendations obtained can be put forward for their structure. The EGRA poses the question whether additional, or disease specific criteria are needed for CEs dealing with chronic non-malignant haematological disorders. To answer the question a review of the special needs of such disorders is presented here in order to identify the specific services needed which must be catered for in the case of centres for RA. The special criteria are divided into the laboratory/diagnostic requirements and the requirements for clinical management. In this section we examine the separate requirements for red cell disorders prior to reporting on the findings of the ENERCA questionnaires
- A Desk research was undertaken to set up the facilities that should be provided by a CE in part or all the different RAs ; Thalassaemia, Sickle cell anaemia and very RA. On the light of the results obtained, the EGRA investigated, through questionnaires, whether these criteria were met by centres in Europe, whether they are officially recognised as such by local authorities or whether are self designated.
- The definition of these facilities was obtained from published documents in the field by the European Commission (EC) and the review of the literature :

a) Documents published by the EC Review overlapping with background chapter by AE

- In July 2004 the Commission High Level Group (HLG) on Health Services and Medical Care was established to bring together experts from all MS to work on practical aspects of collaboration between national health systems an the EU.

One of this HLG was focusing on European Reference Networks (ERN) for rare diseases (RD). Some criteria and principles for ERNs have been developed. Later in December 2006 an expert group of the RDTF outlined the importance of identifying centres of expertise (CEs) and the roles that such centres should fulfil **(Annex)**.

• In October 2011, the European Union Committee of Experts on RDs (EUCERD) , that replaced the Rare Disease Task Force (RDTF), produced recommendations on quality criteria for national Expert Centres in RDs that have been used by the EGRA for identifying the peculiar needs of patients with rare anaemias and the corresponding facilities to be offered by the expert centres.

• **The final list of the EC documents selected as the basis of ENERCA recommendations are the following:**

- Rare Disease Task Force set up by DG Sanco (http://ec.ewuropa.eu/health/ph_threats/non-com/docs/contribution_policy.pdf).
- Council Recommendation (2009/C 151/02) of 8 June on an action in the field of rare diseases

  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2009:151:0007:0010:EN:PDF
- Directive (EC 2011/24/EU) of the European Parliament and of the Council on the application of patients' rights in cross-border health care

  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:088:0045:0065:EN:PDF *EUCERD Recommendations on Quality Criteria for National Centres of Expertise for Rare Diseases (For adoption on 24 October 2011)* 4
- Work of the HLG on Health Services and Medical Care during 2005

  http://ec.europa.eu/health/archive/ph_overview/co_operation/mobility/docs/highlevel_2005_013_en.pdf
- RDTF Report: Overview of Current Centres of Reference on rare diseases in the EU (September 2005)

  http://www.eucerd.eu/upload/file/Publication/RDTFECR2005.pdf

- RDTF Report: Centres of Reference for Rare Diseases in Europe – State-of-the-art in 2006 and Recommendations of the Rare Diseases Task Force (September 2006)

  http://www.eucerd.eu/upload/file/Publication/RDTFECR2006.pdf

- RDTF Report: European Reference Networks in the field of Rare Diseases: State of the art and Future Directions (July 2008)

  http://www.eucerd.eu/upload/file/Publication/RDTFERN2008.pdf

- EUCERD Workshop Report: Centres of expertise and European Reference Networks for Rare Diseases (8-9/12/2010)

  http://www.eucerd.eu/upload/file/WorkshopReport/EUCERDWorkshopReportCECERN.pdf

- EUCERD Workshop Report: National centres of expertise for rare diseases and networking between centres of expertise for rare diseases (21-22/03/2011)

  http://www.eucerd.eu/upload/file/EUCERDReport220311.pdf

- EUCERD Report: Preliminary analysis of the experiences and outcomes of ERNs for rare diseases (May 2011)

  http://www.eucerd.eu/upload/file/Reports/2011ERNAnalysis.pdf

- EUROPLAN: Recommendations for the development of National Plans and Strategies for rare diseases

- http://www.europlanproject.eu/public/contenuti/files/Guidance_Doc_EUROPLAN_20100601_final.pdf

c) Review of the literature:

Identification and location of appropriate information: such information may be found in textbooks, medical journals and through direct internet enquiries. To identify both past and current experience, so that a state of the art picture of a CE can be constructed, the most productive method is to identify the databases of medical references. The key words of the literature review are defined as “Expert Centres for Chronic Disorders” and “Disease specific centres for the management of anaemias such as “thalassaemia”, “sickle cell disease” and “other rare anaemias”.

• Medline – as service of the U.S. National Literary of Medicine and the National Institutes of Health. Search was made by using the PubMed service, which includes over 18 million citations of biomedical literature. Search can be made by keywords, authors, journal etc.

- EMBASE –is a database that includes more than 23 million validated biomedical records.

The Cochrane Library –is a collection of databases that contain high quality, independent reviews, abstracts, clinical trials etc. The most useful database for the purposes of EGRA is the Cochrane Reviews which are evidence based reviews on which to base clinical decisions. The Cochrane library can be accessed on the following site: www.wiley.com/cochrane and the reviews on: www.cochrane.org/reviews.

- The Database of Abstracts of Reviews of Effects (DARE) – is a database of abstracts of systematic reviews focused on the effects of interventions used in health and social care. This Database is owned by the Centre of reviews and Disemination of the National Research Institute of Health Reseach of the NHS of the UK. Access: http://www.crd.york.ac.uk
- CINAHL Journals database – The Cumulative Index to Nursing and Allied Health Literature (CINAHL) was originally an index to nursing literature but has now developed into a comprehensive bibliographic index and includes abstracts and full text materials from selected journals. It can be accessed through http://www.cinahl.com/library/journals.htm and through http://www.ebsco.com

Reading and critically evaluating the information collected: literature results were filtered and the relevant articles shared with all the EGRA members involved.

Results from the search: the evidence base for the designation standards for rare and very rare anaemias

General: Standards, Guidelines and Quality requirement

- WHO resolution on birth defects 2010

Resolution WHA63.17. Birth defects. In: Sixty-third World Health Assembly, Geneva, 21 May 2010 (WHA63/2010/REC/1)

- Belgium: Recommendations and Proposal in view of the Belgian plan for Rare Diseases 2010 *http://www.kbs-frb.be/uploadedFiles/KBS-FRB/05)_Pictures,_documents_and_external_sites/09)_Publications/PUB_2024_PlanBelgePourLesMaladiesRares_DEF.pdf*
- France: Plan for Rare Diseases 2005-2008 *http://www.orpha.net/actor/Orphanews/2006/doc/plan_national.pdf*

- United Kingdom: The Specialised Services National Definitions Set - *http://www.specialisedservices.nhs.uk/info/specialised-services-national-definitions*
- Italy: Regulation for the institution of the Italian National Network for rare diseases. Ministerial decree 279/2001 no www.iss.it/cnmr/ http://www.iss.it/binary/ccmr/cont/DM279.2001.1235501223.pdf

Haemoglobinopathies: standards, guidelines and quality requirement

International

- WHO resolutions (and publications) on haemoglobin disorders
- WHO, 2006. Executive Board Resolution on Sickle Cell Anaemia. EB117.R3. WHO, Geneva, Switzerland
- WHO, 2006. World Health Assembly Resolution on Sickle Cell Anaemia. WHA59.20. WHO, Geneva, Switzerland
- WHO, 2006. Executive Board Resolution on Thalassaemia and Other Haemoglobinopathies. EB118.R1. WHO, Geneva, Switzerland
- WHO, 2007. Report of Joint WHO/TIF Meeting. Management of Haemoglobin Disorders, Nicosia, Cyprus, 16-18 November 2007.
- Prevention of Thalassaemia and other Haemoglobin Disorders vol 2 Laboratory Methods. Published by TIF - 2005
- Guidelines for the Clinical Management of Thalassaemia 2nd Revised Edition. Published by TIF – 2008

Europe

- Best Practice Guidelines published by the European Molecular Genetics Quality Network (EMQN): www.emqn.org/emqn.php

United Kingdom

- Specialised Haemoglobinopathy Services (all ages) - Definition No. 38 - http://www.specialisedservices.nhs.uk/doc/specialised-haemoglobinopathy-services-all-ages
- Sickle cell and Thalassaemia – Handbook for laboratories 2009
- Standards for the Clinical Care of Children and Adults with Thalassaemia in the UK 2008 – second edition. First edition, 2005.
- Standards for the Clinical Care of Adults with Sickle Cell Disease in the UK - 2008.
- Standards for the Linked Antenatal and Newborn Screening Programme (Sickle Cell and Thalassaemia) -2006

France

- Prise en charge de la drépanocytose chez l'enfant et l'adolescent – 2005 http://www.has-ante.fr/portail/upload/docs/application/pdf/Drepanocytose_reco.pdf
- Protocole national de diagnostic et de soins (PNDS) ALD n° 10 - Syndromes drépanocytaires majeurs de l'adulte. 2010. http://www.has-sante.fr/portail/jcms/c_938884/ald-n-10-syndromes-drepanocytaires-majeurs-de-l-adulte
- Protocole national de diagnostic et de soins (PNDS) ALD n° 10 - Syndromes thalassémiques majeurs et intermédiaires. 2008. http://www.has-sante.fr/portail/jcms/c_680242/ald-n-10-syndromes-thalassemiques-majeurs-et-intermediaires

Italy

- Regional Network for Rare diseases. Diagnostic, therapeutic and care pathways (PDTA) for thalassemias: http://malattierare.marionegri.it/images/downloads/PDTA/PDTA_schede/talassemie.pdf

- Associazione Italiana Ematologia ed Oncologia Pediatrica (AIEOP). Linee guida per la gestione della malattia drepanocitica in eta' pediatrica in Italia. http://www.aieop.org/files/files_htmlarea/Linee%20guida%20per%20la%20gestione%20della%20malattia%20drepanocitica%20v2%20del%2017.05.2012.pdf

ENERCA

- ENERCA recommendations and training courses see http://www.enerca/org and De Montalembert M et al ENERCA clinical recommendations for disease management and prevention of complications of sickle cell disease in children Am J Hematol 2011

Rare and very rare anaemias: standards, guidelines and quality requirement

- Leitlinien der Gesellschäft für Pädiatrische Onkologie und Hämatologie. www.awmf.org/uploads/tx_szleitlinien/025-018l_S1_HereditäreHereditaere_Sphaerozytose.pdf
- Leitlinien der Deutschen Gesellschaft für Hämatologie und Onkologie (Hämatologische Erkrankungen - nichtmaligne dgho-onkopedi.de
- Kinderblutkrankheiten.de
- Linee guida per la prevenzione e cura del favismo ed altre sindromi emolitiche correlate a carenza di glucosio-6-fosfato deidrogenasi.

http://www.deficitg6pd.it/Convegno_16-12-2004_Roma/Linee_Guida.htm

- Recommended methods for the characterization of red cell pyruvate kinase variants. International Committee for Standardization in Haematology. BrJHaematol. 1979 Oct;43(2):275-86. PubMed PMID: 41566.
- G6PD deficiency Favism Association - http://www.g6pd.org/
- Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ; General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis and management of hereditary spherocytosis-2011 update. Br J Haematol. 2012 Jan;156(1):37-49. doi: 10.1111/j.1365-2141.2011.08921.x. Epub 2011 Nov 5. PubMed PMID: 22055020
- **ENERCA recommendations and training courses see http://www.enerca/org**
- **Vigifavisme website:** www.vigifavisme.com

Summary report: The EGRA members involved made their comments and conclusion in a summary report that was the basis for the questionnaire. Annexes

Once the facilities were established two questionnaires were developed to assess the accomplishment of the European centres to the identified facilities:

a) Questionnaire 1 - Services for general lab and clinical management for RA: Includes services for general lab and clinical management common for all the categories of Rare anaemias
b) Questionnaire 2 - Specific laboratory tests for RA except haemoglobinopathies: Includes speficic test for the "very rare anaemias" due to the high heterogeneity of laboratory procedures performed in the diagnosis of this group of rare anaemias

Some questions related to specific relevant issues have been selected as examples of the methodology used for preparing the questionnaire:

1. **Are there existing Centres of reference, of expertise?**
2. **What is the expertise covered and how is it defined?**
3. **How is the expertise recognized?**
4. What are the number and geographical distribution of centres R/E in each EU country?

1. Are there existing Centres of reference, of expertise?

The concept of centres of reference for rare diseases has been already adopted in six EU countries: Bulgaria, Denmark, France, Italy, Spain and Sweden.

Is it the case for rare anaemia?

The concept of official centres of reference has been adopted but outside a national policy for rare diseases in eight EU countries: Belgium, Croatia, Czech Republic, Finland, Ireland, Greece, Portugal, UK

Or

The concept doesn't exist in the other EU countries (nevertheless, sometimes, centres of expertise exist): Austria, Cyprus, Estonia, Germany, Hungary, Latvia, Lithuania, Luxembourg, Malta, Norway, Netherlands, Poland, Romania, Serbia, Slovakia, and Slovenia

What has been done in the case of rare anaemia?

Existing data:

Orphanet

An attempt to identify expert clinics in Europe has been made by Orphanet (www.orpha.net), the European database of specialised services in the area of rare diseases. The selection was based on quality indicators including national reputation, high volume of relevant activity, appropriate capacity to manage patients, and a high level of expertise as documented through publications, grants and international collaborations.

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Self declaration of expert centres on rare anaemias

2. What is the expertise covered and how is it defined?

Even if the national health systems are heterogeneous, the expertise should be divided in different fields :

- Diagnosis
- Follow-up
- Management
- Research
- Adapted for children only, adults only or both

Each CR/E should not necessaryly cover all the different fields; a collaboration with other expert centres at the national and international levels are encouraged.

3. How is the expertise recognized?

The CR/E should comply with the following criteria

- Appropriate capacity to diagnose, to follow-up and manage patients with evidence of good outcomes when applicable
- Sufficient capacity to provide expert advice, diagnosis or confirmation of diagnosis, to produce and adhere to good practice guidelines and to implement outcome measures and quality control

- Demonstration of a multi-disciplinary approach
- High level of expertise and experience documented through publications, grants or honorary positions, teaching and training activities
- Strong contribution to research
- Involvement in epidemiological surveillance, such as registries
- Close links and collaboration with other expert centres at the national and international levels and a capacity to network
- Close links and collaboration with patients associations where they exist

All those criteria, asked with the questionnaire, have to be adapted regarding the particular disease covered.

4. What are the number and geographical distribution of centres R/E in each EU country?

The full address (+ website) should be asked trough the questionnaire.

d) External Quality Assessment for laboratories

The low prevalence of rare anaemias makes the provision of EQA for the specialist laboratory tests a challenge for national EQA providers in individual member states since good EQA requires a minimum number of participating laboratories for financial and statistical viability.
Accordingly, the starting point was:

- To identify the list of core laboratory procedures that are consider essential for the diagnosis of rare anaemias
- To assess the use of these core laboratory procedures across European laboratories

A first list of core laboratory procedures linked with the list of RA already available in ENERCA website was prepared. It was produced based on a research of the specific and general laboratory tests advised in the literature for the laboratory diagnosis of each condition. The list was agreed by EGRA and led to a questionnaire aiming to assess the use of these core laboratory procedures across European laboratories. This questionnaire was delivered electronically to the ENERCA network of centres (ENERCA partners). (Annexes)

Based on the results from this questionnaire, a final core list of laboratory tests that are used for the diagnosis of rare anaemias was established. This includes non-specific, general laboratory investigations used in the diagnosis and monitoring of a wide range of conditions and specialist investigations used primarily for the diagnosis of individual diseases. (Annex)

e) Patient's Expectations

- The basis for identifying the items for the patient questionnaire has been the first version drew on the PACIC (Patient Assessment of Care for Chronic Conditions) questionnaire which was created by the Wagner group to assess the patient experience of the services for chronic diseases that they are receiving.

- This questionnaire was adapted by adding new questions which were derived from a questionnaire prepared by patients of the United Kingdom Thalassaemia Society (UKTS) and divided into the following sections:

2. Section 1 has the purpose of describing the patient who is responding, without identifying. Information collected includes age, gender, marital status, education, employment, ethnic origin and country of residence. This section is optional although all responses completed the section.
3. Section 2 collects medical information about the patient and is also optional. The questions include the diagnosis, the current transfusion regime, iron chelation and information about sickle cell disease.
4. Section 3 is not optional and seeks to describe the medical services the patient is currently receiving and whether these services are accessible and convenient. It includes questions such as where the patient is treated, in a specialised centre or in general hospital services, whether services interfere with normal living needs (such as long waiting time and available at times which interfere with work and education), access to centres, financing of treatment, availability of specialists for complications, availability of information and whether the patient feels that they are receiving correct treatment. Section 3a also assesses the patient responses to services and concentrates on quality of services based on the PACIC questionnaire.
5. Section 4 is the part which assesses the patients' expectations of a specialised centre. In this part 19 questions suggest to each patient elements or features of an ideal centre and they are asked to grade them from 'not necessary' to 'essential'. The final part of the section asks the patient to describe their expectations from the service, the doctors, the nurses and the associations. Comments are invited in all sections.

- Two partner patient organisations were asked to contribute questions and review the questionnaire. These were the UK Thalassaemia Society (UKTS) and the Cyprus Anti-anaemia Association (PAS). Many questions were contributed by UKTS and members of PAS also reviewed as a patient focus group.

3.3. Performing the surveys

Based on the analysis performed in the previous tasks a catalogue of surveys was conducted in order to check in practice how to translate these relevant issues into recommendations in a realistic way.

a) Legal and ethical

- As a result of the questionnaire sent to the centres asking for their practices dealing with patients's samples and data, it was concluded that there was no a homogeneous criteria in this area. With the aim of facilitating the networking, we wanted to know if there were some legal criteria that could help in this sense. The goal of this stage was the analysis of the situation of current trans-national regulations in the EU for patients, biological samples and data exchanges between the different Member States. We focused in these points, as we considered that the circulation of samples and data is the most suitable way to facilitate the networking in patient's diagnosis and treatment, although some considerations concerning patients's circulation were also adressed.
- A second protocol, including a questionnaire has been prepared aiming to analyze the trans-national regulations currently existing between MS. This questionnaire includes a list of legal and ethical target points, that is the main

situations or practices from which legal or ethical issues could arise. The questionnaire deals with very similar items that the first one sent to the centres and it was sent to legal experts of ten MS, who use them as a basis for a legal study that should have a similar structure.

The questionnaire has been sent to experts selected so that all geographical areas of the European Union (EU) should be represented. All of them have experience in medical law and in the participation in international projects.
They analysed how the different national regulations and ethical rules are considered in each MS and prepared report (answering the questionnaire) containing information about all the relevant documents existing in each MS.
With the analysis of the answers of the questionnaires / reports, we had the basis to undertake a comparative analysis of the situation between the different MS and propose recommendations.

A final ethical - legal report had been elaborated, included the national reports and the Pan European comparative analysis. It was presented to the steering committee in the 5th Executive meeting held in Sitges, Spain (20th June 2011) and sent to the EGRA for revision.

- The global report shows all the work done by the experts, identified in a color code by country, as well as a conclusion by item. In order to make the information easily comprehensible, the report was structured the following way: firstly, we ordered its results on the basis of the questions; then the answers were inserted following a color code, so as it was easy to distinguish which answer corresponds to each country. Prior to the body of the answers, it was included a summary table, highlighting the most relevant aspects of the issues in each country and a brief conclusion. It should be underlined that at the moment the national reports were received, the Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross-border healthcare was not approved.
- These are the experts who have contributed with their reports:

SPAIN (S)	Carlos Romeo and Pilar Nicolás. Interuniversity Chair in Law and the Human Genome, University of Deusto, University of the Basque Country, Bilbao.
GERMANY G)	Jürgen Simon, University Lüneburg.
BELGIUM (B)	Herman Nys Faculteit der Geneeskunde Centrum voor Bio-Medische Ethiek en Recht Katholieke Universiteit Leuven.
THE NETHERLANDS (N)	Andre Den Exter, Erasmus University Rotterdam.
ITALY (I)	Carlo Casonato, Fabio Cembrani and Simone Pensa, School of Law, DSG Trento University.
PORTUGAL (P)	Helena Pereira de Melo, Faculty of Law, New University of Lisbon.
FRANCE (F)	Myriam Blumberg Mokri, Biomedical expert Lawer, Paris.
CZECH REPUBLIC (CR)	Alena Pejcochova, Interuniversity Chair in Law and the Human Genome, University of Deusto, University of the Basque Country, Bilbao.
CYPRUS (C)	Michael Angastiniotis, Medical Advisor of Thalassaemia International Federation.
UNITED KINGDOM (UK)	Claudia Pitz and Lisette Bongers, Maastricht University.

b) Clinical and laboratory

Based on the previous activities a catalogue of surveys was produced aiming to assess in a realistic way the adequacy of the different services identified involving diagnosis, clinical care and external quality assessment for Rare anaemias:

a) Questionnaire 1 - Services for general lab and clinical management for RA: Includes services for general lab and clinical management common for all the categories of Rare anaemias
b) Questionnaire 2 - Specific laboratory tests for RA except haemoglobinopathies: Includes speficic test for the "very rare anaemias" due to the high heterogeneity of laboratory procedures performed in the diagnosis of this group of rare anaemias
c) Questionnaire 3 - External Quality Assessment availability: Includes the assessment of the availability of EQAS for RA diagnosis from EQAS providers across Europe

Questionnaires 1 and 2 were distributed among ENERCA centres (ENERCA partners) and spread among known European centres treating patients with RA via:

- Centres identified through the ENERCA Project. Information about these centres is available on http://www.enerca.org

- Centres listed by each ENERCA associated or collaborating partner, either in their own countries or in other countries known through literature or previous collaborations.
- National and local scientific societies.

Questionnaire 3 - External Quality Assessment availability

According to the list of core procedures used in the diagnosis of rare anaemias, a questionnaire was elaborated to determine the availability of EQA provision for the investigations in the core list and the willingness of EQA provider organisations to collaborate across national boundaries

The use of higher order reference methods, when available, to determine target values in EQA and to calibrate In-vitro diagnostic devices (IVDDs) has also been examined as part of the survey.

