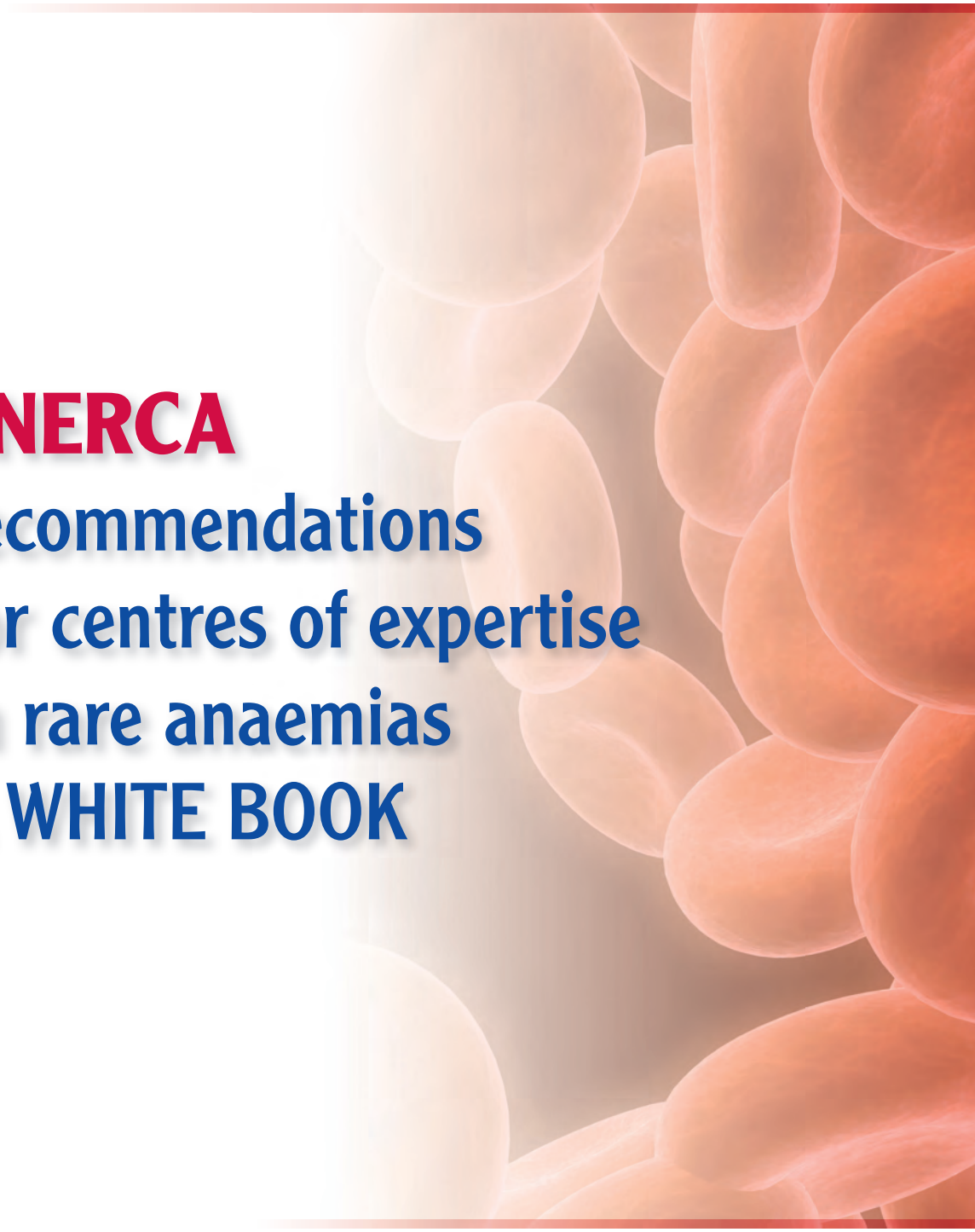

ENERCA
recommendations for centres of expertise in rare anaemias
A WHITE BOOK

enerca
European Network for Rare and Congenital Anaemias

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2. *The institutions that support the contributors that have actively contributed to this White Book project;*
3. *All the ENERCA partners and centres who have participated in the different surveys performed across Europe; and*
4. *All the patients who have participated in the Patient Expectations Survey*

This book is dedicated to all patients suffering from a rare anaemia

6. ENERCA RECOMMENDATIONS

7. IN SUMMARY: FROM RECOMMENDATIONS TO PRACTICE

PREFACE

Rare and congenital anemias are an increasing health problem all over Europe. The most frequent forms are hereditary hemoglobinopathies: sickle cell disease and thalassemia syndromes. These diseases originated in Africa, the Middle East and Asia, and spread all over the world because of ongoing migration from South to North and from East to West.

Although they are associated with considerable mortality and morbidity, including in economically developed countries, these severe diseases have long been neglected by healthcare policy makers.

The only known curative treatment for hemoglobinopathies is hematopoietic stem cell transplantation. Despite this, very few patients are transplanted. A recent survey of Eurocord/ European Blood and Marrow Transplant Group showed that amongst the 70.000 patients who have been diagnosed with Sickle Cell Disease (SCD) in Europe, only 700 have received a transplant.

The reasons for this huge gap between the number of transplants and the number of patients who could be cured by a transplant are multifactorial. They include socio-economic factors, a lack of coordination between local health centers and multidisciplinary medical structures, insufficient awareness on the part of health professionals leading to an absence of information provided to patients and their families, and a lack of a common registry for epidemiological studies and health policy planning.

This White Book aims to bridge the gap between this unmet need and the patient's expectations. To achieve this goal, the book presents ENERCA's recommendations for centers of expertise in rare anemias.

Important conclusions can be drawn from the ENERCA White Book. The book emphasizes the importance of the role played by the laboratories involved in the diagnosis of rare anemias. It underscores the need for patient-orientated multidisciplinary care at the center, and for coordination and cooperation between centers of excellence and primary care facilities. Emphasis is placed on the need to provide information to patients and healthcare professionals. Importantly, the White Book also aims to inform and actively involve public authorities at the national and European levels.

If they are implemented, these recommendation will be a major step to improve the welfare and long-term quality of life of an ever-increasing population of patients who suffer from chronic, debilitating diseases that are too often neglected.

Eliane Gluckman, MD, FRCP
Professor Emeritus University Paris Diderot. Head Eurocord Hopital Saint Louis, Paris. President of the European School of Hematology

PREFACE

The good health of citizens is both a cornerstone and a prerequisite for a fulfilling life and for a well-functioning society. The European Commission is committed to improve the health and well-being of European Union citizens by supporting the Member States in areas where a European response can add value.

Rare diseases represent a field that is especially susceptible to benefit from European cooperation. Affecting less than 5 in 10,000 persons, rare diseases pose specific challenges to the health systems in EU Member States due to a scarcity of resources and expertise limiting the provision of accurate diagnoses, research funding and the development of treatments. While the prevalence of a specific disease in a specific Member State may be limited, rare diseases in the EU affect between 27 and 36 million people, illustrating the importance of a common response.

The European Commission identified rare diseases as a priority action area already in the nineties. Since then, the different EU initiatives addressing rare diseases have been predominantly focusing on bringing together scattered resources and expertise across Member States. Within this framework, the European Reference Networks, as introduced by Directive 2011/24/EU on the application of patients' rights in cross-border healthcare, is a step towards the creation of clearer structures and networks in particular in the area of rare diseases, by bringing together highly specialised providers across the EU.

In the preparation for the establishment of the European Reference Networks within the framework established by Directive 2011/24/EU, the Commission supported ten pilot networks, one of them being the European Network for Rare and Congenital Diseases.

The European Commission congratulates the European Network for Rare and Congenital Diseases for its persistent and assiduous contributions to raising and disseminating awareness, recognition and knowledge about rare anaemias since 2002. The present volume, "ENERCA Recommendations for Centres of Expertise in Rare Anemias: A White Book", is proof of the extensive work that ENERCA has undertaken for over a decade for the benefit of rare anaemia patients. The position paper represents a major contribution towards the creation of a much needed European infrastructure of expertise around rare anaemias.

Through comprehensive legal and ethical frameworks, detailed technical and quality criteria for the establishment of centres of expertise and a strong patient-focus, the volume constitutes a comprehensive and accessible tool and a crucial point of reference for patients and their relatives, health authorities, care providers and other stakeholders across the European Union. As such, it is a crucial step on the way towards allowing

rare anaemia patients to have their disease recognized, diagnosed and to obtain adequate treatment – in all Member States.

This document provides as well a vast amount of expertise which will be of great value for other clinical areas in rare diseases willing to build European Networks to improve patients' diagnosis and treatment.

I am confident that this White Book will become a key reference document for everyone interested in this important area.

John F Ryan
Acting Director of the European Commission Public Health directorate

PREFACE

Parenthood teaches you things about yourself as a human being, as a "member of the human species" or better yet, about your most primitive instincts. You have your whole life organised, have found the perfect wife, a good job and bought a house; you are something like a happy person. But one day a little stranger, weighing less than three pounds, appears in your life; you don't know what kind of person he will become, if he will grow up to love or hate you, if he will become a Nobel Prize winner or a convict, if he will be a football player or will want to enter Big Brother. But from the first moment you see his face you would be willing to give up everything for him, you would sell your house, you would change your job, your lifestyle. You are willing to trade your dreams for his. Without a second thought. That primitive instinct is what has led to the perpetuation of humanity for thousands of years.

Our story starts like most: in a doctor's office. Something happens to your new born son, something goes wrong and you still do not know what it is. All you know is that he needs a blood transfusion.

— A blood transfusion? But…why? What does he have?

— We really don't know. But he needs a blood transfusion. He might have a congenital anaemia.

— Anaemia?

Vertigo, doctors, fears, abyss, hospitals, Google, more doctors, a lot of fears. Transfusions every month, every three weeks analytical tests, doubts, more doctors, more doubts, terror… Months pass and no one explains what is happening. Even worse: they don't know what is happening.

You feel like you're reeling at the top of a cliff and have no idea how deep the drop is. Nobody knows very well how to measure its depth and when you look down all you see is a thick fog. There are hundreds of anaemias, and you have thousands of questions: how can I tell if my son's anaemia is one of the worst? How do I know that my son is going to be a normal child? What is his life expectancy? How can I help him?

— When will we know exactly what he has?

— Later ... there's no hurry. We need more time. Probably when the boy is older we will find out.

—Okay, but how do I know my child is going to be older?

No response. Only silence.

Please, a light, a door, a path!!!!!

But one day you sit in front of a new doctor. But he is "The Doctor", this is "The Hospital" and he is part of "The Team". That doctor, that hospital, that team lead you, guide you, put you on the right track. They tell you about an organisation called "ENERCA". Google again. It looks like a serious organisation. You call. They pick up the phone, they listen to you,... "we're going to help you" And they do help you. Not only do they help you but they also take your hand, guide you, understand you. They say "first we need a diagnosis as soon as possible."

Samples traveling from Madrid to Barcelona, from Barcelona to Utrecht ... and then comes a diagnosis. Finally!! A type of rare anaemia "Pyruvate Kinase enzymatic deficiency". Well, at least it has a "sound" name. There are few cases in Spain, very few. It would have been easier to win the lottery, but for us, this is "our" lottery. Google, books, publications, magazines ... but you already begin to feel better: now you know how high the cliff is, and the fog is clearing. You know what your enemy looks like; you know what you are facing, what you can expect from it, in the best and the worst case scenarios. Relief. You know that you have a long fight ahead, but also feel that the first step, the most important, has already been taken.

What would have happened without a network like ENERCA, without specialised reference centres, in such a rare and infrequent case as ours? How long did it take to reach a diagnosis? What happens to other parents who have not found ENERCA, who have never heard the names of "The Doctor", "The Hospital", and "The Team"? I don't even want to think about it. What I want to know now is how I can help other parents who are going through what I did 5 years ago. How can we be "militants" of information? How can we inform all levels of our society about the importance of these organisations? How can we ensure that these organisations have the necessary support? Isn't it logical for any citizen, patient, parent, healthcare professional, patient organisation to have access and knowledge of the existence of these organisations? Why did none of first doctors know about ENERCA? Why has my son found ENERCA while other children will not be so lucky? After all, isn't this a basic right for every patient?

I hope to get some answers in this book and sharing our experience is our small contribution. Five years later our life focuses on our "friend", the anaemia. But now we know each other. And we watch each other.

Meanwhile, our son, Juanito, dreams of being a football player and sometimes, he also says he would like to be a doctor.

Juan Antonio Jiménez González
Father of a child with Pyruvate Kinase deficiency

EXECUTIVE SUMMARY

The Community added value of Centres of Expertise and European Reference Networks is particularly high for rare diseases due to the rarity of these conditions, which implies both a small 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 clinical care and follow up for patients with rare diseases. This applies particularly to rare anaemias due to the high number of different rare diseases that constitute this group.

In this context, the European Network for Rare and Congenital Anaemias (ENERCA), co-financed by the European Commission, was created in 2002 with the aim of prevention and management of rare anaemias and the development and promotion of policies to improve the well-being of European Union citizens.

The ENERCA White Book is a position paper, developed as a deliverable of the ENERCA 3 project that intends to contribute to the creation of a European Reference Network in rare anaemias by elaboration of the recommendations and, in particular, the definition of the criteria that Centres of Expertise, local centres and their interrelations have to fulfil as healthcare providers wishing to join this network. It has been informed by all the activities that have been performed over the past ten years within the framework of ENERCA.

The White Book is addressed to authorities in charge of the identifying Centres of Expertise, as an essential requirement for the official recognition of the European References Networks, to European and national health authorities, centres and professionals, as well as to all other stakeholders interested in rare anaemias. It is also addressed to the patients, as a way to empower their community in this process.

One particular characteristic of the White Book is the integration of the three main aspects of a Centre of Expertise: a) ethical and legal frameworks to ensure the non-discrimination and non-stigmatisation of rare disease patients across Europe, within their sphere of competencies; b) clinical and laboratory frameworks for defining technical and quality criteria including scope, general and disease specific elements currently defined as technical and professional standards for the diagnosis, treatment and follow-up of patients with rare anaemias; and c) the expectations patients have of Centres of Expertise.

Conceived as a working tool directed to a broad range of stakeholders, the White Book has been designed and structured to be comprehensible even to non-technical and /or non-professional audiences. The reader will find an up-to-date description and epidemiological information on rare anaemias as well as the European Union background

policies for defining Centres of Expertise and European Reference Networks in rare anaemias.

A working group was created with experts of different profiles, known as the European Working Group on Rare Anaemias (EGRA). In order to achieve its objectives, the methodology used by EGRA, was characterised by three main principles: Interdisciplinary work, European coverage and evidence-based principles. Work has been developed into four sequential steps: 1. Analysis of the current situation of rare anaemias in Europe by healthcare professionals in order to identify the most relevant issues that have to be addressed by a centre in order for it to be recommended as Centre of Expertise. 2. Preparation of questionnaires to perform surveys on how the relevant issues identified in step 1 can be translated into practical recommendations. 3. Analysis of the questionnaire results by face to face meetings, feedback and consensus evaluation, and 4. Preparation of a final report on ENERCA policy recommendations for Centres of Expertise. This report is presented in a user-friendly document, easy to understand and available through the ENERCA website (**www.enerca.org**).

Several important conclusions can be drawn from the ENERCA White Book, including the importance of laboratories involved in the diagnosis of rare anaemias, patient oriented and multi-disciplinary care at the centre, the need for coordination and cooperation within and outside the centre, the provision of information to patients and health professionals and the involvement of public authorities at the national and European levels. Official recognition of this structure and assurance of its long term sustainability will only be achieved if public authorities work hand in hand with both professionals of different disciplines and patients.

Finally, the ENERCA White book aims to be a practical tool for health authorities of Member States that are preparing their national directory of formally designated Centres of Expertise. For this, it is important that Member State authorities recognise rare anaemias as an important health component to be included within the National Plans or Actions for Rare Diseases.

1. MISSION, VISION AND SCOPE

Rare diseases (RDs) are probably the area in public health in which joint efforts among European Member States (MS) is most justified and crucial. This is because a common European approach would be more rational, efficient and effective than 27 individual national approaches. According to the European Commission (EC), a disease is considered "rare" in Europe when it affects less than 1 in 2000 individuals, whereas in the USA a "rare disease" affects less than 200,000 inhabitants, at any given time. Fifty percent of RDs affect children, and in more than 80% of cases a genetic origin has been identified, while the remaining 20% are the result of different causes such as infections (bacterial or viral), allergies, environmental factors, and degenerative or proliferative diseases, among others.

The definition of "rare disease" first appeared in European Union (EU) legislation in 1999 through Regulation (EC) No 141/2000, December 1999 on Orphan Medicinal Products. After this a Community Action on RDs was taken in the field of public health for the period 1999 to 2003

It is estimated that 5,000 – 8,000 distinct RDs exist, affecting 6% to 8% of the European population, that is, 27 to 36 million people. RDs, are sub-classified into "rare", "very rare" and "ultra rare" depending on if they affect less than 1 in 2000 persons, less than 1 in 100,000 persons and less than 1 in 2,000,000 persons, respectively .

The specificities of RDs, including a limited number of patients and the scarcity of knowledge and experience, certainly single them out as an extremely special area of very high European added-value. The fact that often no effective cures exist adds to the high level of pain and suffering endured by patients and their families. In addition, the lack of specific health policies for RDs and the scarce and scattered research performed in highly specialised laboratories and centres throughout the EU, makes it difficult of access clinical care and consequently leads to a delayed diagnosis. Therefore, a call for European and International cooperation, to ensure this knowledge is shared so that everyone can benefit from combined resources, has become an important public health challenge. The ongoing implementation of a better comprehensive approach to RDs has led to the development of appropriate public health policies, and important gains continue to be made with the increase of international cooperation and the development of new diagnostic and therapeutic procedures.

"Rare Anaemias" constitute an important and relatively homogeneous group of RDs where "anaemia" is the first and most relevant clinical manifestation of the disease. This importance was recognised for first time by the EC in 2002 when it approved the co-financing of the DG-SANCO Project: "European Network for rare congenital diseases" (ENERCA). Interestingly, this project started shortly before the creation of the High Level Group (HLG) in 2004, which brought together experts in several areas of

expertise from all the MS. For RAs this was a great advantage because it facilitated the gradual development of the ENERCA Project in parallel to the development of the different HLG areas of action: a) patient safety and quality of care, b) health impact assessment and health systems, c)health technology assessment, d) European workforce for health professionals, e) European reference networks, f) information and e-health and, more recently, g) cross-border healthcare purchasing and provision.

Before ENERCA, RAs were almost unknown in Europe, including some health professionals, because in many cases, the cause of the anaemia was not known and/or there is no treatment available. Moreover, for many years anaemias in general have been underestimated by public health providers, due to their frequent misdiagnosis as iron deficiency anaemia, the most frequent cause of anaemia worldwide. ENERCA definitively changed this situation by developing three consecutive phases with a total duration of 10 years. The first ENERCA Project (ENERCA 1), starting in 2002, was devoted to congenital rare anaemias only (*European Network for Rare Congenital Anaemias*) and allowed the establishment of the necessary background for sustainable coordination in the area of health information, collection of epidemiological data, comparability issues, exchange of data and information within and between MS. At this time, it also facilitated a rapid reaction to RAs diagnosis and treatment. In addition to congenital anaemias, the second ENERCA Project (ENERCA 2) starting in 2005, covered other rare causes of anaemia, either hereditary or acquired (*European Network for Rare and Congenital Anaemias*) and dedicated more and stronger activities to health information, patient data collection, education and training and quality assessment of the procedures used to diagnose special RAs. This has provided a first and unique approach for the prevention, diagnosis and treatment of RAs in the word. Finally, the third ENERCA Project (ENERCA 3), starting in 2009, was co-financed by the EC through its Executive Agency for Health and Consumers (EAHC) and its objectives were parallel to the strategic objectives of the EU Health Programme 2008-2013, consisting in the creation of a European Reference Network (ERN) of Centres of Expertise (CEs) in RAs.

Accordingly, the establishment of a ERN of CEs in RAs is a key strategy to improve the clinical management of these patients and to reduce health inequalities across the EU and tackling RDs through a Europe-wide network. This will accomplish 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. For this, after 2012, the EC accepted co-financing a ENERCA extension (e-ENERCA) for the development of an e-health platform that will facilitate the diagnosis and clinical management of severe RAs through the use of new telediagnosis and telemedicine methodologies.

As established in the **Directive 2011/24/EU of 9 March 2011 on the *application of patients' rights in cross-border healthcare,*** RD Networks are a very important tool that should be promoted within that general framework. Some specific benefits of an **ERN of CEs in RAs** will be:

- Providing patients and health professionals access to experts and expertise throughout all European MS , regardless of the country of origin or practice, thereby reducing inequalities and maximising the cost-effective use of resources;
- Implementing epidemiological surveillance throughout the EU that gathers comparable data on patients affected by RAs and launching preventive programmes for tackling RAs;
- Fostering best practices for prevention, diagnosis and clinical management;
- Promoting the dissemination knowledge, sharing expertise and supporting research, and increasing awareness about RAs;
- Facilitating 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 is a main point of interest of the directive, especially for RDs. 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 for establishing national contact points (Article 6 of the Directive).

In this context, the ENERCA White Book aims to be a policy document that addresses the specific criteria to be considered in the process of identification and recognition of healthcare providers as Centres of Expertise (CEs). These, in turn, will constitute the nodes of the future European Reference Network (ERN) that will act as a focal point for medical training and research, information, dissemination and evaluation in RAs.

Accordingly, the ENERCA White Book is first addressed to European and National authorities, health centres, health professionals and other agents in charge of recognizing CEs and ERNs. It is also addressed to the patients as a way to empower the patient community in this important process. These target groups are the following:

- **European Commission:** As a pilot experience, the ENERCA Project offers a great deal of experience in networking activities.
- **National authorities:** The results of surveys performed and published in the ENERCA White Book, will facilitate the task of national agents and health authorities in the recognition and designation of ECs in RA for implementing the objectives of the Directive 2011/24/EU that recognises the important value of networking in RDs, within the framework of each national plan or action for RDs.
- **Health professionals:** The ENERCA White Book is a practical, informative document for the self-assessment of health professionals in RAs and in the different services provided by the network.
- **Patient community:** Patients Associations have actively participated in the preparation of the ENERCA White Book recommendations, which is a clear demonstration of empowering patients in the field of rare anaemias.

In summary, the ENERCA White Book is a policy document structured to be comprehensible beyond a technical and professional level. It has been prepared by a ENERCA Working Group (EGRA), and is addressed to a broad range of stakeholders in a friendly user format, easily understood by non-experts and patients. The reader will find up-to-date information on RAs, including definitions, individual descriptions and epidemiological data, in addition to important information on the EU background policies for defining CEs and ERN. An important objective has been to provide a practical document containing specific material and methodology that can be used to establish a consensus on what criteria should be considered in the process of identification and designation of CEs by national health authorities. The methodology used is presented in detail in a separate chapter and explained by integrating three interdisciplinary approaches: a) a legal and ethical perspective; b) a clinical and laboratory approach concerning specific methodology and quality criteria for diagnosis, treatment and clinical follow up of each rare anaemia; and c) patient expectations. In fact, the document is expected to be a dynamic working tool that can be modified over time, if necessary.

2. THE ENERCA PROJECT

The ENERCA White Book is a deliverable of ENERCA 3, a Project co-financed by the European Commission (DG-SANCO) whose objective is to prevent rare anaemias (RAs) by promoting policies that lead to a healthier lifestyle for the well-being of European Union (EU) citizens. Accordingly, two pivotal aspects have been promoted: 1. A specific framework for cross-border healthcare and 2. European cooperation on health services. As in many other rare diseases (RDs), a large-scale network of experts and specialists working in the field of RAs was non-existent, creating a serious lack of information and knowledge-related difficulties for both patients and health professionals. The ENERCA Project started in 2002, with the purpose of providing health services diagnostic help, training, information dissemination and evaluation in RAs. The ENERCA website (www.enerca.org) has definitely proven to be a focal point for the implementation of such services.