EQA providers within Europe were contacted via the European Quality Assessment in Laboratory Medicine (EQALM) organisation. The organiser of each EQA organisation registered with EQALM was sent the questionnaire ENQUE H2, which had been developed with the assistance of the ENERCA Executive Committee.

Each EQA provider was sent a copy of the Core List of Laboratory Tests and the questionnaire. The questionnaire, which is displayed in full in Annex X, sought information on the provision of EQA services for each test on the core list, including:

- Analytes covered,
- Frequency of distribution,
- Type of survey material,
- Number of participants,
- Performance monitoring,
- 'Wishlist' of new provision,
- Potential for collaboration,
- Accreditation status.

c) Patient's expectations

Methodology used to develop the patients questionnaire

This is a composite questionnaire based on an original one developed by the United Kingdom Thalassaemia Society (UKTS) and supplemented with additional questions, from the PACIC questionnaire which was developed to examine patients' responses to the Chronic Care Model.

Two partner patient organisations were asked to contribute questions and review the questionnaire. These were the UK Thalassaemia Society (UKTS) and the Cyprus Anti-anaemia Association (PAS). Members of PAS also served as a patient focus group to initially test the questionnaire.

The draft of the questionnaire was reviewed by the ENERCA partners, from WPs 1, 3, 4, 5 and 6.

The sections of the questionnaire were designed to address the following questions:

Section 1: The demographics and patient characteristics.

Information collected includes age, gender, marital status, education, employment, ethnic origin and country of residence.

Section 2: The treatment currently received.

Section 3: The access to treatment centres.

Section 3a: Patient assessment of the services that they received.

This section assesses the patient evaluation of services and concentrates on quality of services.

Section 4: Patient preferences

- The questionnaire has been translated into different languages and distributed among at least 14 thalassaemia patient associations (i.e. TIF's existing European members) and European sickle cell patients' associations (identified through internet search and with help from the UK Sickle Cell Society). The final list of associations will represent the great majority of SCD and thalassaemia patients in Europe.

Translations to local languages have been made using the method of translation and retranslation by a different person. The languages so far are Italian, Greek, Bulgarian, Turkish, Romanian, Portugese, French, German and Spanish.

The final questionnaire was distributed to patients throughout Europe, via associations and clinics and by Enerca partners. The answers were collected by courier or by post

3.4 Surveys' results

• The results of the questionnaires were analyzed in order to achieve useful conclusions as the bases for our last goal: the ENERCA consensus recommendations

a) Legal and Ethical

• The meaning of some legal terms varies from a country to another and is under evolution and revision, including in the EU legislation (eg. The term "personal data"). Thus, the comparative analysis is not always 100% reliable. There are several concrete matters which show a lack of legal regulation in some countries (eg.The period of conservation of the samples). Consequently there was no legal basis to settle the recommendation needed. Although the countries included in the study were chosen following a geographical basis criteria, the number of them was just ten.

• The reports were compiled into a comparative one, organized by questions numbers. The answers were resumed in schematic tables with conclusions. This final report was sent back to the experts as well as to the EGRA for review. These were the items proposed to the experts and a brief conclusion after comparing their answers:

1. Introduction. Legal framework

1.1. Specific or general provisions about patients' circulation in Europe.

In general, when citizens receive medical treatment abroad their country, they will be reimbursed only in certain circumstances. A condition is that the treatment is offered in their own country.

1.2. Specific or general provisions about data or biological samples exchange.

There are some legal requirement for the international transfer of data and samples that are not the same in all countries (subject consent, authorization of an administrative authority, agreement between parts…)

1.3. Specific regulation of electronic clinical records.

In general, no specific regulation, but there are plans for the future implementation of the electronic clinical records and further legislation could be enacted

1.4. Specific regulation of genetic counselling.

Different answers. In some countries there are no specific provisions; in others few rules that have to be develop, others have more detailed requirements referred to the professionals involved, organization of the service, information to patients, etc.

1.5. Does accreditation of genetic counsellors exist in your country?

There is a need to clarify the working field. In most countries accreditation for Clinical genetics exists as a medical specialty. The Commision Regulation (EU) No 213/2011 of 3 March 2011 has amended Annexes II and V to Directive 2005/36/EC of the European Parliament and of the Council on the recognition of professional qualifications. The Regulation includes medical genetics in the list on the recognition of professional qualifications.

1.6. Specific or general provisions about quality standards for clinical department/ laboratories.

In general there are national provisions about quality standards. In some cases special requirements are established for genetic laboratories. In other cases further legal development is foreseen. In the international Level ISO 15189:2003 establishes particular requirements for quality and competence of Medical Laboratories.

1.7. Is there any regulation about the Ethical Committees?

Ethical committees are regulated in all the countries following the Directive Directive of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

According to UNESCO International Declaration on Human Genetic Data, 2003 "Independent, multidisciplinary and pluralist ethics committees should be promoted and established at national, regional, local or institutional levels, in accordance with the provisions of Article 16 of the Universal Declaration on the Human Genome and Human Rights. Where appropriate, ethics committees at national level should be consulted with regard to the establishment of standards, regulations and guidelines for the collection, processing, use and storage of human genetic data, human proteomic data and biological samples. They should also be consulted concerning matters where there is no domestic law. Ethics committees at institutional or local levels should be consulted with regard to their application to specific research projects"

According to the Recommendation Rec(2006)4 CE on research on biological materials of human origin: 2. Member states should apply the provisions concerning ethics committees contained in chapter III of the Additional Protocol concerning biomedical research (CETS No. 195, 2005) to the review of research within the scope of this recommendation. This Protocol states that "Every research project shall be submitted for independent examination of its ethical acceptability to an ethics committee. Such projects shall be submitted to independent examination in each State in which any research activity is to take place".

1.8. Is there any legal distinction in this area between private or public centres?

There is no legal distinction between private or public centres

2. Patient's Personal Data

2.1. Definitions: personal data / codified data/ anonymous data.

There is a common definition (Directive on data protection) but there are not specific criteria to determine the meaning of the possibility of identification.

2.2. Period of storage the clinical data.

In general there is no a concrete period of time previewed for the storage

2.3. Security measures for the storage of health data (paper or electronic format)

The person responsible of the data file has the duty to implement high level security measures. The responsible of the data file shall implement appropriate technical and organizational measures required by the Directive and national laws. These measures shall guarantee an appropriate level of security. Health data must be protected by high level guarantees.

2.4. Provisions about the transfer of clinical data: national or international

Clarification of the implementation of article 8 of the Directive 95/46/EC and the establishment of a harmonized procedure is needed.

According to article 8 (The processing of special categories of data), Member States shall prohibit the processing of personal data revealing racial or ethnic origin, political opinions, religious or philosophical beliefs, trade-union membership, and the processing of data concerning health or sex life. This shall not apply, among other cases, where the data subject has given his explicit consent to the processing of those data, except where the laws of the Member State provide that the prohibition referred to in paragraph 1 may not be lifted by the data subject's giving his consent; Paragraph 1 shall not apply where processing of the data is required for the purposes of preventive medicine, medical diagnosis, the provision of care or treatment or the management of health-care services, and where those data are processed by a health professional subject under national law or rules established by national competent bodies to the obligation of professional secrecy or by another person also subject to an equivalent obligation of secrecy. But it is not clear if the exception of the management of health – care services includes those abroad the country (and not just the National Health system).

2.5. Conditions for using the data personal / codified / anonimyzedanonymized data for research studies: consent, intervention of an ethics committees.....

In the case of research with data, there is no homogeneous rules about the need of consent (in some cases are exceptions) or the need of an ethical revision.

3. Patient's Samples

3.1. Conditions to obtain and analyse the samples with diagnostic purposes (information, consent....).
In some countries there are differences in the requirements based on the procedure to obtain the sample (invasive or not) or the nature of the information that is going to be obtained (genetic / other health information).

In other cases no specific provision has been enacted in this field, so general rules are applicable and consent is required as the analysis is a medical service and personal data are going to be collected

3.2. Period of storage samples for diagnosis purposes.

There are no specific provisions in the European countries examined in general terms. However, some national Laws established a concrete period of time that is between 15 and 30 years.

3.3. Who is the responsible of the storage?

There are few specific provisions. Options: the physician or the centre.

3.4. Does the laboratory have to check that the consent has been obtained by the physician or other health professional?

The checking is not necessary in most of the European countries examined.

3.5. It the consent necessary to send samples to other centres for diagnostic purposes?

In some cases no provision appears, but the general rule, would be to understand that the original consent provides enough guarantees. In some countries this is expressly previewed.

3.6. Which information should be given for using the sample for diagnostic purposes?

There is a high level of consensus concerning these points:

a) Purpose of analysis and type of information you are going to obtain, especially, if DNA or RNA data are going to be obtained

b) Possible inconveniences linked to the collection of samples

c) Location of the undertaking of the analysis and the destination of the sample after the analysis: storage for diagnostic purposes codification, destruction, use for research purposes

d) Guarantee of confidentiality of the information obtained

e) His/Her faculty to take a stance in relation to the communication to him / herself of the data obtained in the research

f) Information regarding the implication of the results for his/her family members

g) The convenience that the person, where appropriate, transmit this information to the family members, in case it would be relevant for their health

h) Indicating the possibility to get in contact with him/her, and the way to do so

i) Information regarding the data will be stored

j) Identification and contact details of the person responsible for the storage of data

k) Procedure to exercise his / her rights regarding to the data storage

l) Provisions about international circulation of data

m) Provisions about international circulation of samples

3.7. Conditions to obtain and analyse the samples with research purposes.

Consent is always required for the removal and subsequent use for research.

3.8. It is necessary the specific consent to use for research a sample that has been obtained for diagnosis?

In this area there is an important distinction between those laws that do not require added consent to the already expressed consent for diagnostic purposes, and laws that require another consent. In general these laws include exceptions

3.9. Which information should be given to the patient before his/ her consent for using the sample for research purposes?

Taking into account the general provisions a high level of consensus exists concerning the following points, unless in detailed points referred to genetic data and to return of results.

a) Purpose of the project research, especially, if DNA or RNA data are going to be obtained.

b) Expected benefits

c) Possible inconveniences linked to the donation and obtaining of the sample

d) Identity and contact details of the person responsible for the research.

e) The patient's rights with regard to revoking the consent, and the effects this may have

f) Location of the undertaking of the analysis and the destination of the sample at the end of the research?: codification, destruction or other research

g) The patient's rights to gain access to the obtained data

h) Guarantee of confidentiality of the information obtained

i) Information regarding the implications of genetic analysis

j) His/Her faculty to take a stance in relation to the communication to him / herself of the data obtained in the research

k) Information regarding the implication of the results for his / her family members

l) The convenience that the person, where appropriate, transmit this information to the family members, in case it would be relevant for their health

m) Indicating the possibility to get in contact with him /her, and the way to do so.

n) Information regarding the data will be stored

o) Identification and contact details of the person responsible for the storage of data

p) Procedure to exercise his / her rights regarding to the data storage

q) Provisions about international circulation of data

r) Provisions about international circulation of samples

3.10. Where should be stored the document/s of consent?

In the department, in the patients clinical record stored in the centre, other. There is no a clear and unanimous provision, but in everycase the document is stored.

3.11. For how long should the samples be stored?

In general there is no specific period established

4. Implications of the Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross border healthcare.

This Directive has been adopted on 9 March by the European Parliament and Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 25 October 2013. They shall forthwith inform the Commission thereof.

The European Court of Justice had already addressed some issues related to cross-border healthcare, in particular concerning the reimbursement of healthcare provided in a Member State other than the residence of the recipient of such assistance. With this Directive is to achieve the most effective and general application of the principles settled down by the Court.

The Directive aims to 'Establish rules for facilitating access to safe and high-quality cross-border health care in the Union and to ensure patient mobility in accordance with the principles established by the Court of Justice and to promote cooperation on health care between Member States, whilst fully respecting the responsibilities of the Member States for the definitions of social security benefits relating to health and for the organization and delivery of health care and medical care and social security benefits, in particular for sickness'.

According to the European Parliament, 'the aim is absolutely not to encourage cross-border health care as such, but to ensure its availability, safety and quality when it is of use or necessary'.

The Directive establishes the criteria according to which all European Union citizens are entitled to receive healthcare in any State of the Union (cross-border healthcare). The option is a system of reimbursement, not a direct payment of treatment, perhaps to prevent "health tourism".

In general, the State must reimburse the costs of cross-border healthcare to which citizens are entitled under the laws of the State in which the citizen is affiliated. In some cases, prior authorization of that State is required. The authorization may be denied in some cases, as when the healthcare can be provided on its territory within a time limit which is medically justifiable.

There is a unanimous opinion about the importance of the directive for the States (patient coming from abroad) and for the citizens (possibilities of going to other country). (In the case of the Netherlands national rules have been

adopted yet in this sense)3. However, it is important to underline that the movement of patients is just one of the alternatives in health border health care. Sharing data and samples with other professionals could be a better option. In this sense, networking mechanisms should be implemented to include this possibility in the concept of cross border health care.

- **E-Health**

A key point to ensure the quality of care of patients receiving treatment in several countries, as well as to share data with experts in other countries, is the preparation of an integrated medical record accessible to all professionals involved in it. This objective (and the implementation of the measures designed to make it effective) should be achieved in a way which ensures the protection of health data.

On this purpose, some important initiatives in relation to the new Directive have already been adopted. Those initiatives have crystallized in the creation of a unique system of electronic health prior to the end of year 2015 (e-health). All countries should develop in their territories digital systems that support health in Europe. The creation of an area of e-health should follow the guidelines set forth in the Recommendation of the European Commission of July 2, 2008 on cross-border interoperability of electronic health records.

[3] Under the new Health Insurance Act (HIA 2006) EU patient mobility rules and jurisprudence has been incorporated. Article 13 HIA facilitates cross-border health care (conditionally: covered by the healthcare scheme; prior authorization in case of in-patient health care). Prior authorization for in patient health care abroad is not necessary when the foreign hospital has been contracted by the Dutch social health insurance fund. In case of serious waiting times (health damage), prior authorization for inpatient treatment abroad cannot be refused.

In case of out-patient care, cross border services will be covered according to Dutch tariffs. Prior authorization is not necessary.

These rules have been incorporated under the new Act in line with ECJ's rulings on cross border care.

This recommendation aims to create the basis for the generation of a system which allows health professionals to have access to patients' health data (medical history, medical treatment, emergency data, etc..), while ensuring a high level of protection of health data. A Working Group has been settled in order to advise EU on how to promote this system of e-Health. This group has met for the first time on 10 May this year 2011.

- **Particular interest for the case of rare diseases**

The Directive pays special attention to the diagnosis and treatment of rare diseases, taking into account that patients in these cases face huge difficulties, as recognized in the recommendation of the Council of 8 June 2009 on action in the field of rare diseases.

In this context, the Directive states that patients, health professionals and funders must be awarded of the necessity to refer patients with rare diseases to other Member States. The singular interest of the creation of European reference networks (Article 13a and 12) is also highlighted.

The European Union Committee of Experts on Rare Diseases (EUCERD) has published and Opinion on the options for the implementation of this Directive focused on the specific issues of Rare Diseases.

CONCLUSIONS

- The creation of a European network of diagnosis is legally accepted at the EU level. Moreover, it is a priority and reinforced issue since the adoption of Directive 2011/24/EU.
- **The success of a Network dedicated to improve the diagnosis and treatment of patients with Rare Diseases (RD) depends of the feasibility of an easy communication** between the involved MS not only by Internet but also by other means such as translational referral procedures. It is well known that for this, clear differences in legislation exist between the different MS which sometimes make this practice very difficult.
- The transnational health care assistance system is very similar in all EU countries and a further harmonization is expected, but its practical application has not developed in a way adapted to diagnostic networks for rare diseases.
- However, the creation of the network is essential to harmonize the procedures and the adoption of common criteria to achieve efficiency in its managing.
- Two of the most important factors for the good going of the network are the transference of data and samples, and, in more exceptional cases, the circulation of patients.
- There is a general EU regulatory framework for the management of data which has been integrated into the legal framework of all States. However, regulation relating to very specific topics and specific differences shows severe differences between the different national laws (eg in the regulation of genetic testing and genetic counseling).

- There are international tools of larger geographical areas with significant impact on this subject: however, some of them have not been adopted by all Member States of the Union (see section 6) or, even if adopted, imply a different normative level, that is, a different power to obligate.
- It is therefore necessary to establish specific and realistic common criteria which respect national laws, so as professionals may know what the protocols of acting are. The Network itself (Enerca) can acquire these criteria from the normative framework reflected in this study. This way, respect for national laws could be guaranteed and a "maximum guide", ie a model summarizing the best way of accomplishing with all applicable rules, delivered.

b) Clinical and laboratory

Rationale

In view to contribute to the empowerment of patients with rare anaemias, one of our objectives was the mapping of existing centres that take care of patients with rare anaemias in Europe. Another goal was to obtain a directory of facilities available per centre for patients with rare anaemias. Finally, we thought that it could realistically help to define a consensus regarding the criteria to be recognised as a centre of expertise for haemoglobinopathies and very rare anaemias.

Limitations

- It is certain that all European centres have probably not been informed of this initiative.
- Some countries, like France or England, have already organized centres of expertise or excellence at a national level. For this reason many expert centres in France did not answer the questionnaire
- The results should be taken with caution. Indeed, although some questions were asked in different ways, an error of interpretation is always possible. An example is the number of patients followed. One centre alone reported more patients than the number estimated in the whole country. It seems that the minor haemoglobinopathies were reported and not the major ones.

The main sections of the questionnaire

General overview of the centre and its activity

- Reference or general centre
- Type of patients followed
- Number of patients followed annually
- If a laboratory, average number of samples tested annually

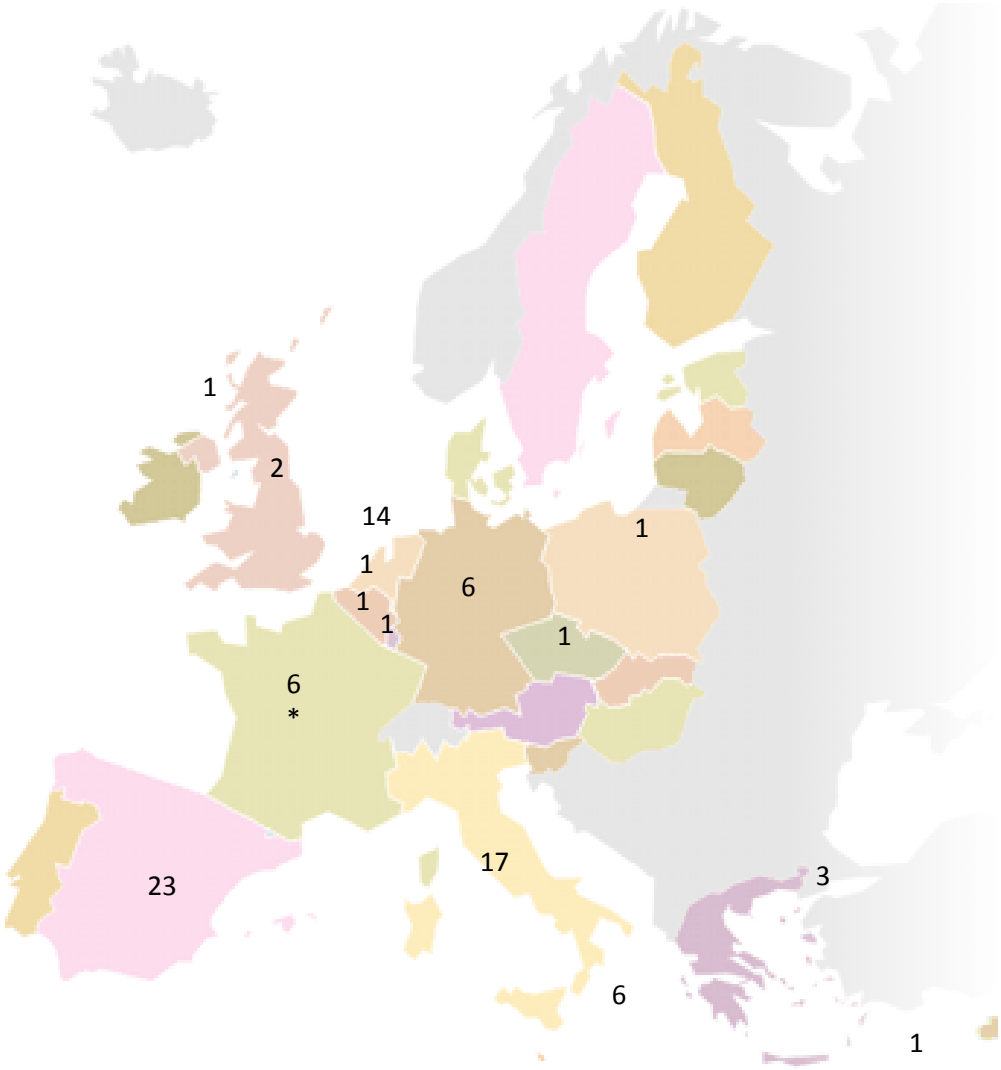
The expertise covered in the centre

- Diagnosis and prevention
- Follow-up/case management
  - Acute and chronic events: allocated services and staff
- Criteria (Proof) of expertise
  - Availability of
    - Specialised services
    - Specific treatments
    - Patients services
    - Decision supports (recommendations)
    - Registries
  - Link with research
  - Publications, grant, teaching and training activities

Results

Number of answers: 93

** Six centres participated; see complete list of recognized centres on limitations section*



PART I: HAEMOGLOBINOPATHIES

GENERAL OVERVIEW

Centres:

- Concerned by the haemoglobinopathies: 75/93 (95%)
- Concerned only by the laboratory section: 6/75 (8%)
- Concerned only by the clinical section: 61/75 (81%)