Since 2002, ENERCA has also taken an active role in cooperation within the EU by helping health professionals and improving patient 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").* **ENERCA** has developed through three consecutive phases:

First phase (2002-2004) dedicated to developing a website (www.enerca.org) addressed to both health professionals and patients, with the aims of:

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

Second phase (2005-2008) for covering both hereditary and acquired RAs with the aims of:

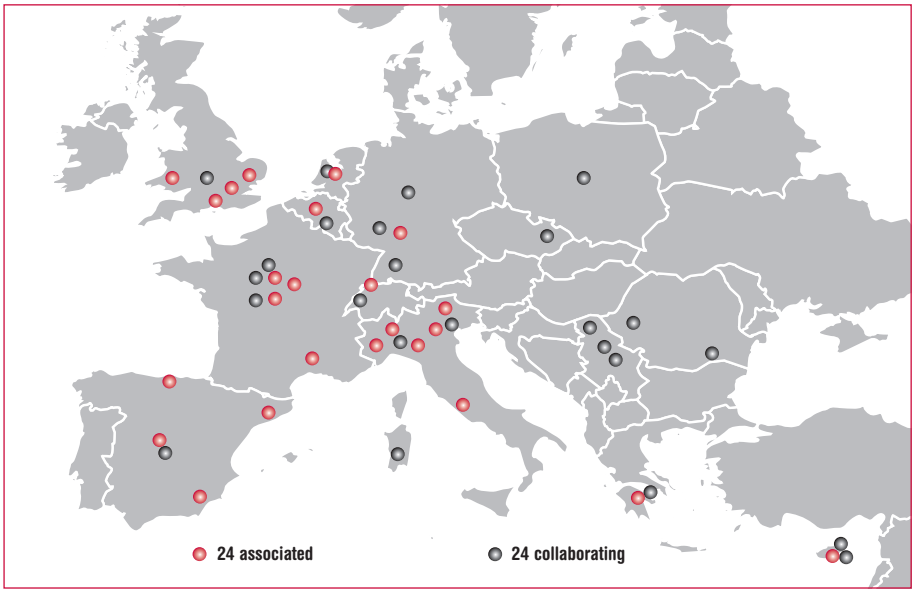
- Developing a pilot study to initiate the mapping of existing individual experts and expert centres for RAs in Europe.
- Collecting clinical and laboratory data on haemoglobinopathies as a pilot experience for the future development of the European registry of RAs.
- Increasing the epidemiological information on RAs in Europe by promoting prevention programmes (new born screening of haemoglobinopathies) in European countries where this information was scarce or non-existent, such as Catalonia and Latvia.

- Establishing a pilot external quality assessment scheme (EQAS) for the general diagnosis of RAs through red blood cell (RBC) morphology examination and thalassemia through the quantification of haemoglobins A_2 and F.
- Disseminating ENERCA knowledge across Europe via the website, approaching national and European scientific societies and the organisation of the first European Symposium on RAs (Barcelona, 2007).

Third phase (2009-2012) for creating a European Reference Network (ERN) in RAs as a platform to establish communication between the individual experts and centres of expertise (CEs) in RAs and for providing information and health services to professionals, patients and any stakeholder interested in RAs. The specific objectives of ENERCA 3 were:

- Involvement in the European Commission (EC) plan for Pilot ERNs for the final recognition of ENERCA as a RD ERN after 2013.
- The promotion of the EQAS procedures for the diagnosis of very rare anaemias (VRAs).
- To foster guidelines and recommendations for prevention, diagnosis and clinical management of RAs.
- The improvement of health professionals' knowledge of RAs by organising topic-focused training courses at the European and national levels and by the organising the 2nd and 3rd European symposiums on RAs, with the involvement of the patients' community.

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



- Increase social awareness of RAs by means of developing educational material in the most widespread European languages.
- The promotion of research and cooperation among experts in RA.

ENERCA3 involved 48 partners (24 associated and 24 collaborating) distributed in 15 different European countries (see **Figure 1**). Most of the partners have been working together since 2002 and all of them are well known and recognised experts in their respective fields. Contact person and institution details are listed in **Annex 1**.

Partners were distributed in different working groups on the basis of six Work Packages (WPs); 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).

3. RARE ANAEMIAS IN EUROPE

3.1. Rare Anaemias: The concept

Anaemia is a clinical manifestation defined as the decrease of haemoglobin (Hb) concentration in blood. It is a very common condition in human pathology and may result from a wide variety of causes, either congenital or acquired. Anaemia is always the manifestation of an underlying disease, and never a disease by itself. In general, there are three primary causes of anaemia: 1) Bone marrow erythropoietic defects associated with or without reduced haemoglobin synthesis. 2) Hemolysis or excessive destruction of mature red blood cells, and 3) blood loss or 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 analysers 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 anaemia in Europe. However, there is 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 origins.

3.1.1. General Diagnostic approach

More than 80% of the rare anaemias (RAs) 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. As in other rare diseases (RDs), the low number of patients creates the need to mobilise resources and their study can only be efficient if done in a coordinated manner on the European level.

Among hereditary anaemias, haemoglobinopathies are the most common 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 5 per 10000 individuals. For this reason, in Europe, haemoglobinopathies are considered a particular group of RDs or RAs. Whereas thalassaemia syndromes are inherent in the autochthonous European at-risk groups (Mediterranean anaemia), other haemoglobinopathies have been imported by immigration (Sickle-cell anaemia). As in any anaemia, the diagnosis of a RA is often prompted by pallor, noticed by the patient, the family, and/or the General Practitioner (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)

The first general diagnostic test is the complete blood count (CBC) that includes four main parameters: a) haemoglobin concentration (Hb), the key of anaemia diagnosis, b) RBC count or concentration of RBCs, given as number of cells per litre 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 anaemias. The following three main indices are automatically measured by modern haematology analysers: 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 RBC 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 especially useful to 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 characterised by increased reticulocyte count (*regenerative* anaemias), whereas anaemias due to erythropoietic insufficiency (aplasia or dyserythropoiesis) are characterised by a lower than expected reticulocyte count from the severity of the anaemia (*aregenerative* anaemias). In thalassaemias, where erythropoietic insufficiency coexists with some degree of haemolysis, the reticulocyte count may be variable.

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 thalassaemia, being the most frequent haemolysis and erythropoietic failure. Here, the reticulocyte count is the most useful test to differentiate these two conditions. In clinical practice, the most frequent cause of anaemia is iron deficiency (ID), characterised by a low MCV (microcytic anaemia). In southern European countries, with larger "at risk" thalassemia populations (Mediterranean Basin), this hereditary disorder can be misdiagnosed as 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 recovers its normal value. However, if after treatment the MCV remains low, the coexistence of a thalassaemic gene has to be investigated. It should be mentioned that there are a number of conditions where the MCV can falsely rise, masking the main indicator of thalassaemia diagnosis. This is the case in some patients with thalassaemia who co-inherit another cause of haemolytic anaemia leading to an increased reticulocyte count. This can falsely increase the value of MCV and mask 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 highlighted 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 and in the differential diagnosis of macrocytic anaemias. RBC morphology examination provides in some cases (e.g. red blood cell membrane disorders, sickle cell anaemia) 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 also has the advantage of speed that may be important in severe anaemias such as those mentioned before.

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

These tests are the next step for the identification of the cause of the anaemia or of its mechanism. They include a group of laboratory procedures depending on clinical or laboratory diagnostic orientation of the anaemia (Dacie and Lewis. Practical Haematology, Elsevier, 11th Edition, 2012). In order to provide an initial 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). The ENERCA website (www.enerca.org) provides practical flowcharts for the diagnostic orientation of anaemia. For this, three data from the patient have to be provided: sex, Hb concentration 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 the final diagnosis always requires the advice of a health professional, but they provide the basic information on how the investigation of anaemia causes can be undertaken in routine clinical practice. Using these flowcharts the most frequent RAs (haemoglobinopathies, thalassaemias and haemolytic anaemias) can be easily recognised. Depending on the results of the recommended basic tests, more specific tests (including molecular biology) can be performed. Some of these specific tests can also be performed in general haematology laboratories but other tests must be performed in specialised laboratories. In all 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 specialised laboratories, local (national or regional) EQAS organisations cannot establish a specific EQAS for these procedures due to its high cost. Accordingly, the EQAS for these procedures have to be promoted at the European level as ENERCA 3 has done with some rare diagnostic tests for RAs.

3.1.2. Classification

RAs can be classified, according to their pathophysiology, into two main groups: 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, have no curative treatment, except for 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 RDs are associated with anaemia, moderate or severe. One example of this is the Rendu-Osler disease (hereditary telangiectasia), a relatively well known RD where anaemia due to iron deficiency is very common and sometimes the first clinical manifestation of the disease. Furthermore, some anaemias due to rare chronic inflammatory diseases, vitamin deficiencies, immune diseases, malignancy or other rare disorders may probably also be considered RAs, although they have not been included in this group.

ENERCA is actively contributing to the WHO Update Platform ICD-10 of blood and blood forming organs. In this classification, anaemias, are classified into three main groups: D50-D53 (nutritional anaemias), D54-D59 (haemolytic anaemias), and D60-D64 (aplastic and other anaemias). This ICD classification includes all kind of anaemias, hereditary, acquired, common and rare. ENERCA has extracted the RAs that have been individually listed its web page. For practical purposes according to their mechanism, prevalence and/or relevant clinical and/or social impact on the European population, these RAs have been classified into ten 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 non-sideroblastic anaemias due to iron 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 origin (AUO). The underlying cause of rare anaemias remains unexplained in about 30% of patients, almost one third of which might be accounted for as myelodysplastic syndromes. AUO can also be 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 area for clinical and biological research to examine.

3.1.3 Epidemiology

Introduction

Originally confined to the global problems of infectious diseases, over the past 50 years clinical epidemiology has become the basis of modern evidence-based medicine. No medical stakeholder is able to oversee the vast amount 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 on rare diseases, which entered into the scope of medical stakeholders in the last decade. ENERCA is concerned with the special problems of RAs, and aims to obtain valid epidemiological evidence of their prevalence, life expectancy and effects of therapies.

In RAs, as in other rare and very rare disorders, only supranational networks can provide valid data. ENERCA has concentrated on data from the MS of the EU (although also including patients from Switzerland and other Non-EU European countries) on a subset of RAs. However, the methodological experience gained by ENERCA can be (and has been) 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 difficulties in bundling the results of the different general and medical cultures found in different countries. For example, it is often overlooked that social-economic conditions and medical practices vary among MS, resulting in different recognition of the epidemiological data studied.

Methods and definitions

Frequency is a general term used 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 (such as what were formerly referred to as races) in a given region. The latter became of paramount importance with the ongoing immigration of people from the Mediterranean Basin to northern- and central

Europe and from Asia and Africa to all MS, as mentioned above. The frequency is a useful parameter in any first approach of disease characteristics and a useful tool to decide on the methodology to estimate more precise issues. No one would challenge the fact, that thalassemia is much more frequent in the Mediterranean area or in immigrants within the northern European countries. However, mathematically defined parameters are needed for research and healthcare planning.

Prevalence, or more exactly prevalence proportion, is the main indicator used for any epidemiological study of congenital diseases, representing the vast majority of conditions considered in a disease category. Paroxystic Nocturnal Haemoglobinuria (PNH) is an exception, with prevalence being of major significance, as is the case with cancers. 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 **point prevalence** in common diseases. Another useful measure is the number of affected children among 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 proportion is obtained with post-natal screening programs, as described for SCD.

Ideally, all cases of a given disease or disease category in a population at a given 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 depending on socio-economic conditions, and the same is true for the proportion of misclassifications. These data are important for ENERCA's attempts to harmonize the diagnosis of rare anaemias in European countries.

Sources of data

Registries are among the main sources for epidemiological data in rare anaemias. They are structured collections of cases of one of the disease categories considered by ENERCA3in a digital databank. 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 in national regulations for data protection, as well as time and resource limitations, prevented us from achieving this goal. However the work done by several ENERCA working parties, and intensive discussions during the Executive Committee meetings and symposia yielded very useful experiences on suitable strategies, methodological problems, pitfalls and risk of biases in supranational rare disease epidemiology projects, as shown paradigmatically for the rare anaemias.

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 resources of much longer duration than available today.

Clinical trials, even though primarily directed at improving therapeutic measures, and often dependent on industrial interests, are another source of epidemiological data. Only a few such trials are performed in the group of very rare anaemias and none are supported by ENERCA. However, data from such trials are available for some of the disease categories considered by ENERCA, such as PNH.

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 priori* known that these disorders are endemic in the MS surrounding the Mediterranean Basin, due to the holoendemic malaria still present up to the 20th century. Prevalence in the MS of central and northern European MS, is largely the result of a history of migration and the influence of Turkish dominance on the Balkans and on what is today the territory of Hungary and Austria. Considering the new migration starting after World War II, and the ongoing immigration from south to north and from east to west, data from the central and northern European MS are of particular interest for healthcare planning in the EU.
2. Very rare anaemias (VRAs) are a group of RAs with an even lower prevalence. They include enzyme disorders of the red blood cell, red blood cell membrane disorders, Congenital Dyserythropoetic Anaemias (CDA), Diamond-Blackfan anaemia (DBA) and Fanconi Anaemia, Primary iron metabolism disorders, including Hereditary Sideroblastic Anaemia (HSA) and other VRAs, such as Congenital defects of vitamins B12 and folic acid and Paroxysmal Nocturnal Haemoglobinuria (PNH). Very little data on regional distribution were available, although 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 the rate of new mutations, of consanguinity and on detection rates, at least in disorders with autosomal recessive heredity.

3.2. General description

It is not the objective of this chapter to provide exhaustive scientific information of every RA, but rather to provide an overview to health professionals, experts on other RD, policymakers, patients and other stakeholders that may be interested in the main categories of RA, the heterogeneity of these disorders and the most common clinical features and complications that affect the patients.

A list of up to 62 different RAs has been collected on the ENERCA website (www.enerca.org). Specific disease profiles have been prepared and translated into different languages. For all of them, the ORPHANET, OMIM and ICD codes, definition, treatment and hereditary pattern, as well as health professionals dealing with these disorders and patient associations are provided.

3.2.1. Sickle cell disease (SCD)

- ORPHANET code: 232
- OMIM code: 603903
- ICD-10 code: D57.0 / D57.1

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 new-borns 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 alpha-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).

Epidemiology

Sickle cell disease in Europe is predominantly a disorder seen in immigrant communities. The gene in its carrier state form, as it offered some degree of protection against malaria, 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.

Incidences come from five neonatal screening programmes financed by the local or national public health authorities and implemented in five EU countries: England, France, Belgium (Brussels, Liège), Spain (Madrid, Extremadura, Comunidad Valenciana and Pais Vasco), and The Netherlands (2–6) **(Table 1)**.

Table 1. 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	Streetly, 2010
France	1992	Targeted	±1:700	Bardakjian, 2009
Brussels (Belgium)	1994	Universal	±1:1600	Gulbis, 2009
The Netherlands	2007	Universal	±1:4200	Bouva, 2010
Madrid (Spain)	2007	Universal	±1:6250	Cela de Julian, 2007

When a systematic neonatal or new born screening programme was implemented (all countries except France), the prevalence of sickle cell disease ranged from 1:2000 to 1:6250 live births.

Registries are the best tools to find prevalence numbers but very few exist in Member States. But prevalence is expected to substantially increase in the near future due to decreased mortality rate; and mobility and migration flows can also be suggested as a contributing factor to their increase.

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 a clinical event whose aetiology may be SCD.

Management

The first symptoms may be expected a few months after birth, when HbS level rises. While in less severe sickle cell disorders, clinical problems may develop later in life.

SCD is a chronic disease characterised by anaemia and damage to a number of organs, such as the 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 their prevalence is highly variable. That means that the management of patients with sickle cell disease has to be offered by a multi-disciplinary team of health and social workers. Specialist and local centres working together in networks offer the best chance of providing a full range of services, including 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 with patients in order to identify those at risk for certain adverse outcomes such as:
  - For stroke by monitoring them by transcranial Doppler scanning.
  - 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 a 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 due to 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 providing a full range of services within a network. 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 the organisation.

Key messages

- *Sickle cell disorders might be very rare or rare conditions in the different countries of the European Union. They have been introduced from southern Europe and sub-Saharan Africa and Asia.*
- *Each sickle cell disorder is a multi-organ disease requiring lifelong specialised care by a multi-disciplinary team.*
- *Care is best provided in Expert Centres and networks that include 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.*

3.2.2. Beta-thalassaemia

- ORPHANET code: 848, 231214
- OMIM code: 613985
- 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 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 multiorgan 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 that

limit the chain imbalance such as those reducing alpha chain production (alpha-thalassaemia) or those increasing HbF production (e.g. hereditary persistence of foetal 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.

Epidemiology

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 Southeast 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 results in early death of many affected individuals. Global epidemiology for beta-thalassemia is shown in **Table 2**. For Europe the data are shown in **Table 3**.

Table 2. 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

Data 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 3. 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

Diagnosis

The carriers of beta-thalassaemia have no clinical manifestations. In the homozygous and compound heterozygous 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 and compound heterozygous 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 to tailor the treatment to individual patient needs.

Prevention

Reduction of affected births can be 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 chronic nature of the condition is also the basis of the proposal for expert care centres. 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 is a 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 that 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 on 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. In areas where thalassaemia is rare problems in patient care may arise.*
- *Thalassaemia is a multi-organ disease requiring lifelong specialised care by a multi-disciplinary team.*
- *Blood transfusion and iron chelating therapy are the standard treatment modalities but haemopoietic stem cell 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.*

3.2.3. Alpha thalassaemia

- OPHANET code: 846, 93616
- OMIM code: 604131, 613978
- ICD-10 code: D56.0

Introduction

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 molecular defects affecting 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 in 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 anaemia will cause heart failure in the foetus with swelling of tissues including the placenta, and problems of toxaemia and delivery to the mother. This is known as Hydrops fetalis and fetal death is the usual outcome. If not recognised early there is also danger to the life of the mother. In a few cases intrauterine transfusion has allowed the baby to be born alive, although transfusion dependent and requiring lifelong 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 two different kinds of alleles (chromosomes): $\alpha^{+,}$ where only one gene out of 2 is missing or inactive and α^{0} alleles where no functional alpha genes is present.

The resulting genotypes are associated with the following conditions:

- Heterozygous 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.
- Homozygous α^{+} 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).
- Heterozygous 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 an α^{+} carrier If both parents are α^{0} carriers, 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 (Hydrops foetalis).

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 do not diagnose alpha thalassaemia, especially if the haematological findings suggest an α^0 carrier or if prenatal diagnosis is requested. DNA analysis is most often performed when there is a risk of having an affected child (severe HbH disease or hydrops fetalis) and if prenatal diagnosis is requested.

Epidemiology

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 Southeast 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 results in early death of many affected individuals. Global epidemiology for alpha-thalassemia.

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 alpha zero thal carriers.

HbH disease is a mild to moderate haemolytic anaemia; it is usually 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 on 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).*

3.2.4. Hereditary red blood cell enzyme defects

- ORPHANET code: 32, 57, 362, 371, 712, 713, 766, 868, 33574, 35120, 86817, 90030, 90031, 621, 139373, 139380, 97234
- OMIM code: 102730, 138300, 190450, 230450, 231900, 235700, 266120, 266200, 300653, 305900, 610681, 611881, 612631, 613470, 300908, 134700, 615512, 250800, 250790, 261670
- ICD-10 code: D55.0, D55.1, D55.2, D55.3, D74.0, E74.0, E74.1

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 anaemia. 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 anaemia (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 4). 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 anaemia also occurs as a consequence of other enzyme deficiencies, the most common of which is pyruvate kinase (PK) deficiency. Deficiencies of glucose-phosphate 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 defects are expressed throughout the body. Neurologic symptoms, myopathy and other symptoms constitute a prominent part of the clinical syndrome.

Table 4. Red blood cell enzyme disorders associated with hemolytic anemia.

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
Glutathione reductase (GSR) deficiency	Glutathione metabolism	Acute	AR
Glutamate-cysteine ligase (GCLM) deficiency	Glutathione metabolism	Chronic	AR
Glutathione synthetase (GSH-S) deficiency	Glutathione metabolism	Chronic	AR
Adenosine deaminase (ADA) hyperactivity	Nucleotide metabolism	Chronic	AD
Pyrimidine 5'-nucleotidase (P5N) deficiency	Nucleotide metabolism	Chronic	AR

AR, autosomal recessive; XL, X chromosome linked; AD, autosomal dominant.

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 4),** while deficiencies of G6PD and PGK are X linked. Adenosine deaminase (ADA) hyperactivity is a very rare disorder which appears to be inherited in a dominant manner.

Epidemiology

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 (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-characterised database of patients with PK deficiency, the true frequency was, 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 most frequently 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 provided in **Table 5** Enzymes Epidemiology.

Table 5. The currently available data regarding the worldwide number of families/cases diagnosed with anaemia due to very rare enzyme disorders.

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

Clinical Picture and Diagnosis

Red cell morphology in red cell enzyme deficiencies is, in general, unremarkable except for P5N deficiency, which is characterised 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 anaemia 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 counselling. It may also be helpful in recommendations for treatment, since patients with some enzyme deficiencies tend to respond more favourably to splenectomy than do 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 anaemia in adults and neonatal icterus in infants. Usually the anaemia 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 anaemia of variable degree, ranging from severe transfusion-dependent hemolytic anaemia to compensated hemolysis with a

normal steady-state hemoglobin concentration. Chronic jaundice is common, 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 anaemia 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 PK6, 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 anaemia is designated hereditary nonspherocytic hemolytic anaemia.*
- *Hemolysis may be either chronic or limited to periods of increased oxidative stress (specific drugs, infection, ingestion of fava beans).*
- *Some enzyme disorders are characterised by additional prominent non-haematological 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.*

3.2.5. 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
- OMIM code: 109270, 130600, 141700, 179650, 225450, 235370, 266140, 611804
- ICD-10 code: D58.1

Southeast Asian Ovalocytosis (SAO)

- ORPHANET code: 98868
- OMIM code: 166900, 166910
- ICD-10 code: D58.1

Hereditary stomatocytosis (HSt)

- ORPHANET code: 3202, 3203
- OMIM code: 194380, 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, result in hereditary elliptocytosis. Mutations responsible for HS are mainly localised 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 characterised 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 characterised 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 characterised 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 unrelated families.

Other rarer and heterogeneous 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) or ABCB6 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.

Epidemiology

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 realistically 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 black Africans and patients of Mediterranean descent. 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.

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 assessed 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 a positive EMA-binding test are almost constant findings in hereditary spherocytosis, although they are not informative in HE or HSt.

In severe/atypical HS and HE cases, or when HSt is suspected, the diagnostic workup 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 the 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 HSt 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 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, particularly in case of 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 (such as ENERCA) is 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 always be considered.*
- *Creation of European EC networks are required to increase knowledge of these rare disorders.*

3.2.6. Congenital dyserythropoietic anaemias

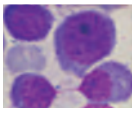
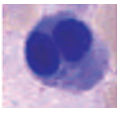
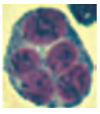
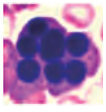
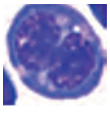
Definition

Congenital dyserythropoetic anaemia (CDA) is a disease category consisting of a group of hereditary anaemias 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 6.**

Pathophysiology and common 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 anaemias, disorders of haemoglobin synthesis.

Table 6. Congenital dyserythropoietic anemias: overview.

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
Morphology					
Gene Locus	CDAN1 15q (15.1.3)	SEC23B 20p11.23-20p12.1	Unknown 15p (21-15)	Unknown Unknown	Unknown Unknown
Dysmorphologies	Skelton, Others	Variable, rare	B-Cells Retina	Variable	CNS Others

Even though symptoms are present in infancy or new-borns, 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) or Ulm (DE). The key feature to CDA from haemolytic anaemias 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, 615631
- 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.

Recently, a homozgous mutation of the C15ORF41- Gene was found in a Pakistani family without any mutation of the *CDAN1*-Gene.

CDA type II

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

The 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 standardised 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 sufficient 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.

A novel mutation of the *KIF3* –gene was associated with all affected members of the autosomal dominant Swedisch and Californian families.

CDA –variants

- ORPHANET code: 85
- 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.

Epidemiology

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 ENERCA's 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. There are large differences in prevalence 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 CDA diagnosis, publication bias and consanguinity rates are most probably the cause of the geographic differences estimated.

Point prevalence and number of affected children of all live births are close to this figure, with a median life expectancy over 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.

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 their lifetimes. 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 lifelong 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 7.**

Table 7. 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 crises, 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 lifelong follow up. Information 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 to comply, the cornerstone to maintaining quality of life and improving life expectancy.

Key messages

- *Congenital dyserythropoetic anaemia (CDA) consist of a group of hereditary anaemias 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 life can be achieved by specific and palliative therapy.*
- *Assurance of the physician's expertise and cooperation with expert centres are mandatory for the patient's compliance, the cornerstone to maintaining quality of life and improving life expectancy.*

3.2.7. Diamond-Blackfan anaemia

- ORPHANET code: 124
- OMIM code: 105650, 606129, 606164, 610629, 612527, 612528, 612561, 612562, 612563, 613308, 613309, 614900, 615550
- ICD-10 code: D61.0

Definition

Diamond Blackfan anaemia (DBA) is a rare bone marrow failure syndrome characterised by severe normochromic macrocytic anaemia and reticulocytopenia, typically appearing 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 haematological 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 anaemia have not been completely elucidated. A generally recognised 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 also recently supported 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.

Epidemiology

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 a 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 better definition of 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.

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 8.**

Table 8. Diagnosis criteria for DBA.

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
Major • **Gene mutation described in "classical" DBA** • **Positive family history** **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 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 an 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.*

3.2.8. Fanconi Anaemia

- ORPHANET code: 84
- OMIM codes: 227650; 227646; 227645; 300514; 605724; 609054; 610832; 600901; 614083; 613390; 614082; 614087; 613951; 603467; 609053, 615272
- ICD-10 code: D61.0

Definition

Fanconi anaemia (FA) is a rare inherited syndrome characterised 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, and the risk of BMF is 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 (anaemia) or during development (malformations).

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% in most countries) have mutations in FANCA, FANCC and FANCG. Approximately, 2/3 (EEUU) to 3/4 (southern European countries such as Spain) of patients belong to the FANCA subtype.

Epidemiology

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 ethnic groups such as Spanish Gypsies, to 1:209 in the overall Caucasian population. Other ethnic groups with higher incidence of FA are Ashkenazi Jewish and white Afrikaners from South Africa due to founder mutations in FANCC and FANCA respectively.

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 hypersensitive 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 haematology 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 reconfirm 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 at 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 mobilised 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 characterised 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.*

3.2.9. Hereditary or Congenital Sideroblastic Anaemia

- ORPHA code: 480, 699, 1047, 2598, 2802, 3463, 75563, 98362, 255132
- OMIM code: 205950, 530000, 600462, 613561, 222300, 598500, 604928, 614296, 301310, 557000, 300751
- 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.

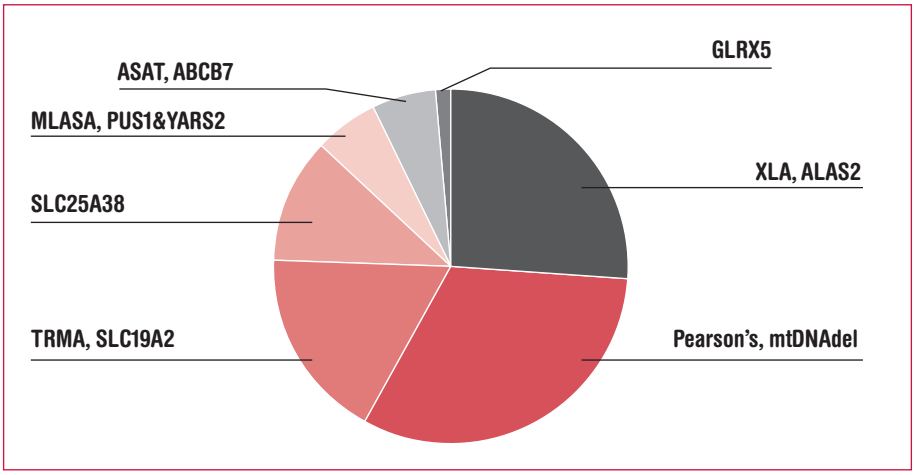
Epidemiology

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 have been collected from the literature by a single individual, or by personal communication from other diagnostic laboratories, and are shown in **table 9.** A consensus view among ENERCA colleagues has yet to be reached 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 a specific registry.

Table 9. Reported cases of hereditary or congenital siderobloastic anaemia.

	Reported cases	Families
All	310-330	255
Non- syndromic X- linked sideroblastic anaaemias (ALAS2)	124	92
Pearson's Syndrome	90-100	90-100
Thiamine responsive megaloblastic 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

Figure 1. 314 HSA cases: subtype distribution.



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% of 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.

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.

Special clinical management aspects

All HSA patients require a multi-disciplinary 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, 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.*

3.2.10. Hereditary non-sideroblastic anaemias due to iron metabolism defects

Definition

Hereditary non-sideroblastic anaemias due to iron defects are a group of disorders sharing the following features: 1) the presence of a mild to severe hypochromic microcytic anaemia 2) the absence 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 these diseases presents specific particularities, but all of them are inheritance as autosomal recessive genetic diseases.

Aceruloplasminemia

- ORPHANET Code: 48818
- OMIM Code: 604290
- ICD-10 code: G23.0

Atransferrinemia

- ORPHANET Code: 1195
- OMIM Code: 209300
- ICD-10 code: D50.8

DMT1-deficiency Anaemia

- ORPHANET Code: 83642
- OMIM Code: 206100
- ICD-10 code: D50.8

Iron Refractory Iron Deficiemcy Anaemia (IRIDA)

- ORPHANET Code: 209981
- OMIM Code: 206200
- ICD-10 code: D50.8

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 to have 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 resulting in iron deficiency. *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-sideroblastic anaemias due to iron defects 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 due to iron defects

Hereditary non-sideroblastic anaemia should be considered a cause in any hypo non 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.

Epidemiology

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 characterised 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 less than 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 exist and are waiting for further clinical and genetic characterization.

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 common causes have been excluded, 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 variations are private, requiring full sequence analysis of the relevant gene that requires contacting a genetic diagnostic expert centre (see GeneTest or ENERCA for expert laboratories).

Special clinical 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. Recent papers have suggested that iron chelation therapy may have some positive effect on the development of neurological complications.

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 anaemia is poorly responsive to iron treatment and the patient may develop severe liver iron overload, these treatments (iron supplementation or transfusions) should be given with care to these patients. In contrast, 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 regularly, especially during growth.

Key messages

- *Hereditary non-sideroblastic anaemia is a heterogeneous group of rare and non-syndromic conditions that occur within all populations and 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 less than 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.*

3.2.11. Paroxysmal Nocturnal Haemoglobinuria (PNH)

- ORPHA code: 447
- MIM code: 300818, 615399
- ICD code: D59.5

Introduction and Pathophysiology

Paroxysmal nocturnal haemoglobinuria (PNH) is a unique disorder frequently affecting young individuals and was first described as a distinct clinical entity in 1882. The disease is characterised by complement mediated intravascular haemolysis leading to anaemia, significant fatigue (usually out of proportion to the anaemia) and organ dysfunction such as renal impairment and pulmonary hypertension. The most feared complication is thrombosis which may be life-threatening. Thromboses occur within both the arterial and venous systems. PNH arises on the background of a bone marrow failure such as aplastic anaemia or myelodysplasia. In 1963, Dacie first suggested that PNH is an acquired clonal disorder resulting from a somatic mutation in a haematopoietic stem cell. PNH arises through a somatic mutation of the *phosphatidylinositol glycan A* (*PIG-A*) gene in bone marrow stem cells resulting in a clonal expansion of these haematopoietic stem cells. The *PIG-A* gene codes for a protein, which is critical in the catalysis

of the first step of glycosylphosphatidylinositol (GPI) biosynthesis. A single mutation disrupting GPI biosynthesis results in a deficiency of all GPI-anchored proteins on the cell membrane and in the PNH phenotype. Deficiency from PNH red cells can be complete (giving rise to PNH type III cells) or partial (PNH type II cells). Type I cells have normal levels of GPI-linked proteins on their surface. This variability in the severity of the deficiency, as well as in the proportion of the affected cell population, defines the clinical manifestations of the disease.

There is conclusive evidence that PNH only develops in individuals who have a predisposition to the development or, more likely, the expansion of the GPI-deficient haematopoietic clones. This predisposition is probably the presence of an underlying bone marrow failure, usually aplastic anaemia but may also be myelodysplasia (MDS) or myelofibrosis, either preceding or co-existent with the diagnosis of PNH. This led to the conclusion that patients with PNH are permissive for the expansion of GPI-deficient haematopoietic clones. GPI-deficient cells with *PIG-A* mutations occur very frequently at low levels in normal individuals but do not expand in competition with the normal haematopoietic cells. It appears that normal haematopoiesis is suppressed by the immune system, presumably either directly or indirectly through one or more GPI-linked proteins, and that this attack spares the GPI-deficient PNH clone.

Among the deficient GPI linked proteins are the complement regulatory proteins CD55 (previously known as Decay Accelerating Factor) and CD59 (previously known as Membrane Inhibitor of Reactive Lysis). The resulting increased complement sensitivity of PNH cells leads to intravascular haemolysis, thrombosis and many of the clinical manifestations of the disease. Ongoing haemolysis and/or insufficient haematopoiesis often result in transfusion dependence. Haemolysis in patients with PNH can be monitored by levels of the enzyme lactate dehydrogenase (LDH). LDH is a standard biochemical measure of intravascular haemolysis and probably the most sensitive. Levels are frequently elevated in patients with PNH, exceeding 20 times the upper limit of normal during severe paroxysms.

CD55 inhibits C3 and C5 convertases, whereas CD59 is the sole membrane regulator of membrane attack complex (MAC) assembly (20;21). CD59 prevents terminal complement components from forming the haemolytic membrane pore, C5b-9 (the membrane attack complex). As a result of complement-mediated attack, the survival of PNH erythrocytes *in vivo* is shortened to about 10% that of normal red cells (24).

The platelets in PNH patients also lack GPI-anchored proteins. The absence of CD59 renders platelets susceptible to attack by complement. When this occurs, the platelets are not destroyed but rather they undergo morphological changes, which result in the release of vesiculated membrane attack complex. These vesicles or microparticles are very procoagulant *in vitro* and are present at significantly elevated levels in the blood of patients with PNH. This is likely to be one of the principle mechanisms of thrombosis in PNH.

Consequences of intravascular haemolysis

The lack of complement regulation on the PNH red cell surface renders these cells extremely sensitive to complement-mediated lysis resulting in systemic haemoglobin release. Free haemoglobin consumes nitric oxide (NO). NO normally acts to maintain vessel tone through smooth muscle relaxation and inhibit platelet aggregation and activation and reductions in nitric oxide plasma levels lead to smooth muscle dystonias, including hypertension, gastrointestinal contractions leading to abdominal pain, dysphagia and erectile dysfunction, as well as clot formation. In addition, systemic release of haemoglobin is associated with pulmonary and systemic hypertension, decreased organ perfusion, renal failure and increased mortality.

NO depletion, as well as direct complement activation of PNH platelets, has been implicated in the most feared complication of PNH - thrombosis. Thrombosis occurs in approximately 50% of patients with haemolytic disease and is the cause of death in at least one-third. There is a predilection for the intra-abdominal and cerebral veins but a common site of thrombosis is also the simple deep vein thrombosis and pulmonary embolus. Arterial thromboses are also increased in patients with PNH. While studies have reported a strong correlation between a larger PNH type III neutrophil clone and the occurrence of thrombosis (56;59;63), thrombosis appears to also be elevated in patients with smaller clones as low as 10% when compared to the normal population. (64) Patients with thrombosis at presentation have only a 40% survival rate at 4 years.

Diagnosis

Testing for PNH should be considered in patients with conditions listed in **Table 10**, including those with Direct Antiglobulin Test (DAT)-negative haemolytic anaemia, unexplained cytopenia and unexplained thrombosis.

Table 10. When to test a patient for PNH.

Consider testing for PNH in patients with the following conditions
Patients with Aplastic Anaemia and Myelodysplasia
DAT-negative (or complement only-positive DAT) haemolytic anaemia
Haemoglobinuria
Recurrent abdominal pain or dysphagia with high LDH
Thrombosis (venous or arterial) at an unusual site or associated with haemolysis or a cytopenia
Unexplained cytopenia

Flow cytometry is the principle investigative tool to diagnose PNH and has replaced the biochemical assays that assessed the sensitivity of red cells to complement-mediated lysis, such as the Ham Test, used previously. Each laboratory tends to have a local preference for the reagents used and includes monoclonal antibodies against CD55 and CD59 for red cells and CD14, CD16, CD24 and the reagent FLAER (fluorescein-labelled proaerolysin) for granulocytes and monocytes. The FLAER reagent binds

directly to the GPI anchor and is certainly highly effective in the identification of small leukocyte PNH clones. At least two antibodies against two different GPI-anchored proteins should be utilised to diagnose PNH to distinguish from rare inherited deficiencies of single GPI-anchored proteins. Monitoring of the clone size by flow cytometry is also required in managing the disease and is most sensitive and specific when granulocytes are assessed, as this lineage is unaffected by hemolysis and transfusion. Once detected, PNH clones should be closely monitored, usually every 6-12 months.

Epidemiology

PNH is known to be a rare disorder with figures of incidence quoted by PNH information websites range between 1 per 100,000 to 5 per million population. In a study performed to accurately report the incidence and prevalence of PNH in a given population of 3,742,835 in a well-defined geographical area, an incidence of 0.13/100,000/year and estimated 15-year prevalence of PNH of 1.59 per 100,000 was found. It is important to note that these figures report the incidence and prevalence of a PNH clone of any size (from 0.01% to 100% PNH granulocytes). The finding of any size of PNH clone requires ongoing monitoring and potentially management, ideally with a haematologist with experience treating this rare disease.

Management

As complement mediated lysis or activation is responsible for the majority of the clinical manifestations in PNH, targeting complement is an attractive strategy in these patients. In PNH, this has been achieved with use of the complement inhibitor, eculizumab. Eculizumab is a recombinant humanised chimeric monoclonal antibody that specifically targets the complement protein C5 and prevents its cleavage. All patients who receive this drug should be vaccinated against *Neisseria meningitidis*. Reductions in thrombosis rate and improvements in renal function as well as pulmonary hypertension have been reported. Patients also report significantly improved quality of life and reduction in symptoms such as fatigue, abdominal pain and dysphagia. All these positive effects have also translated into significantly improved survival for patients with PNH on eculizumab therapy.

Prior to eculizumab therapy, options principally relied on supportive measures with transfusions as required and the treatment of complications, such as thrombosis, when they occur. It is conventional to give all patients with evidence of haemolysis, folic acid supplementation as they will have increased red cell turnover. Many patients often also require supplementation with iron therapy, despite being transfusion dependent, due to the chronic haemosiderinuria and haemoglobinuria which may lead to iron deficiency. Corticosteroids have been used to reduce the hemolysis in PNH but high doses are required with its attendant side effects and therefore do not have a role in the long-term management of patients with PNH. Anticoagulation prophylaxis can be of benefit to reduce thrombosis risk in patients who are not able to receive eculizumab and where the risks of antico-

agulation are justified i.e. proportion of PNH granulocytes greater than 50%, platelet count greater than 100 x 10^9/L and no other contraindications to anticoagulations, e.g. varicose veins.

The only curative strategy remains allogeneic stem cell transplantation but this carries a considerable risk of mortality. In view of the fact that a proportion of patients will eventually experience a spontaneous remission of PNH and with the advent of effective novel therapies, stem cell transplantation should only be considered for the indication of severe aplastic anaemia or progressive bone marrow failure.

Data collected from the Global PNH Registry will hopefully answer many unanswered questions in this rare disorder and haematologists are strongly encouraged to enrol all PNH patients, regardless of clone size or therapy, into this registry (www.pnhregistry.org).

Conclusions

PNH remains a fascinating disease in which huge advances in understanding has been made in the last few decades. This has led to the first effective therapy targeting the cause of many of the complications - complement mediated activation, with the drug, eculizumab.

Key messages

- *PNH is a potentially disabling disease.*
- *Nitric oxide depletion contributes to smooth muscle dystonia (abdominal pain, dysphagia, erectile dysfunction), severe lethargy, renal impairment, hypertension and thrombosis.*
- *Consider testing for PNH in patients with AA or MDS, DAT-negative haemolytic anaemia, haemoglobinuria or unexplained thrombosis.*
- *Gold standard test for diagnosis is flow cytometry.*
- *FLAER can detect small leukocyte PNH clones by flow cytometry.*
- *Clone size should be monitored regularly once found.*
- *All patients should receive folic acid supplementation.*
- *Many patients require additional iron replacement.*
- *Allogeneic stem cell transplantation remains the only curative strategy but indications have diminished in light of cases of spontaneous remissions of PNH and the advent of the effective therapy, eculizumab and should only be considered for the underlying bone marrow failure and not PNH itself.*
- *Eculizumab (Soliris) blocks the complement cascade at C5 preventing terminal complement activation. It effectively, safely and significantly prevents intravascular haemolysis thereby reducing (or abolishing) symptoms and transfusion requirements in patients with PNH.*

- *Eculizumab significantly reduces thrombosis rate in patients with PNH.*
- *Eculizumab improves renal function, reduces nitric oxide consumption and pulmonary hypertension in patients with PNH.*
- *Eculizumab has safely been used in a small number of pregnant patients with PNH*
- All patients who receive Eculizumab should be vaccinated against *Neisseria Meningitidis.*
- *Eculizumab has been demonstrated to significantly improve survival for patients with PNH.*

All patients with a PNH clone, regardless of size or therapy, should be enrolled in the Global PNH Registry (www.pnhregistry.org).

3.3. Laboratory diagnosis and quality assessment

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 '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'.

Certification of clinical laboratories requires following the ISO developed quality management system requirements; some of them are listed below:

- 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.

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. 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 by 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.

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 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 patient 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 intelligible 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 inter-laboratory comparability in some other way, e.g. by the interchange of patients' material with other centres providing a similar service.

ENERCA contribution to EQAS in Rare Anaemias

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). (see annex 1).

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 (see annex 2). This includes non-specific, general laboratory investigations used in the diagnosis and monitoring of a wide range of conditions and specialist investigations.

Within the ENERCA project, two 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.

3.4. Good medical practice in the management of rare anaemias

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 part. 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 organisations 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 politeness, 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 organisational 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 organisation 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 anaemias, 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 healthcare are subject to Quality Assessment. "QA and Improvement" (QA/I) programs are the tool

by which each organisation 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 anaemias (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 organisations 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 analysing 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 anaemias 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 behaviour. It is all these qualities which, along with good training, ensure the best clinical practice in the field of rare anaemias.

3.5. Treatment of patients with severe rare anaemias

Most therapeutic approaches for the management of rare anaemias, as described under each diagnostic category, are able to provide patients with symptomatic relief, the possibility to prolong life and improve quality of life. Until recently only supportive care, mainly based on transfusion therapy, was available for patients with rare anaemias. Other approaches have been developed in the last decades, depending on the precise diagnosis of the anaemia, and as a result of advances in scientific research. One example is the treatment now proposed for PNH (see chapter 3.2.11). For a more detailed description of the treatments of each type of anaemia, please refer to the corresponding chapter of this book or to the ENERCA website.

There are still two general 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.

3.5.1. 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 anaemia 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 Human Leukocyte Antigen (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 a HLA identical sibling donor in 1982 more than 3000 successful transplantations have been reported.

The patient's 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 under 30, cure rates are 70-90% if there is a sibling donor, whereas transplant related mortality reaches 50% in patients who are 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 are the responsibility of the centre of expertise in collaboration with the transplant centre. The follow up is also a combined responsibility of the multi-disciplinary team and the transplant centre.

3.5.2. Gene therapy

This is an attractive treatment for 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 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 5 years. Currently more patients have entered a trial in the USA and results are awaited. The technique involves the introduction a functional beta globin gene to the patient's cultured haemopoietic stem cells, these are then re-introduced to the patient in a 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.

4. CENTRES OF EXPERTISE AND EUROPEAN REFERENCE NETWORKS

Since the 1990s at both the European Union (EU) and Member State (MS) level political concepts and initiatives concerning rare diseases have emerged **(Figure 1).** **ENERCA** started in 2002, and therefore it fed on all the content and initiatives surrounding rare diseases in Europe from that year until present, and actively contributed to inputting many of these developments first as a member of the Rare Disease Task Force (2002-2009) and later in the European Committee of Experts in Rare Anaemias (2009-2012). During the 10 years that ENERCA has existed, a number of countries have led the way to the first European legislation concerning rare diseases, the Orphan Medicinal Product Regulation of 16 December 1999, and the ensuing Commission Communication (2008) and Council Recommendation (2009). Sweden, for example, established the first centres of expertise for rare diseases in 1990 and a rare disease database and information centre in 1999; Denmark established an information centre in 1990 and then centres of expertise for rare diseases in 2001; in Italy, a decree on rare diseases came into force in 2001; and in France, Orphanet was established in 1997 with the support of the French Ministry of Health as the portal for information on rare diseases and orphan medicinal products, followed by the first national plan/strategy for rare diseases in Europe (2004). A number of other countries (Bulgaria, Greece, Portugal, and Spain) elaborated a national plan/strategy for rare diseases at the very same time as EU policy in the field was defined through the Commission Communication and Council Recommendation. In 2011, policy at Member State level gathered momentum in the wake of EU policy, in particular the elaboration of national plans or strategies for rare diseases, in response to the recommendation of the Council to "elaborate and adopt a plan or strategy as soon as possible, preferably by the end of 2013 at the latest, aimed at guiding and structuring relevant actions in the field of rare diseases within the framework of their health and social system".

For the period 2008-2013 the Commission adopted the White Paper COM (2007) 630 final "Together for Health: A Strategic Approach for the EU 2008-2013" of 23 October 2007 developing the EU Health Strategy. Actions under Objective 1 of this EU Strategy cover a Communication on European Action in the Field of Rare Diseases and in point 4.1 of this EU Strategy it is suggested to put forward EC-level structured cooperation mechanisms to advise the Commission and to promote cooperation between the Member States.

As a consequence, rare diseases are now one of the priorities in the Second EU Health Programme 2008-2013 and according to the DG SANCO Work Plans for the implementation of the Public Health Programme, main lines of action and priorities are chosen every year.

4.1. The European Commission policies for rare diseases: EU Public Health Policy

A Community action programme on rare diseases, including genetic diseases, was adopted for the period of 1 January 1999 to 31 December 2003, with the aim of ensuring a high level of health protection in relation to RDs. As the first EU effort in this area, specific attention was given to improving knowledge and facilitating access to information about these diseases. Orphan Medicinal Product Regulation (Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products, was proposed to set up the criteria for orphan designation in the EU and describes the incentives (e.g. 10-year market exclusivity, protocol assistance, access to the Centralised Procedure for Marketing Authorisation) to encourage the research, development and marketing of medicines to treat, prevent or diagnose rare diseases

The ***Council Recommendation on an action in the field of rare diseases 2009/C 151/02)*** adopted on 8 June 2009, outlined the specificities of rare diseases, great heterogeneity of diseases and of expression of diseases without predominant symptoms, limited number of patients and a scarcity of relevant knowledge and expertise) and singled them out as a unique domain of very high added value of action at Community level. This added value can be achieved by gathering national expertise on rare diseases, which is scattered throughout the Member States (MS) and organising collaboration between centres of expertise (CEs), healthcare providers, laboratories, patients and individual experts within and between MS to offer optimal cross-border services to all EU citizens. The recommendation engages the responsibility of Member States and concentrates on supporting and strengthening the adoption before the end of 2013 of national plans and strategies for responding to rare diseases, on improving recognition and visibility of rare diseases, on encouraging more research into rare diseases and forging links between centres of expertise and professionals in different countries through the creation of European reference networks in order to share knowledge and expertise and, where necessary, to identify where patients should go when such expertise cannot be made available to them. The role of patient organisations is also highlighted as particularly important. The Council Recommendation on a European action in the field of rare diseases recommended actions at national level to implement the EU action. The seven key themes of the Council Recommendation are the following:

I. *Plans and strategies in the field of rare diseases* – calls on the MS to elaborate and adopt a plan or strategy by the end of 2013.
II. *Adequate definition, codification and inventorying of rare diseases* – evokes the common definition of a rare disease as a condition affecting no more than 5 per 10 000 persons; aims to ensure that rare diseases are adequately coded and traceable in all health information systems based on the ICD and in respect of national procedures; and encourages MS to contribute actively to the inventory of rare diseases based on the Orphanet network.