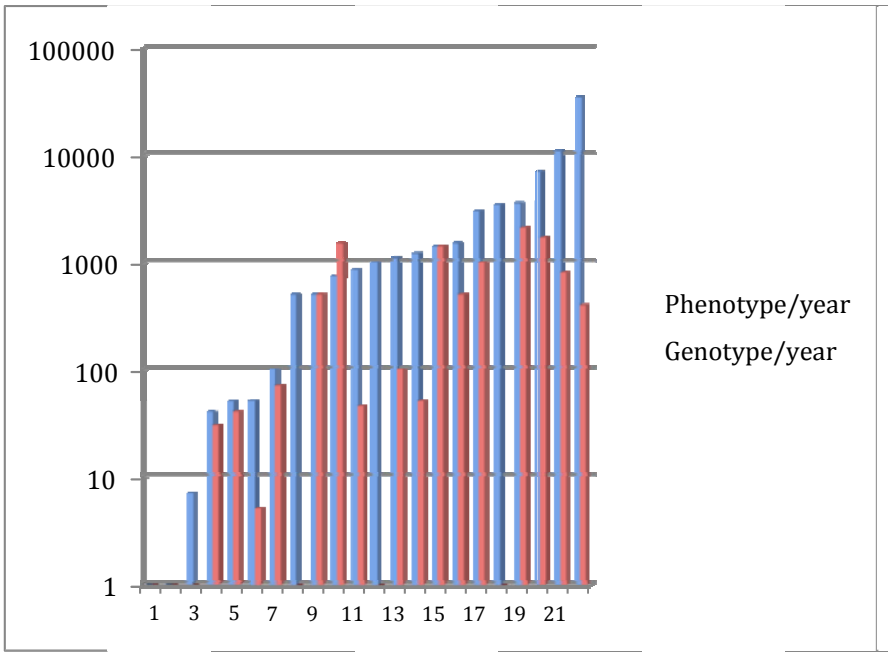
Centres involved in the diagnosis (phenotype/genotype):

Self declaration as a:

- Reference centre: 22/75
- General centre: 7/75

	Reference centre (n= 22)	General centre (n= 7)
External quality control used for phenotyping	14/22 (63%)	2/7 (28%)
External quality control used for genotyping	3/22 (14%)	0/7 (0%)
Average number of samples received for advice	2 to 10,000	0 - 200

Reference centre, number of phenotype/genotype per year:


100000
10000
1000
100
10
1
1 3 5 7 9 11 13 15 17 19 21
Phenotype/year
Genotype/year

Centres involved in the clinical care:

Self declaration as a:

- Reference centre: 39/69
  - *The Netherlands* *7/13*
  - *Belgium* *3/9*
  - *Spain* *8/22*
  - *Greece* *4/6*
  - *Italy* *8/10*
  - *Luxembourg* *0/1*
  - *North Ireland* *0/1*
  - *Cyprus* *1/1*
  - *Germany* *3/3*
  - *France* *3/3*
  - *Czech Republic* *0/1*
  - *Poland* *1/1*
  - *Bulgaria* *1/3*

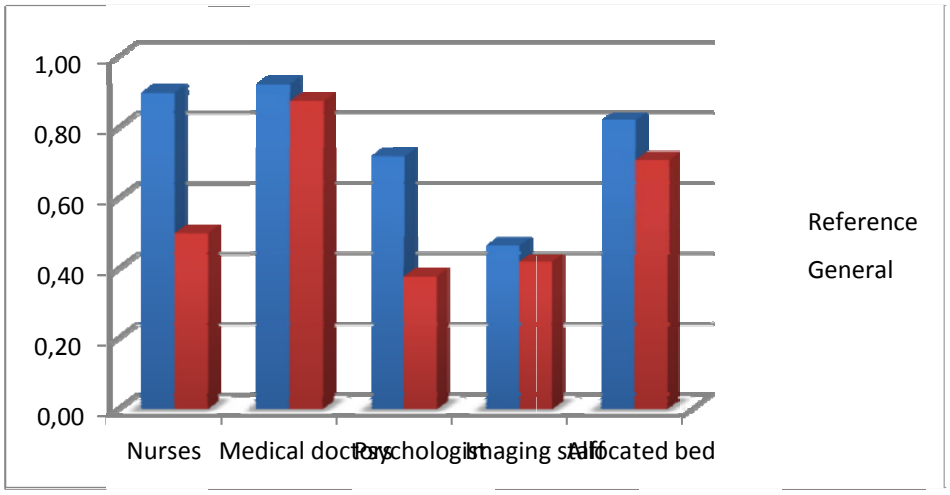
- General centre: 24/69

THE EXPERTISE COVERED IN THE CENTRE

Acute events: allocated staff and beds

Number of centres where the service is

offered/Total



Availability of specialized services able to deal with sickle cell disease/thalassaemia adverse events:

	Reference ce tre (n= 39)	General centre (n= 24)
Intensive care unit	31/39 (79%)	13/24 (54%)
Transcranial Doppler	32/39 (82%)	13/24 (54%)
MRI	36/39 (92%)	18/24 (75%)
Angio MRI CT	34/39 (87%)	14/24 (58%)
Angiofluorography	35/39 (90%)	19/24 (79%)
Audiometry	25/39 (64%)	4/24 (17%)
Assessment of cardiac iron by T2*MRI	38/39 (97%)	17/24 (71%)
Measurement of liver iron by	28/39 (72%)	8/24(33%)

Biopsy	30/39 (77%)	11/24 (46%)
MRI	32/39 (82%)	12/24 (50%)
SQUID	1/39 (2.6%)	0/24 (0%)

Availability of treatments:

In a reference centre

Blood transfusion is offered in all the reference centres. An extended immune-phenotype is performed in the majority of the centres (36/39; 92%) where there is also an access to donor red blood cell units with rare phenotypes (32/36; 89%). Exchange blood transfusion, either manual or automated, is offered (35/39; 90%) and sometimes as a 24-hour service (29/39; 74%).

Hydroxyurea (37/39; 95%) as well as iron chelation (100%) is offered to the patients. Stem cell transplantation is offered only in more than two-thirds of the centres (27/39; 69%).

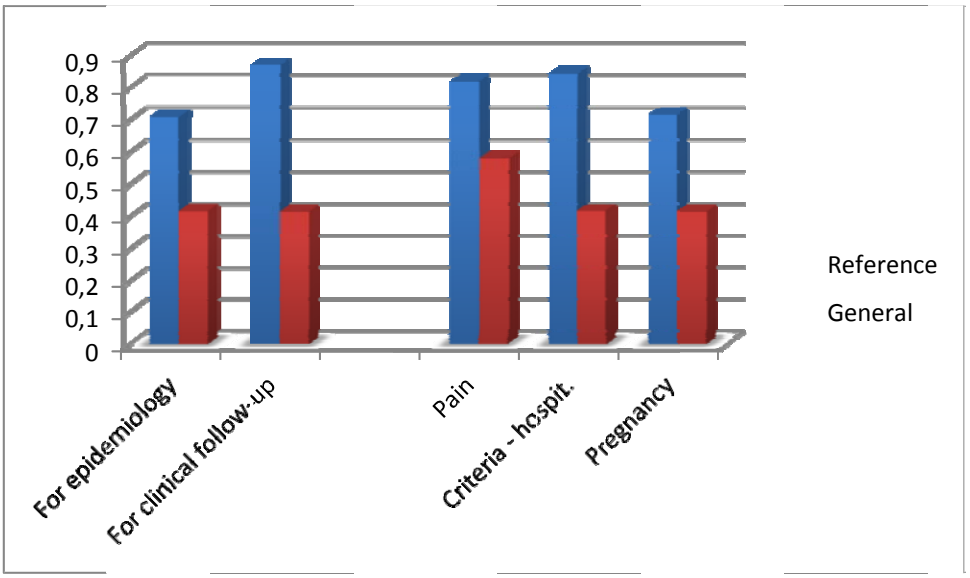
In a general centre

Blood transfusion is offered in all the centres. An extended immune-phenotype is performed in the majority of the centres (22/24; 92%) where there is also an access to donor red blood cell units with rare phenotypes (22/24; 92%). Exchange blood transfusion, either manual or automated, is offered in more than half of the centres and sometimes as a 24-hour service (14/24; 58%).

As in the reference centres, hydroxyurea as well as iron chelation (20/24; 83%) is offered to the patients; stem cell transplantation is offered only in few centres (5/24; 21%).

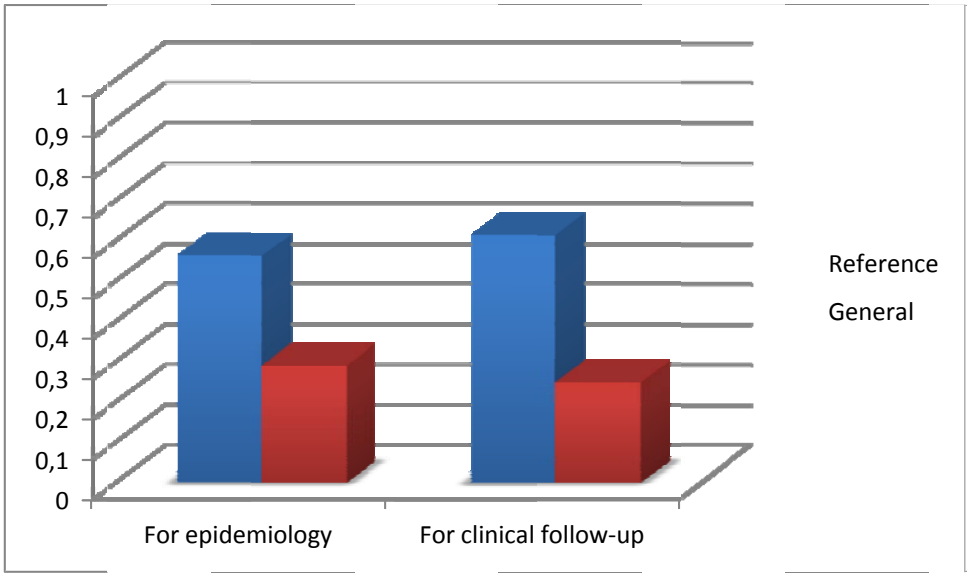
Availability of decision supports

Number of centres where the service is offered/Total



Availability of a registry

Number of centres where the service is offered/Total



In a reference centre

Fifteen and seventeen centres have less than 20 patients with sickle cell disease and a thalassaemia major or intermedia, respectively in their registries.

Seven of them have less than 20 patients registered for both types of haemoglobinopathy.

Link with research, publications, grant, teaching and training activities

In a reference centre

Publications and teaching activities are performed in most of the centres (31/39; 79%). Accessibility to research (25/39; 64%) and more particularly to grants (14/39; 36%) is less frequent.

In a general centre

Publications (10/24; 42%), teaching (4/24; 17%), link with research (5/24; 21%) or access to grant (0%) is rare.

PART II: VERY RARE ANAEMIAS

Anaemias considered:

- Red blood cells membrane disorders
- Red blood cells enzyme disorders
- Congenital dyserythropoietic anaemia
- Diamond Blackfan Anaemia
- Paroxysmal nocturnal haemoglobinuria
- Hereditary sideroblastic anaemia
- Very rare anaemias due to defective iron utilization

Seventy of the 79 centres are concerned by this part of the questionnaire.

Self declaration as a:

- Reference centres: 31/70
- General centres: 39/70

Some centres self-declare as reference centre for a specific disease but not for others.

Disease/Disorders	Number of centres concerned	Total number of patient registered:		
		reference centres	general centres	Total
RBC membrane	57	1546	335	1881
RBC enzymes	56	432	191	623
CDA	36	104	25	129
DBA	38	115	50	165
PNH	38	202	32	234
Hereditary sideroblastic anaemia	30	29	27	56
Very rare anaemia – defective iron utilization	20	93	8	101
Other anemia (ie: FA, AA, Rh null)	11	173	9	182

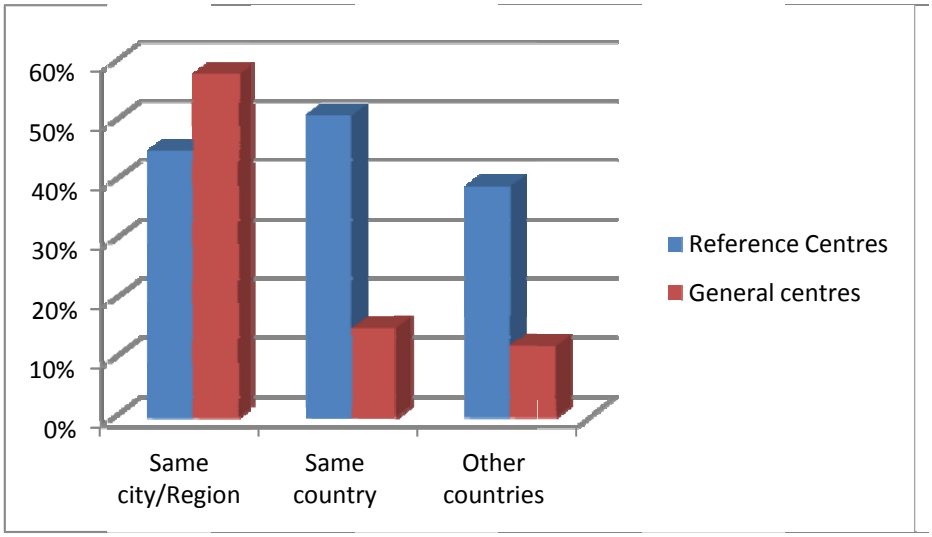
- There is a good representation of all the considered diseases, although the total number of patients registered for each pathology is drastically lower that the expected on the basis epidemiologic data.

- In spite of the number of patients, in most cases the median number of patients registered by a single centre is low. As regard red cell membrane disorders, for example the median is 26, in fact 11/24 Reference Centres have less than 20 patients in their registers.

This situation is much more evident in very rare anemias (RBC enzyme defects, CDAs, PNH, DBA, HSA) where the median number of cases registered for each pathology ranges from 2 to 10.

In General centres this observation become more evident (the median of patients registered for each Centre in all the diseases considered ranges from 1 to 7).

- Residence of the patients studied in Reference and General Centres



Due to the rarity of these disorders half of the Reference Centres are dealing not only with patients originating from their own country, but 39% of centres follow patients coming from abroad.

Distribution of self-declaration of the 31 reference centres among different countries.

	Reference centres/ Centres in each country
o *The Netherlands*	*8/13*
o *Belgium*	*2/9*
o *Spain*	*7/23*
o *Greece*	*1/3*
o *Italy*	*5/9*
o *Luxembourg*	*0/1*
o *North Ireland*	*1/1*
o *Cyprus*	*0/1*
o *Germany*	*3/3*
o *France*	*2/2*
o *Czech Republic*	*0/1*
o *Poland*	*1/1*
o *Bulgaria*	*1/3*

GENERAL OVERVIEW

Centres:

- Concerned by the very rare anemia 70/79 (88%)
- Concerned only by the laboratory section: 21/70 (30%)
- Concerned only by the clinical section: 25/70 (35%)

LABORATORY DIAGNOSIS

21 centres are involved in the diagnosis (phenotype/genotype)

	Reference centres n.10	General Centres n.11
Centres involved in diagnosis – phenotype	8/10 (80%)	11/11(100%)
Centres involved in diagnosis – genotype	7/10 (70%)	1/11 (9%)
Average number of samples received for advice	5 to 3,000	2 to 2,000
Centres involved in prenatal diagnosis	7/10 (70%)	1/9 (11%)
Centres involved in pre-implantation diagnosis	1/10 (10%)	1/11 (9%)

-Genotype diagnosis is mostly performed in reference centres.

-Genetic counselling – available in 17/31 Reference Centres and in 14/39 General Centres- is mostly performed in collaboration with Genetic Centres.

-Prenatal diagnosis (performed in Reference centres except for 1 General) is performed in cases of severe pyruvate kinase deficiency, other rare red cell enzyme defects, CDAII, fanconi anemia, severe cases of instable Hb.

The number of samples received in one year for phenotype diagnosis is extremely variable ranging from 5 to 3000 (median: 42) in Reference centres, and from 2 to 2000 in general centres (median: 25)

	Reference centre (n= 10)	General centre (n=11)
External quality control used for phenotyping	4 (4 %)	1 (9%)
External quality control used for genotyping	1 (1 %)	0 (0%)

Due to the rarity of these disorders external quality control are adopted in a very low number of centres and for a limited number of pathologies.

External quality control for phenotyping used are: SKML (The Netherlands, 2 Centres); SEHH (Spain, 1 centre); UK NEQAS (Belgium, 1 Centre)

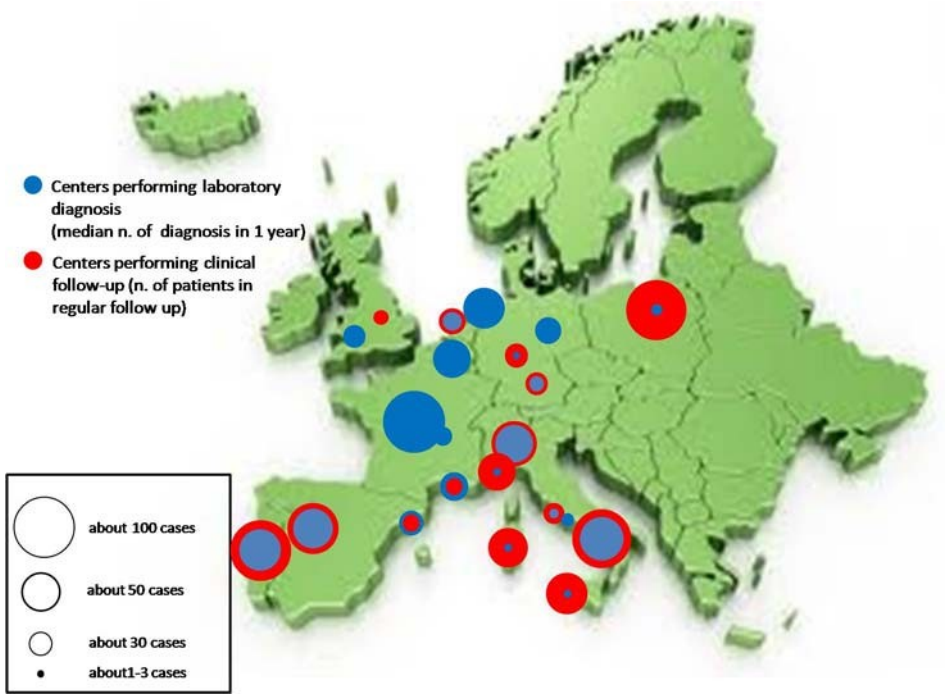
External quality control programmes for genotyping of VRA do not exist at the moment.

The only program used (performed only in 1 Centre in the Netherland) is EMQN (European Molecular Quality Network).

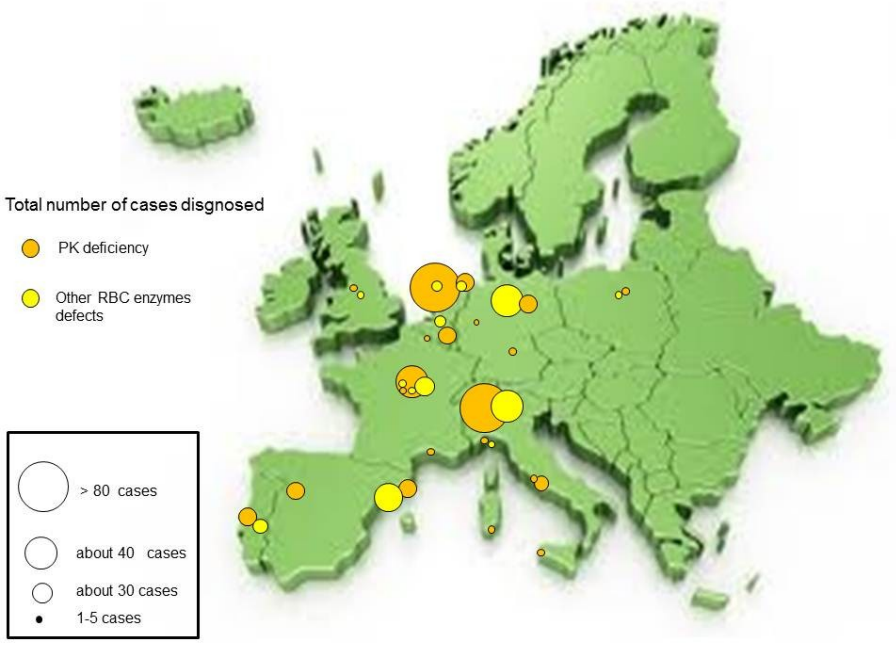
RBC membrane disorders and rare enzyme defects: diagnostic aspects.

Because of the wide heterogeneity of these two groups of defects, a specific technical questionnaire was distributed among centers. A response was received from twenty-six centers, some of them already involved in the questionnaire "Facilities for patients with rare and very rare anaemias".

Distribution and activity of 26 Centres involved in the diagnosis and follow-up of red cell membrane defects which answered to the technical survey.