III. *Research on rare diseases* – calls for the identification and fostering of rare disease research at all levels.
IV. *Centres of expertise and European reference networks for rare diseases* – asks the MS to identify and facilitate networks of expertise based on a multi-disciplinary approach to care, and foster the diffusion and mobility of expertise and knowledge.
V. *Gathering the expertise on rare diseases at European level* – calls on MS to share best practices, develop medical training relevant to the diagnosis and management of rare diseases, coordinate European guidelines, and, to minimise the delay in access to orphan drugs, as well as to share clinical/therapeutic added-value assessment reports at the Community level.
VI. *Empowerment of patient organisations* – calls on MS to consult patient representatives on policy development; facilitate patient access to updated information on rare diseases; promote patient organisation activities.
VII. *Sustainability* – highlights that long-term sustainability in the field of information, research and healthcare of infrastructures must be ensured.

The **European Union Committee of Experts on Rare Diseases (EUCERD)** , was formally established via the European Commission Decision of 30 November 2009 (2009/872/EC) to aid the European Commission with the preparation and implementation of Community activities in the field of rare diseases . The EUCERD has already adopted a set of recommendations on quality criteria for CEs in MS considering that CEs are the future nodes of European Reference Networks (ERNs). CEs have to work with other healthcare providers, laboratories and other experts who are the closest competent point of care for patients to ensure that:

1. Expertise travels rather than patients themselves when appropriate: through the national healthcare systems there can be very different structures organised by regions, treatments, development and adoption of e-tools for tele-expertise and tele-consultation.
2. Exchange of data, biological samples, radiological images, other diagnostic procedures and all offers of materials, occurs appropriately when needed to improve diagnosis and care, to improve knowledge and contribute to the development of new therapies.

The **European Project for Rare Diseases National Plans Development (EUROPLAN**) was founded by the European Commission between 2008-2011. The main goal of the project was to provide national health authorities with supporting tools for the development and implementation of national plans and strategies for rare diseases as recommended by the Council. The supporting tools included a Guidance document on recommendations for the definition and implementation of national plans and strategies for rare diseases; a joint report with the RDTF on initiatives and incentives in the field of rare diseases in Europe; and a document on the recommended set of indicators for monitoring and evaluating the implementation of national initiatives. In the context of the EUROPLAN project, national conferences and workshops on the subject of national plans and strategies, took place throughout

2010 in 15 EU MS: these national conferences were organised by National Alliances of rare disease patient organisations under the supervision of EURORDIS. The conferences aimed both to raise awareness of the Council Recommendation and to move forward the process of developing a national strategy for rare diseases in each particular country. The support activities of Europlan will continue in the context of the EUCERD Joint Action: Working for Rare Diseases N° 2011 22 01 from March 2012 for a 3 year period.

The Directive 2011/24/EU on the application of patients' rights in cross-border healthcare, was approved in early 2011. Highly relevant to rare disease patients suffering from scarce and scattered resources for care and diagnostics, the Directive seeks to facilitate access to healthcare for EU citizens and encourage cooperation between EU Member States in the field of health. Member States will have 30 months to put the provisions of the Directive into national legislation following the publication in the Official Journal of the European Union. The Directive will have no impact on the rights of each Member State to determine which health benefits they will provide. Thus, if a particular treatment is not reimbursed in a patient's home country, it will not be reimbursed if accessed in another Member State. Member States would be able to require prior authorisation for "hospital care" and reimbursement would match the amount that patients would receive in their home country. However, Article 13 of the Directive specifically addresses the commitment of the Commission on behalf of rare disease patients: *"The Commission* shall support Member States in cooperating in the development of diagnosis and treatment capacity in particular by aiming to:

(a) Make health professionals aware of the tools available to them at Union level to assist them in the correct diagnosis of rare diseases, in particular the Orphanet database, and the European reference networks.
(b) Make patients, health professionals 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 Member States even for diagnosis and treatments which are not available in the Member State of affiliation."

Since 2012 the Directive has been implemented by the **Cross-Border Healthcare Expert Group,** a legal forum of all 27 Member States where they meet to vote on implementing acts and discuss general issues concerning the transposition of the directive. Delegated and implementing acts will, by defining the criteria as provided in the Directive, establish the methodology of the whole process of deciding which European Reference Networks (ERNs) to support, including the process of selection and designation of the healthcare providers to be considered members of the ERNs and several categories of criteria for the adequate management, monitoring and evaluation of the networks.

It is clear that after 2013, The ERN for Rare Diseases will have a strategic role in the improvement of quality treatment for all patients throughout the European Union as called by the patients' organisations.

From the Rare Disease Task Force (RDTF) to the European Union Committee of Experts in Rare Diseases (EUCERD)

The ***Rare Diseases Task Force (RDTF)*** was established in January 2004 via Commission Decision 2004/192/EC of 25 February 2004 on the programme of Community action in the field of public health (2003 to 2008). The members of the RDTF included current and former rare disease research project leaders, elected experts from Member States, and representatives from relevant international organisations (DG Research, DG Enterprise, EuroStat, EMA, WHO, OECD). Over 6 years, the RDTF played a pivotal role instigating key collaborative rare diseases initiatives in Europe and many key topics were brought forward for discussion in relation to rare disease research, policy and actions. The activities of the RDTF were published in OrphaNews Europe, the official newsletter of the Rare Diseases Task Force. Various working groups were identified and constituted to meet the specific objectives of the RDTF: this included the Standards of Care working group, the Public Health Indicators working group, and the Coding, Classification and Data Confidentiality Group. These working groups produced various reports, recommendations and scoping papers, all available on the archived site of the former RDTF. One of the most notable contributions of the RDTF was its pivotal role between June and October 2007 in drafting the Communication Rare Diseases: Europe's Challenges, in close collaboration with the European Commission. The process ultimately culminated in the adoption of the European Council Recommendation on an Action in the Field of Rare Diseases in June 2009.

The ***European Union Committee of Experts on Rare Diseases (EUCERD)*** replaced the RDTF via European Commission Decision of 30 November 2009 (2009/872/EC). EUCERD is charged with aiding the EC with the preparation and implementation of Community activities in the field of RDs, in cooperation and consultation with the specialised bodies in Member States, the relevant European authorities in the fields of research and public health action and other relevant stakeholders acting in the field including patients. The EUCERD will foster exchanges of relevant experience, policies and practices between these parties. Specifically, the EUCERD is charged with the following responsibilities:

1. Assisting the Commission in monitoring, evaluating and disseminating the results of measures taken at Community and national level in the field of rare diseases.
2. Contributing to the implementation of Community actions in the field, in particular by analysing the results and suggesting improvements to the measures taken.
3. Contributing to the preparation of Commission reports on the implementation of the Commission Communication and the Council Recommendation.
4. Delivering opinions, recommendations or reports to the Commission either at the latter's request or on its own initiative.
5. Assisting the Commission in international cooperation on matters relating to rare diseases.

6. Assisting the Commission in drawing up guidelines, recommendations and any other action defined in the Commission Communication and in the Council Recommendation.
7. Providing an annual report of its activities to the Commission.

The EUCERD comprise 51 members and the corresponding alternates, namely: (a) one representative per Member State from ministries or government agencies responsible for rare diseases (b) four representatives from patients' organisations; (c) four representatives of the pharmaceutical industry; (d) nine representatives of ongoing and/or past Community projects in the field of rare diseases financed by the programmes of Community action in the field of health including three members of the pilot European Reference Networks on rare diseases; (e) six representatives of the ongoing and/or past rare diseases projects financed by the Community Framework Programmes for Research and Technological Development; (f) one representative of the European Centre for Disease Prevention and Control (ECDC).

As an ongoing and past Community project in the field of rare diseases financed by the Community Action on Health, ENERCA is officially represented in EUCERD by its Head of Project. This has allowed ENERCA to be nourished by all the experiences, policies, practices and initiatives surrounding RDs developed in Europe during its 10 years of existence.

EUCERD activities have been supported by Joint Action Nº 2008 119 (RDTF/EUCERD). EUCERD sources of information include EC websites and documents, OrphaNews Europe, EUCERD publications and meetings, reports on orphan drugs, EURORDIS websites of Patient Organisations and national alliances, EUROPLAN questionnaire and Conferences final reports, and Orphanet web portal.

4.2. The European Centres of Expertise (CEs)

Centres of expertise (CEs) in the field of RD are mentioned in the High Level Group on Health Services and Medical Care Report (November 2005), the Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of Regions on Rare Diseases: Europe's challenges (2008) and the Council Recommendation on an action in the field of rare diseases (2009), as well as in the recommendations for National Plans and Strategies for Rare Diseases (EUROPLAN) and in the Directive for cross-border healthcare (2011).

The EC RDTF has held two workshops dedicated to centres of expertise (2005 & 2006) and has produced two reports *"RDTF Report: Overview of Current Centres of Reference on rare diseases in the EU - September 2005"* and *"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"*. These workshops and reports helped reach the following consensus:

1. Centres of expertise have a European added-value; there is a need to disseminate information on centres of expertise.
2. Networks of centres should be encouraged.
3. There should be no hierarchy between European centres and national/regional centres.

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 for 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.

Additional criteria for National CEs were suggested in the EUCERD workshop of 2012 on the Cross Border Health Care Directive, which aimed to clarify better standards and indicators needed to evaluate and audit national CEs and ERNs. These criteria included:

- National centres must be open to European collaboration.
- National centres must accept being 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 reducing delays to diagnosis and for building healthcare pathways from primary care.
- Centres should have a quality management system in place to assure quality of care that takes into account European norms.
- Centres should adhere to good practice guidelines where they exist.
- Centres should be responsible for establishing healthcare 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 they 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 Member States (MS) to identify, in time, individual centres of expertise, otherwise patients will unnecessarily seek care abroad, introducing new and extremely challenging problems in the implementation of the Cross Border healthcare. In terms of ERNs the highlighted observations include:

- There is a clear distinction between the missions of CEs and of ERNs.
- The current networks are mainly experts networks not necessary officially recognised as national centres.
- Expert clinical care and clinical research always go 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.

Finally four aspects have to be taken into consideration when defining an CE.

1. There cannot be two parallel systems for designating CEs. In order to avoid confusion and duplication of efforts, nationally designated CEs and ERNs should be the same entity.
2. The definition of a CE has to be clarified in respect to individual expertise: i.e. is a centre of expertise a centre where an expert works, or is it a physical structure/team which provides expert services? Although a centre of expertise can be defined by the expertise of one person, there is an element of instability that must be considered, as this expert may change jobs, retire etc., and the centre would cease to exist.
3. A periodic re-evaluation process should be incorporated into systems of designation of centres of expertise. A discussion also took place on the indicators that should be put into place to monitor implementation and outcomes in the field.
8. Expertise needs to be identified at a national level before networks of expertise at a European level can be built.

4.3. The European Reference Networks (ERNs)

The Directive (EC 2011/24/EU) of the European Parliament and of the Council on the application of patients' rights in cross-border healthcare establishes that the Commission

should support the continued development of European reference networks (ERNs) between healthcare providers and centres of expertise (CEs) in the Member States (MS). ERNs can 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. This Directive should therefore give incentives to MS to reinforce the continued development of ERNs which are based on the voluntary participation of their members, but the Commission should develop criteria and conditions that the networks should be required to fulfil in order to receive support from the Commission. For this article 12 of the Directive established that:

1. The Commission shall support MS in the development of ERNs between healthcare providers and CEs the MS, in particular in the area of rare diseases. The networks shall be based on voluntary participation by its members, which shall participate and contribute to the networks' activities in accordance with the legislation of the Member State where the members are established and shall at all times be open to new healthcare providers which might wish to join them, provided that such healthcare providers fulfil all the required conditions and criteria (see paragraph 4).
2. European reference networks shall have at least three of the following objectives:

    a) To help realise the potential of European cooperation regarding highly specialised healthcare for patients and for healthcare systems by exploiting innovations in medical science and health technologies.
    b) To contribute to the pooling of knowledge regarding sickness prevention.
    c) To facilitate improvements in diagnosis and the delivery of high-quality, accessible and cost-effective healthcare for all patients with a medical condition requiring a particular concentration of expertise in medical domains where expertise is rare.
    d) To maximise the cost-effective use of resources by concentrating them where appropriate.
    e) To reinforce research, epidemiological surveillance like registries and provide training for health professionals.
    f) To facilitate mobility of expertise, virtually or physically, and to develop, share and spread information, knowledge and best practice and to foster developments of the diagnosis and treatment of rare diseases, within and outside the networks.
    g) To encourage the development of quality and safety benchmarks and to help develop and spread best practice within and outside the network.
    h) To help Member States with an insufficient number of patients with a particular medical condition or lacking technology or expertise to provide highly specialised services of high quality.

3. Member States are encouraged to facilitate the development of the European reference networks:

a) By connecting appropriate healthcare providers and centres of expertise throughout their national territory and ensuring the dissemination of information towards appropriate healthcare providers and centres of expertise throughout their national territory.
b) By fostering the participation of healthcare providers and centres of expertise in the European reference networks.

4. For the purposes of paragraph 1, the Commission shall:

a) Adopt a list of specific criteria and conditions that the European reference networks must fulfil and the conditions and criteria required from healthcare providers wishing to join the European reference network. These criteria and conditions shall ensure, inter alia, that European reference networks:
    i. Have knowledge and expertise to diagnose, follow-up and manage patients with evidence of good outcomes, as far as applicable.
    ii. Follow a multi-disciplinary approach.
    iii. Offer a high level of expertise and have the capacity to produce good practice guidelines and to implement outcome measures and quality control.
    iv. Make a contribution to research.
    v. Organise teaching and training activities; and
    vi. Collaborate closely with other centres of expertise and networks at national and international level.

b) Develop and publish criteria for establishing and evaluating European reference networks;
c) Facilitate the exchange of information and expertise in relation to the establishment of European reference networks and their evaluation.

5. The Commission shall adopt the measures referred to in paragraph 4(a) by means of delegated acts in accordance with Article 17 and subject to the conditions of Articles 18 and 19.
6. The measures referred to in points (b) and (c) of paragraph 4 shall be adopted in accordance with the regulatory procedure referred to in Article 16. Measures adopted pursuant to this Article shall not harmonise any laws or regulations of the Member States and shall fully respect the responsibilities of the Member States for the organisation and delivery of health services and medical care.

The Work Plan 2006 for the implementation of the EU public health programme, introduced, for the first time, as a priority in the area of rare diseases to develop **Pilot European Networks of Centres of Reference for Rare Diseases**. According to this priority, 10 Projects have been selected for funding:

1. European Centres of Reference Network for Cystic Fibrosis with the Klinikum der Johann Wolfgang Goethe-Universität (DE) as Project Leader.

2. European Network of Centres of Reference for Dysmorphology with The University of Manchester (UK) as Project Leade.
3. Patient Associations and Alpha1 International Registry with the Stichting Alpha1 International Registry (NL) as Project Leader.
4. European Porphyria Network: providing better healthcare for patients and their families with the Assistance Publique - Hôpitaux de Paris (FR) as Project Leader.
5. Establishment of a European Network of Rare Bleeding Disorders, with the Università degli Studi di Milano (IT) as Project Leader.
6. European network of paediatric Hodgkin's lymphoma – European-wide organisation of quality controlled treatment with the University of Leipzig (D) as Project Leader.
7. European Network of Reference for Rare Paediatric Neurological Diseases (NEUROPED) with theEuropean Network for Research on Alternating Hemiplegia (AT) as Project Leader.
8. A reference network for Langerhans cell histiocytosis and associated syndrome in EU with Assistance Publique Hôpitaux de Paris (FR) as Project Leader.
9. Improving Health Care and Social Support for Patients and Family affected by Severe Genodermatoses –TogetherAgainstGenodermatoses (TAG) with Fondation René Touraine (FR) as Project Leader.
10. European Reference Network of expert centres in rare anaemias (ENERCA 3) with Hospital Clínic de Barcelona (ES) as Project Leader.

The purpose of EU actions to develop national/regional centres of expertise is the establishment of Europedan Reference Networks (ERNs) and to establish a procedure for designation and accreditation methodology of ERNs for Rare Diseases according to the Directive on Crossborder Health Care in order to:

1. Provide adequate, long-term public funding to ERNs of Centres of Expertise in order to ensure their sustainability and continuity of care for patient.
2. Recommend inclusion in the National Plan for Rare Diseases provisions on the recognition and funding of Centres of Expertise and their participation in ERN.
3. Recommend the adoption of national initiatives in the National Plans for Rare Diseases on specialised social service.
4. Provide financial support to networks of specialised social service.

In the context of Centres of Expertise (CEs) and of European Reference Networks (ERNs), the work performed by RDTF on 'Standards of Care' produced between 2005 - 2008 has been of particular value. This activity was promoted by the High Level Group (HLG) on Health Services and Medical Care created 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 the following 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).

Through the analysis of pilot ERNs funded by DG-SANCO or by DG-Research programme 2008-2013, despite the wide heterogeneity noted in the activities and geographical coverage, it was possible to recognise various benefits, but also weaknesses and needs for further action and coordination both at the EU level (Directorate General for Research and Innovation (DG RTD) and DG SANCO) and at the MS level. Valuable resources developed and worth mentioning include:

- Shared database/registries/biobanks at the disposal of community.
- Guidelines/best standards of diagnosis and care and information packages.
- Training tools and training sessions covering both the medical and the social dimension of care.
- Shared tools for tele-expertise at the disposal of the medical community.

A major concern worth noting is 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 put 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 the presentation of such work in different European countries, it was highlighted that:

- National CEs in terms of disease coverage are closely related to the organisation of healthcare in each country - The definition of a national centre of expertise differs from one country to another: some have specialised centres (by disease or group), and/or generally a list of centres (for all RDs) – reflecting differences in the size of the countries. Some CEs 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 CEs may do more than one of the above activities. Therefore, missions of National CEs 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 CEs. 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 CEs 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 CEs.

Core to the move from the pilot ERNs to RD ERNs under the terms of the *Cross-Border Healthcare Directive* is the possibility to embed RD ERNs in the healthcare systems of the EU so that the sustainability of such networking is ensured and no longer driven by short term projects. Further consideration may need to be given to the various different possible network structures to be considered depending on the outcomes of the deliberation of the CBHC Expert Groups:

i. Horizontal – CEs at the same level such as the expert centres across the different Member States.
ii. Vertical – the different "points of care" of a healthcare pathway from primary care through to the CE providing the expertise.
iii. Diagonal – when different specialties such as social, rehabilitative, psychological, physiotherapists etc., work together.

The general concept and the method of implementing ERNs are defined in *Article 12 of the Cross-Border Healthcare Directive (Directive 2011/24/EU)*.

These recommendations will help focus on the specificities of rare diseases and the criteria for the establishment and evaluation of ERNs within the field of rare diseases, as well as the exchange and dissemination of information.

The document is based on iterative review and discussion at workshops and EUCERD meetings. These recommendations are designed to be complementary to the advice of the Cross-Border Healthcare Directive Expert Group on ERNs and develop the specific vision of RD ERNs. Therefore, generic issues are not addressed and the following recommendations focus on the networking dimensions specific for RD, assuming that MS have established and designated national CEs, or will do so in the framework of their national plan or strategy for RD.

EUCERD and the EUCERD Joint Action will continue to stimulate the establishment of CEs in countries where there are none and identify where there is the need for improved capacity building in this area. Therefore it excludes recommendations on the care pathway dimension that is linked to the functioning of CEs, not of ERNs. Due to the parallel discussions of the CBHC Expert Group, EUCERD may need to revisit these recommendations at a later date as the conclusions of the CBHC Expert Group become established. In addition, work of other groups (for example conclusions on RD registry development) may need to be taken into account as plans for RD ERNs evolve.

4.4. The EUCERD Recommendations for CEs and ERNs

Member States, European Commission, other EC initiatives (e.g. other projects and joint actions, Cross-Border Healthcare Expert Group, EUnetHTA, EPAAC), Centres of expertise in the field of rare diseases healthcare providers, RD experts and existing RD network coordinators and partners, patient organisations will the target groups of the following recommendations.

1. RD ERNs will provide the framework for healthcare pathways for RD patients through a high level of integrated expertise. ERNs will provide networking of centres on a European level, and ensure that individual healthcare professionals are in touch with the networks. This will cover in a step-wise approach all rare disease patients as well as undiagnosed RD patients and provide high community added value addressing the challenges of rarity of patients and heterogeneity of rare diseases.
2. Nationally designated centres of expertise (CE) are the core participants in ERNs. In the context of rare diseases, such centres should be compliant with the *EUCERD recommendations for quality criteria for CE in rare diseases*, or working towards compliance.
3. An RD ERN needs to be flexible enough to accommodate working with different national CE structures. Depending on the national healthcare system, CEs can be very different structures organised by regions, treatments, or diagnostic procedures, offering services in one location or through an established network. The differences between national CEs for RDs may be more marked than for other areas of medical expertise.
4. An RD ERN should cover essential core tools and activities. The detailed scope of each ERN will vary between medical areas. However some core components of an RD ERN can be identified, and the tools required to facilitate the delivery of some of these transversal components could be shared between different ERNs to allow interoperability. The core components of a RD ERN should include:

   i. Registry platform
   At a minimum the registry platform for an RD ERN should be capable of providing epidemiological data, data for clinical outcomes, elements of data for health economic analysis and patient reported outcomes.

   ii. Quality assurance mechanisms
   Quality assurance programmes for laboratory testing may be shared between several RD ERNs and more broadly e.g., EuroGentest, EMQN. Supporting the establishment of QA schemes for the techniques performed in a very limited number of centres should be within the scope of ERNs.

   iii. Mechanism for information flow on guidelines/best standards of diagnosis and care amongst MS
   Good practice guidelines for RD generated by particular CEs in an RD ERN or by a network should be shared within an ERN and more broadly, as applicable. RD ERNs should have a mechanism whereby this information can be shared between MS for implementation as applicable within their specific healthcare setting.

iv. Training and education tools. Mechanisms for evaluation and clear indicators of performance
The groups providing evaluation of RD ERNs should be multi-stakeholder and include patient organisations. Indicators should form the basis for evaluation across all ERNs for RD, covering short, medium and long term outcomes, generic and specific issues and cover process, outcomes (many of which will be able to be measured utilising the output of the registries) and impact such as through the utilisation of patient reported outcomes.

v. Communications infrastructure to ensure visibility of the ERNs and their processes and accessibility.

vi. Cross-border referral mechanisms.

vii. Telemedicine core

In all of these areas where disease specific resources and tools have already been generated and demonstrated to be of high quality (e.g. by previous networks) RD ERNs should be required to utilise these in order to maximise the value of previous investments.

5. An RD ERN will need to be clear on the definition of transparent and seamless healthcare pathways for:

   i. Patients with
      - A clear diagnosis but no CE covering this diagnosis in their country,- A suspicion of diagnosis without a CE in their country and
      - No diagnosi
   ii. CEs when
      - A patient requires a suitable CE outside their country
      - Support, training and consultation is required

   Decisions on these pathways should be the responsibility of the governing body of an RD ERN.

6. A particular problem in the field of rare diseases is that for some patients a complete diagnosis is not possible even with the highest levels of medical knowledge. The concept of an RD ERN sharing, improving and providing the highest levels of medical knowledge on the European level provides a unique opportunity to reduce the uncertainties for the patients that remain undiagnosed. Therefore, patients who may have a rare disease but who are undiagnosed require a pathway into the most appropriate ERN or ERNs to have the best possible chance of achieving a precise diagnosis and to access appropriate care once a diagnosis is achieved. Models to provide such a mechanism for undiagnosed patients should be further explored.
7. An RD ERN should have the responsibility and capacity to follow such patients, manage their care according to medical need and to re-evaluate the diagnosis as new testing becomes available. For this purpose, RD ERNs might require access to research expertise, though any diagnoses achieved in a research setting would need to be confirmed in an accredited laboratory thereafter. Due to fast advancing technologies, the RD field is frequently on the border between research and care.

Therefore, RD ERNs will need to address how to deal with increasing capacity following research advances.