Distribution of centres involved in the diagnosis of rare red cell enzymes disorders



Most centres perform the diagnosis of RBC membrane disorders (23/26 centres) and pyruvate kinase deficiency (18/26) but only in a few Centres the diagnosis of very rare enzyme deficiencies is available, this is particularly evident for genotyping of enzyme defects:

Disease	N. Centers involved in: Phenotyping	Genotyping	Total cases registered
Enzymes of glycolysis			
Hexokinase (HK)	10	2	6
Glucosephosphate isomerase (GPI)	10	6	23
Phosphofructokinase (PFK)	7	3	22
Phosphoglycerate kinase (PGK)	5	1	10
Pyruvate kinase (PK)	18	9	361
Triosephosphate isomerase (TPI)	7	3	31
Aldolase	7		10
Enzymes of nucleotide metabolism			
Adenylate kinase (AK)	3	2	5
Pyrimidine-5' nucleotidase	6	3	25
Enzymes of hexose-monophosphate shunt and glutathione met.			
6-phosphogluconate dehydrogenase (6-PGD)	5		2
Gamma-glutamylcysteine synthetase (GCS)	5	2	4
Glutathione synthetase (GSH-S)	3		1
Glutathione reductase (GR)	6	2	5
Glutathione peroxidase (GSH-Px)	2		1
Glutathione S-transferase (GST)	2		1
Other red blood cell enzyme activities			
NADH diaphorase	2	2	13
NADPH diaphorase	2		6

PATIENTS FOLLOW-UP/CASE MANAGEMENT

Centres involved in the clinical care:

Self declaration as :

- Reference centre: 23/31

Reference centres for care /Reference Centres in each country

o	*The Netherlands*	*5/8*
o	*Belgium*	*2/2*
o	*Spain*	*4/7*
o	*Greece*	*1/1*
o	*Italy*	*4/5*
o	*Luxembourg*	*0/0*
o	*North Ireland*	*1/1*
o	*Cyprus*	*1/1*
o	*Germany*	*3/3*
o	*France*	*2/2*
o	*Czech Republic*	*0/0*
o	*Poland*	*1/1*
o	*Bulgaria*	*0/1*

- General centre: 27/39

General centres for care / general centres in each country

o	*The Netherlands*	*2/5*
o	*Belgium*	*4/7*
o	*Spain*	*11/16*
o	*Greece*	*1/2*
o	*Italy*	*5/5*
o	*Luxembourg*	*0/1*

- *North Ireland* *0/0*
- *Cyprus* *0/0*
- *Germany* *0/0*
- *France* *0/0*
- *Czech Republic* *1/1*
- *Poland* *0/0*
- *Bulgaria* *2/2*

THE EXPERTISE COVERED IN THE CENTRE

Acute events: allocated staff and beds

Availability of specialized services able to deal with very rare anaemia adverse events:

	Reference centre (n= 31)	General centre (n= 39)
Intensive care unit	26/31 (83%)	29/39 (74%)
Magnetic Ressonance Imaging (MRI)	26/31 (83%)	32/39 (82%)
Computed Tomography (CT)	26/31 (83%)	32/39 (82%)
Audiometry	26/31 (83%)	33/39 (85%)
Assessment of cardiac iron by T2*MRI	21/31 (67%)	16/39 (41%)
Measurement of liver iron by	24/31 (77%)	23/39 (59%)
Biopsy	21/23 (67%)	16/39 (41%)
MRI	23/31 (74%)	21/39 (54%)
SQUID	01/31 (3%)	1/39 (3%)

Availability of treatments:

In a reference centre

Blood transfusion is offered in all the reference centres. An extended immune-phenotype is performed in the majority of the centres (83%) where there is also an access to donor red blood cell units with rare phenotypes (26/31; 83%). In 24/31 centres (77%) exchange blood transfusion, either manual (64%) or automated (39%), is offered and sometimes as a 24-hour service (21/31; 68%).

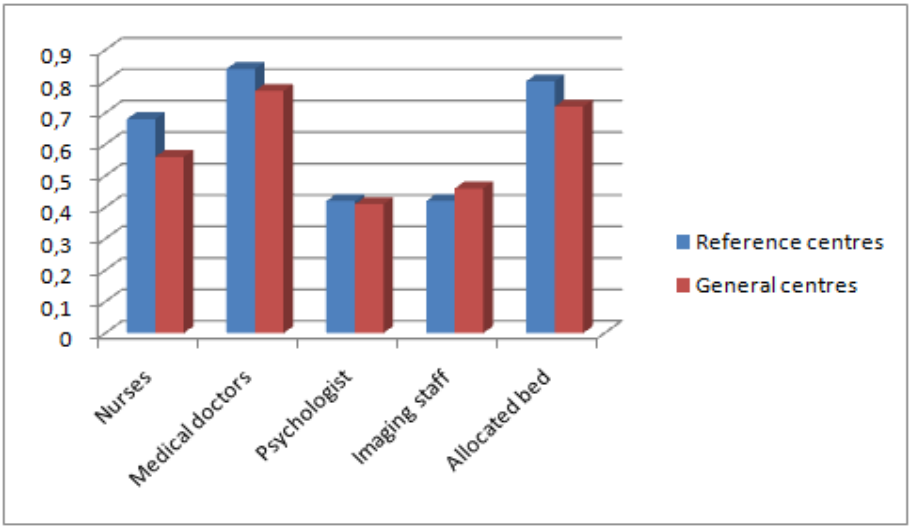
Monitoring for iron overload and iron chelation is always offered to the patients. Stem cell transplantation is offered only about 90% of centres (28/31).

In a general centre

Blood transfusion is offered in all the centres. An extended immune-phenotype is performed in the large number of the centres (33/39; 85%) where there is also an access to donor red blood cell units with rare phenotypes (29/39; 74%). Exchange blood transfusion, either manual or automated, is offered in about 60% of the centres and sometimes as a 24-hour service (18/39; 46%).

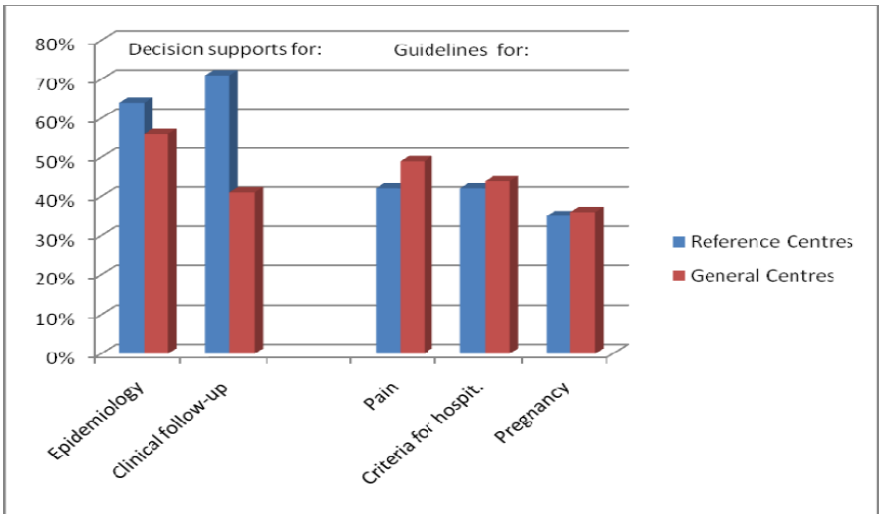
As in the reference centres, iron chelation is offered to the patients (34/39; 87%); stem cell transplantation is offered only in few centres (12/39; 30%).

Availability of decision supports



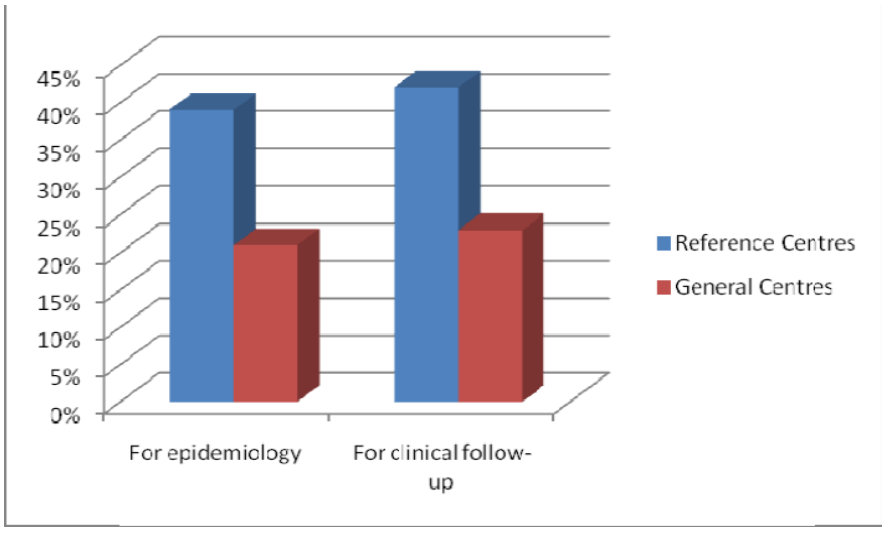
In Reference Centres guidelines (electronically and/or in a booklet form, for patients or for health professionals) are available in about 60% of Centres. 70% of Centres have standards for clinical follow-up. For very rare anemias indications for management of chronic pain, criteria for hospitalisation and pregnancy are available in less than half of Centres.

Availability of a registry



Link with research, publications, grant, teaching and training activities

Research activities (Publications, Grants, training activities)



Publications and teaching activities are performed in a large number of the Reference Centres. Accessibility to research, performed regularly or at least occasionally, is also a common practice

In General Centres the link with research is occasional: publications, teaching, or access to grant are rarely encountered.

CONCLUSIONS

Services to be offered

Availability of specialized equipments and treatments are satisfactory. But our results show that for laboratories as well as for clinical centres necessary tools to give a diagnosis, to follow and manage the patients are not always available. If it exists, the dedicated and specialized teams are not always implemented.

It is obvious that with a rare disease, few patients and sometimes geographic isolation, it is very complex to provide all the expected services.

Our results show also that, even in reference centres, a registry is not always implemented. Nevertheless, **collection of a core data set will** support the continuous improvement of clinical care. Decision supports for the health workers and for the patients are also missing in several centres. In view to improve those points, difficulties to create and collect data should be investigated.

Accessibility to grants is not frequent even in reference centres. A proposal should be that more grants and funds could be dedicated to rare diseases.

Finally, in view to share tools and all aspects in the management of patients with rare or very rare anaemias as well as to improve the knowledge of these diseases at all levels, networking should be encouraged.

Centres that responded to the questionnaire (See Annex XXX)

QUESTIONNAIRE 3 - EQAS AVAILABILITY

The responses to this questionnaire were received from a limited number of EQA providers; however, it included several large providers with a comprehensive range of services and also reflects the provision listed in other catalogues, such as that provided by the College of American Pathologists (www.cap.org) or the Centre for Disease Control (www.cdc.org).

Number and location of responses received

The questionnaire was distributed to 31 member organisations within EQALM and responses were received from 16 (52%). This included a supplier from Canada.

Table 1. Responses received

Country	EQA provider organisation
Canada	QMP-LS
Croatia	Croatian Society of Medical Biochemists - Committee for External Quality Control
Czech Republic	SEKK
Denmark	DEKS
France	AFSSAPS

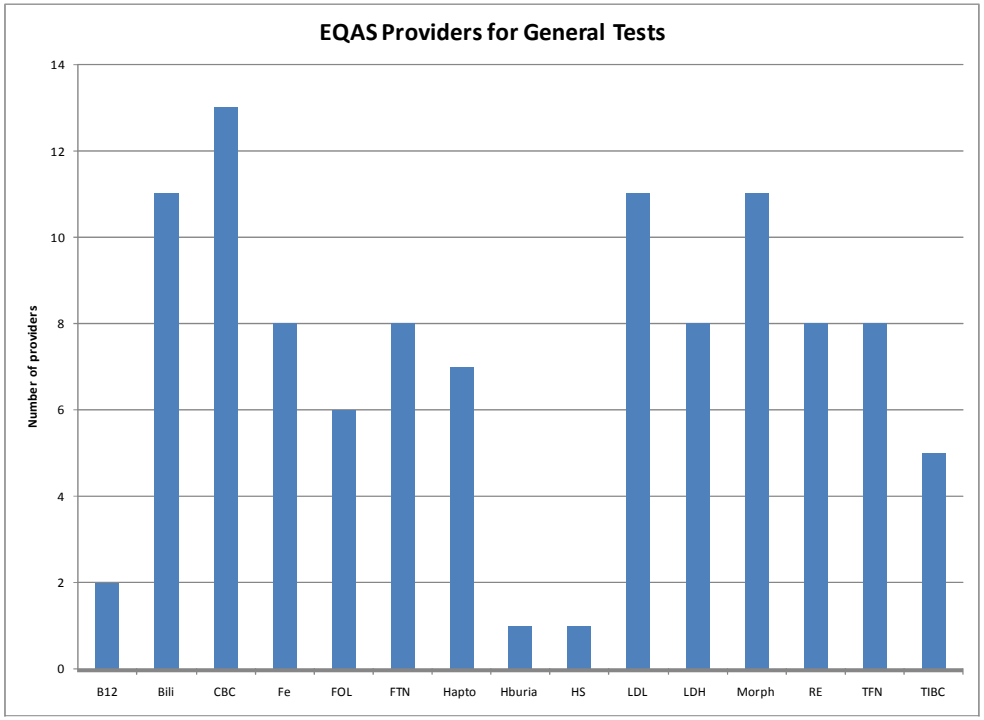
	CTCB
Ireland	Irish EQAS
	RIQAS
Norway	NOKLUS
Romania	RoEQALM
Russia	National Centre for EQA in Laboratory Medicine
Slovenia	SNEQAS
Spain	Sociedad Espanola de Hematologia y Hematorapia
Sweden	EQUALIS
Switzerland	CSQC
United Kingdom	UK NEQAS

EQA provision for 'General' tests

The provision of EQA for tests from the General section of the Core Tests list was good amongst the EQA providers that responded to the questionnaire, with 17/22 (77%) of tests covered.

Figure 1. EQAS provision for tests from the General section of the core list of laboratory tests.

Key: B12: vitamin B12 assay; Bili: bilirubin; CBC: complete blood count; Fe: serum iron; FOL: serum &/or red cell folate; FTN: serum ferritin; Hapto: haptoglobin; Hburia: Haemoglobinuria; HS: urinary haemosiderin and bone marrow iron stain; LDL: low density lipoprotein; LDH: lactate dehydrogenase; Morph: peripheral blood/bone marrow morphology; RE: reticulocyte count; TFN: transferrin; TIBC: total iron binding capacity.



Five tests not covered by the EQA organisations that responded were: urine ferroxamine iron, serum transferrin receptor, liver iron, myocardial iron and zinc protoporphyrin.

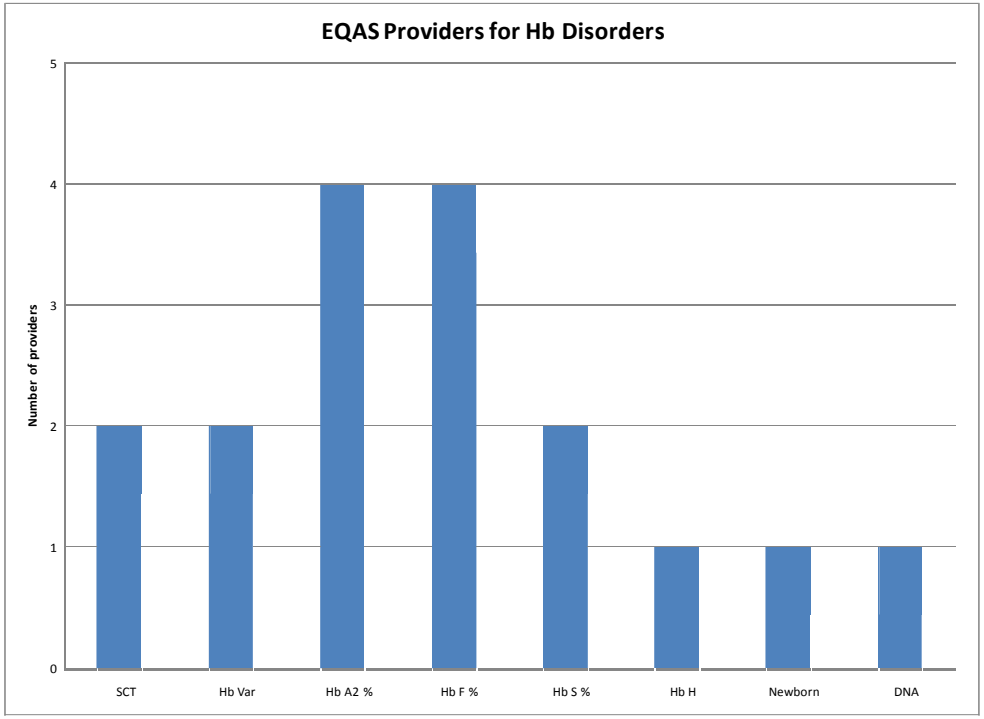
EQA provision for diagnostic tests for haemoglobin disorders

Only 4 of the 16 EQA organisations provided EQA services for diagnostic tests associated with the haemoglobin disorders, and just 3 of these provided their services outside their own countries. The tests covered included sickle solubility; haemoglobin (Hb) variant identification; quantification of Hb A_2, Hb F and Hb S; Hb H bodies; newborn sickle screening and molecular haemoglobinopathies.

EQA services were not available for unstable haemoglobins, Heinz bodies, oxygen affinity (p50) or globin chain synthesis among the organisations that responded.

Figure 2. EQAS provision for tests for the diagnosis of haemoglobin disorders.

Key: SCT: sickle screening test; Hb Var: Hb variant identification; Hb A2%: quantification of Hb A2; Hb F%: quantification of Hb F; Hb S%: quantification of Hb S; Hb H: Hb H inclusion bodies; NB sickle: newborn sickle screening; DNA: molecular haemoglobinopathies.



EQA provision for tests for the diagnosis of red cell enzymopathies, membrane disorders and other rare anaemias

There is little provision of EQA for the specialist diagnostic tests of other rare anaemias. Of the core list of laboratory tests for red cell enzymopathies, red cell membrane disorders and paroxysmal nocturnal haemoglobinuria (PNH), EQA is only provided amongst the responders to the questionnaire for glucose-6-phosphate dehydrogenase (G6PD) activity, Hb F by flow cytometry, Kleihauer (acid elution) slides, methaemoglobin and PNH by flow cytometry. In all cases, there was a single EQA provider for each test.

EQA service provision across national borders

Service provision across national boundaries was available from 10/16 (60%) of EQA service providers. In addition, 11/15 (73%) that responded would be prepared to offer new specialist EQA services in collaboration with other EQA providers.

Frequency of service provision

There was a large variation in the frequency and number of specimens provided by different EQA organisations. For full (complete) blood count (FBC or CBC), for example, the number of specimens varied from 1 to 26 annually. A similar variation is seen for bilirubin (from 'as requested' to 52 specimens annually) and for Hb A_2 quantification (1 to 18 specimens annually).

EQA providers' 'wishlist'

The EQA providers were asked which tests they thought would most benefit from the development of new EQA services. EQA is available for the majority of the tests on the wishlist, through the organisations within EQALM that responded to the questionnaire. Only 4/16 tests listed did not have EQA provision by an alternative organisation.

Table 2. Diagnostic tests that respondents feel would most benefit from the provision of EQA, and the availability of EQA services from another EQALM member organisation.

Tests for which EQA is available within EQALM questionnaire respondents	Tests for which EQA is not available within EQALM questionnaire respondents
Hb variant detection	Unstable haemoglobins
Hb A_2, Hb F and Hb S quantification	Heinz bodies
G6PD activity	Serum transferrin receptor
Kleihauer	Pyruvate kinase activity
Flow cytometry for Hb F	
Reticulocyte count	
Red cell folate	
Serum folate	
Cobalamin	
Serum ferritin	
Serum haptoglobin	
Blood Film Morphology	

The EQA providers reported the availability of a higher order reference method in less than 5% of analytes tested and this was not consistent between providers.

In nearly all cases, target values were derived from a consensus of participants' results. For tests such as morphology, a consensus of expert laboratories was also used. Over 90% of tests were subject to performance assessment.

It was encouraging to note that 7/16 of the EQA providers offered accredited services.

CONCLUSIONS

This questionnaire has demonstrated that the provision of EQA for general or routine diagnostic tests utilised in the diagnosis of rare anaemias is adequate amongst the EQA provider organisations within EQALM. These tests are used in the diagnosis and monitoring of a greater range of disorders other than the rare anaemias, are widely available and hence have good EQA provision. The provision of EQA for more specialist tests however is not as good.

This relatively poor provision of EQA for RA diagnostic tests reinforces the need for collaborative operation where possible. The choice of EQA programme is made difficult by the differences in the service offered between providers, for example the very wide variation in the frequency of provision. The responsibility for the selection of an EQA scheme appropriate to the laboratory's needs lies with the laboratory and this requires a diversity of EQA provision; however, patients have the right to expect that the EQA services conform to a recognised quality standard and that the scope of the EQA programme is clear. For this reason, EQA providers should work to improve their services through accreditation to international standards, such as ISO17043.

This is particularly important if EQA services are provided across national boundaries.Of the EQA wishlist items, ENERCA has identified pyruvate kinase (PK) activity as the diagnosis procedure for which it is most feasible to develop a pilot EQA scheme. Relatively few centres may provide qualitative and/or quantitative PK assay within any one EU member state and the interpretation of the results is challenging, meaning that an effective EQA programme would have an impact. The most effective model would be to develop a Europe wide EQAS, using the expertise of a consortium of experts in EQAS provision, laboratory diagnosis and clinical management of PK deficiency. A suggested protocol for a pilot EQA scheme has been developed between ENERCA Executive Committee partners.

The provision of high quality EQA, coupled with educational support, is an important component of a quality management system by which laboratory performance can be improved and maintained, and key to the development of laboratory services for patients with rare anaemias. The majority of the EQA providers indicated a willingness to provide their services across national borders or to collaborate in the development of new, specialist services. This is important where the EQA programme may only be statistically viable if participants are recruited from a number of countries. However, the availability of sufficient volumes of stable survey material, funding models that restrict the provision of EQA to a single country, the cost of transportation, restrictive customs rules, language and differences in local medical practice all pose barriers to cross border service delivery

International accreditation standards and support from professional bodies with an international profile, such as ENERCA, the International Federation of Clinical Chemistry (IFCC), the International Council for Standards in Haematology (ICSH) and the World Health Organisation (WHO), will be required to overcome the barriers to the cross-border provision of EQA for RA.

The use of molecular techniques is widely used in the diagnosis of RA, although EQA for such tests was not well represented among the EQA
providers responding to the questionnaire. Reference centres should be referred to the European Molecular Quality Network (EMQN). EMQN provides EQA for a diverse range of molecular diagnoses or the information on other providers in the field. EMQN also develops international guidelines for diagnosis, for example, in the field of haemoglobinopathies, with particular emphasis on the use of molecular techniques.

A networking organisation such as ENERCA has a key role in the facilitating collaborative working between experts in the development of EQA services and in publicising their availability to laboratory professionals.

c) Patient's expectations

Rationale

An important aspect of the effort to describe the centre of expertise for rare anaemias is the need to understand patient expectations of the ideal treatment centre. All patients experience their treatment centre as a second home due to the chronicity of the condition and the need for frequent visits and the long hours spent on the centre, for example to have blood transfusions and see the several doctors who make up the multi-disciplinary team. Patient opinion on the services provided is therefore pivotal to planning or upgrading any expert centre.

Limitations to the study

The responses were mainly from patients with transfusion dependent anaemias and mainly from beta-thalassaemia patients (91.5 %). Only few patients with other rare anaemias responded and most were patients with sickle cell anaemia (4 %). The reason for this was probably that the distributor (TIF) was more likely to receive responses from its members and that transfusion dependent patients are more likely to be interested in the quality of services since they are the most frequent users of such services. This has however created a bias in some the responses. In addition there is a very variable representation of European countries.

Results of the survey

Section A: General information

- **Demographics**

The questionnaire was answered by 415 patients across Europe. 85% were answered by patients and 14% were answered by parents, while 1% did not state who answered. In those questionnaires that were answered by parents, 71% were on behalf of patients under the age of 18 years, and in 21% the patients were over 18 years, while in 8% there was no explanation as to why parents answered.

Table 1. Demographics:

Age (years)	2-66 range	29 mean		
Sex	Male 44%	Female 54%	No answer 1.4%	
Marital	Married	Single	Cohabiting	Divorced

status (adults)	27%	52%	2.4%	5.5%
Education	50% no response	21% university graduates	26% attending school	3.1% pre-school
Employment	35% no response (55.5% under 18y)	32% full time	13.5% part time	19.5% unemployed

Conclusion: A significant proportion of adult patients are married and others are divorced or cohabiting. This is the first indication from this questionnaire that patients are fulfilling 'normal' life demands and ambitions. This proportion is satisfactory considering that the median age of the sample is 29 years.

- Origin of respondents: Order by number of respondents or alphabetic

Table 2. Country of residence:

- Table 2a. Ethnic origin

Country of Residence	**N**
Italy	74
Bulgaria	62
Turkey	37
Portugal	26
Greece	24
Cyprus	24
Germany	5

199

France	2
UK	111
Belgium	7
Spain	2
Albania	1
Romania	39
Malta	1
Total	**415**

Ethnic Origin	**N**
Italian	79
Bulgarian	63
Romanian	38
Turkish	37
Pakistani	22
Greek	27
Cypriot Greek	25
Cypriot Turkish	17
Sub Sahara African	12
British Indian	4
Portugese	14
Spanish	2
Algerian	2
British	8
British Asian	8
Bangladeshi	4
SE Asia	3
Asian	3
Indian	12
Chinese	4
Iran	2
German	2
Maltese	1
Middle East	1

Macedonia Skopje	1
Venezuela	1
Iraq	1
Russia	1
Albania	2
Not stated	19
Total	**415**

- **Table 3. Diagnosis of the patients included in the survey:**

Diagnosis	**Number**	**percentage**
Thalassaemia major	350	84.34%
Thalassaemia intermedia	30	7.23%
HbH Disease	2	0.48%
Sickle Cell Anaemia (Hb SS)	18	4.34%
Sickle cell disease (Hb S/ beta thalassaemia)	8	1.93%
Other	4	0.96%
No answer	3	0.72%

Conclusions: The vast majority of responders were multitransfused thalassaemia patients. Since this group of patients presents the significant demands on services their needs may be considered as representative of any other group of chronic anaemia. Their responses to this questionnaire may be regarded as

representing the expectations of patients with rare anaemias in Europe, especially those under chronic transfusion regimen. On the other hand, patients with sickle cell syndromes in whom vaso-occlusive episodes and other recurrent events cause different very serious complications may have other expectation but are poorly represented in this sample.

- **Table 4. Clinical Care:**

4.1. Access to treatment

Location of centre compared to residence	Number of patients	Percentage
• Local/near home	268	64,58%
• Another region/city	136	32,77%
• Another country	1	0,24%
• No answer	10	2,41%

Access to treatment centre	Number of patients	Percentage
• Very easy	90	21,69%
• Easy	193	46,50%
• Difficult	80	19,28%
• Very Difficult	11	2,65%
• Too expensive to reach	30	7,23%
• No answer	11	2,65%

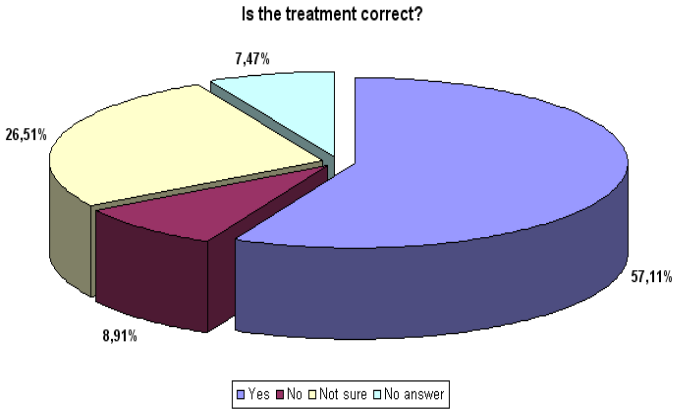
It is clear that around 33% of patients need to travel to reach their regular treatment centre and around 30% find it difficult to reach. The need for networking between secondary centres and an expert or reference centre must be considered essential so that patients can all have equal access to expert

services. Networking and shared care must be planned with shared patient records and periodic visits to the expert centre for review.

4.2. Confidence in the care received

This is an important quality measure rarely acknowledged by expert centres. The results of this survey are as follows:

Is your treatment correct?	Number of patients out of 415	Percentage
• Yes	237	57%
• No	37	8,9%
• Not sure	110	26%
• No answer	31	7,5%



The proportion of patients with no confidence in their treatment is small however there are a significant number of patients who are uncertain, which makes a total of 35% who cannot state that they are sure of their treatment. This should be included in the audit of an expert centre. Patients should be asked and not just health officials when assessing or re-evaluating a centre.

4.3. Quality of clinical care

The specific questions asked were the following:

a) Almost half (44%) of patients are given choices about treatment. Of those who are not given choices, around half (48%) are treated in specialised haemoglobinopathy centres and another 40% in haematology departments. This is an indication that specialised departments in general may have a paternalistic approach to patient care and give little attention to patient involvement in their own care.

b) Patients asked to talk about problems with their medication (234/415) are more likely to be treated in specialised centres (62%) compared to those treated in haematology centres (32%) or paediatric departments (5.5%) or oncology units (2.4%).

c) Only 35% of patients are given a written copy of their treatment plan. Those who are given such a copy are again more likely (71%) to be followed in a specialised haemoglobinopathy centre.

d) Care is estimated by 63.4% of patients to be well organised. Again the majority 70% who answered positively are treated in specialised haemoglobinopathy centres.

e) Only 35% of responders agreed that they were encouraged to talk their goals in treatment.

f) The majority (60%) however believe that their doctor has considered their beliefs, habits etc in prescribing a treatment regime. Most who answered positively (66%) are treated in a specialised haemoglobinopathy centre. Also 60% believe that the doctor made a treatment plan that they can carry out, and again 67% of these are treated in specialised centres

g) Discussion with the patient concerning how the disorder affects their daily life is unusual since most (59%) have never or only sometimes been asked the question.

h) 75% of patients have never or rarely been contacted at home to see how they are following a clinic visit.

i) Most patients (54%) are not encouraged to join a support association. Those who are, are more likely to be treated in a

specialised centre (58.33%) and much less if treated in a general haematology department.

4.4. Financing chronic patient care

Who pays for the treatment	Number of patients	Percentage
• Myself/Family	99	24%
• Private health insurance	36	8.7%
• State health insurance or free state care	280	67.5%
Total	415	100%

In this survey, one third of European patients with chronic anaemias claim to pay for their treatment. This is claimed by 23% of Italian responders (not immigrants), 21.5% of Turkish patients, 48% of Bulgarian patients, 74% of Portuguese patients (all with sickle cell and half are immigrants from Africa). This means that coverage by health services is only partial in several locations or some patients opt for out of pocket expenditure in the private sector to secure the level of care that they trust.

- **5. Treatment**

1. Blood transfusion:

5.1. Blood transfusion regime

Current transfusion regime	Number of patients	Percentage
• Not transfused	24	5,78%
• Transfused regularly	374	90,12%
• Transfused occasionaly	10	2,41%
• No answer	7	1,69%

5.2. Range of pre-transfusion Hb in regularly transfused patients

Range of pre-transfusion Hb	Number of patients (374)	Percentage
• Less than 7 g/dl	3	0,8%
• 7-9 g/dl	33	8,8%
• 9-10 g/dl	120	32%
• Over 10 g/dl	42	11%
• No answer	176	47%

Comment: Only 3 patients maintain an Hb <7g/dl. Of the 198 who were able to define their pre-transfusion haemoglobin level 162 (82%) were above 9g/dl and so within international guidelines for thalassaemia major. The remaining 18% of regularly transfused patients are either being treated outside agreed protocols or are sickle cell patients who are transfused with more caution, considering the danger of stroke.

5.3. Waiting time for transfusion and timing of services

This question was prompted by the patients and their associations, complaining that they have to wait for long hours for treatments such as the regular transfusions, which are necessary once or twice per month. These services are only available during the morning and coincide with school or working hours. Lack of consideration of these needs by centres, is in their estimation, an indication of the quality of care provided, since such timing severely handicaps the patients' ability to integrate and lead a 'normal' life. They regard this timing of service provision as an indication of indifference from health providers to their 'real' needs. The responses to relevant questions concerning the timing of transfusions are seen in the tables:

Waiting time for transfusion	Number	Percentage
Under 30 minutes	154	37%
30-60 minutes	83	20%
1-2 hours	73	18%
2-3 hours	41	9.9%
Longer	35	8.4%
No answer	29	7.0%
Total:	415	100%

5.4. Time of transfusion

Time of transfusion	Number of patients	Percentage
Morning	256	62%
Afternoom	117	28%
Evening	9	2,2%
Overnight	10	2,4%
Weekend	8	1,9%
Other (hospitalised for 3-5 days)	1	0,24%
No answer	14	3.4
Total	415	100

These responses show that although most patients do not have to wait for longer than an hour before their blood transfusion is set up and running, the majority of transfusions are in the mornings and some in the afternoon, indicating that the majority will not be helped in their desire to for regular uninterrupted schooling or employment.

5.5. Educational status of patients who go for transfusion the morning

Patients who go for transfusion the morning (176 patients)		
Educational status	**Number of patients**	**Percentage**
• University	38	22%
• School Graduates/attendees	50	28%
• No answer	88	50%

5.6. Employment status of patients who go for transfusion the morning

Patients who go for transfusion the morning (176 patients)		
Employment status	**Number of patients**	**Percentage**
• Working full time	23	13%
• Working part time	54	31%
• Not working	23	13%
• No working through choice	15	8,5%
• No answer	61	35%

From the above figures it can be seen that especially employment can be affected in many patients by the timing of transfusions.

Conclusion: availability of services outside school and working hours is a quality of care consideration for a dedicated centre providing services for chronic anaemias and must be included in the ENERCA recommendations

5.7 Iron chelation - -

372 patients are regularly chelated.

5.7.1 Age started:

Age range	Number of patients	percentage
0-4 years	94	25.2%
4-8 years	58	15.6%
8-12 years	33	9%
12+ years	23	6.2%
No answer	164	32 44%
Total :	372	100.

The mean age of starting chelation in this group is 6.7 years. This means that many patients who started transfusions early were given chelation late in the past, even though some of the late starters were patients with anaemias which are not initially transfusion dependant. This may explain the damage due to iron overload in patients who are apparently well chelated in their current

5.7.2 Which chelation regime:

Chelating agent	Number of patients	percentage
Desferrioxammine	117	32%
Deferiprone	30	10%
Deferasirox	152	30%
Combination	73	20%
No answer	43	8%
total	415	100

5.7.3 Regularity of chelation:

Only 190 patients responded to the question concerning the regularity of chelation. Of these the majority (166) stated that they take their chelation regularly and only 24 admitted being erratic in their compliance. The 225 who failed to respond may represent many non-adherents. An interesting
observation is that the vast majority (93%) who are taking their chelation regularly are being treated in specialised **Centre of care**

The majority of patients are treated in specialised centres.

Many patients are still followed at general haematology departments and transfused in haematology day units. Of 415 patients who responded 195 (47%) stated that they received care in a specialised haemoglobinopathy centre while
220 (53%) are treated in other departments, mainly haematology day-care units or paediatrics. Assuming that currently paediatrics has officially taken over adolescent patients up to the age of 18 years, 11 older patients are followed by a paediatric department and 6 of these are actually receiving transfusions in such departments, including a patient aged 50 years. Transition from paediatric to adult care is still an issue in some centres.

- **Multidisciplinary care**

As patients with chronic and congenital anaemias grow their condition becomes, from a haematological disorder, a multi-organ condition. The head of a haemoglobinopathy or for rare anaemias centre of expertise is also the head and coordinator of the multi-disciplinary team which includes the main specialties of cardiology, endocrinology and hepatology as well as psychology and many others in the case of SCD i.e. ophthalmology, pneumology…. Nursing as an essential part of the team is assumed to be present in any centre. Are patients in Europe under regular supervision by the main specialities? This is the question addressed to the patients in this questionnaire:

Cardiology: The multi-transfused patients are 350 in this survey. Of these: 218 (62%) are followed by a cardiologist according to guidelines i.e. at least once per year. Another 10 patients are seen at wider intervals (all are under 12 years of age). 69 are not followed by a cardiologist and 53 did not respond to the question. These 69 (around 20%) patients age range 13-46 years, are not monitored for a condition which is the major cause of mortality in iron loaded multitransfused patients. This is a major concern and must be noted in any classification of a centre as a centre of expertise in Europe.
Endocrinology: Only 221/415 (53%) of patients responded to the question concerning follow up by an endocrinologist. Of the 194 patients who did not answer and are presumably not followed by an endocrinologist, 91 were not transfusion dependent or had a sickle cell syndrome or are under the age of 10years. There is still a high degree of non-responses (103) which is probably indicative of no monitoring by an expert in a sizeable proportion of patients.
167/221 (75.6%) are followed annually according to guidelines while another 11 are seen at rarer intervals. An expert centre is expected to do much better. In this cohort of patients 120 of the 167 seen regularly (72%) are patients who state that they are looked after in a specialised haemoglobinopathy centre.

Psychology: Of the 415 responders only 26 (6.27%) responded and admitted visits to psychologist. 7 (27%) of these were seen frequently, every 3 months or less and so may have been on specific treatment. 8 (31%) are seen routinely annually or occasionally and 11 did not specify.

Hepatology: The necessity for a specialised consultation and follow up by a hepatologist arises when there are signs that specific treatment is required especially for liver infection and for fibrosis, cirrhosis or the development of hepatoma. For this reason only a proportion of patients will be seen by a specialist according to the incidence hepatitis virus infections and neglected iron overload in the liver. In this survey only 91 of the 415 patients were followed by a liver specialist. This is probably a reflection of the low incidence of hepatitis infections in the European setting.

Access to multidisciplinary care: A little over half of the patients who responded to this question (53.7%), were seen in the same hospital. Around 31% had to go to another hospital and a smaller proportion (13%), visited some specialists in the same hospital and for some they had to travel. Five patients (1.7%) visited specialists in private clinics..

Specialist care is only partially covered by state free services or state health insurance for 90 patients. This means that 90/415 (21.7%) of patients with chronic anaemias in Europe have to pay totally or partially for specialist care. This makes access even more difficult and raises questions on whether quality of care is not affected.

- **6. Days lost from education or work**

Patients with chronic anaemias lose days from work because of ill health but also because of health service working hours and the need for appointments for services. In this survey the responses are as follows:

Days lost from education or work per year?	Number of patients	Percentage
• None	45	11%
• 1-5 days	37	9%
• 6-10 days	20	4.8%
• 11-15 days	48	11.6%
• 16 or more days	213	51.1%
• No answer	52	12.5%
• Total	415	100

Patients who lose more than 16 days per year have a higher probability of poor health or of receiving poor quality services. In considering the criteria of centres of expertise, this parameter can be used as an outcome measure for periodic re-evaluation of national centres of excellence and auditing even though, in this survey it is self reported by patients retrospectively and so results may be inaccurate.

- **7. Talking about the condition**

The ease with which a patient with chronic disease talks about his/her condition to peers outside the family is a quality measure of social adjustment which however is influenced by the culture and degree of knowledge of the social environment. In this survey it seen from the responses to the question that patients are almost equally divided in their responses:

How easy is to talk to a friend about having Thalassaemia or Sickle Cell Disease?	Number of patients	Percentage
• Very easy	71	17,1%
• Easy	137	33,%
• Difficult	129	31.1%
• Very difficult / impossible	45	10,8%
• No answer	33	8
• Total	415	100

Most patients, around 60%, who talk easily about their condition are those looked after in specialised haemoglobinopathy centres.There might be different reasons for this, including a better quality of the clinical management, taking into account the psychological aspects of the disorder.

- **8. Obtaining knowledge about the disease**

Transfer of knowledge about the disease is the duty of every treating physician and medical team. Patients do consult and seek answers from other sources and it is important to know what these sources are, how reliable they are, and whether it is the duty of the expert centre to inform and educate these other sources so that the information that they provide is accurate. In this survey we obtained the following responses:

Find out about the correct treatment	**Number of patients out of 415**	**Percentage**
• From own doctor	296	71%
• From other doctor	46	11%
• Reading the protocol	47	11%
• From the patient association	117	28%
• From other patients	83	20%
• From the internet	71	17%

From these results it emerges that the patient support association is the most important source after the patient's doctor. This emphasises the need of the close relationship that the expert centre needs to maintain with the support association and the need to provide information and educational material so

That correct information is ensured. The internet also needs to be monitored for accuracy. The importance of peer communication in chronic disease is also demonstrated.

B. COMPARISION EXPERT CENTRES VS OTHER CENTRES

In the previous analysis of the patient questionnaires an overview of the responses was given. In this second review we present the differences in response between patients treated in specialised haemoglobinopathy centres, compared to patients receiving care in general haematology departments, paediatric departments or internal medicine departments.

- **1. Centre category**

Of 415 patients who responded 195 (47%) stated that they received care in a specialised haemoglobinopathy centre while 220 (53%) received care in other departments, as detailed in the following table:

Receive treatment in	Number of patients	Percentage
• General Haematology Department	176	42%
• General Paediatric Department	36	9%
• Private clinic	0	0%
• Other (mostly oncology)	8	2%
• Specialised haemoglobinopathy centre	195	47%
Total	415	100%

2.The patients were asked if they take their medications (chelating agents) regularly:

3.Adherence to treatment is regarded as an indicator of quality of care.

In this series, of 126 patients who claimed regularity in taking medication 91 (72%) were being treated in specialised centres, while 35 (28%) in other departments. This might be an indication of the difference in care and attention provided by the specialised services.

Blood transfusion:

The vast majority of patients receive their blood transfusions in a specialised haemoglobinopathy centre or in a haematology day unit:

Where transfused	Number of patients	Percentage
• Haematology day unit	176	42%
• Adult Haematology ward	32	7,7%
• Children's ward	35	8,4%
• Accident & Emergency	4	0,96%
• Specialised Haemoglobinopathy Centre	141	34%
• Home	1	0,24%

Patients transfused in haematology day care units may have to share staff attention with other less chronic but more dramatic conditions such as haematological malignancies. Also 6 patients over the age of 18 years were receiving blood transfusions in a paediatric

department, including one patient aged 50 years. At least for chronically transfused patients a specialised day unit is recommended. However the specialised centres are not more helpful than the other centres since 66% of those who are required to be transfused in the morning are treated in such centres, interfering with other commitments. Availability of hospital services outside school or working hours is a quality consideration for dedicated centres.

- **4. Multidisciplinary care**

Multidisciplinary care is a feature of all centres dealing with chronic disease as well as chronic and rare anaemias. Iron overload is only one of the possible
complications with effects on vital organs. Over time all anaemias become multi- organ disorders, including the sickle cell syndromes, Fanconi's anaemia, Paroxysmal Nocturnal haemoglobinuria and others. In this survey it was found that the multi-transfused patients who are followed according to guidelines were mostly patients followed by a specialised centre:

Regular follow up of at-risk patients	**Specialised Centre**	**Other centres**	**No answer**
Cardiology follow up	66.3%	36.4%	1.3%
Endocrinology follow up	70.6%	30.6%	1.2%

One question which is important to the effective care of multi-transfused patients is whether they are followed from an appropriate age by a specialist for early detection of complications or whether they are referred only once a complication has arisen. From this questionnaire a clear answer cannot be given

5. The ease with which patients talk about their condition

Of those that answered that they find it easy or very easy to talk to a friend about their condition, 60% are treated in specialised centres, while 40% are treated in other centres. This result may indicate that patients treated in centres are more able to discuss their problems so the support they receive is probably stronger.

6. Trust of patients concerning treatment

7. 147 patients answered that they were not receiving correct treatment or at least that they were not sure. 78 (53%) of the patients who answered negatively are treated in other hospital units while the rest are treated in specialised centres. Patient anxiety is evident in whichever environment they are cared for. **Relationship with services** The patients were asked a series of questions aimed to detect whether quality of care was better in specialised centres. A superiority of the specialised centres was demonstrated in the following fields:

- Patients are asked about problems with medication.
- Patients are given a written copy of treatment plan (mostly or always)
- Patients regard their care as well organised
- Doctor considers patients beliefs, habits etc in choosing treatments
- Doctor makes a treatment plan that the patient can carry out
- Patients are encouraged to join a patient association

Where the specialised centres perform less well than other centres, is in giving patients choices concerning their treatment and discussing goals. These findings may be a reflection of a more authoritative approach by doctors in these centres where specialised doctors feel that their expertise cannot be challenged.