8. As with all ERNs, RD ERNs should have robust and clearly defined governance and oversight structures with clear and comparable methods for evaluation. Due to the specific role of patient organisations in RDs, they should play an integral role in these functions for RD ERNs where patient organisations exist that have the capacity to do so.
9. ERNs require strong leadership and the coordinating site should be chosen on the basis of proven ability to coordinate a network and its shared tools as well as to lead the medically relevant activities in the disease field. The best coordinating partner is not automatically the best centre of expertise or the one with the largest number of patients, rather the one that has the capacity to fulfil all the key functions of coordination and to expand the network as necessary.
10. All ERNs will be required to deliver added value in at least three of the objectives listed in *Article 12 of the Directive on patients' rights in cross-border healthcare*. RD ERNs will be composed of existing CEs but they will need to collaborate with each other, patient groups, health and social care providers and diagnostic laboratories. Because of the specificities of rare diseases – great heterogeneity of diseases and of expression of diseases without predominant symptoms, a limited number of patients and a scarcity of relevant knowledge and expertise – sharing knowledge between different healthcare providers is an overarching goal of an RD ERN. It also has to ensure that centres not fulfilling the EUCERD quality criteria and not nationally designated, as well as individual healthcare professionals are in touch with the networks and recognise the opportunities they provide for their patients.
11. Therefore different forms of affiliation to an RD ERN (association, collaboration) should be allowed to ensure inclusivity. Existing sources of information (Orphanet, pilot ERNs, EUCERD, information from patient organisations) should be used to identify key experts who can play a valuable role in RD ERNs. In smaller countries for example, where CEs may be limited we recommend that other healthcare providers can become affiliated members of an ERN in order to have access to the best standards of care and diagnosis. Such affiliated members might have co-operative agreements for onward referrals, and would be required to attend network-training meetings and contribute to the overall data collection of the network. This would allow an RD ERN to reach out to as many MS as possible. Within the on-going national planning for rare diseases (national plans or strategies for rare diseases), there should be a process for designation of national CEs or affiliated experts in view of their membership into future ERNs.
12. Funding mechanisms for RD ERNs need to be adequate and long-term. Sustainable and long-term funding processes are needed, as RD ERNs are likely to remain necessary for the foreseeable future. Based on satisfactory evaluation against agreed indicators, funding should be for at least 5-year periods.
13. The funding for RD ERNs should include support for co-ordination and networking. The costs of the CEs and affiliated centres in delivering healthcare are the responsibility of the MS. However, there are specific costs for networking, which should

be part of a sustainable funding support mechanism from EC funds. Such funds should be available for:

- Coordinator time.
- Project management.
- Registry coordination.
- IT and communication platform.
- Support for network meetings within an ERN and between ERNs for patients with no diagnosis.
- Training and education packages both online and face to face.
- Networking activities with other ERNs.
- Board activities with their bureau.

14. Funding for core network activities should be at comparable levels between RD ERNs in terms of the numbers of centres integrated and numbers of diseases covered but may not always be comparable with the support necessary for non RD ERNs.
15. Ahead of the designation process for ERNs, consideration should be given to the possible economies of scale of developing shared platforms across RD ERNs such as core components for registries, QA etc. (as listed in recommendation 4).
16. A clear process for the designation of RD ERNs should be established. Criteria for the evaluation of prospective ERNs should include their inclusiveness and plans for expansion, excellence of the network, leadership qualities of the proposed coordinator, and numbers of MS involved, amongst others.
17. A step-wise strategy for RD ERN designation should be delineated so that all patients with a rare disease will have access to an appropriate ERN in a defined period of time. This should include access to an ERN for those rare disease patients still seeking a diagnosis.
18. As it will only be possible to establish a limited number of RD ERNs at the beginning of the process, it is recommended to give priority to ERNs which meet the following 3 priority criteria as a robust starting point: 1. Existing formal or informal networks of experts have reached maturity; 2. There are patient registries established and willing to interoperate; and 3. There are existing networks of patient groups. Each thematic RD ERN would still need to expand over the course of its first five years of designation to include other centres, expert groups, patient groups and ultimately diseases.
19. Based around the concept of medical specialties and body systems, diagnostic and therapeutic areas can be identified each covering a wide range of rare diseases. Comparison of the systems in place in MS with well developed services for rare diseases shows that the number of diagnostic and systemic areas which might cover the majority of diagnoses could be approximately 20-30. By the end of the Health for Growth Programme (in 2020), the 20 to 30 ERNs should be established and covering a wide range of RD. These first established ERNs will be the ones meeting the 'priority criteria' as defined above and will then progressively expand in order to cover all RDs by the end of the two next EU Public Health Programmes (by 2025), through integration of appropriate centres and expertise.

20. A formal system for networking across all RD ERNs and sharing expertise should be defined and implemented. Good practice and common methodologies on the common areas of ERN work should be shared (e.g., registry development, utilisation and sharing of data and banked tissue resources, good practice guidelines etc.). For future ERNs, as high quality systems to implement common tools are defined, the utilisation of these standards and methodologies should be a condition for designation.
21. Working groups under the auspices of the EUCERD Joint Action should be established as necessary to help oversee the establishment and implementation of the RD ERNs within the scope of *Article 12 of the Cross-Border Healthcare Directive (Directive 2011/24/EU)* and to align progress in ERN developments with other ongoing RD initiatives.

4.5. The World Health Organization (WHO) Position

Since 2000, non-communicable diseases (NCDs) and especially chronic conditions (chronic diseases) have been set as 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 several 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 globalisation. 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 life for patients. 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; and 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; and 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 healthcare 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 healthcare, with an emphasis on maternal and child health.
- Education and training for health care providers, particularly those in primary healthcare.
- Establishment of effective mechanisms to foster development of patient–parent support organisations, 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 emphasizing that the WHO Director General is committed to their 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 (ENERCA 2) and third (ENERCA 3) phases of the project. 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 work package on education and training. Leaders of WHO Collaborating Centres have been involved in other work packages of ENERCA 2 and 3. Moreover, in 2007, ENERCA with the participation of the WHO and the Thalassaemia International Federation (TIF) organised a Symposium on Immigration and Haematology: "Towards the prevention of haemoglobinopathies in Europe" (Budapest, 30 August, 2007) where a joint action was proposed to call on public authorities and governments to follow up on the WHO recommendations and draw attention to the need for targeted policies to tackle severe haemoglobinopathies, immigration and mobility flows in Europe. during the Madrid 2010 ENERCA symposium (communication by Dr Patricia Aguilar Martinez and press release), ENERCA 3 included a communication on WHO resolutions on haemoglobin disorders This cooperation and partnership greatly contributed to the promotion of the WHO relevant resolutions as described in the ENERCA-TIF health and migration policy report: "Haemoglobinopathies on the Move: Is Europe ready?" (August 2013, http://www.enerca.org/activities/training/haemoglobinopathies-on-the-move.html). ENERCA has also been 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 ENERCA 3 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.

4.6. Special Remarks

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. A draft recommendation was discussed in June 2012 (in the context

of EUCERD Join Action for ERNs) and adopted by EUCERD in November 2012. So far a number of recommendations have a consensus, including:

- ERNs are established between CEs designated at MS level. Accordingly, CEs 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 and tele expertise 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, develop tools for tele-expertise.
  - Prepare guidelines, updated information, training tools.
  - Provide training and expert opinions.

In the meantime MS are urged to work on designation of CEs using EUCERD Recommendations on quality criteria for CsE as adopted in 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 CEs 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 in the correct diagnosis or rare diseases, in particular the Orphanet database, and the European reference networks.
- Make patients, health professionals 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.

5. ENERCA RECOMMENDATIONS: METHODOLOGY

5.1. ENERCA Working Group on Rare Anaemias (EGRA)

First of all, a working group was created to prepare CE recommendations for rare anaemias (RAs), integrating different expert profiles: a) physicians, b) molecular biologists, c) legal-ethical experts and d) patient associations. The methodology followed for achieving the final objective was based on the following principles:

- **Interdisciplinary,** integrating experts and professionals from clinical and laboratory science, ethical and legal knowledge, and patient expectations.
- **European coverage** including experts from eight different European countries: Belgium, Cyprus, France, Germany, Italy, Spain, The Netherlands and United Kingdom (see Annex 3). Involvement of other MS was assured by broadening the different surveys performed, seeking experts from different countries and the involvement of TIF, the international umbrella for patient associations.
- **Evidence based,** ensuring, as much as possible, a realistic approach to achieving the final results by using the current situation of real practice as starting point.

Using these principles, the following consecutive steps were covered:

1. **Analysis of current situation** in Europe, in order to identify and select the "hot topics" that have to be addressed into recommendations for criteria to recognise centres of expertise.
2. **Performing surveys** based on the results of Step 1 that were based on questionnaires that check how the "hot spots" (most relevant issues) can be translated into practical recommendations.
3. **Evaluation of the responses to the questionnaires** to achieve a consensus.
4. **Preparation of a final report** with ENERCA's recommendations on criteria to recognise CEs.

5.2. Analysis of the current situation in Europe

5.2.1. Legal and ethical perspectives

A survey was conducted using a questionnaire (see Annex 4) sent to the 48 ENERCA centres (ENERCA partners, see Annex 1) in order to identify a) the way by which the centres manage in clinical practice the issues related to patients' rights: consent, data and samples; and b) to identify harmonised procedures. This survey was a tool to approach and learn about the practices in the centres they are managing, going a step

further than the already authorised information provided to the ENERCA Expert Group. The questionnaire included a list of items that were proposed by the legal experts and the content of the questionnaire was structured in seven issues:

- Checking quality standards in the circulation of samples or data through Europe.
- Organisational issues involved in data transfer and patient mobility;
- Personal data Issues.
- Obtaining, use, transfer, storage and destruction of biological samples;
- Referring patients.
- Genetic counselling.
- Knowledge/awareness of the applicable legislation.

Responses to the questionnaires were received from 23 centres (48%): Belgium (1), Cyprus (1), Denmark (1), France (6), Germany: (1), Greece (1), Italy (4), Netherlands (1) Portugal (1), Romania (1), Serbia (2), Spain (1), UK (2). These centres included private and public clinical departments, diagnostic laboratories, research laboratories and others (e.g., blood transfusion service). These responses have been analysed in order to compare the criteria and procedures followed by these different centres and to identify the items that should be addressed from a legal and ethical perspective. The main conclusions were that the criteria used for practice procedures were heterogeneus and that there was a general awareness of ethical and legal principles in clinical practice by all participants.

Two representative examples were the following. First, the responses to the question concerning the period of storage of samples were: 45% "years", 27% did not reply, and the remaining were a broad range of periods: "6 months" (5%), "1-10 years" (5%), "unlimited period" (4%), "no time established" (9%)", "other" (5%).. Second, the responses to the question on the requirement of the patients consent to send blood samples to other centres for diagnostic purpose was: 54% "yes," 32 % "no", and 14% did not reply.

It was concluded that recommendations are necessary to harmonise the criteria for patient data and sample circulation within the network. These conclusions have been sent to national legal experts of ten Member States (Table 1), in order to use this information for a legal study that should have a similar outline.

5.2.2. Clinical and laboratory requirements

Services to be provided by each CE dedicated to one specific rare anaemia or a group of rare anaemia have been established by EGRA's "state of the art" of professional practice, using different criteria mainly based on key documents published by the European Commission (EC) and obtained from the literature review.

1. Key documents published by the European Commission (EC)

- 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-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:088:0045:0065:EN:pdf
- 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
- Work of the High Level Group 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
- Recommendations on Quality Criteria for Centres of Expertise for Rare Diseases in Member States
  http://www.eucerd.eu/?post_type=document&p=1224
- 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/EUCERDWorkshopReport-CECERN.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/ERN2011Analysis.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 COMMISSION: Results of Public Consultation on the implementation of European Reference Networks (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) Brussels, June 2013 http://ec.europa.eu/health/cross_border_care/consultations/cons_implementation_ern_en.htm

2. Literature review

Information from textbooks, medical journals and direct internet searches. The most productive method is to identify the databases of medical references using the following key words: "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 performed by using the PubMed service, which includes over 18 million citations of biomedical literature. Search can be done 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 Cochrane Reviews which is a collection of 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 DARE (Database of Abstracts of Reviews of Effects) – 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 Dissemination of the National Research Institute of Health Research of the NHS of the UK (http://www.crd.york.ac.uk).
- CINAHL (Cumulative Index to Nursing and Allied Health Literature) Journals database – 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. Access: http://www.cinahl.com/library/journals.htm and http://www.ebsco.com

Reading and evaluating the information collected resulted in the preparation of evidence based standards required for the diagnosis and follow-up of patients with rare and very rare anaemias. This was discussed in the context of speciality practice by ENERCA experts and the current state of the art information. This information was used to prepare the survey's questionnaires.

5.2.3. Patient expectations

Identification of the items for the patient questionnaire was based on the first version of 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 divided into four sections:

1. Section 1 has the purpose of describing the patient who is responding, without identifying them. Information collected includes age, gender, marital status, education, employment, ethnic origin and country of residence. This section is optional, although all respondents completed the section.
2. 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.
3. 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 scheduling that interferes 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 the correct treatment. Section 3a also assesses the patient responses to services and concentrates on quality of services based on the PACIC questionnaire.
4. Section 4 is the part which assesses the patient's 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 of 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.

5.3. Performing the surveys

Several surveys were conducted to check how to translate the collected information in section 5.2 into recommendations for CE recognition.

5.3.1. Legal and ethical survey

The objective of this survey was to analyse the situation of current trans-national regulations on patients, biological samples and data exchanges between different Member States (MS). The questionnaire (see Annex 5) included a list of legal and ethical target points dealing with similar issues previously sent to the centres and it was sent to legal experts in Biolaw of 10 MS, who used them as a basis for a legal

study that should have a similar structure (**Table 1**). All these experts had experience in medical legislation and participation in international projects. They analysed how the different national regulations and ethical rules are considered in each MS included in the survey, and prepared a report with information on relevant documents existing in each MS**.** The analysis on the questionnaire responses allowed to perform a comparative analysis of the situation between the different MS, and to prepare a consensus recommendation proposal.

In order to make the information easily comprehensible, the report was structured the following way: firstly, results were tabulated on the basis of the questions; then the responses were inserted following a colour code to easily distinguish responses corresponding to each country. Prior to the body of the answers, a summary Table was included, highlighting the most relevant aspects of the issues in each country and a brief conclusion. It should be mentioned that when national reports were received, 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 yet published.

Table 1. List of National experts in BioLaw participating in the survey.

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.

5.3.2. Clinical and laboratory surveys

Based on the desk research described in section 5.2.2, three surveys were undertaken to assess the adequacy of the different professional services identified involving basic diagnosis, clinical management of patients and external quality assessment (EQA) for RAs:

1. **Survey 1:** General laboratory requirements for basic diagnosis and clinical management of patients with RA. (see Annex 6).
   For this survey, the questionnaire contained the following sections:

   a. General overview of the centre
      - Self-declared as a: Reference Centre (SDRC) or general centre (SDGC).
      - Type of patients attended.
      - Number of attended patients per year.
      - Average number of samples tested per year.

   b. Centre of expertise
      - Diagnosis and prevention.
      - Patient 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 availability.
        – Link with research:
            – Publications and research grants.
            – Teaching and training activities.

2. **Survey 2:** Specific laboratory tests for RA including specific tests for the "very rare anaemias". (see Annex 7).
3. **Survey 3:** External Quality Assessment (EQA) availability for RA to determine the provision capacity and willingness of EQA provider organisation s to collaborate across national boundaries. (see Annex 8).

For **Surveys 1 and 2,** the questionnaires were sent to:

1. ENERCA partners (associated and collaborating). (see Annex 1).
2. European centres dealing with the diagnosis and treatment of patients with RA (see Annex 9 y 10):
   1 – **SDRC through ENERCA Membership Information** http://www.enerca.org
   2 – Centres provided by ENERCA partners either in their own countries or in other countries known through literature or previous collaborations.
   3 – Centres provided by national and/or local scientific societies.

For **Survey 3,** the questionnaire was sent to European EQA providers listed by the European Quality Assessment in Laboratory Medicine (EQALM) organisation.

5.3.3 Patient expectations

The questionnaire for patient expectations was prepared to address questions from the following sections (see Annex 11):

1. **Section 1:** Patient demographics and characteristics.
   Information collected includes age, gender, marital status, education, employment, ethnic origin and country of residence.
2. **Section 2:** The treatment currently received.
3. **Section 3:** The access to treatment centres.
   Patient assessment of the services that they received. This section assesses the patient evaluation of services and concentrates on quality of services.
4. **Section 4:** Patient preferences.

This questionnaire was prepared in eight languages (Italian, Greek, Bulgarian, Turkish, Romanian, Portuguese, French, German and Spanish) and distributed among thalassaemia patient associations (i.e., TIF's existing European members) and European sickle cell patient associations (identified through internet search and with help from the UK Sickle Cell Society). See Annex 12.

5.4 Evaluation of the responses

5.4.1. Legal and Ethical

Limitations

- Although the countries included in the study were chosen following a geographical basis criteria, the number was just ten.
- There are several concrete matters which show a lack of legal regulation in some countries (e.g., the period of conservation of the samples). Consequently, there was no legal basis to settle the recommendations needed.
- The meaning of some legal terms varies from a country to another and is under evolution and revision, including in EU legislation (e.g. the term "personal data").

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 was adopted on 9 March 2011 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. The objective of 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 organisation 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.

In general, the State must reimburse the costs of cross-border health care to which citizens are entitled under the laws of the State in which the citizen is affiliated. In some cases, prior authorisation of that State is required. The authorisation 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). 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.

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. (See 4.1. The European Commission policies for rare diseases: EU Public Health Policy).

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 availability of an integrated medical record accessible to all professionals involved. 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.

Regarding this purpose, some important initiatives in relation to the new Directive have already been adopted. Those initiatives have crystallised 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.

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 organised in order to advise the EU on how to promote this system of e-Health. This group met for the first time on 10 May 2011.

Comparative Report

The reports from the national experts were compiled into a comparative report, organised by questions numbers. The answers were summarised in schematic tables with conclusions. This final report was sent back to the experts as well as to the EGRA for review.

a) Legal framework

The items proposed to the experts and the responses received led to the following conclusions:

Specific or general provisions about patient circulation in Europe

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

Specific or general provisions about data or biological samples exchange

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

Specific regulation of electronic clinical records

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

Specific regulation of genetic counselling

Different answers. In some countries there are no specific provisions; in others a few rules have to be developed, while others have more detailed requirements regarding the professionals involved, organisation of the service, information to patients, etc.

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 Commission 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.

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.

Is there any regulation about the Ethics Committees?

Ethics committees are regulated in all the countries following Directive 2001/20/EC 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's International Declaration on Human Genetic Data, 2003 "Independent, multi-disciplinary 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".

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

There is no legal distinction between private or public centres.

b) Patients' Personal Data

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.

Period of storage of the clinical data.

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

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

The person responsible for the data file has the duty to implement high level security measures. The person in charge of the data file shall implement appropriate technical and organisational 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.

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 harmonised 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 received abroad (and not just those received from the national health system).

Conditions for using the data personal / codified / anonymised data for research studies: consent, intervention of an ethics committee.

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

c) Patient Samples

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

Period of storage samples for diagnostic purposes

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

Who is the responsible for the storage?

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

Does the laboratory have to verify that consent was obtained by the physician or other health professional?

Verification is not necessary in most of the European countries examined.

Is 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 reviewed.

What information should be given for using the sample for diagnostic purposes?

There is a broad 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 where the analysis is performed and the destination of the sample after the analysis: storage for diagnostic purposes, codification, destruction, use for research.
d. Guarantee of confidentiality of the information obtained.
e. Patient's right to choose whether or not they are informed about data obtained in the research concerning their sample.
f. Information regarding the implication of the results for his/her family members.
g. The right for the person, where appropriate, to transmit this information to the family members if it is relevant for their health.
h. Indicating the possibility to get in contact with the patient, 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 for the patients to exercise their rights regarding data storage.
l. Provisions about international circulation of data.
m. Provisions about international circulation of samples.

Conditions to obtain and analyse samples for research purposes

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

Is specific consent required to use a sample that has been obtained for diagnosis for research?

In this area there is an important distinction between laws that do not require consent beyond that already given for diagnostic purposes and laws that require further consent. In general these laws include exceptions

What information should be given to the patient before they consent to allow their sample to be used for research purposes?

Taking into account the general provisions, there is a broad consensus concerning the following points, except for those regarding genetic data and returning 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 donating and obtaining of the sample.
d. Identity and contact details of the person responsible for the research.
e. The patient's right to revoke 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 right to gain access to the obtained data.
h. Guarantee of confidentiality of the information obtained.
i. Information regarding the implications of genetic analysis.
j. Patient's right to choose whether or not they are informed about data obtained in the research concerning their sample
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 for the patients to exercise their rights regarding data storage.
q. Provisions about international circulation of data.
r. Provisions about international circulation of samples.

Where should consent document/s be stored?

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

How long should the samples be stored?

In general there is no specific period established.

Main conclusions

- The creation of a European network for diagnosis is a priority 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 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 clear differences in legislation exist between the different MS that sometimes make this practice very difficult.
- The transnational health care assistance system is very similar in all EU countries and a further harmonisation 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 harmonise the procedures and the adoption of common criteria to achieve efficiency in its managing.
- Two of the most important factors for the proper operation of the network are the transfer 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 (e.g., in the regulation of genetic testing and genetic counselling).
- 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 to national laws, so professionals may know what protocols they must follow. 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", i.e., a model summarising the best way of accomplishing with all applicable rules, delivered.

5.4.2. Clinical and laboratory

Limitations

- Not all European centres have been informed of this initiative.
- France and England have already established national centres of expertise or excellence for RD. For this reason many expert centres in France did not respond to the questionnaire. (see Annex 13).
- The results should be viewed with caution. Although some questions were asked in different ways, an error of interpretation is always possible.

Results

- **Survey 1:** General laboratory requirements for basic diagnosis and clinical management of patients with RA.

  A total of 93 responses were received. (see the list of participants on Annex 9).

Figure 1. 6 centres participated from France; see complete list of recognized centres in Annex 14.

1. Haemoglobinopathies

a) General overview

Total Centres	75/93 (95%)
Only with the laboratory section	6 /75 (8%)
Only with the clinical section	61/75 (81%)
Centres concerned with phenotyping/genotyping	29/75
Self-declared Reference Centre (SDRC)	22/29
Self-declared General Centre (SDGC)	7/29

Figure 2. Number of Phenotype/genotype requests per year in the SDRC.

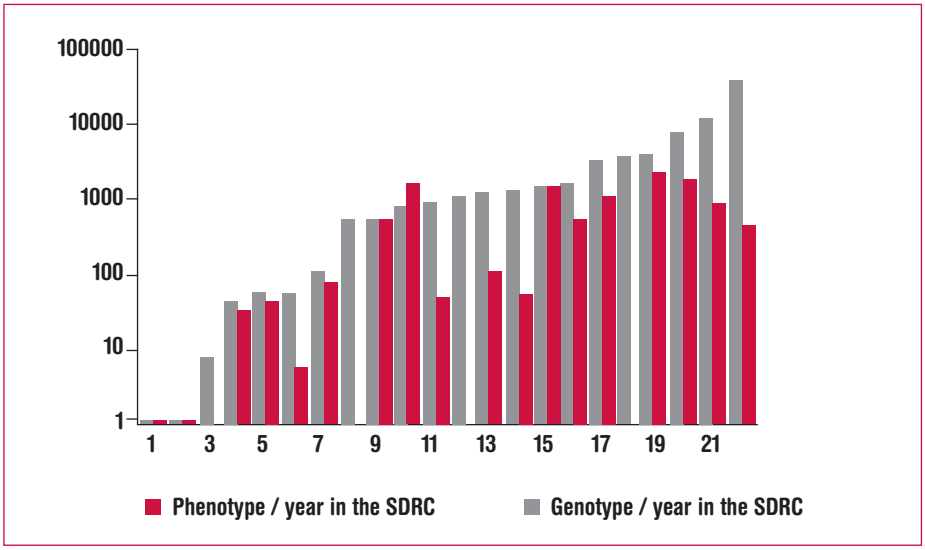


Table 2. Centres using EQAS.

	SDRC (n= 22)	SDGC (n= 7)
EQAS for phenotyping	14/22 (63%)	2/7 (28%)
EQAS for genotyping	3/22 (14%)	0/7 (0%)
Average number of samples received for diagnosis (per year)	2 to 10,000	0 - 200

b) Centres involved in patient's clinical care and follow-up

SDRC	36/69
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
SDGC	24/69

Expertise covered (SGRC Reference: 39/ SDGC General: 24):

Figure 3. Acute events coverage (staff and beds).

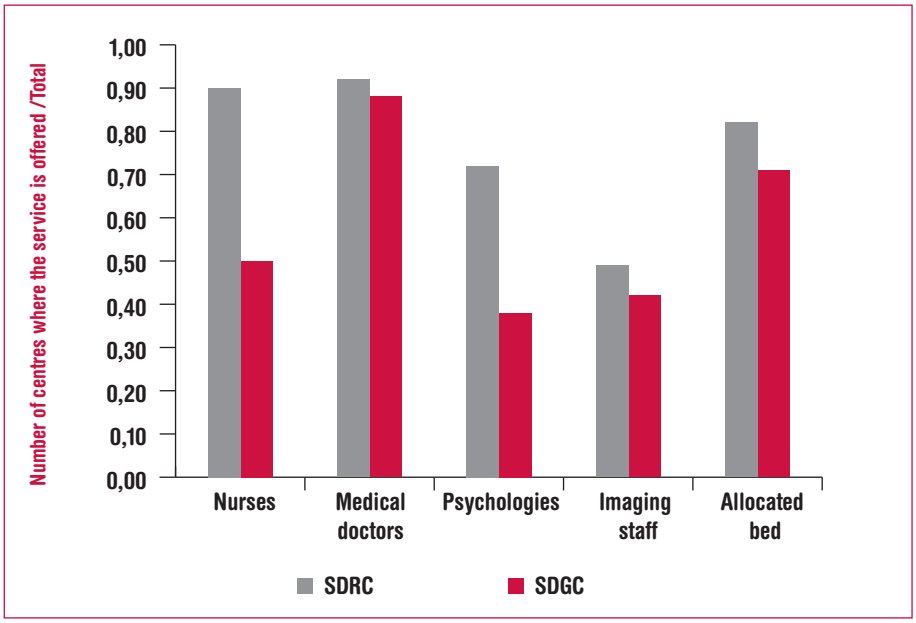


Table 3. Availability of specialised services dealing with sickle cell disease/thalassaemia adverse events.

	Reference centre (n= 39)	General centre (n= 24)
Intensive Care Unit (ICU)	31/39 (79%)	13/24 (54%)
Transcranial Doppler (TCD)	32/39 (82%)	13/24 (54%)
Magnetic Resonance imaging(MRI)	36/39 (92%)	18/24 (75%)
Angio MRI	34/39 (87%)	14/24 (58%)
Computed Tomography (CT)	35/39 (90%)	19/24 (79%)
Angiofluorography	25/39 (64%)	4/24 (17%)
Audiometry	38/39 (97%)	17/24 (71%)
Assessment of cardiac iron by T2*MRI	28/39 (72%)	8/24(33%)
Measurement of liver iron by		
Biopsy	30/39 (77%)	11/24 (46%)
MRI	32/39 (82%)	12/24 (50%)
SQUID*	1/39 (2.6%)	0/24 (0%)

*Superconducting quantum interference device.

Treatment availability

In an SDRC

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 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 in only two-thirds of the centres (27/39; 69%).

In an SDGC

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%).

Figure 4. Decision support availability.

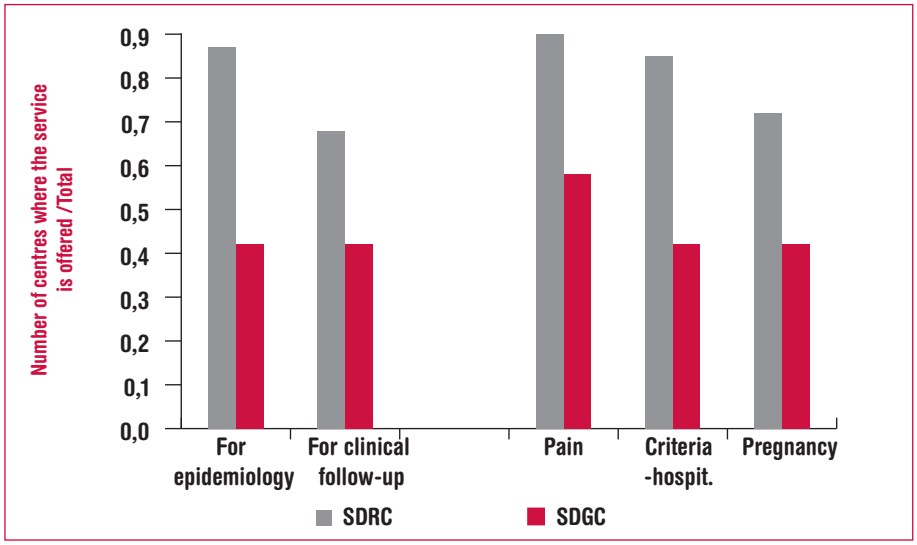
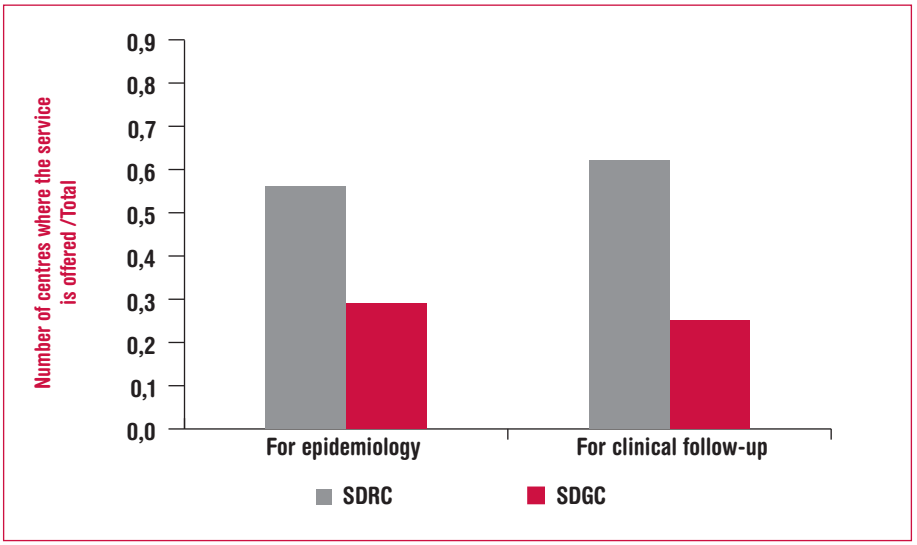


Figure 5. Registry availability.



Comments

Among SDRC, 15 and 17 have less than 20 patients with sickle cell disease and thalassaemia (major or intermedia), respectively in their registries. For both types of haemoglobinopathies, seven of them have less than 20 patients registered.

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

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

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

2. Very rare anaemias (VRA)

a) General overview

Total Centres	70/93 (75%)
Only with laboratory section	21/70 (30%)
Only with clinical section	25/70 (35%)
SDRC	31/70
SDGC	39/70

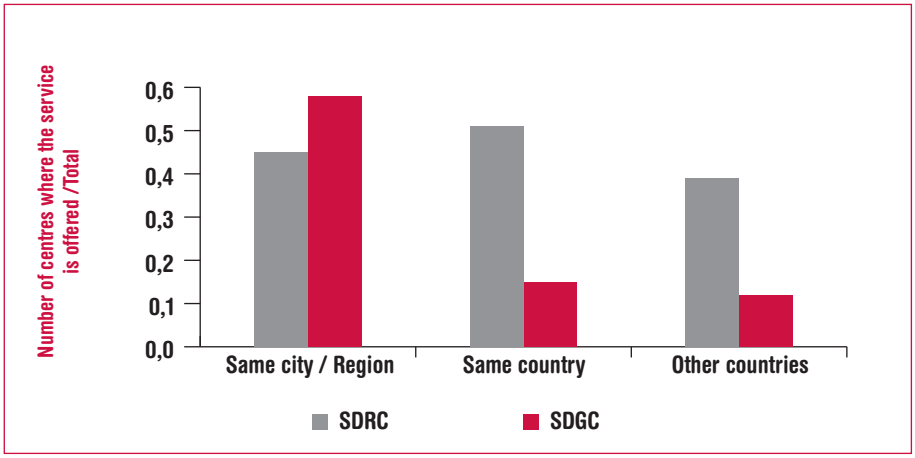
Distribution of the 31 SDRC in the different European countries:	
The Netherlands	8/13
Belgium	2/9
Spain	7/23
Greece	1/3
Italy	5/9
Luxembourg	0/1
North Ireland	1/1
Cyprus	0/1
Germany	3/3
France	2/2
Czech Republic	0/1
Poland	1/1
Bulgaria	1/3

Table 4. VRA coverage and number of patients registered.

Very Rare Anaemia (VRA)	Centres: 70	Registered patients		
		SDRC	SDGC	Total
RBC membrane disorders	57	1543	335	1878
RBC enzyme disorders	56	432	191	623
CDA	36	104	25	129
DBA	38	115	50	165
Fanconi Anaemia and other (AA, Rh null, etc.)	11	173	9	182
Hereditary sideroblastic anaemia)	30	29	27	56
VRA due to defective iron utilization	20	93	8	101
PNH	38	202	32	234

CDA: Congenital dyserythropoietic anaemias.
DBA: Diamond-Blackfan Anaemia.

Figure 6. Residence of the patients studied in SCRC and SCGC.



Comments:

- There is good representation of all the diseases considered, although the total number of patients registered for each pathology is drastically lower than expected on the basis of epidemiologic data.
- In spite of the number of patients, in most cases the median number of patients registered by a single centre is low. In the case of red cell membrane disorders, for example, the median is 26, but 11/24 reference centres have less than 20 patients in their registers.

This situation is much more evident in VRA (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 becomes more evident (the median of patients registered in each centre for all the diseases considered ranges from 1 to 7).

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

b) Centres involved in the laboratory diagnosis (phenotyping/genotyping)

SDRC	10/21
SDGC	11/21

Table 5. Number of Phenotype/genotype requests per year.

Laboratory diagnosis	SDRC:10	SDGC: 11
Phenotype	8(80%)	11(100%)
Genotype	7(70%)	1(9%)
Average number of samples received for diagnosis	5 to 3,000 (median:42)	2 to 2,000 (median: 25)
Prenatal diagnosis Pre-implantation diagnosis	7(70%) 1(10%)	1 (11%) 1 (9%)

Comments:

- Genotype diagnosis is mostly performed in reference centres.
- Prenatal diagnosis is performed in Fanconi anaemia , CDAII and severe cases of pyruvate kinase deficiency, other rare RBC enzyme defects and instable Hb.
- Genetic counselling is available in 17/31 of the SCRC and in 14/39 of the SCGC. It is mostly performed in collaboration with genetic centres.

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).

Table 6. Number of centres using EQA.

Centres using EQA	Reference centre (n= 10)	General centre (n=11)
EQA for phenotyping	4 (4 %)	1 (9%)
EQA for genotyping	1 (1 %)	0 (0%)

Due to the rarity of these disorders and limited EQA available for most of very rare anaemias, EQA are adopted in very few centres and for a limited number of pathologies.

EQA organisations for phenotyping are: SKML (The Netherlands, 2 Centres); SEHH (Spain, 1 centre); UK NEQAS (Belgium, 1 Centre).

EQAS for genotyping: There is only one centre in The Netherlands subscribed to the European Molecular Quality Network (EMQN).

c) Centres involved in clinical care and follow-up

Total Centres:	55
SDRC	24/55
SDGC	26/55

SDRC in the different European countries	
The Netherlands	5/8
Belgium	2/2
Spain	4/7
Greece	1/1
Italy	4/5
Luxembourg	0/0
North Ireland	1/1
Cyprus	1/1
Germany	3/3
France	2/2
Czech Republic	0/0
Poland	1/1
Bulgaria	0/1

SDGC in the different European countries	
The Netherlands	2/5
Belgium	4/7
Spain	11/16
Greece	1/2
Italy	5/5
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

Expertise covered by the centres involved in the patient's clinical care (Reference: 24/ General: 26) was the following:

Figure 7. Acute events coverage: allocated Staff and beds.

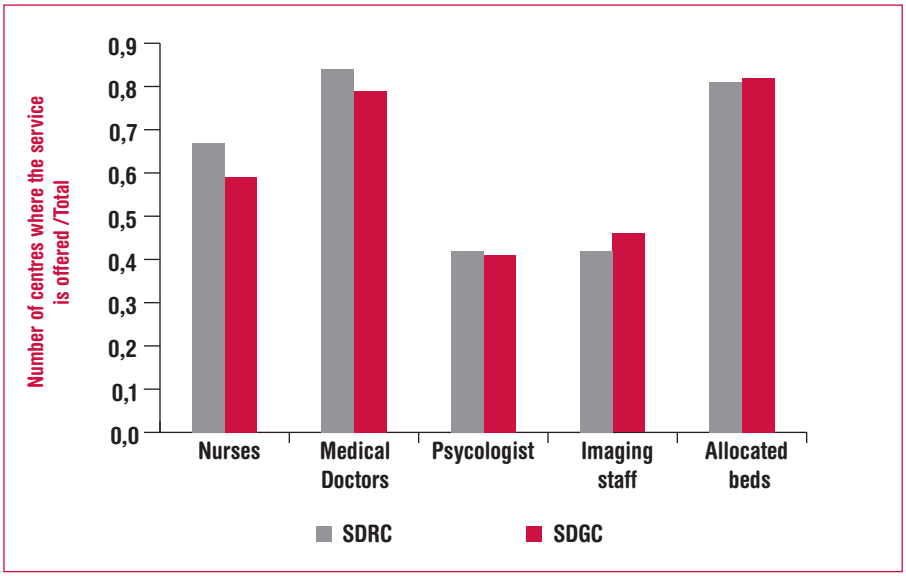


Table 7. Availability of specialised services dealing with VRA adverse events.

	Reference centre (n= 31)		General centre (n= 39)	
Intensive care unit	26/31	(83%)	29/39	(74%)
Magnetic Resonance 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%)	01/39	(3%)

*Superconducting quantum interference device

Availability of treatment

In an SDRC

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, sometimes as a 24-hour service (21/31; 68%).

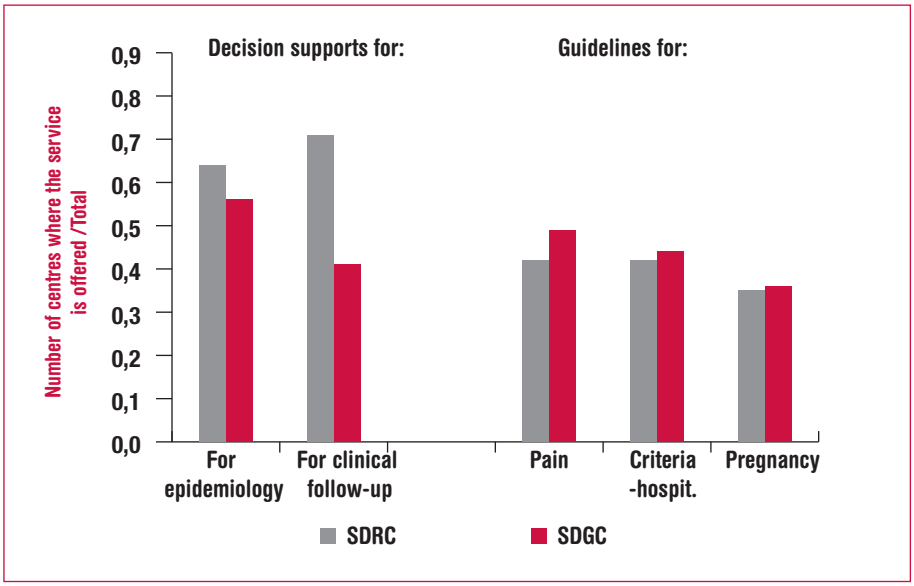
Monitoring for iron overload and iron chelation is always offered to the patients. Stem cell transplantation is available in about 80% of centres (25/31).

In an SDGC

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%).

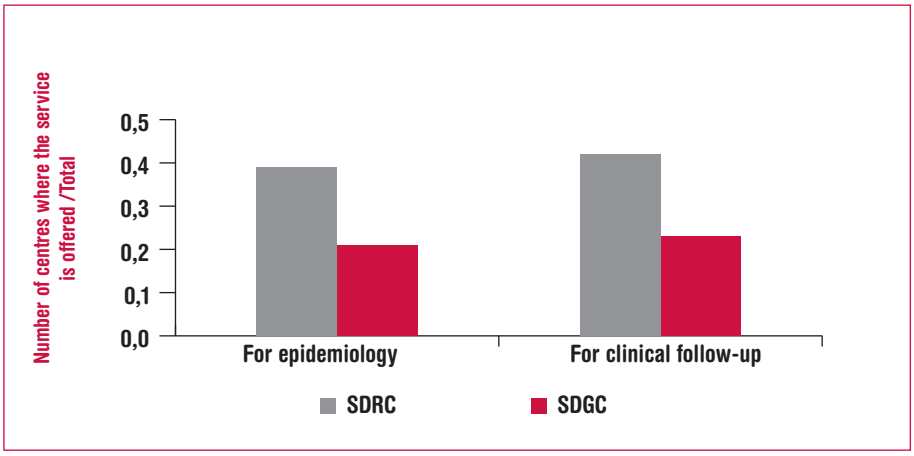
Figure 8. Availability of decision supports.



Comment

SDRC 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 anaemias indications for management of chronic pain, criteria for hospitalisation and pregnancy are available in less than half of centres.

Figure 9. Registry Availability.



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

In an SDRC

Publications and teaching activities are performed in a large number of the centres (22/31; 71% for both). Accessibility to research, performed always or at least occasionally, is also frequent (27/31; 87%); accessibility to grants (11/31; 35%) is less frequent.

In an SDGC

Publications (11/39; 28%), teaching (2/39; 5%), link with research (6/39;15%) or access to grants (5%) are rare.

SURVEY 2. Laboratory test for RBC membrane and enzyme defects.

A response was received from 26 centres (see Annex 10), some of them already involved in the questionnaire "Facilities for patients with rare and very rare anaemias".

a) RBC membrane defects

Total Centres involved: 26

Figure 10. Geographical distribution of centres dealing with the diagnosis of RBC membrane defects.

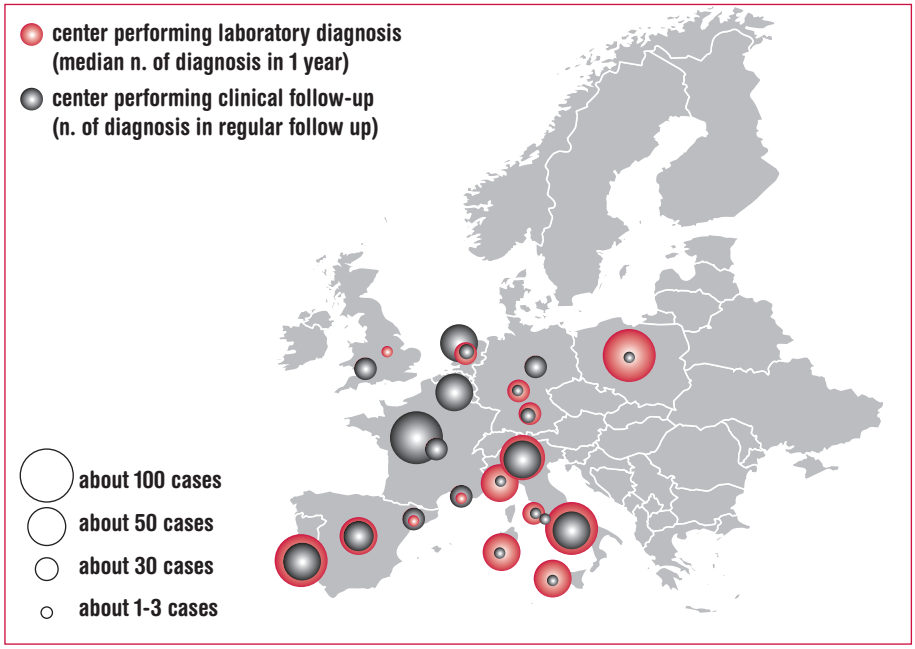
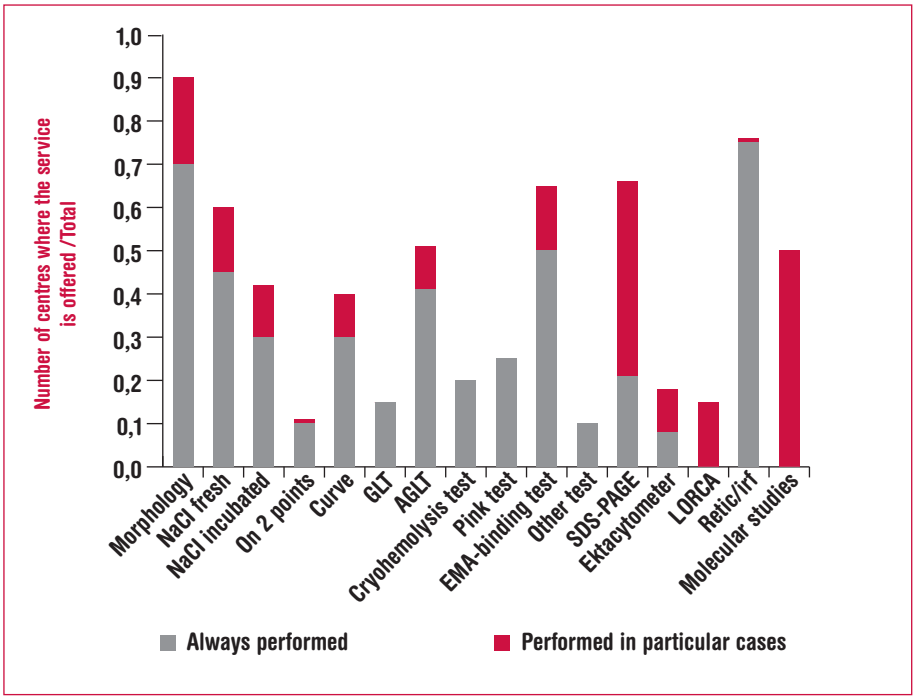


Figure 11. Diagnostic tests for RBC membrane defects.



Comments

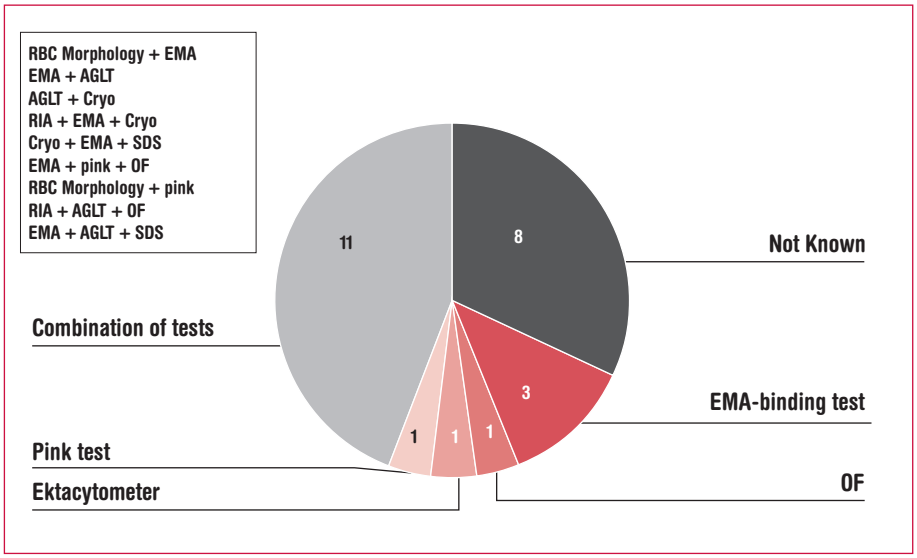
The most frequently adopted tests are the EMA-binding test, NaCl curve on fresh blood (i.e., OF, osmotic fragility test), and AGLT (acidifyed glycerol lysis test). Less than 20% of the centres used the cryohemolysis test. The ektacytometry, SDS-PAGE and molecular analysis are generally performed in particular cases.

Best specificity and sensitivity: There is a wide heterogeneity of opinions about the best test (or combination of tests) to use in the diagnosis of red cell membrane disorders. The majority of centres indicated the use of a combination of tests, variable from centre to centre, rather than relying on a single method.

Comment

The majority of Centres use of a combination of tests rather than relying on a single method. The most common combined tests are: EMA-binding test, NaCl curve on fresh blood (i.e., OF, osmotic fragility test), and AGLT (acidifyed glycerol lysis test).