- **8. What do patients expect and want to see in a specialised centre**

The final part of the questionnaire was devoted to series of 20 questions, each one expressing possible characteristics of an expert centre, and the patients were asked grade these characteristics according to whether they regard them as essential or not. The most essential characteristics, chosen as essential by over 55% of patients, are the following:

- Experience and technical support to diagnose and assess complications: regarded as essential by 61% and useful by 29%
- Following good clinical practice guidelines: regarded as essential by 58% and useful by 34%
- A centre should have a coordinated team with an experienced doctor in charge: chosen by 71% and regarded as useful by 22%
- A multi-disciplinary team of specialist working within the centre: essential for 60% and useful for 32.5%
- A doctor who understands the patient's needs: essential for 67.5% and useful for 27.5%
- Doctors who discuss treatment plans and give choices: essential for 62% and useful for 33%

In contrast patients regarded the following as less:

- The centre should be a separate unit from other hospital departments: regarded as essential by 48% and as unnecessary or of little use by 16%
- The presence of a psychologist or a social worker in the centre: regarded as essential by only 28.4% and of little use or not necessary by another 28.6%
- The centre to be involved in research: regarded as essential by 40% although another 47% thought it useful
- Staff taking time to teach self care: regarded as essential by 44.8%
- Staff informing about patients rights: essential for 45%
- Staff guiding the patients towards social services, educational and other activities: this was regarded as essential by only 26.75%. However around 50% felt that it is useful.

- Centre to be in contact with the general practitioner or local health services to give advice: this was regarded as essential by 37%
- The centre networking with centres abroad is essential for 48% and within the country for 50%
- Maintaining close links with the patients' support association is regarded as essential by 41% but also useful for 43%
- Patients voice in advisory committees: essential for 46%

C. SUMMARY OF CONCLUSIONS

A. Patients treated in specialised centres, according to their responses seem to have better results compared to those treated in other centres in the following:
    - They have better adherence to prescribed treatment
    - They have shorter waiting times for transfusions
    - Timing for transfusions is more likely to consider the patients' educational and employment needs
    - They are more likely to be followed by a cardiologist according to guidelines
    - They are more likely to followed by an endocrinologist according to guidelines
    - They are more likely to talk about their condition with friends. This indicates better social adjustment and possible support
    - Patients treated in specialised centres acknowledge better quality of services since they are more likely to be asked about more problems with medication, the doctor considers their beliefs and habits in prescribing and makes a treatment plan that they can carry out and is more likely to provide a written copy of the treatment plan. However they are less likely to be given choices or to discuss their goals in treatment. They generally regard their care well organised.
    - They are more likely to be directed to join a patient support association

B. Patients with severe anaemias, who responded to this questionnaire, have accepted all the suggestions concerning the qualities of a specialised centre. However they regard as the most essential features of an expert centre the following;
    - Experience of the centre in diagnosis and assessment of complications
    - Following good clinical practice guidelines
    - A coordinated, multi-disciplinary team, within the centre, with an experienced doctor in charge.

- A doctor who understands the patients needs
- Discussion of treatment plans and to be given choices in treatment

They gave their 'least necessary' (=acceptable/desirable but secondary) votes to the following:

- A separate unit from other hospital departments
- The presence of a psychologist or a social worker in the centre or to be guided to such services
- Involvement of the centre in research
- Teaching self care
- Information about patient rights
- Contact and networking with primary care or other centres, nationally or internationally
- Links with support associations
- A patients voice in advisory committees

4. ENERCA RECOMMENDATIONS

4.1. ENERCA recommendations for Centres of Expertise on rare anaemias.

The consensus recommendations is the outcome of a proposal of general agreement between the different ENERCA partners involved in different process of labelling or recognition as centres of expertise for rare and very rare anaemias in their respective countries; ditto for the local centres.

For countries where no such process is running, this consensus could be an example, for countries where this process is running, as this consensus offers an ideal level of services to be provided, it may serve to raise the current level.

Based on the three reports resulting from the surveys analysis, proposals of recommendations were elaborated and circulated among EGRA in order to be discussed in a multidisciplinary way and reach a final consensus.

The interactions between the different proposals were a key point to be analyzed. Accordingly, a meeting was held on 1st February 2012 in Paris. Two external advisers were invited:

- Phil Darbyshire- Adviser on UK criteria for expert centres recognition.
- Raffaela Colombatti- Italy - Adviser on Haemoglobinopathy criteria

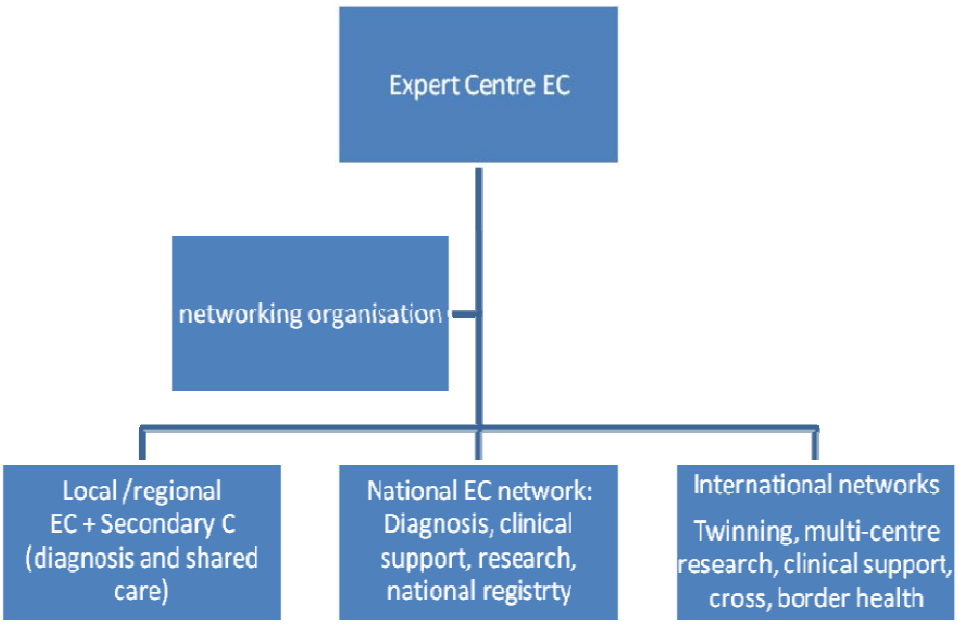
Final proposal of ENERCA consensus recommendations

The proposal presents a set of recommendations as the minimal required identifying a centre as one out of the following two categories:

“Centre of expertise “ is as a centre with an appropiatte capacity to address the complex and diverse conditions of RAs within a multidisciplinary approach. A centre of expertise provides expert advice, produces guidelines and has links with other centres of expertise building a European Reference Network.
“Local centre” is a centre that offers health care in a defined catchment area. Local centres are bounded to a centre of expertise

The relations between this two categories of centres are integrated in a supra structure represented in figure

Figure


Expert Centre EC
networking organisation
Local /regional EC + Secondary C (diagnosis and shared care)
National EC network: Diagnosis, clinical support, research, national registrty
International networks
Twinning, multi-centre research, clinical support, cross, border health

Recommendations for centres of expertise for Rare Anaemias

Legal and ethical recommendations

The harmonization concerning the procedures of the management of data and samples would help to remove obstacles for the collaboration between centres in different countries and for the creation of a network. Although there is a common european regulation of data protection in Europe (Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data, under revision), its implementation or interpretation differ among States. Besides, there are very specific but important points that are not regulated in some States.

This is why a minimun consensus would be very positive and the recommendations try to underline some important issues that should be adressed form this perspective.

There are general recommendations, recommendations for the clinical activity and recommendations for the area of research (even though this is not the framawork of this WP).

Sometimes the recommendations are just a wake up call and reproduce a mandatory requirement (eg. The implementation of security mesures for data files) and in other cases, the recommendation goes deeper than the regulation in some concrete points, when the harmonization is considered specially usefull (eg. Stablishment of a minimun period of 15 years for the storage of “non active” patients). These recommendations are suggestions that would help the creation of a network and could lead for benefits for the patients.

Concerning data, it is recommended to inform the patient about that the data and samples could be send to other centers outside the National Health System, including the description of the objectives of the management (clinical, epidemiology or research)and to ask for her appropriate consent. An adequate information and consent, as well as the establishment of a procedure to share the data and samples, would be a good basis for further uses of this resources in the benefit the patient, her family and also the scientific research.

Finally, when samples or data obtained for health purposes are going to be used with research purposes, specific consent of patient should be required. Some concrete items are purposed:

a) Purpose of the research, especially, if DNA or RNA data are going to be obtained.

b) Expected benefits

d) Identity of the person responsible for the research, or provision about the possibility of information about this.

e) The patient's rights with regard to revoking the consent, and the effects this may have

f) Location of the undertaking of the analysis and the destination of the sample at the end of the research?: codification, destruction or other research

g) The patient's rights to gain access to the obtained data

h) Guarantee of confidentiality of the information obtained

i) Information regarding the implications of genetic analysis

j) His/Her faculty to take a stance in relation to the communication to him / herself of the data obtained when the data are relevant for his /her health.

k) Information regarding the implication of the results for his / her family members

l) The convenience that the person, where appropriate, transmit this information to the family members, in case it would be relevant for their health

m) Indicating the possibility to get in contact with him /her, and the way to do so.

n) Information regarding the data will be stored

o) Identification and contact details of the person responsible for the storage of data

p) Procedure to exercise his / her rights regarding to the data storage

q) Provisions about international circulation of data

r) Provisions about international circulation of samples

Medical and Techinal recommendations

Any centres of expertise, which serves patients with RAs, must comply with the standards and conditions that have been specified by RDTF and EUCERD, as previously described. At the same time the services provided should be adapted to the needs for diagnosis and management of the specific haematological disorders. The diverse conditions, which are, classified as RAs share common service needs which include the requirement for a correct diagnosis, the need to control the anaemia, the need to monitor and manage complications and the need for holistic and supportive care. RAs are conditions, which are chronic and often manifest in infancy. Many are lethal without the provision of optimal treatment and this makes life-long multidisciplinary care a necessity.

The rarity of these conditions means that most centres treating haematological disorders will not gain experience and so diagnosis and management will be concentrated in centres which have, for reasons of prevalence or academic interest, made special studies and have contributed to research and case management. It has been demonstrated that survival is improved when patients are treated in an expert centre (GL Forni et al 2009).

It is recommended that an expert centre for RAs, in order to fulfil the RDTF and EUCERD criteria, should meet the following standards and be able to offer the following services:

1. ***The capacity to provide expert diagnosis or confirmation of diagnosis***
   For the diagnosis of RAs the basic requirement is a competent haematology laboratory equipped for all routine tests, including automated red cell counters, morphology, and tests for haemolysis and haemoglobin fractionation. However no single centre has the capacity to make a definitive diagnosis of all 62 listed RAs. There should be availability in each centre of special diagnostic tools for some RAs according to the special interest of the laboratory or the disorders prevalent in its geographical locality. For those conditions where diagnostic experience is lacking then the expert laboratory should have knowledge of the laboratories that can offer assistance so that networking becomes immediately a necessary feature of expert centres.

Examples of necessary specialised tests available to an expert centre are the molecular tests and specific tests, the enzymatic assay of at least the the most frequent RBC enzyme defect (G6PD) Algorithms should be developed for the diagnosis of each of the anaemias. All methods used should have external quality control, as described in the following section.

Diagnosis includes the identification and early detection of complications in patients. This requires that the expert centre be supported by hospital services such as general biochemistry and virology but especially by radiology. The radiology department must provide the following services necessary for monitoring haematology patients: abdominal ultrasound, trans-cranial Doppler, echocardiography, magnetic resonance imaging including angio-MRI and means to assess iron concentration in vital organs (such as MRI-T2*), bone mineral density (DEXA), and computerised tomography. These tests are means to support the need for expert case management.

2. ***The capacity to provide expert case management***

In these chronic blood disorders, case management requires several conditions and standards, which include:

a. Adherence to <u>good practice guidelines</u>, as listed in page 109-11. Through these evidence based guidelines the following recommendations are regarded as essential:

- The availability of adequate supply of safe blood for transfusions is a necessity for many forms of RAs and so close links with the blood bank and the hospital transfusion committee are necessary. For regularly transfused patients the possibility of adverse reactions is increased and so special protective measures such as extended red cell genotyping genotyping, pre-storage filtration and nucleic acid testing (NAT) must be implemented.
- The availability of all essential drugs, such as iron chelating agents, and the adequate supply of these drugs is a necessity.
- RAs become, over time, multi-organ diseases. In order to address this there is need for the availability of a coordinated multidisciplinary team of specialists and the preventive

monitoring of patients by haematologists and/or internists, cardiologists, endocrinologists, psychologists, hepatologists and others according to the expected complications. Specialised nurses are also a necessary component of a multi disciplinary team. In many of these disorders pain management is necessary and close collaboration with a pain control team is essential. Hypersplenism and splenic sequestration are features of several RAs and splenectomy is often considered essential so that collaboration with the surgical team is necessary. It is recommended that leadership and coordination of the multi-disciplinary team should provide for team meetings and joint consultations.

- The provision of free treatment with no out of pocket expenses from these chronically affected families.
- Providing holistic care. Treating every facet of the person – physical, emotional, psychological, educational, financial and vocational
- Maintaining a dedicated clinical record for each patient preferably on an electronic form
- Staff to follow previously agreed practical procedures or activities such as blood transfusion and haemovigilance, iron chelation, pharmacovigilance, growth monitoring, infection prevention.
- Specialized facilities for patient care are required. In many hospitals care for non-malignant haematology is provided in the same settings as malignant cases, which may have dangerous neutropenia and its consequences. For RAs it is recommended to have separate outpatient and day care services, where blood transfusions and other procedures can be given. These day care units are essential for patient safety and privacy and should provide services at times compatible with the patients' needs for education and employment.

b. Provide expert advice, including information to patients and genetic counseling. Patients with chronic conditions should receive detailed explanations concerning their disorder, their treatment options including information on side-effects. This is a prerequisite to good management which allows patient involvement, self management, and promotes adherence to treatment. The process of educating patients about their

condition begins with the first patient/doctor encounter but may continue with other members of staff. Counseling is a skill that requires training and should adhere to standards, respecting the autonomy of the individual or couple, privacy and confidentiality. This means giving enough time at each interview. It is obvious from the Enerca patient questionnaire that patients value this information and the time taken to provide it, especially by the doctor. The uncertainty of almost 40% of the patients concerning the correctness of their care, is probably a reflection of the lack of adequate information and patient involvement. There is evidence of a discrepancy between the physician's treatment goals and patients' expectations (Nagl M, Farin E 2011). Enerca strongly recommends that the degree of communication should be sufficiently high to allow for better understanding of each patient's beliefs and concerns, especially about medications. Assistance in this process may be provided by an expert patients' programme, in which already well informed and experienced patients can share concerns and experiences with other patients. This is supported by the patients' questionnaire which showed that patients received information from sources other than their treating physician. The expert centre should ensure that such information is accurate by 'training' these other sources (support associations, other patients) and to review the information on the internet.

c. Psychosocial support is vital in any chronic disorder and should be integrated in the global management. Medical and nursing staff should be trained in providing emotional support to patient and family. Special attention must be given to non-adherence to prescribed treatment. There must be availability of mental health professionals. One aim is to encourage self care and management. This requires educational activities and materials for the patients.

d. Staff patient ratio – The staff required for an expert centre is difficult to estimate and several factors must be considered. The first is the need for continuity of care which is a recommendation for all chronic disorders. This means that senior staff should stay constant even if junior doctors will probably rotate as part of their training. Another factor determining the number of staff, concerns the duties which may be allocated to doctors. Duties, of a haematologist for example, may include laboratory duties as well as clinical and even administrative. In addition there may be teaching and research duties. Decisions on staffing must be estimated according to local conditions. For

haemoglobinipathy centres the staff/patient ratio was arbitrarily set as 1 doctor per 50 patients by a WHO advisory group several years ago. In addition it is advised to have 1 nurse for 33 patients and 1 psychologist per 100 patients. Secretarial support must not be neglected and 1 per 100 patients is recommended. More detailed studies at local level are needed since where haemoglobin disorders are rare or other rare anaemias are present, these levels may not be reached The staff patient ratio should be further discussed the need for staff experience must be satisfied.

3. Implementation of outcome measures and quality control – the importance of a patient registry:

An expert centre should be ready to provide evidence of good outcomes in terms of morbidity (complication rates) and mortality. Such data can only be available if an updated registry of patients is maintained with enough clinical information to allow regular evaluation of results. The kind of data that is collected should include age distribution of patients, survival data, auditing disease related deaths, monitoring population screening and prenatal diagnosis programmes, the number of new annual affected births and quality of life outcomes. A list of indicators of monitoring evaluating outcomes should be agreed by the expert centres. Patient interest outcomes and patient driven questionnaires are recommended. It is important to identify 'care gaps' (Montague TJ et al Can J Cardiol 2007 Gagnon MP et al Implementation Science, 2011) which are the difference between best care and usual care, covering all aspects including access, diagnosis, prescription and treatment adherence.

Methodologies for quality control and auditing are therefore necessary for any centre labelled an expert centre and a periodic re-evaluation process and regular auditing are included in the 2011 Eucerd Recommendations on Quality Criteria for National Centres of Expertise. In addition quality of care includes measures of patient satisfaction, such as confidence in the correctness of treatment they receive, the quality of information that they are given, time spent in communication with staff, timing of services and several others which are included in the patient questionnaire of thisENERCA project.

It is recommended that the registry is electronically based with the facility of statistical analysis of patient data. It should also conform to accepted European standards for confidentiality and patient informed consent. A list of indicators for measuring outcomes is should be agreed on a national or international level so that uniform reporting will assist in comparing results.

4. Sufficient activity and capacity to provide relevant services at a sustained level of quality see comment above

Quality cannot be assured if a clinic sees few patients and a laboratory few requests. What, however, is the minimum throughput required to designate a clinical and/or laboratory haemoglobinopathy centre expert is not clarified. It is suggested that the minimum number of patients should be not less than 50 although this number may be less for the very rare anaemias. This figure is arbitrary and subject to debate. Concerning the laboratory samples, after consultation with a group of laboratory scientists, a minimum of 500 samples per year was suggested, depending on the type of test considering also the rarity of the anaemia. For very rare anaemias reference laboratories networking across Europe may be used for confirmation of the diagnosis.

5. High level expertise and experience

To determine this it is necessary to investigate such parameters as:

a. The grades and specialities of doctors/nurses.
b. The number of years they have been involved in managing patients with rare anaemias. This is ensured if continuity of care is implemented.
c. Strong contribution to research. Research should be patient orientated and ethically conducted. Adequate and accurate patient information is necessary, in simple understandable lay terms, so that informed consent may be obtained. This is particularly needed where drug trials are concerned. Research should be supported by grants and lead to relevant publications.
d. Teaching and training activities.
e. Involvement in Epidemiological surveillance

It is recommended that these parameters are considered in the designation of an expert centre.

6. Close links and collaboration with other expert national and international centres and capacity to network.

Networking is a major necessity in rare diseases involving both secondary centres and centres of expertise. This has been a concern of the DG SANCO High Level Group on health services and medical care, who have recommended the development of European reference networks (ERNs) since 2004. Emphasis on ERNs has also been given by the recent EUCERD recommendations. The Enerca project has from the beginning of the project been investigating the possibility creating networks for expert centres dealing with rare anaemias and creating tools to facilitate networking. Electronic tools are basic to successful

communications between network partners. Legal and ethical considerations must be considered for the exchange of information and patient samples and clinical data, which is another aspect which has been central to the Enerca project. All these aspects networking are further discussed in other parts of this book.

7. Close links with patients associations

Patients with RAs should be well supported by patient driven organisations which are able to promote services and provide patient support. The treating expert centre should be aware of the activities of the associations and be able to collaborate and even guide the associations to assist in the needs of patients.

Further reading

1. Forni GL, Puntoni M, Boeri E, Terenzani L, Balocco M. The influence of treatment in specialized centres on survival of patients with thalassaemia major. Am J Hematol. 2009; 84(5): 317-8
2. Nagl M, Farin E. Congruence or discrepancy? Comparing patients' health valuations and physicians' treatment goals for rehabilitation for patients with chronic conditions. Int J Rehab Res 2011
3. Montague TJ, Gogovor A, Krelenbaum M. Time for chronic disease care and management. Can J Cardiol 2007. 23(12): 971-5
4. Gagnon MP, Labarthe J, Legare F, Ouimet M, Estabrooks CA, Roch G, Ghandour EK, Grimshaw J. Measuring organizational readiness for knowledge translation in chronic care. Implementation Science 2011, 6: 72
5. EUCERD Draft Recommendations for European reference Networks for Rare Diseases. EUCERD Meeting 20-21 June 2012.

Expert laboratories services

a) General recommendations:

- The laboratory must be appropriately accredited with a nationally approved accreditation scheme based on the ISO 15189 standard.
- The laboratory must have all the tools/techniques necessary to obtain a final diagnosis (in its own environment **or via a national or European network)**
- The laboratory must participate in accredited national or European External Quality Assessment Schemes (EQAS) for the tests (or diagnosis) performed and must be able to demonstrate satisfactory performance as defined by the criteria specified by the EQA scheme organisers. If national or European EQA doesn't exist, inter laboratory evaluations on patients' samples should be implemented.
- High level of expertise and experience of the laboratory must be documented through publications, grants or honorary positions, teaching and training activities.
- There must be a strong contribution to research
- The laboratory must be involved in epidemiological surveillance, such as registries.
- The laboratory must have close links and collaboration with other expert laboratories at the national and international levels and capacity to participate in networks.
- The laboratory must have close links and collaborations with clinical centres of expertise.

b) Recommendations for laboratory diagnosis of a rare or very rare anaemia

- The laboratory must use a testing algorithm to determine those individuals or pregnancies at risk of severe **rare or very rare anaemia**. This testing algorithm sets out the conditions to be tested for and the analytical methods that can be used.
- The laboratory must have a standard operating procedure for the screening service, describing the process of laboratory testing from initial receipt of the specimen until dispatching of the report.
- The laboratory must provide guidelines for the standardised reporting of antenatal screening results.
- There must be a documented risk management policy for the laboratory aspects of the screening service. This should describe the steps in the testing protocol where mistakes could occur and the procedures that have been implemented to minimize the risk of the mistake occurring.

c) Recommendations for Genetic Counselling

Trained and competent counsellors are needed in order to provide:

- Clear and non-directive information with the possibility of informed choice by the at-risk couple.
- Explanation on the genetic risk in a manner easily understandable by a lay person.
- Clear information on the disease and its management, including the likely outcomes of treatments.
- Clear description of the risks of prenatal diagnosis as well as its benefits where applicable.
- Clear description of all the available reproductive options for the couple.

Consensus recommendations proposal: Specialised services for rare and very rare anaemias

LEGAL AND ETHICAL	***Description***	***Expert centre and Network of care***	***Local centre linked to providers of services***
	GENERAL		
Security measures for data files	Implementation of security measures according to national law (transposition of Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data). The security measures refer to files with patient's health data. Among others security measures, it is remarkable that health data should be transferred using an encryption process.	**X**	**X**
Electronic clinical records (Non mandatory. Recommended to be part of the ERN)	Implementation of electronic clinical records	**X**	**-**
Ethical and legal training for professionals	Professionals receive training about the ethical and legal requirements involved in their activities	**X**	**-**
Quality requirements of centres in order to transfer data and samples	Samples and data are sent only to centres that comply with quality standards	**X**	**X**

LEGAL AND ETHICAL	***Description***	***Expert centre and Network of care***	***Local centre linked to providers of services***
	DIAGNOSIS AND TREATMENT		
Period for the storage of "non active" patient's data	Minimum period of 15 years to keep the data in the clinical record is determined.	**X**	**X**
Transfer of clinical data and samples	Clinical data and samples will be sent to other centres after codification process. Means should be implemented in order to assure that it will be no reasonable way to identify the patient in the centre that receives the data or samples.	**X**	**X**
Information and consent about the transfer of identified data or samples	In case identified data or samples are going to be transferred to a centre outside the National health system, information and consent is previously obtained to the patient.	**X**	**X**
Information about health care in other centres	Centre informs patients about the possibility of receive appropriate health care in other centres in the country or abroad	**X**	**X**
Procedure to transfer of data / samples / patients	There is a established procedure to transfer data, samples or patients to other centres	**X**	-
Requirement for professional proof or evidence of expertise	In the case of absence of national regulation, professional expertise has to be demonstrated by sufficient merits.	**X**	-
Quality standards regulations	Centres comply with the quality standards required by national legislation for the services that provide.	**X**	**X**

LEGAL AND ETHICAL	***Description***	***Expert centre and Network of care***	***Local centre linked to providers of services***
	RESEARCH		
Subject consent for the use of samples or data for research purposes.	When samples or data obtained for health purposes are going to be used with research purposes specific consent of patient is required. (see concrete items in the introductory remarks to these remmendations) Information have to be given about the transfer previewed to other centres with research purposes.	**X**	**X**

MEDICAL AND TECHNICAL	***Expert centre and Network of care***	***Local centre linked to providers of services Minimum services *In collaboration with expert centre(s)***
LABORATORY		
Phenotype:		
Screening methods + External QC (if available)	**X**	**X**
Extensive phenotyping + External QC (if available)	**X**	-
Genotype + External QC (if available)	**X**	-
Prenatal diagnosis (if applicable)	**X**	-

SPECIALIZED EQUIPMENT FOR ** can be shared in networks		
Management of severe and life-threatening complications	**X**	**-**
Management of acute, uncomplicated events	**X**	**X**
Management of chronic complications**	**X**	**X**
Routine out-patient monitoring	**X**	**X***
Routine day case transfusion	**X**	**X***
Blood transfusion	**X**	**X***
Bone marrow transplantation	**X**	**-**
Specific treatments (e.g. Iron chelation)	**X**	**X***
DIAGNOSIS, FOLLOW-UP, MANAGEMENT	***Expert centre and Network of care***	***Local centre linked to providers of services Minimum services *In collaboration with expert centre(s)***
SPECIALIZED TEAM FOR ** can be shared in networks		
Management of severe and life-threatening complications	**X**	**-**
Management of acute, uncomplicated events	**X**	**X**
Management of chronic complications**	**X**	**-**
Routine out-patient monitoring	**X**	**X**
Management of surgical procedures	**X**	**X***
Management of pregnancy	**X**	**-**
Routine day case transfusions	**X**	**X**

Blood transfusion **	**X**	**X***
Bone marrow transplantation**	**X**	**-**
Specific treatments (e.g. Iron chelation)	**X**	**X***
MULDISCIPLINARY APPROACH		
Consultations	**X**	**-**
Genetic counselling	**X**	**-**
Link with screening programmes (if applicable)	**X**	**-**
Recommendations, standards of care	**X**	**X***
Network with peripheral hospitals, GPs …	**X**	**-**
From paediatric to adulthood	**X**	**X**
DIAGNOSIS, FOLLOW-UP, MANAGEMENT	***Expert centre and Network of care***	***Local centre linked to providers of services Minimum services *In collaboration with expert centre(s)***

PROOF (EVIDENCE) OF EXPERTISE

Certification (Accreditation)	X	**-**
Anonymous annual data collection of key outcomes	X	**X***
Number of patients (annual regular follow-up)	X	**-**
Rate of mortality	X	**-**
Event-free survival	X	**-**
…	X	**-**

Publications	X	-
Research or link with research	X	-
Grants	X	-
Teaching	X	-
Training	X	**X**
Network (National/international)	X	**X***
Network inside the Institution	X	**X**

SERVICES FOR PATIENTS (specialist nurses) evidence of patients/families interactions and perspectives

Structured information (leaflet, website…)		
Responsibility in preparation	**X**	-
Availability of the information	**X**	**X**
When applicable, proof of informed consent	**X**	**X**
Support for home management, discharge from hospital	**X**	-
Education programmes	**X**	**X***

PATIENT PREFERENCES	***% Strong support and***	***Expert centre Network of care***	***Local centre linked to providers of services***
To follow best practice guidelines	58	**X**	**X**
Experience / technical support for diagnosis and complications	61	**X**	-
Separate unit for rare anaemias	48	**X**	-
Coordinated team with experienced doctor in charge	71,1	**X**	**X**
Multidisciplinary care	60	**X**	-
Doctor who understands patients' needs	67,5	**X**	**X**
Presence of a psychologist	28	**X**	-

Presence of a social worker	28	**X**	**X**
Continuity of care	52,5	**X**	-
Involvement in research	40	**X**	-
Adequate staff /patient ratio	49	**X**	**X**
Reducing waiting time	49	**X**	**X**
More time with the doctor		**X**	-
Staff attention to patient concerns	50,6	**X**	**X**
Proving information to patients		**X**	**X**
Assist self management	45	**X**	**X**
Discuss treatment plans	62,4	**X**	-
Inform about patients' rights	45	**X**	-
PATIENT PREFERENCES	***% Strong support***	***Expert centre and Network of care***	***Local centre linked to providers of services***
Expert centre to contact GP/2ry centre	37	**X**	-
Network of expert centres nationally	50,4	**X**	-
Network internationally	48	**X**	-
Close links with support associations	41	**X**	-
Patient voice in advisory committees	46	**X**	-
Transfusion after working /school hours		**X**	-
Full financial support of treatment		**X**	**X**

4.2.ENERCA Recommendations as an open self assessment dynamic tool

The ENERCA WhiteBook has been designed as a dynamic tool that can be evaluated and adapted according to the new needs and stakeholders' expectations. It aims to reach a critical mass of stakeholders, namely European and MS Health authorities, health professionals, scientific community, patients community and other providers of health care, in order to improve and upgrade the services offered.

ENERCA's objective is the recognition and long-term sustainability of the European Reference Network of Centres of Expertise in Rare Anaemias. For this reason, ENERCA has to promote geographical expansion in order to cover as many European countries as possible. This is a main consideration in the dynamic and long term evolution of the ERN and this will increases its European added-value.

ENERCA geographical expansion will be achieved not only by involving new expert centres into the ERN, but also by establishing collaborations with additional scientific societies and patients' organisations. This will 41 allow to share new experiences and will improve networking capacities and skills of the network.

The objective is the recognition by MS of the expert centres already identified and to allow the geographical expansion of the network to other expert centres, mainly those existing in Eastern countries.

An On-line aplication in the ENERCA website

One of ENERCA's main tool for the dissemination of the project results has been the ENERCA website. This Whitewebsite will be used to disseminate the WB and ENERCA recommendations. The White Book is freely available on the ENERCA website. In addition, an on-line application, based on ENERCA recommendations, is foreseen to allow centres dealing with RA to assess their level of accomplishment with the ENERCA recommendations. An evaluation of the agreement of participants to the recommendations published will be also assess

The ENERCA web portal – www.enerca.org

- The website is an open door to ENERCA. It is intended to be a tool for both patients and health professionals looking for reliable information and centres specializing in RA around Europe.

The website is open to professionals, patients and the general public. A restricted area is only for exchanges of ENERCA partners.

<u>Cross bar Contents</u>

- **About ENERCA:** you will find a description of ENERCA project, its main objective and outcomes, the partners that are involved in ENERCA project and the working groups.

- **Anaemias:** you will find a list up to 62 Rare Anaemias that we have in the database. If you click in one of them, sickle cell anaemia (SCA) for instance, you will go to the specific disease card, which includes:

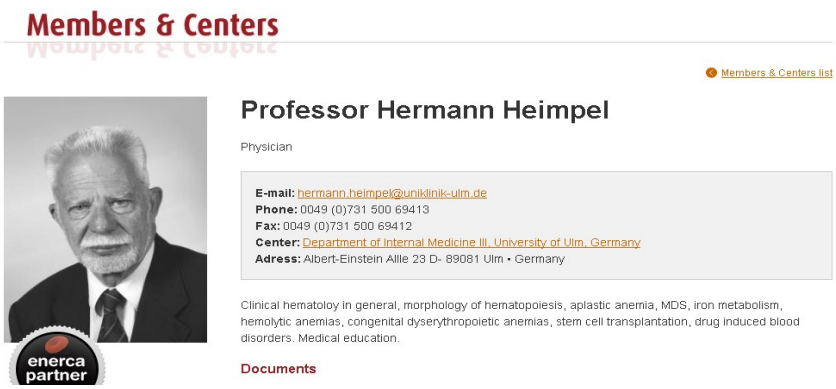
General info: Acronym, synonym, ICD classification, and links to orphan number and OMIM, group and subgroup and a general description in English prepared by ENERCA partners.

Members and centres dealing with SCA across Europe: You can also select those centres listed in your own country.

Patient Associations only for SCA: then you can choose your country and look for more information.

A list of *documents*, such as the disease's descriptions translated into seven languages by ENERCA partners.

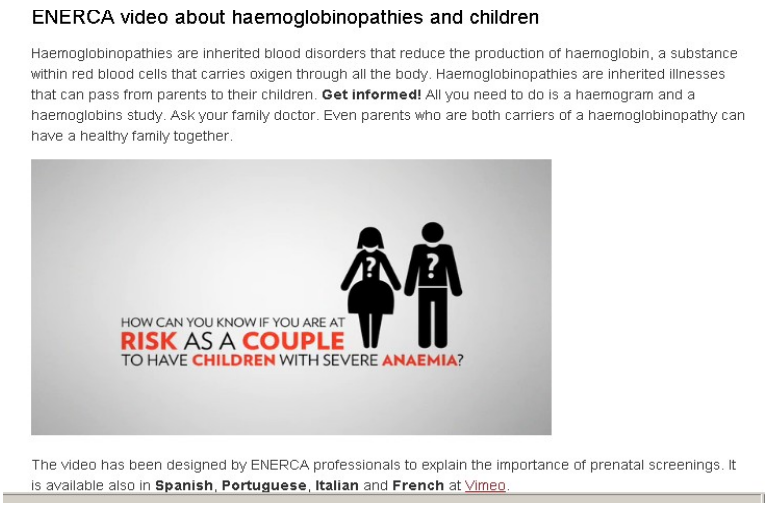
- **Members and centres:** you will find a map of Europe where you will be able to select your country, see all the centres across Europe or select a specific RA. Centres will be displayed by alphabetic order; you will also find the ENERCA members already registered in each centre and the diseases covered. In addition, the ENERCA member profile includes contact data, area of expertise, diseases and scientific publications.



- **Patient's Associations:** a similar structure that created for centres is used for patient's associations. Patients will be able to find out the patient's associations existing in their own country or in other country but focussed in their specific RA.

- **Documents:** In document section you will find all the documents listed by alphabetic order, and you can list them only for one specific RA or one language.

- **Activities:** you will find in this section different documents related to education and training activities performed in the framework of ENERCA, such as the descriptions of the RA, the speakers' presentations presented in the different topic focused courses organized by ENERCA, and in the ENERCA Symposium in RA, addressed to both patients and health professionals. You will also find educational material addressed mainly to patient's but also for health professionals of primary care and the general population, such as a video created in English and translated into Spanish, Portuguese, Italian and French aiming to increase the awareness about the preventive programmes for couples affected by a RA.



Other activities include the creation of comics focused on specific topics and address to teenagers and their parents.

- **News and events:** In this section you will find news that could be of interest for everyone involved in the field of RA; patients, health professionals, public health authorities, students. Includes progress of ENERCA project, scientific congresses, advances made by other RD associations…etc

- **Professional section:** In the professional section you will find a basic diagnosis flowchart for RA classification, the Medical Alert Card (MAC) that can be printed by the health professional for being presented by the patient when is moving to another centre or country, and a search engine to facilitate finding a health professional by her/his name.
- If you are a patient/professional and/or your association/centre is not listed: **join ENERCA!**

5. CONCLUSIONS: From the recommendations to the practice

The following conclusions are focused in the final goal of the EGRA, wich is the creation of a European Reference Network of centers of expertice in Rare Anaemias.

1. **The Community added value of European Reference Networks is particularly high for rare diseases** because of the rarity of these conditions, which implies both a limited number of patients and a scarcity of expertise within a single country. Gathering expertise at European level is therefore paramount to ensure equal access to accurate information, appropriate and timely diagnosis and high quality care for rare disease patients.

2. The **efficiency of ENERCA as a pilot network has been demonstrated** in relation with all its objectives. Specifically in the case of rare anaemias, one of the main problems is the high number of undiagnosed or not correctly diagnosed patients. In this sense some successful experiences have been achieved. e.g. networking for genetic diagnosis of enzyme deficiency disorders

3. In order to assure sustainability of the network on the long term it is mandatory to **officially acknowledge of the European Reference Network**

4. **The involvement of national authorities is fundamental to the development of the networks in two main areas**: the recognition of centers of expertice and the sustainability of the networks. The strategy should be a global one, as stated in the Council Recommendation of 2009.

5. The work done regarding recommendations included in this White Book could be a useful tool for Member States use in their national plans for the **recognition of centers of expertice.** The methodology used could be applied for other rare diseases. Member States should take into account the efforts already done. In this sense the White Book will be also presented to National Authorities involved in the Rare Diseases National plans.

6. The other major prerequisite for the development of National plan/strategies on rare diseases including centres of expertise and European Reference Networks is financial support for their **sustainability**.

The existing heterogeneity between Member States in health infrastructures and technical capacities of NHS systems to support such programmes and the resources available at Member State level to allocate for them has to be taken into account. It is not adequated to understand these networks as an academic exersice for the research community, but within a public health strategy.

7. **The Euopean Comission also plays an important role** with regard to the international dimension of this strategy. In this sense, the EUCERD Join Action is developing its activity in two directions, the establisment of a common framework for the creation of European Reference Networks for Rare Diseases and the implementation of mechanisms to assure their sustainability.

8. ENERCA as a pilot network funded by the Europen Commission contributes to the establishment of this framework by providing the experience in **key point regarding networking**.

- The initial experiences of ENERCA prove that **telemedicine and telediagnosis** serve the needs of the multi-disciplinary team which is especially necessary in the care of rare anaemias since complications may affect all vital organs or lifes. Networks shall be supported through information systems with interoperability including electronic health records (EHR), e-health systems and sharing of data and images. Electronic networking is the most cost-effective method of collaborative exchange; however, the need for patient travel and for local assessment by an expert should not be completely eliminated in order to achieve the best level of health care provision. Cross border health, as a recognised right of European citizens, must be respected and supported.

- Registry of patients is a fundamental tool in order to achieve the objectives of European Reference Network in rare anaemias, in both clinical practice and research. The ENERCA experience shows that the creation of the registry needs a national and cross border policy in this direction that takes into account all the facts implied: patient's rights, standardization of procedures (including diseasease codification), professional implication and technical support.

- Auditing of clinical and laboratory practice in the context of Rare Diseases is essential. The implementation of External Quality Assesment in the case of rare diseases needs a European approach to reach a minial critical mass

of laboratory performing the tests for its validation. ENERCA has identified gaps in this context and has promoted the creation of new External Quality Assessment schemes to assure the high quality in the diagnosis of patients with rare anaemias. Networking will allow easier identification of laboratories working on the same diseases, facilitating audit, the sharing of best practice and information on external quality assessment across national boundaries.

- In the context of rare diseases, European Reference Networks are the adequate platform to produce **Best Practices Guidelines**. Accorging to ENERCA experience, experts in the Networks are committed to the creation of Recommendations regarding clinical practice. The cumulative knowledge and experience of these experts represents a resource that must be upgraded to the status of guidelines.

- Networks have a **huge potential in educational activities**. ENERCA has had great succeess in the organization of several international and national events focused on specific aspects of rare anaemias. Therefore Networks serve as a tool to assure the inclusion of Rare Diseases in the Continual Medical Education.

- The need for **research** in multi-centre settings has already been emphasised and is an integral part of expert centre networking. Without such networks the development of new drugs in rare anaemias would be almost impossible.

- It is imperative that there is professional oversight and coordination of the Network, to ensure the maintenance of agreed standards and the dissemination of information about the Network to all European member states.

9. The **multidisciplinary approach** of ENERCA WhiteBook that includes not only the medical and technical issues, but also the legal and ethical ones, as well as the patients' expectations for centres of expertise has not been done before for an European Reference Network. This pioneer activity is an added value of ENERCA project that could be useful for any other network in rare chronic diseases.

10. Since the exchange of samples and the transfer of personal data is imperative in networking, in both clinical management and research, **legal**

and ethical issues must be taken into account in the European reference network. Althouhg a common European regulation concerning the management of personal data exists; there are some differences in specific and relevant points in national law. Similar differences can be found regarding samples management and biobanking. These differences hamper networking. A key point looking for a solution is to provide patients with adequate information before consent to the clinical procedure or the research involvement. This information should include the possibility of integrating the clinical procedure in a network and its consequences, as well as the descripción of the future research previewed. The importance of this harmonization will be increased taking into account the coming transposition of Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 regarding the application of patients' rights in cross-border healthcare.

11. **Involvement of patients** in ENERCA has resulted in several patient-based recommendations for the recognition of centres of expertise. Involving the patient's community **is essential** in the context of Rare Diseases. The patient's contribution leads to a more complete and realistic approach and assures that the quality of care matches the patient's expectations.

CONCLUSION: From recommendations to the practice

The Community added value of ERNs is particularly high for rare diseases by reason of the rarity of these conditions, which implies both a limited number of patients and scarcity of expertise within a single country. Gathering expertise at the European level is therefore paramount in order to ensure equal access to accurate information, appropriate and timely diagnosis and high quality care for patients with rare disease. This applies particularly to rare anaemias (RA) due to the high number of diseases involved in this group.

ENERCA, as a pilot network funded by the European Commission, has been contributing to the establishment of this framework by providing its experience in some key **points regarding networking**. These include the following:

1. **I**n the context of RD, networks are the adequate platform to produce **Best Practices Guidelines**. According to the ENERCA experience, experts in the Networks are deeply committed to the elaboration of Recommendations regarding clinical practice. The cumulative knowledge and experience of these experts represents a resource that must be upgraded to the status of guidelines.
2. **Auditing clinical and laboratory practices** in the context of RA (and RD) is essential. The implementation of External Quality Assessment in the case of rare diseases requires a European approach to reach a minimal critical mass of laboratories performing the tests. ENERCA has identified some gaps in this context and has promoted the creation of new EQAs to assure high quality diagnosis for patients with rare anaemias. Networking will allow easier identification of laboratories working on the same diseases, facilitating audit, the sharing of best practice and information on external quality assessment across national boundaries.
3. **Registries of patients are fundamental tools** in order to achieve the objectives of a ERN in RA (RD), in both, clinical practice and research. The ENERCA experience is that the creation of registries requires national policies in this direction. These have to take into account all the involved parameters, including patient's rights, standardization of procedures (including diseases codification), professional implication and technical support.
4. Networking has a **huge potential in educational activities**. ENERCA has successfully organized several international and national events focused on specific aspects of rare anaemias. Networks are also a useful tool to assure the inclusion of RD in Continuing Medical Education programmes.

5. The initial experiences of ENERCA prove that **telemedicine and telediagnosis** serve the needs of the multi-disciplinary team, which are necessary in the care of rare anaemias since complications may affect all vital organs. Networks shall be supported through information systems with interoperability including electronic health records (EHR), e-health systems and the sharing of data. Electronic networking is the most cost-effective method of collaborative exchange; however, the need for patient travel and for local assessment by an expert should not be completely eliminated in order to achieve the best level of health care provision. Cross border health, as a recognised right of European citizens, must be respected and supported.

6. The need for **research** in multi-centre settings has already been emphasised and is part of expert centre activities and EC networking. For example, the development of clinical trials on new drugs would be almost impossible in rare anaemias and other RD, without such networks.