Figure 12. Method with best Specificity and sensitivity (number of Centres).



b) RBC Enzyme defects

Figure 13. Geographical distribution of centres dealing with the diagnosis of RBC enzyme defects.

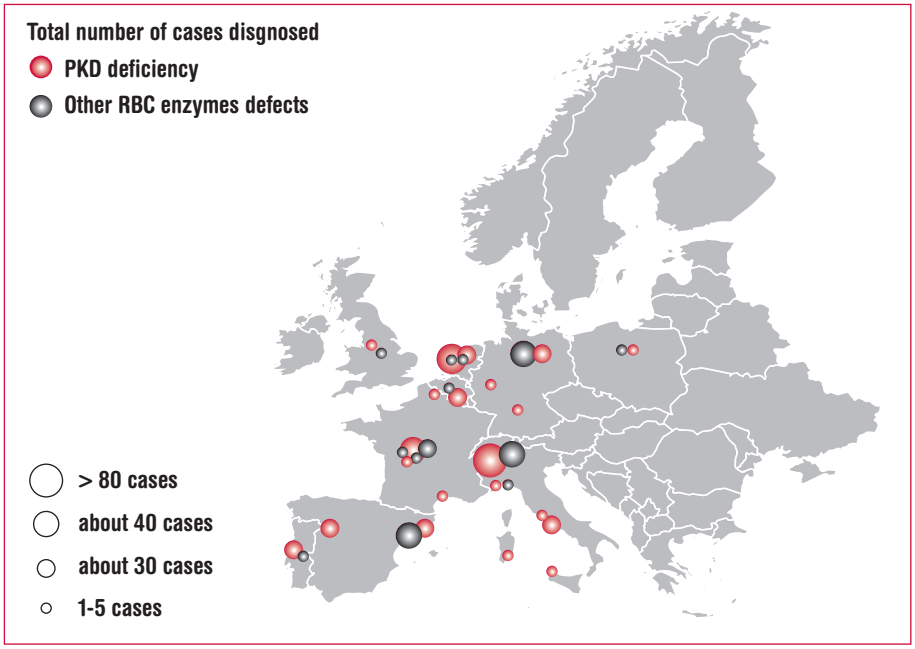


Table 8. Centres involved in phenotyping/genotyping and registered cases.

Enzymopathy	Centres involved in: Phenotyping	Genotyping	Total 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 metabolism			
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

Main conclusions from Survey 1 and 2

Availability of specialised equipment and treatments are satisfactory. But our results show that for laboratories as well as for clinical centres necessary tools to provide a diagnosis and to follow and manage the patients are not always available. If they exist, dedicated and specialised teams are not always implemented.

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

Our results also show 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 order to improve those points, the difficulties involved in creating and collecting data should be investigated.

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

Finally, in order 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.

Survey 3: External Quality Assessment (EQA) availability

The responses to this questionnaire were received from a limited number of EQA providers; however, it included several large providers that provide 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).

a) 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. Responders are listed in **Table 9**.

Table 9. 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	ANSM (The French National Agency for Medicines and Health Products Safety, (formerly AFSSAPS)
	CTCB
Ireland	Irish EQAS
	RIQAS
Norway	NOKLUS
Romania	RoEQALM
Russia	National Centre for EQA in Laboratory Medicine

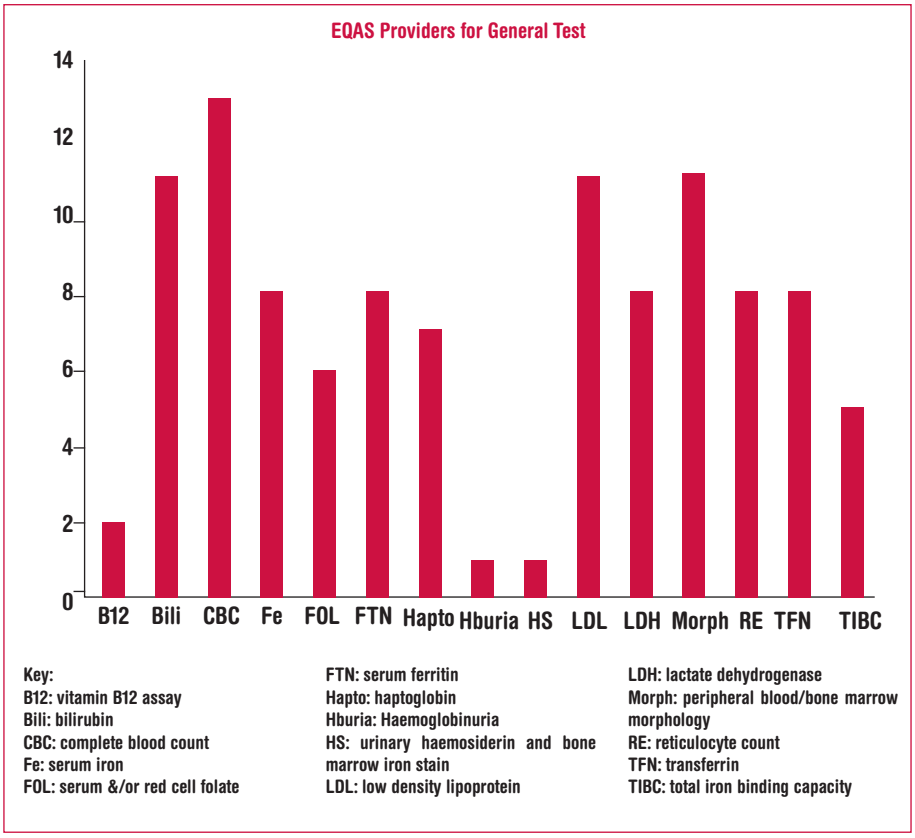
Table 9. Responses received (cont.).

Country	EQA provider organisation
Slovenia	SNEQAS
Spain	Sociedad Espanola de Hematologia y Hematorapia
Sweden	EQUALIS
Switzerland	CSQC
United Kingdom	UK NEQAS

b) EQA provision for general anaemia diagnostic 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 as shown in **Figure 13.** Five tests not covered by the EQA organisations that responded were: urine ferroxamine iron, serum transferrin receptor, liver iron, myocardial iron and zinc protoporphyrin.

Figure 14. EQA for general tests used in the diagnosis of anaemias covered by EQAS Organisers.

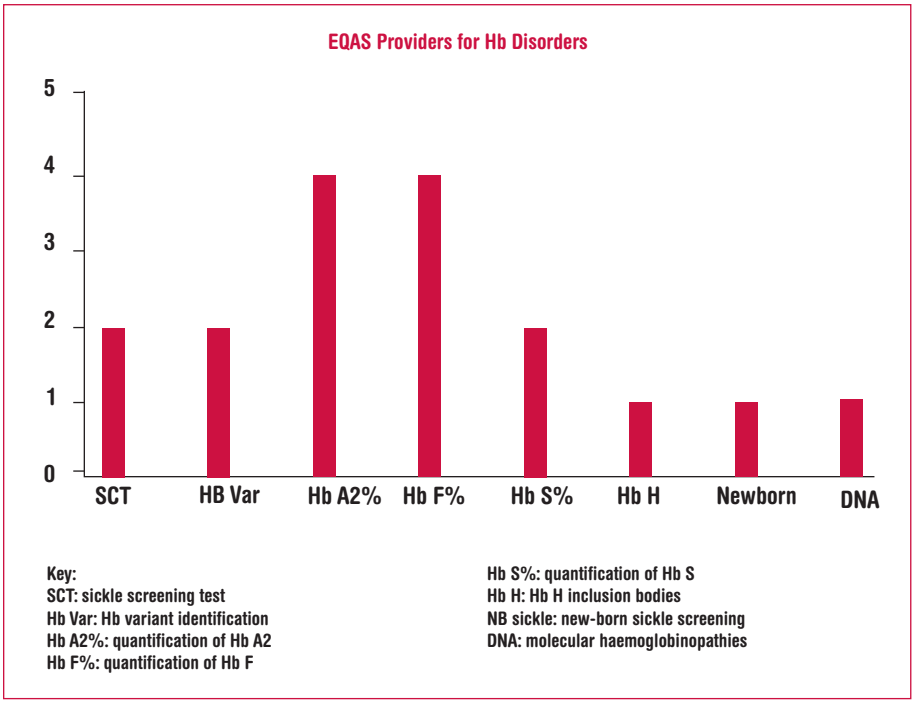


c) EQA for diagnostic tests for haemoglobin disorders covered by EQAS Organisers

Only 4 of the 16 EQAS organisations provided EQA for laboratory tests dealing with the diagnosis of haemoglobin disorders **(Figure 14).** These tests include the sickle solubility test ; haemoglobin (Hb) variant identification; quantification of Hb $A_{2,}$ Hb F and $Hb_{S;}$ Hb H bodies; new-born sickle screening and molecular haemoglobinopathies.

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

Figure 15. EQAS provision for tests for the diagnosis of haemoglobin disorders.



d) EQA for membrane, enzyme and other VRA diagnostic tests covered by EQAS Organisers

There is little provision of EQA for the special diagnostic tests used for VRA such as RBC membrane, enzyme defects, paroxysmal nocturnal haemoglobinuria (PNH) and other. EQA is only provided for glucose-6-phosphate dehydrogenase (G6PD) activity, Kleihauer (acid elution) slides, methaemoglobin, Hb F quantification and PNH diagnosis by flow cytometry. In all cases, there was only one EQA provider for each test.

e) EQA service provision across national borders

Service provision across national boundaries was available from 10/16 (60%) 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.

f) Frequency of service

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 A2 quantification (1 to 18 specimens annually).

g) EQA providers' 'wish list'

The EQA providers were asked which tests they thought would most benefit from the development of new EQA services. Responses are shown in **Table 10.** EQA is available for the majority of the tests on the wish list, through the organisations within EQALM that responded to the questionnaire. Only 4/16 tests listed did not have EQA provided by an alternative organisation.

Table 10. RA Diagnostic tests considered to most benefit from EQAS availability by EQALM questionnaire respondents.

Tests available for EQA	Tests not available
Hb variant detection	Unstable haemoglobins
Hb A2, 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 participant 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.

Main conclusions from survey 3

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 rare anaemias, are widely available and hence have good EQA provision. The provision of EQA for more specialist tests however is not as good.

The relatively poor provision of EQA for RA diagnostic tests reinforces the need for collaboration 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 selecting an EQA scheme appropriate to the laboratory's needs lies with the laboratory and this requires a diversity of EQA options; 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 wish list items, ENERCA has identified the diagnosis procedure for pyruvate kinase (PK) as the 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, laboratory diagnosis and clinical management of PK deficiency. A suggested protocol for a pilot EQA scheme has been developed by 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 barriers and differences in local medical practice all pose challenges 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 facilitating collaboration between experts in the development of EQA services and in publicising their availability to laboratory professionals.

5.4.3. Patient expectations

Limitations

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

General information

Demographics

The questionnaire was answered by 415 patients across Europe **(Table 11).** 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 11. Demographics.

Age (years)	2-66 range	29 mean		
Sex	Male 44%	Female 54%	No answer 1.4%	
Marital status (adults)	Married 27%	Single 52%	Cohabiting 2.4%	Divorced 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

Table 12. Country of residence.

Country of Residence	N
Italy	74
Bulgaria	62
Turkey	37
Portugal	26
Greece	24
Cyprus	24
Germany	5
France	2
UK	111
Belgium	7
Spain	2
Albania	1
Romania	39
Malta	1
Total	415

Table 12a. Ethnic origin.

Ethnic Origin	N
Italian	79
Bulgarian	63
Romanian	38
Turkish	37
Pakistani	22
Greek	27
Cypriot Greek	25
Cypriot Turkish	17
Sub-Saharan African	12
British Indian	4
Portuguese	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

Patient diagnosis

Table 13. 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%

The vast majority of responders were multitransfused thalassaemia patients. Since this group of patients presents 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. Unfortunately, patients with sickle cell syndromes for whom vaso-occlusive episodes and other recurrent events cause different very serious complications may have other expectations are poorly represented in this sample.

Clinical Care

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.

Confidence in the care received

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

Are you receiving the correct treatment?	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.

Quality of clinical care

The specific questions asked were the following:

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

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

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.

Treatment

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%

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%

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.

Waiting time for transfusion and appointment times for services

This question was prompted by the patients and their associations, complaining that they have to wait many 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 hours that service is provided 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%

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 for regular uninterrupted schooling or employment.

Educational status of patients who go for transfusions in the morning

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

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%
Not 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 time of day that transfusions are provided.

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

Iron chelation

372 patients are regularly chelated.

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 regime.

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

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 a specialised **centre.**

The majority of patients are treated in specialised centres.

Many patients are still followed by 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 50 year old patient. 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 changes from a haematological disorder to a multi-organ condition. The head of a haemoglobinopathy or rare anaemias centre of expertise is also the head and coordinator of a multidisciplinary 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, etc. 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: There were 350 multi-transfused patients who participated 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 longer intervals (all are under 12 years of age). Sixty-nine are not followed by a cardiologist and 53 did not respond to the question. These 69 (around 20%) patients who range from 13-46 years old, are not monitored for a condition that is the major cause of mortality in iron loaded multitransfused patients. This is a major concern and must be noted in any classification of 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 longer 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 to visiting a psychologist. Seven (27%) of these were seen frequently, every 3 months or less and so may have been on specific treatment. Eight (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 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 of 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 Europe.

Access to multi-disciplinary 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 some had to travel. Five patients (1.7%) visited specialists in private clinics.

Specialist care is only partially covered by free state 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.

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.

Talking about the condition

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

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 looked after in specialised haemoglobinopathy centres. There might be different reasons for this, including better clinical management, which takes into account the psychological aspects of the disorder.

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 for a close relationship between the expert centre and the support association and the need to provide

information and educational material to ensure patients receive the correct information. The internet also needs to be monitored for accuracy. The importance of peer communication in chronic disease is also demonstrated.

Main conclusions

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 are more likely to follow the prescribed treatment.
- They have shorter waiting times for transfusions.
- Appointments for transfusions are 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 service since they are more likely to be asked about problems with medication, the doctor considers their beliefs and habits when 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 to be well organised.
- They are more likely to be directed to join a patient support association.

Patients with severe anaemias, who responded to this questionnaire, have accepted all the suggestions concerning the qualities of a specialised centre. However they regard the most essential features of an expert centre to be 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.
- Patient representation in advisory committees.

6. ENERCA RECOMMENDATIONS

The consensus recommendations are the outcome of a proposal of general agreement between the different ENERCA partners involved in different processes of labelling or recognition of centres of expertise for rare and very rare anaemias in their respective countries; likewise 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 analysing the surveys, proposals of recommendations were elaborated and circulated among EGRA in order to be discussed in a multi-disciplinary way. The interactions between the different proposals were a key point to be analysed. In addition, two external advisers were invited to review the draft: one expert on UK criteria for recognising expert centres and the other for Haemoglobinopathy criteria on paediatrics.

Here a set of recommendations are proposed as the minimal required criteria to recognise a healthcare provider as a centre of expertise (CE) in one of the following categories:

1st. "Centre of expertise" is a centre with the appropriate capacity to address the complex and diverse conditions of RAs using a multi-disciplinary approach. A centre of expertise provides expert advice, produces guidelines and has links with other centres of expertise building a European Reference Network.

2nd. "Local centre" is a centre that offers health care in a defined catchment area. Local centres are bounded to a centre of expertise.

6.1. Legal and Ethical recommendations

The harmonisation of procedures to manage 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 differs among MS. Besides, there are very specific but important points that are not regulated in some States.

This is why a minimum consensus would be very positive and the recommendations try to underline some important issues that should be addressed from this perspective.

There are general recommendations, recommendations for the clinical activity and recommendations for the area of research (even though this is not the framework of this WP).

Sometimes the recommendations are just a wakeup call and reproduce a mandatory requirement (e.g., the implementation of security measures for data files) and in other cases, the recommendation goes deeper than the regulation in some concrete points, when harmonisation is considered especially useful (e.g., the establishment of 15 years as a minimum period for the storage of the samples of "inactive" patients). These recommendations are suggestions that would help the creation of a network and could lead to benefits for the patients.

Concerning data, it is recommended that the patient is informed about the data and samples that could be sent to other centres outside the National Health System, including a description of the objectives of the management (clinical, epidemiology or research) and to obtain their consent. 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 use of these resources for the benefit of patients, their families and also for scientific research.

Finally, when samples or data obtained for health purposes are going to be used for research, specific consent of patient should be required. Some concrete items are:

a. Purpose of the research, especially if DNA or RNA data are going to be obtained.
b. Expected benefits.
c. Identity of the person responsible for the research, or provide the possibility to be informed of who it is.
d. The patient's rights to revoke the consent, and the effects this may have.
e. Where the analysis will be undertaken and the destination of the sample at the end of the research: codification, destruction or other research.
f. The patient's right to gain access to the obtained data.
g. Guarantee of confidentiality of the information obtained.
h. Information regarding the implications of genetic analysis.
i. The ability of patients to decide whether or not they receive information about the data obtained when it is relevant to their health.
j. Information regarding the implication of the results for a patient's family.
k. The convenience that the person, where appropriate, transmit this information to the family members, in case it would be relevant for their health.
l. Indication of the possibility to get in contact with the patient, and the way to do so.
m. Information regarding the data that will be stored.
n. Identification and contact details of the person responsible for the storage of data.
o. Procedure for patients to exercise their right regarding to the data storage.
p. Requirements about international circulation of data.
q. Requirements about international circulation of samples.

6.2. Clinical and laboratory recommendations

Centres of expertise, dealing with RA patients, 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 rare anaemia. The diverse conditions that are classified as RAs share common service needs, including a correct diagnosis, controls for the anaemia, procedures to monitor and manage complications and provide holistic and supportive care. RAs are chronic diseases, in general, and often manifest in infancy. Many are lethal if not properly treated and this makes lifelong multi-disciplinary care an essential requirement.

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. Experience has demonstrated that survival is improved when patients are treated in a **centre of expertise.**

Taking into account this general framework, the recommendations in this section are divided into four categories: a) laboratory recommendations, b) clinical recommendations (patient care and follow-up), c) evidence proof of expertise and d) services for patients.

6.2.1. Recommendations for laboratory practice

- **Laboratory accreditation** based on the ISO 15189 standard, by an official national organism
- **Necessary resources** (human, technical and management) for achieving a diagnosis (in house or via a national or European network):
  - Routine tests: 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.
  - Special diagnostic tests: for VRA No single centre has the capacity to make a definitive diagnosis of all 62 listed RAs. Accordingly, Table 12 contains a list of the tests considered to be essential for the diagnosis of VRA : a) Membrane defects, b) Enzyme defects, c) Hemoglobinopathies and d) Erythropoietic defects Each centre should have special diagnostic tools for some RAs according to the special interest of the laboratory or the disorders prevalent in its geographic location.

Table 1. List of tests for RA diagnosis.

General Use	Centre of expertise	Local centre
Blood film morphology	x	x
CBC (complete blood count)	x	x
Reticulocyte count	x	x
Total and indirect serum bilirubin	x	x
Serum haptoglobin	x	x
Serum LDL and LDH levels	x	x
Urine haemosiderin (Haemosiderinuria)	x	x
Haemoglobinuria/ Myoglobinuria	x	
Urine ferrioxamine iron	x	?
Serum and RBC folate	x	x
Serum Cobalamin (Vitamin B12)	x	x
Serum ferritin	x	x
Serum Transferrin	x	x
Serum transferrin receptor (STR)	x	?
Transferrin saturation index (TSI)	x	x
Serum iron and total iron binding capacity (TIBC)	x	x
Zinc-protoporphyrin	x	-
Bone marrow examination and iron stain (Perls stain)	x	-
Hb disorders		
Hb electophoresis: cellulose acetate membrane or agarose gel electrophoresis (alkaline pH)	x	x
Hb electophoresis: citrate agar or agarose gel electrophoresis (acid pH)	x	
Iso-electric focusing	x	
Capillary electrophoresis	x	
High Pressure Liquid Chromatography (HPLC)	x	
Globin chain electrophoresis	x	
Hb S detection: whole blood sickling test	x	x
Hb S detection: sickle solubility test	x	x
p50 measurement (oxygen affinity) for altered O2 affinity Hbs	x	
Hb M detection using absorption spectra	x	
2,6 - Dichlorophenolindophenol test for Hb E	x	
Unstable haemoglobins: isopropanol stability test	x	
Unstable haemoglobins: heat stability test	x	
Microcolumn chromatography Hb A2, Hb S quantitation	x	x

Table 1. List of tests for RA diagnosis (cont.).

General Use	Centre of expertise	Local centre
Hb disorders		
Alkali denaturation Hb F quantitation	x	
Kleihauer test (HbF distribution)	x	
Flow cytometry for Hb F cells	x	
Hb H bodies	X	
Heinz bodies	X	
GENETICS	x	
Beta gene sequence analysis	X	
Alpha gene sequence analysis	X	
Gamma gene sequence analysis	X	
ARMS HbS, Hb C, Hb D, HbE	X	
ARMS beta thalassaemia common mutations	X	
Multiplex PCR for commom alpha mutations	X	
MLPA beta and alpha gene	X	
PCR for commom deltabeta mutations	X	
PCR for Hb Lepore mutations	X	
Red cell enzyme disorders		
Glucose-6-phosphate dehydrogenase (G6PD) deficiency		
NBT spot test	x	x
Fluorescent spot test	x	x
Cytochemical demonstration of G6PD deficiency		
Quantitative assay for G6PD activity	x	
Gene sequence analysis	x	
Pyruvate Kinase deficiency		
Spot test		x
Quantitative assay for PK activity	x	
Gene sequence analysis	x	
Pyrimidine-5'-nucleotidase deficiency		
Colorimetric assay	x	
Radiometric assay	x	
31P-NMR	x	
HPLC	x	
Spectrophotometric assay	x	
General Use	Centre of expertise	Local centre

Table 1. List of tests for RA diagnosis (cont.).

Red cell enzyme disorders		
Other red cell glycolytic enzyme deficiencies		
Quantitative assay: HK, GPI, PFK, Aldolase, TPI, PGK, BPGM, GSR, ADA, AK and other	x	
Reduced glutathione (GSH) assay	x	
Glutathione stability	x	
Molecular diagnosis - PCR & direct sequencing	x	
Methemoglobinemia		
Quantitative assay for metHbreductase activity	x	
Molecular diagnosis and gene sequencing	x	
RBC membrane disorders		
Demonstration of red cell membrane proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (HS, HE, Stomatocytoses)	x	
Osmotic gradient ektacytometry (HS, HE, Stomatocytosis)	x	
Proportion of spectrin dimers and tetramers in red cell membranes (HE)	x	
Tryptic digestion of spectrin (HE)	x	
Osmotic fragility test (OFT) (especially HS)	x	x
Autohaemolysis test (HS)		
Acidified glycerol lysis test (AGLT) (HS)	x	
Pink test (HS)	x	
Cryohaemolysis test (CHT) (HS, positive results also for SAO)	x	
Eosin-5-maleimide (EMA) binding test (HS, positive results also for SAO)	x	
Molecular diagnosis (SSCP, RFLP, direct sequencing) (membrane defects in general)	x	
PNH		
Ham test (acidified serum lysis test)		x
Sucrose lysis test (sugar-water test)		x
Flow cytometry for CD55 and CD59	x	
FLAER assay (fluorescently labeled aerolysin test)	x	
Iron metabolism disorders		
Hepcidin quantification	x	
Related genes sequencing	x	
Erythropoietic disorders (CDA, FA ,DBA and other)		
Related gene sequencing	x	

- **Reference laboratories** must have a list of alternatives where the samples can be sent if final diagnosis cannot be achieved with its own or local resources. When diagnostic experience is lacking the expert laboratory should know what other laboratories can offer assistance, so networking is a necessary feature of expert centres. Examples of necessary specialised tests available to an expert centre are molecular tests and specific tests, the enzymatic assay of at least the most frequent RBC enzyme defect (G6PD and PK).
- Participation in **External Quality Assessment Schemes (EQAS**) for diagnostic tests. The laboratory must demonstrate a satisfactory performance by participating in accredited local, national or International EQAS. When such a facility is not available in the country, inter laboratory evaluations on patient samples will be necessary.
- **Professional expertise and experience** of the laboratory practice must be documented by publications, honorary positions, university teaching or education and training activities.
- **Strong contribution to research.**
- **Epidemiological surveillance** involvement, such as registries.
- **Close collaborative links** with other expert laboratories at the national and international levels and capacity to participate in networks.
- **Close collaborative links** with clinical practice of haematology, paediatrics and internal medicine.
- **Testing algorithm** (i.e., decision tree for screening and diagnostic tests to determine individuals or pregnancies at risk of severe rare or very rare anaemia.) This testing algorithm should enable a logical approach towards the specific analytical tests required to reach a diagnosis.
- **Standard operating procedure (SOP)** for the whole screening process from initial specimen receipt until report delivery.
- **Guidelines for the standardised reporting of antenatal screening results**.
- **Documented risk management policy** describing the possible mistakes that may occur in the different steps of the testing protocol where and the procedures to be implemented to minimize the risk of the mistake occurring.