7. Since the exchange of samples and the transfer of personal data are imperative in networking, in both clinical management and research, **legal and ethical issues must be taken into account in a European reference network.** Despite the existence of a European regulation concerning the management of personal data, some differences exist in specific and relevant points of national laws. In the same way, differences can be found regarding sample management and biobanking. These differences can become a barrier to networking. Providing patients with adequate information before they give their consent to the clinical procedure or the research involvement seems to be a key point to overcome this problem. This information should include the possibility of integrating clinical data in a network and its consequences, as well as the description of the future research previewed. The importance of this harmonization will rise, taking into account the coming transposition of Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 regarding the application of patients' rights in cross-border healthcare.

8. **Involvement of patients** in ENERCA has resulted in several patient-based recommendations for the recognition of centres of expertise. Involving the patient's community **is essential** in the context of RD. The patient's contribution leads to a more complete and realistic approach and assures that the quality of care matches the patient's expectations.

9. It is imperative that there is professional oversight and coordination of the Network, to ensure the maintenance of agreed standards and the dissemination of information about the Network to all European member states.

To our knowledge, this is the first time that a **multidisciplinary approach** such as the one of ENERCA White Book is used for an ERN. Indeed, it includes not only medical and technical issues, but also legal and ethical ones, as well as patients' expectations for centres of expertise. This pioneer activity is an added value of the ENERCA project that could be useful for any other network on rare chronic diseases. The **efficiency of ENERCA as a pilot network has been demonstrated** in relation to all its objectives. Specifically in the case of RA one of the main problems is the high number of undiagnosed patients. Some successful experiences have been achieved in that sense.

The involvement of national authorities is fundamental to the development of networks in two main areas: the recognition of centres of expertise and the sustainability of the networks. The strategy should involve both aspects, as stated in the Council Recommendation of 2009.

The work done regarding recommendations included in this White Book is a practical tool that States could use for the **recognition of centres of expertise** in their national plans**.** The methodology used could be transposed to other rare diseases. Member States should take into account the efforts already done. In that sense, it is a commitment for EGRA

members to present the WB to National Authorities involved in National Plans for Rare Diseases.

In order to assure long term sustainability of the **network** there is also a need for an **official recognition** of this structure.

The major prerequisite for the development of National plan/strategies on RDs, including CsE and ERNs, is financial support for their **sustainability**.

The existing heterogeneity between MS in health infrastructures and technical capacities of NHS systems to support such programmes, and the resources available at MS level to allocate for them, has to be taken into account. It is not adequate to consider these networks as an academic exercise for the research community, but rather to completely integrate them within a public health strategy.

This is why **the EC also plays also an important role** regarding the international dimension of these strategies. In this sense, the EUCERD Join Action is developing its activity in two directions, the establishment of a common framework for the creation of ERNs for RD and the implementation of mechanisms to assure their sustainability.

CBC: COMPLETE BLOOD COUNT

CD: CHRONIC DISEASE

CE: CENTRE OF EXPERTISE

DNA: DESOXYRIBONUCLEIC ACID

EAHC: EUROPEAN AGENCY FOR HEALTH AND CONSUMERS

EC: EUROPEAN COMMISSION

ENERCA: EUROPEAN REFERENCE NETWORK OF RARE AND CONGENITAL ANAEMIAS

ERN: EUROPEAN REFERENCE NETWORK

EGRA: ENERCA GROUP ON RARE ANAEMIAS

HB: HAEMOGLOBIN

MS: MEMBER STATE

MCH: MEAN CORPUSCULAR HAEMOGLOBIN

MCV: MEAN CORPUSCULAR VOLUME

PCV: PACKED CELL VOLUME

RA: RARE ANAEMIA

RBC: red blood cells

RD: RARE DISEASE

SCA: SICKLE CELL ANAEMIA

SCD: SICKLE CELL DISEASE

VRA: VERY RARE ANAEMIA

WB: WHITE BOOK

WP: WORKING PACKAGE

Useful sources

Executive Agency for Health and Consumers

European Commission DG Sanco

Eucerd TIF

Orphanet

Eurordis

Annex Ces France

1/ Situation of expert centres in rare anaemias in France

In 2005, the French government launched a national plan for rare diseases, considering rare diseases one of the five major priorities of the 2004 *Law relating to public health policy.* The main objective of this plan was "*to ensure equity in the access to diagnosis, to treatment and to provision of care*" for people suffering from a rare disease. Ten strategic priorities where defined, including epidemiology, information for patients, health professionals and the general public, education, screening and access to diagnostic tests, treatment and quality of healthcare provision, orphan drugs, support for patients' associations, research and innovation, national and European partnerships[1].Among the concrete actions undertaken, successive calls for bids were launched in the fields of both, reference laboratories (2005-2006) and clinical centres of expertise (2004-2006-2008).

In the domain of rare anaemias, six reference laboratories were selected. Reference laboratories were requested to form a national network in order to develop harmonized diagnostic steps, propose recommendations, organize training, set quality controls, give expert advice on atypical cases and ensure scientific and technology watch. The six labelled laboratories were mainly specialized in the diagnosis of haemoglobin disorders. In order to cover all the rare red cell disorders, they have progressively involved in the network non-officially labelled laboratories working in other fields of red cell disorders.

On the clinical field, the policy was to label two types of expert centres: only a few number of reference centres and then, in order to ensure a comprehensive coverage of the care all over the country, to build a network of so-called "competence centres" at the regional level. In the field of rare anaemias, two national "reference" centres on sickle cell disease and one on thalassaemia were labelled in 2004 and 2006. Fourteen centres of "competence" were then designated in 2008. They are all included in regional University Hospitals. Both, reference and competence centres had to fill in a detailed application form and were nominated officially by the French health authorities following a review process. The centres should be able to provide dedicated care on rare red cell disorders and should offer a number of facilities, including expert clinical consultations, dedicated hospitalisation, expert laboratory diagnosis (available in the centre itself or through the national network), high level imaging tools etc. Reference and competence centres are now organized in a network with periodical meetings.

Table XX France: list of officially labelled reference laboratories, centres of reference and centres of competence[2]

Reference laboratories
1. Laboratoire d'hématologie, Hôpital St-Eloi, CHU de Montpellier, Dr Patricia Aguilar Martinez.
2. Génétique moléculaire et biochimie, Hôpital de la Timone, CHU de Marseille. Pr Catherine Badens.
3. Génétique moléculaire médicale, CHU d'Amiens. Pr Jacques Rochette
4. Biochimie génétique, Hôpital Robert-Debré (Paris). Pr J Elion,
5. Laboratoire de biochimie de l'hôpital, Edouard-Herriot (Lyon). Dr Alain Francina, Dr Philippe Joly.
6. Biochimie génétique, Centre hospitalier Henri-Mondor (Créteil). Dr Serge Pissard.

Centres of reference on rare anaemias

1. Centre de référence des syndromes drépanocytaires majeurs. (2004). AP-HP Hôpital Henri Mondor, 51, avenue du Maréchal de Lattre de Tassigny, 94010 Créteil cedex. Coordonnateur : Pr Frédéric Galacteros. Tel : 33 (0)1 49 81 24 43
2. Centre de référence des thalassémies, (2006) AP-HM Hôpital des enfants de la Timone. Service d'hématologie pédiatrique 13385 Marseille cedex 5. Coordonnateur : Dr Isabelle Thuret, Tel : 33 (0)4 91 38 67 76.
3. Centre de référence de la drépanocytose, (2006) CHU de Pointe à Pitre/Abymes Hôpital Ricou, 97110 Pointe à Pitre. Coordonnateur : Dr Maryse Etienne-Julan. Tel : 33 (0)5 90 91 68 08

Centres of competence on rare red cell disorders

1. Service de pédiatrie 3, CHU Hôpital de Hautepierre, Avenue Molière, 67098 STRASBOURG CEDEX, Tel : 33 (0)3 88 12 80 91
2. Service d'hémobiologie, CHU de Bordeaux - Hôpital Pellegrin, Place Amélie Raba Léon, 33076 BORDEAUX CEDEX, Tel : 33 (0)5 56 79 59 62
3. Service d'oncologie et hématologie, pédiatriques, CHU de Clermont-Ferrand - Hôtel Dieu, Boulevard Léon Malfreyt, 63058 CLERMONT FERRAND cedex 1, Tel : 33 (0)4 73 750 009
4. Service d'hémato-oncologie pédiatrique, CHU de Dijon - Hôpital du Bocage, 2 Boulevard Maréchal de Lattre de Tassigny, BP 77908, 21079 DIJON CEDEX, Tel : 33 (0)3 80 29 36 01
5. Service de médecine de l'enfant et de l'adolescent, CHU de Rennes - Hôpital Sud,16 boulevard de Bulgarie BP 90347, 35203 RENNES CEDEX 2, Tel : 33 (0)2 99 26 71 62
6. Service d'oncologie pédiatrique, CHRU de Clocheville, 49 Boulevard Béranger, 37044 TOURS CEDEX 9, Tel : 33 (0)2 47 47 47 51
7. Service d'immuno-hémato-oncologie pédiatrique, CHU Hôpital Charles Nicolle, 1 Rue de Germont, 76000 ROUEN , Tel : 33 (0)2 32 88 81 91
8. Service d'Hématologie et d'oncologie médicale, CHRU de Montpellier - Hôpital Lapeyronie, 371 Avenue Doyen Gaston Giraud, 34295 MONTPELLIER CEDEX 5, Tel : 33 (0)4 67 33 80 79
9. Service d'hémato-oncologie pédiatrique, CHU de Nancy - Hôpital d'enfants Brabois, 5 Allée du Morvan, 54511 VANDOEUVRE-LES-NANCY, Tel : 33 (0)3 83 15 45 50

10. Unité d'hémato-oncologie, CHU Hôpital des enfants, 330 Avenue de Grande Bretagne TSA 70034, 31059 TOULOUSE CEDEX 9, Tel : 33 (0)5 34 55 86 11
11. Service d'hématologie, pôle Enfant, CHRU de Lille - Hôpital Jeanne de Flandre, Avenue Eugène Avinée, 59037 LILLE CEDEX, Tel : 33 (0)3 20 44 41 05
12. Service de pédiatrie - Unité d'hématologie, Centre Hospitalier de Mamoudzou, BP4 Mayotte, 97600 MAYOTTE, Tel : 33 (0)2 69 61 86 67
13. Hôpital de jour adultes, CH de CAYENNE, Avenue des Flamboyants, 97300 CAYENNE, Tel : 33 (0)5 94 39 51 47
14. Service d'oncologie pédiatrique, Hôpital Mère-Enfant,7 Quai Moncousu, 44093 NANTES CEDEX 1, Tel : 33 (0)2 40 08 36 10

Reference laboratories

7. Laboratoire d'hématologie, Hôpital St-Eloi, CHU de Montpellier, Dr Patricia Aguilar Martinez.
8. Génétique moléculaire et biochimie, Hôpital de la Timone, CHU de Marseille. Pr Catherine Badens.
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Centres of competence on rare red cell disorders

15. Service de pédiatrie 3, CHU Hôpital de Hautepierre, Avenue Molière, 67098 STRASBOURG CEDEX, Tel : 33 (0)3 88 12 80 91
16. Service d'hémobiologie, CHU de Bordeaux - Hôpital Pellegrin, Place Amélie Raba Léon, 33076 BORDEAUX CEDEX, Tel : 33 (0)5 56 79 59 62
17. Service d'oncologie et hématologie, pédiatriques, CHU de Clermont-Ferrand - Hôtel Dieu, Boulevard Léon Malfreyt, 63058 CLERMONT FERRAND cedex 1, Tel : 33 (0)4 73 750 009
18. Service d'hémato-oncologie pédiatrique, CHU de Dijon - Hôpital du Bocage, 2 Boulevard Maréchal de Lattre de Tassigny, BP 77908, 21079 DIJON CEDEX, Tel : 33 (0)3 80 29 36 01
19. Service de médecine de l'enfant et de l'adolescent, CHU de Rennes - Hôpital Sud,16 boulevard de Bulgarie BP 90347, 35203 RENNES CEDEX 2, Tel : 33 (0)2 99 26 71 62
20. Service d'oncologie pédiatrique, CHRU de Clocheville, 49 Boulevard Béranger, 37044 TOURS CEDEX 9, Tel : 33 (0)2 47 47 47 51
21. Service d'immuno-hémato-oncologie pédiatrique, CHU Hôpital Charles Nicolle, 1 Rue de Germont, 76000 ROUEN , Tel : 33 (0)2 32 88 81 91
22. Service d'Hématologie et d'oncologie médicale, CHRU de Montpellier - Hôpital Lapeyronie, 371 Avenue Doyen Gaston Giraud, 34295 MONTPELLIER CEDEX 5, Tel : 33 (0)4 67 33 80 79
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24. Unité d'hémato-oncologie, CHU Hôpital des enfants, 330 Avenue de Grande Bretagne TSA 70034, 31059 TOULOUSE CEDEX 9, Tel : 33 (0)5 34 55 86 11
25. Service d'hématologie, pôle Enfant, CHRU de Lille - Hôpital Jeanne de Flandre, Avenue Eugène Avinée, 59037 LILLE CEDEX, Tel : 33 (0)3 20 44 41 05
26. Service de pédiatrie - Unité d'hématologie, Centre Hospitalier de Mamoudzou, BP4 Mayotte, 97600 MAYOTTE, Tel : 33 (0)2 69 61 86 67
27. Hôpital de jour adultes, CH de CAYENNE, Avenue des Flamboyants, 97300 CAYENNE, Tel : 33 (0)5 94 39 51 47

28. Service d'oncologie pédiatrique, Hôpital Mère-Enfant,7 Quai Moncousu, 44093 NANTES CEDEX 1, Tel : 33 (0)2 40 08 36 10

References and web links

1) http://www.orpha.net/testor/doc/French_National_Plan.pdf

2) http://www.orpha.net/orphacom/cahiers/docs/FR/Liste_des_centres_de_reference_labellises.pdf

Annex Centres questionnaire

HôpitalErasme	Clinical Chemistry	Brussels	Belgium
CHR de la Citadelle	Pediatrie	Liège	Belgium
CHU Liège	Hematology	Liège	Belgium
Hôpital de Jolimont- Lobbes	PediatricHematology	La Louvière	Belgium
HUDERF	PediatricHemato- Oncology	Brussels	Belgium
KUL-UZ Leuven	Hematology (adult)	Leuven	Belgium
CHU Mons		Mons	Belgium
AZ Turnhout, Campus St-Elisabeth	Clinical Laboratory	Turnhout	Belgium
UniversitairZiekenhuis Gent	LaboratroiumKlinischeBiologie	Gent	Belgium

CHEI-IRIS SUD	Paediatrics	Brussels	Belgium
Hôpital de Jolimont	Haematology	Haine St- Paul	Belgium
St Marina Universsity Hospital	Pediatric	Varna	Bulgaria
Nat. Spec. Hosp. Active Treatm. ofHemat.Diseases	Thalassaemia Department	Sofia	Bulgaria
Specialized ChildrensOncohematology hospital		Sofia	Bulgaria
Archbishop Makarios	Cyprus Thalassaemia Centre	Nicosia	Cyprus
Palacky University, Medical Faculty	Peaediatrics	OLOMOUC	Czech Republic
Hôpital Necker	PédiatrieGénérale	Paris	France
APHP, Hôpital Henri Mondor	Unité des Maladies Génétiques du Globule Rouge	CréteilCedex	France

Hospices Civils de Lyon HôpitalEdouard Herriot	Laboratoire de Biochimie et BiologieMoléculaire	Lyon	France
CHU of Montpellier	Laboratoire d'Hématologie	Montpellier	France
AP-HP, Hopital Bicêre	Laboratoire d'Hématologie	Le Kremlin Bicêtre	France
CHU	Laboratoire de génétique moléculaire et biochimie	Paris	France
University of Ulm	Internal medicine III, Department of Pediatrics, Department of blood transfusion	Ulm	Germany
Charité-Universitätsmedizin Berlin	KlinikfürPädiatriem.S.Onkologie/Hämatol./KMT	Berlin	Germany
University Düsseldorf	PediatricHematology / Oncology	Düsseldorf	Germany
University of Wùrzburg and Internal Medicine II (Red cell Lab)	Dep of Pediatrics	Wurzburg	Germany

Universitatsmedizin Gottingen (UMG)	Pediatrics	Göttingen	Germany
Universität Heidelberg	Zentrum fur kinder und Jugendmedizin	Heidelberg	Germany
General Hospital of Trikala	Thalassemia Unit	Trikala	Greece
General Hospital of Chania -– Crete	Thalassemia Unit	Chania	Greece
General Hospital of Corinth	Thalassemie Unit	Corinth	Greece
Hippocration General Hospital	Thalassemia Unit, 1st Depart. ofPediatrics, Auth.	Thessaloniki	Greece
General Hospital of Athens "Laiko"	Thalassaemia and Haemoglobinopathies Centre	Ampelokipi, Athens	Greece
University of Athens	First Dept of Pediatrics	Athens	Greece

U.O. di Pediatria ad indirizzo oncoematologico	Dipart. Integrato materno infantile Policlinico Modena	Modena	Italy
Meyer Paediatric Hospital	PediatricOnco-Haemathology department	Firenze	Italy
University of Catania	PediatricHematology and Oncology	Catania	Italy
Azienda Ospedaliero Universitaria Consorziale Policlinico di Bari	Division of Hematology and Oncology "F. Vecchio"	Bari	Italy
Azienda Ospedaliera-University of Padova	Clin. Of Pediatr. Hematol.- Oncol Departimento Pediatria	Padova	Italy
San Gerardo Hospital	PediatricHaemathology Department	MONZA	Italy
Agostino Gemelli Hospital	Pediatric Haemathology and Oncology Dept.	ROMA	Italy
Azienda Ospedaliero Universitaria	Pediatria	Udine	Italy

Azienda Ospedaliera Maria degli Angeli	Servizio di Pediatria Emato-oncologia, Dipartimento di Pediatria	Pordenone	Italy
Children's Hospital	Dept. of Pediatric	Brescia	Italy
Fond. IRCCS Cà Granda Osp. Maggiore Policlinico	UO Ematologia – UOS Patofisiologia dell'anemia	Milan	Italy
Catholic University of Sacred Heart	Hematology	Rome	Italy
Universita' degli studi di Cagliari		Cagliari	Italy
Ospedale Microcitemico Istituto Superiore di Sanita'	- Dep. Hematology Oncol	Rome	Italy
Azienda Ospedaliera "Ospedali Riuniti Cervello"		Palermo	Italy

Ospedali Galliera – Ematologia Centro Microcitamia	**Ematologia Centro Microcitamia Anemia Congenite**	Genoa	Italy
Università di Napoli	Dipartimento di Pediatria, II Universita'	Naples	Italy
Università Federico II	CEINGE	Naples	Italy
National Health Lab (Labor. Nat. De Santé) NHL	Hematology Division	Luxembourg	Luxembourg
Belfast City Hospital	Haematology	Belfast	Northern Ireland
Warsaw Medical University	Department of Pediatrics, Hematology and Oncology	Warsaw	Poland
Hospital InfantilUniversitario Nino Jesus	Servicio de Hemato-Oncologiapediatrica	Madrid	Spain

Hospital Virgen del Puerto		Plasencia	Spain
H.U. "Puerta del Mar"	Hematologia	Cadiz	Spain
Hospital de Cruces	Hematologia	Baracaldo.Bizkaia	Spain
Hospital de Tortosa Verge DelaCinta	Hematology / Pathology	Tortosat	Spain
Huca	Hematology	Oviedo	Spain
Hospital de Sabadell	Paediatry	Sabadell	Spain
K. General Yagüe (Burgos)		Burgos	Spain
Hospital La Candelaria Tenerife	Hematologia	S/ Cruz de Tenerife	Spain

Hospital Universitario Central de Asturias	Hematology	OUJEDO	Spain
Hospital Valld'Hebron	Pediatrichematology and oncology	Barcelona	Spain
Hospital Germans Trias. Badalona	Hematology	Badalona	Spain
Hospital Son Espases		Palma de Mallorca, IllesBalears	Spain
Hospital UniversitariArnau de Vilanova	Laboratori Clinic ICS Lleida	Lleida	Spain
Hospital Universitari de G.C. Doctor Negrin	Hematolgia	LasPalmasde GranCanaria	Spain
Hospital de Tortosa Verge de la Cinta	Hematology	Tortosa	Spain

IMPPC-Instit. ofPredict.and Pers. Med of Cancer	Genetics and Epigenetics	Badalona.Barcelona	Spain
Hospital Sant Pau	PediatricHematology and Oncology, Ped. BMT Unit	Barcelona	Spain
Hospital Virgen de la Salud	Hematology	Toledo	Spain
Hospital Clinico San Carlos	Universidad Complutense de Madrid	Madrid	Spain
Hospital General Santa Barbara	Haematology	Soria	Spain
Hospital de Txagorritxu	Servicio de Hematologia y hemoterapia	Vitoria	Spain
Hospital General de Granollers	Pediatrics / hematology	Granollers	Spain
Meander MC			The Netherlands
?	?	?	The Netherlands

?	?	?	The Netherlands
Radboud University Nijmegen Medical Centre	Lab.Med., Lab. Genetic, Endocr., Metab. Dis. 830	Nijmegen	The Netherlands
Utrecht University Children's Hospital	Hematology	Utrecht	The Netherlands
LUMC	Hematology	Leiden	The Netherlands
Sanquin	Blood Cell Diagnostics	Amsterdam	The Netherlands
UMC Utrecht	Hematology	Utrecht	The Netherlands
Haga Teaching Hospital	Hematology	The Hague	The Netherlands
University Medical Centre Groningen	Hematology (adult patients only)	Groningen	The Netherlands

AMC	Hematology	Amsterdam	The Netherlands
Erasmus MC	Hematology	Rotterdam	The Netherlands
Emma Children's Hospital AMC	Pediatric department	Amsterdam	The Netherlands
Leiden University Medical Centre	Clinical Genetics	Leiden	The Netherlands
King College Hospital	– Red Cell laboratory	London	United Kingdom
NHS Blood and Transplant		Bristol	United Kingdom