6.2.2. Recommendations for clinical care and follow-up

- **Detection and treating of severe disease complications**: Some RAs are multi-organic diseases and to address eventual complications and special treatments CEs need to be supported by hospital services such as laboratory (haematology, general biochemistry and virology) and image services (radiology and other complementary services) to properly monitor these patients, if necessary. This includes abdominal ultrasounds, 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.
- Capacity to provide expert case management in cases of chronic blood disorders, case management requires several conditions and standards, which include adherence to

good practice guidelines. Through these evidence based guidelines the following recommendations are regarded as essential:

- Availability of a **blood bank** to adequately supply blood for transfusions For regularly transfused patients the possibility of adverse reactions is increased and so special protective measures such as extended red cell genotyping, pre-storage filtration and nucleic acid testing (NAT) must be implemented.
- Availability of **orphan drugs** and other drugs necessary for iron overload treatment such as iron chelating agents, and their adequate supply procedure.
- Availability of a **coordinated multi-disciplinary 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.
- **Free treatment** should be provided with no out of pocket expenses from these chronically affected families.
- **Holistic care should be provided.** Treating every facet of the person – physical, emotional, psychological, educational, financial and vocational.
- **Maintaining a dedicated clinical record** for each patient, preferably in an electronic form.
- **Specialised staff** to follow previously agreed upon practical procedures or activities such as blood transfusion and haemovigilance, iron chelation, pharmacovigilance, growth monitoring, infection prevention.
- **Specialised facilities for patient care and drug administration,** such as day care units, 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 as well as for other non-malignant diseases, it is recommended to have separate outpatient consultation and day care services such as those existing in day care units, where blood transfusions and other procedures can be administrated. 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.

- **Provide expert advice on genetic counselling:** Trained and competent counsellors are needed in order to provide:
  - Clear and non-directive information should be provided to provide at-risk couples the ability to make an informed choice.
  - Explanation of 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.

- **Appropriate staff to 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. A haematologist, for example, may have laboratory duties as well as clinical and even administrative duties. There may also be teaching and research duties.

  Decisions on staff must be estimated according to local conditions. For haemoglobinipathy centres the staff to 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 to patient ratio should be further discussed as the need for staff experience must be satisfied.

- **Evidence Proof of expertise.** The objectives of this section are fundamentally to provide a framework which will support improvements in patient care by:

  - Providing a consensus between the different ENERCA partners involved in defining and promoting recognition as centres of expertise for haemoglobinopathies in their respective countries; and also for the local centres.
  - For countries where no such process is running, this consensus could be an example to support further development.
  - For countries where this process is running, this consensus suggests the optimal level of services to be provided; it should provide local clinicians with a valuable tool to support service improvements in conjunction with health planners. It is accepted that many centres will not have all the components in place on one site to receive expertise recognition. It is vital, particularly in areas of low prevalence, that collaborative networks are created to provide all aspects of care, not necessarily on one site.
  - Another important function of the centre of expertise is to support by education, leadership and clinical advice smaller local centres within their geographic area.

  Several aspects of the consensus recommendations defining a centre of expertise or a **local centre** are highlighted.

- **Implementation of outcome measures and quality control – the importance of a patient registry.** A centre of expertise 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. It is recommended that the registry be digital, and able to provide 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 should be agreed on a national or international level so that uniform reporting will assist in comparing results.

  A list of indicators of monitoring evaluating outcomes should be agreed upon by the centres of expertise. Patient interest outcomes and patient driven questionnaires are recommended. It is important to identify 'care gaps' 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 a centre of expertise 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 the treatment they receive, the quality of information that they are given, time spent in communication with staff, time of day that services are provided and several others, which are included in the patient expectations survey.

- **Efficient activity and capacity to provide relevant services at a sustained level of quality**

  The minimum throughput required to designate a clinical and/or laboratory haemoglobinopathy centre expert is arbitrary and subject to debate. It is suggested that the minimum number of patients should not be less than 50 in the case of haemoglobinopathies, however this number may be less for very rare anaemias. Concerning the laboratory samples, after consultation with a group of laboratory scientists, a minimum of 500 samples per year was suggested in the case of haemoglobinopathies, and a minimum of 20 samples per year for very rare anaemias. For very rare anaemias reference laboratories networking across Europe may be used for confirmation of the diagnosis.

- **High level expertise and experience**
  - The grades and specialities of doctors/nurses.
  - The number of years they have been involved in managing patients with rare anaemias. This is ensured if continuity of care is implemented.

- 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.
- Teaching and training activities.
- Involvement in Epidemiological surveillance.
- It is recommended that these parameters are considered in the designation of centres of expertise.

- **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. Recent EUCERD recommendations have also emphasised the need for ERNs. 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, another aspect which has been central to the ENERCA project. All these aspects of networking are further discussed in other parts of this book.

- **Special services for patients**
  - 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. Counselling 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.
  - 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.
  - Close links with patient associations: Patients with RAs should be well supported by patient driven organisations which are able to promote services and provide patient support. The treating centre of expertise 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.

6.3. Recommendations based on patient expectations

In their responses to the questionnaire, the majority of patients emphasise the following recommendations, which the EGRA team agree are important:

Early diagnosis and management of complications: This is a lifesaving requirement that any centre of expertise must be able to provide and also use to support local or secondary centres. This important requirement implies both clinical experience, following management guidelines but also having the technical means for specialised tests. Listed in the table below are examples of specialised equipment needed for both urgent investigation and long term monitoring. One important example for monitoring of multi-transfused patients who are susceptible to iron overload is the availability of Cardiac MRI – this must be available to all expert centres and must be also made available to patients receiving routine treatment at secondary or peripheral centres through a networking agreement since it is not always possible to have the necessary technology at all centres.

For expert management of complications it is not possible for a single haematology or internal medicine team to function alone. Multi-organ involvement, either due to vascular damage or iron overload, as previously described, can lead to acute, life threatening situations or long term organ damage and malfunction. Such complications require both specialised monitoring, for example routine heart tests by a cardiologist, and the management of complications as they arise. A variety of specialists are therefore needed to collaborate with the core team of doctors and nurses. The availability of specialists is particularly necessary in an expert centre that has networking arrangements with secondary centres.

Expertise is also necessary for providing information and especially for genetic counselling, which requires interpretation and understanding of the laboratory diagnosis, intimate knowledge of the disease consequences, available treatments as well as the choices offered to at-risk couples, following accepted principles of counselling. This service should therefore be provided by experts at a centre of excellence rather than being left to a variety of staff who may easily mislead affected individuals or couples.

Proof of expertise is important and indicators, such as outcome measures, are recommended by the EUCERD Recommendations [1]. In some locations there is an accreditation system and in the UK a team of experts, including expert patients, inspects, assesses and advises treatment centres.

Patient expectations

It is obvious from the ENERCA patient questionnaire that patients value the information given 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 patient expectations. 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 patient 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 centre of expertise should ensure that such information is accurate by 'training' these other sources (support associations, other patients) and reviewing information on the internet.

6.4. ENERCA Consensus Recommendations for Centres of Expertise in Rare Anaemias

6.4.1. Legal and ethical

Legal and ethical	Description	Centre of Expertise and European Reference Network	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 health data. Among others security measures, personal health data should be transferred using encryption.	X	X
Electronic clinical records	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	-

Legal and ethical	Description	Centre of Expertise and European Reference Network	Local centre linked to providers of services
	GENERAL		
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
	DIAGNOSIS AND TREATMENT		
Period for the storage of "inactive" patient 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 in such a way that two aspects can be guaranteed: the confidentiality concerning the patient identity and the tracebility of the samples	X	X
Information and consent for the transfer of identified data or samples	In case identified or identifiable data or samples are going to be transferred to a centre outside the national health system, information and consent is previously obtained from the patient	X	X
Information about health care in other centres	Centre informs patients about the possibility of receiving appropriate health care in other centres in the country or abroad	X	X
Procedure to transfer of data / samples / patients	There is an 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 they provide	X	X
	RESEARCH		
Patient consent for the use of samples or data for research purposes	When samples or data obtained for health purposes are going to be used for research purposes, specific consent of patient is required (see concrete items in the introductory remarks to these recommendations). Information has to be given about the transfer provided to other centres for research purposes	X	X

6.4.2. Clinical and laboratory

Clinical and laboratory	Centre of Expertise and European Reference Network	Local centre linked to providers of Minimum services
A. 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	-
B. CLINICAL (PATIENT FOLLOW-UP/ MANAGEMENT)		
SPECIALISED EQUIPMENT FOR		
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*
SPECIALISED TEAM FOR		
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*
MULTI-DISCIPLINARY APPROACH		
Consultations	X	-
Genetic counselling	X	-
Link with screening programmes (if applicable)	X	-

Clinical and laboratory	Centre of Expertise and European Reference Network	Local centre linked to providers of Minimum services
A. LABORATORY		
MULTI-DISCIPLINARY APPROACH		
Recommendations, standards of care	X	X*
Network with peripheral hospitals, GPs …	X	-
From paediatric to adulthood	X	X
EVIDENCE PROOF 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*

* In collaboration with centres of expertise.
** Can be shared in networks.

6.4.3. Patient expectation

Patient expectations	% Strong support	Centre of Expertise and European Reference Network	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
Multi-disciplinary 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	-
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

* In collaboration with centres of expertise.
** Can be shared in networks.

6.5. ENERCA Recommendations as an open self-assessment dynamic tool

The ENERCA White Book has been designed as a dynamic tool that can be evaluated and adapted according to new needs and stakeholderexpectations. It aims to reach a critical mass of stakeholders, namely European and MS Health authorities, health professionals, scientific community, patients community and other health care providers, 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 patient organisations. This will allow new experiences to be shared and improve the networking capacities and skills of the network.

The objective is for MS to recognise the expert centres already identified and allow the geographic 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 tools for the dissemination of the project results has been the ENERCA website. This website will be used to disseminate the White Book and ENERCA recommendations. The White Book is freely available on the ENERCA website. In addition, the creation of an on-line application, based on ENERCA recommendations, is foreseen to allow centres dealing with RA to assess how many ENERCA recommendations they have incorporated. An evaluation of the agreement of participants to follow the recommendations will also be assessed. **www.enerca.org**

7. IN SUMMARY: From recommendations to practice

The Community added value ERNs is particularly high for rare diseases due to the fact that they affect a limited number of patients and because there is little 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 diseases. This applies particularly to rare anaemias (RA), due to the high number of different diseases that constitute 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. In 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 very 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** to achieve the objectives of a European Reference Network in Rare Anaemias 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 of the parameters involved, including patient rights, standardisation of procedures (including diseases codification), professional involvement and technical support.
4. Networking has a **huge potential in educational activities**. ENERCA has successfully organised 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 teams that 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. In addition, joint research activities are the only way to discover new causes leading to RA in the substantial group of patients that remain undiagnosed.
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 involvement in research seems to be a key way 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 foreseen. The importance of this harmonisation 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 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 patient 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 **multi-disciplinary approach** like that of the ENERCA White Book has been used for an ERN. Indeed, it includes not only medical and technical issues, but also legal and ethical ones, as well as patient

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 made. In that sense, it is a commitment for EGRA members to present the White Book to National Authorities involved in National Plans for Rare Diseases.

In order to assure long term sustainability of the **network,** there is a strong need for **official recognition** of this structure.

The major prerequisite for the development of national plan/strategies on RDs, including CEs 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.

List of acronyms and abbreviations

ADA: Adenosine Deaminase
AFSSAPS: Agence Française de Sécurité Sanitaire des Produits de Santé
ALA: Alpha Lipoic Acid
AML: Acute Myeloid Leukemia
APoGI: Accessible Publishing of Genetic Information
ATP: Adenosine Triphosphate
AUG: Anaemias of Unknown Underlying cause
BMF: Bone Marrow failure
CBC: Complete Blood Count
CD: Chronic Disease
CDA: Congenital Dyserythropoietic Anaemia
CDAs: Congenital Dyserythropoietic Anaemias
CE: Centre of Expertise
CEs: Centres of Expertise
CP: Ceruplasmin
CSA: Congenital Sideroblastic Anemia
CT: Computed Tomography
CTCB: Centre Toulousain pour le Contrôle de Qualité en Biologie Clinique
DBA: Diamond Blacfan Anaemia
DEB: Diepoxybuthane
DEKS : Danish Institute for External Quality Assurance for Laboratories in Health Care
DEXA: Bone Mineral Density
DG-SANCO: Directorate General for Health & Consumers
DHSt: Dehydrated hereditary stomatocytosis
DNA: Desoxyribonucleic Acid
eADA: Erythrocyte Adenosine Deaminase
EAHC: European Agency for Health and Consumers
EC: European Commission
EGRA: Enerca Group on Rare Anaemias
EHR: Electronic Health records
EMQN: European Molecular Genetics Quality Network
ENERCA: European Reference Network on Rare and Congenital Anaemias
EPO: Erythropoietin
EQA: External Quality Assessment
EQALM: European Organisation for External Quality Assurance
EQAS: External Quality Assessment Scheme
EQUALIS: External Quality Assurance in Laboratory Medicine in Sweden
ERN: European Reference Network
ETS: External Transcribed Sequences
EU: European Union
EUCERD: European Union Committee of Experts on Rare Diseases

EUROPLAN: European Project for Rare Diseases National Plans Development
EURORDIS: European Rare Diseases Organization
FA: Fanconi Anaemia
FBC: Full Blood Count
FP6: Sixth Framework Programme
FP7: Seventh Framework Programme
G6PD: Glucose-6-Phosphate Dehydrogenase
GCLM: Glutamate - Cysteine Ligase
GDF: Growth Differentiation Factor
GP: General Practicioner
GPI: Glucosephosphate Isomerase
GVHD: Graft Versus Host Disease
HB: Haemoglobin
HbA: Haemoglobin Adult
HbF: Haemoglobin Fetal
HbS: Haemoglobin S
Hburia: Haemoglobinuria
HE: Hereditary Spherocytosis
HEMPAS: Hereditary Erythroblastic Multinuclearity with Positive Acidified Serum Test
HIV: Human Immunodeficiency Virus
HK: Hexokinase
HLA: Human leukocyte antigen
HLG: High Level Group
HNSHA: Hereditary Nonspherocytic Haemolytic Anaemia
HPFH: Hereditary Persistence of Fetal Haemoglobin
HPLC: High-performance liquid chromatography
HPP: Hereditary pyropoikilocytosis
HSA: Hereditary Sideroblastic Anaemia
HSCT: Hematopoietic stem cell transplantation
HSt: Hereditary stomatocytosis
ICD: International Classification of Diseases
ICSH: International Council for Standards in Haematology
ID: Iron Deficiency
IDA: Iron Deficiency Anaemia
IFCC: Internationl Federation of Clinical Chemistry
IQC: International Quality Control
IRIDA: Iron-refractory Iron Deficiency Anemia
IRP: Iron Regulatory Protein
ISO: International Organization for Standardization
ITS: Internal Transcribed Sequences
LDH: Lactate Dehydrogenase
LDL: Low Density Lipoprotein
LoRRca: Laser-assisted Optical Rotational Cell Analyzer
LPI: Labile Plasma Iron

MAC: Medical Alert Card
MCH: Mean Corpuscular Haemoglobin
MCHC: Corpuscular Haemoglobin Concentration
MCV: Mean Corpuscular Volume
MDS: Myelodysplastic Syndromes
MMC: MitoMycin C
MOH: Ministry of Health
MPI Database: Mid-Pacific Institute Library Database
MRI: Magnetic Ressonance Imaging
MS: Member State
MSs: Member States
mtDNA: Mitocondrial Desoxyribonucleic Acid
NADPH: Nicotinamide Adenine Dinucleotide Phosphate-OXIDASE
NAMSE: Nationales Aktionsbündnis für Menschen mit seltenen Erkrankungen
NAT: Nucleic Acid Testing
NCDs: Non Communicable Diseases
NHAs: National Health Authorities
NHS: National Health Services
NOKLUS:: External quality assurance in Norwegian primary health care
NP: National Plans
NS: National Strategies
OHSt: Over Hydrated Hereditary Stomatocytosis
OMIM: Online Mendelian Inheritance in Man
P5N: Pyrimidine 5'-Nucelotidase
PACIC: Patient Assessment of Care for Chronic Conditions
PCV: Packed Cell Volume
PESPA: Greek Alliance for Rare Diseases
PFK: Phosphofructokinase
PGK: Phosphoglycerate Kinasa
PK: Piruvate Kinase
PMPS: Pearson's Marrow-Pancreas Syndrome
PNH: Paroxysmal Nocturnal Haemoglobinuria
QC: Quality Control
QMP-LS: Quality Management Program - Laboratory Services
R&D: Research and Development
RA: Rare Anaemia
Ras: Rare Anaemias
RBC: Red Blood Cell
RBCs: Red Blood Cell
RD: Rare Disease
RDs: Rare Diseases
RDTF: Rare Diseases Task Force
RDUK: Rare Disease UK
RDW: RBC Distribution Width
RIQAS: Randox International Quality Assessment Scheme

RNA: Ribonucleic acid
RoEQALM: Societatea Romana pentru Asigurarea si Controlul Extern al Calitatii in Medicina de Laborator
RP: Ribosomal Protein
RPs: Ribosomal Proteins
rRNAs: Ribomosal Ribonucleic Acid
SA: Sideroblastic Anaemia
SCA: Sickle Cell Anaemia
SCC: Squamous Cell Carcinomas
SCD: Sickle Cell Disease
SCT: Sickle Cell Screening Test
SDS-PAGE: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SEHH: Sociedad Española de Hematología y Hemoterapia.
SKML: Foundation for Quality Medical Laboratory Diagnostics
SNEQAS: Slovenia National External Quality Assessment Scheme
SOP: Standard Operating Procedure
SQUID: Superconducting Quantum Interference Device
TEC: Transient Erytroblastopenia of Childhood
TFN: Transferrin
THAL: Thalassaemia
THTR1: Thiamine Transporter Protein 2
TIBC: Total Iron Binding Capacity
TIF: Thalassaemia International Federation
TPI: Triosephosphate Isomerase
UK NEQAS: United Kingdom National External Quality Assessment Service
UKTS: UK Thalassaemia Society
UNESCO: United Nations Educational, Scientific and Cultural Organization
UNIAMO: Federazione Italiana Malattie Rare Onlus
VRA: Very Rare Anaemia
VRAs: Very Rare Anaemias
WB: White Book
WG: Working Group
WHO: World Health Organization
WP: Working Package
WPs: Working Packages
XLSA: X-Linked Sideroblastic Anemia

Useful sources

- ENERCA (www.enerca.org)
- Executive Agency for Health and Consumers (www.ec.europa.eu/eahc)
- European Commission DG Sanco (www.ec.europa.eu)
- Eucerd (www.eucerd.eu)
- TIF (www.thalassaemia.org.cy)
- Orphanet (www.orpha.net)
- Eurordis (www.eurordis.org)
- European Union Law (www.eur-lex.europa.eu)

Annex 1. List of ENERCA 3 partners

Associated Partner	Acronym	Country	Name
Hôpital Erasme - Université Libre de Bruxelles	ERASME	Belgium	Béatrice Gulbis
Thalassaemia International Federation	TIF	Cyprus	Androulla Eleftheriou
		Cyprus	Michael Angastiniotis
Centre Hospitalier Universitaire de Montpellier	CHU-MTP	France	Patricia Aguilar Martinez
CHU Henri Mondor	INSERM	France	Josiane Bardakdjian
INSERM-Centre Recherche Biologique Bichat Beaujon	INSERM	France	Carole Beaumont
Centre de la Drépanocytose Necker	CDN	France	Mariane de Montalembert
European School of Haematology	ESH	France	Deirdre Jasmin
Universität of Ulm	UULM	Germany	Hermann Heimpel
"LAIKO" General Hospital Athens	LGHA	Greece	Ersi Voskaridou
Universitá Vita-Salute San Raffaele	UNISR	Italy	Clara Camaschella
University of Verona	UNIVR	Italy	Lucia de Franceschi
Università del Piemonte Orientale	UPO	Italy	Irma Dianzani
Centro di Ricerca per l'Ingegneria Genetica	CEINGE	Italy	Achille Iolascon
Università degli Estudi di Milano	UNIMILANO	Italy	Andrea Mosca
Thalassaemia Centre of the University of Turin	TCUT	Italy	Antonio Piga
Fondazione IRCCS Ospedale Maggiore Policlinico	IRCCS	Italy	Alberto Zanella
	IRCCS	Italy	Paola Bianchi
Centro Hospitalar Coimbra	CHC	Portugal	Leticia Ribeiro
Hospital Clinic de Barcelona	CLINIC	Spain	Juan Lluis Vives Corrons
			Maria del Mar Mañú Pereira
			Laura Olaya Costa
Hospital 12 de Octubre	HDOC	Spain	Florinda Gilsanz
Universidad de Deusto	UD	Spain	Carlos Romeo
Universidad de Deusto	UD	Spain	Pilar Nicolás
Universitair Medisch Centrum Utrecht	UMCU	The Netherlands	Richard van Wijk
UK National External Quality Assessment Service	UKNEQAS	United Kingdom	Barbara De la Salle
Cardiff University School of Medicine	CU	United Kingdom	Alison May
University College London	UCL	United Kingdom	John Porter

Associated Partner	Acronym	Country	Name
King's College London School of Medicine	KINGS	United Kingdom	Allison Streetly
		United Kingdom	Swee Lay Thein

Collaborating Partner	Acronym	Country	Name
Hospital Universitaire des enfants Reine Fabiola	HUERF	Belgium	Alice Ferster
Cyprus Thalassaemia Centre	CTC	Cyprus	Soteroulla Christou
Cyprus Institute of Neurology and Genetics	CING	Cyprus	Marina Kleanthous
Palacky University Olomouc	PUO	Czech Republic	Dagmar Popislova
Institut Universitaire d'Hematologie	IUH	France	Yves Beuzard
Eurordis	EURORDIS	France	François Houyez
Hôpital St Louis	HStL	France	Gerard Socié
Saarland University	SAAR	Germany	Inglof Bernhardt
Universitätsmedizin Göttingen Georg – August – Universität	UGGAU	Germany	Nina Kollmar
University Freiburg	UF	Germany	Charlotte Niemeyer
Biomedical Research Foundation: Academy of Athens	BFR	Greece	Dimitris Loukopoulos
Clinic of Pediatric Hematology Oncology. University of Padova	UPAD	Italy	Raffaella Colombatti
Regional Thalassaemia Hospital	RTHC	Italy	Renzo Galanello †
Universtà degli Studi di Pavia	UdSP	Italy	Giampolo Minetti
Institute of Hematology	IHP	Poland	Jerzy Koscielak
Fundeni Clinical Institute	FCI	Romania	Anca Colita
Universitatea de Medicinasi Farmacie Victor Babes	UMFVR	Romania	Margit Serban
Institute of Haematology – Clinical Centre of Serbia	IH-CCS	Serbia	Milica Colovic
University Children's Hospital	UCH	Serbia	Dragana Janic
Institute of Molecular Genetics Engineering – University of Belgrade	IMGGE	Serbia	Sonja Pavlovic
Complejo Hospitalario de Toledo	CHT	Spain	Angel F Remacha
WHO - Human genetics Programme	WHO	Switzerland	Victor Boulyjenkov
Sanquin Blood Supply Foundation, laboratory of red blood cell diagnostics	SBSF	The Netherlands	Arthur Verhoeven
UK Thalassaemia Society	UKTS	United Kingdom	Chris Sotirelis

Annex 2. Final core list of laboratory tests

Hb Disorders

Condition	Specific Laboratory Test	Reference
Haemoglobinopathy or thalassaemia	High performance liquid chromatography (HPLC)	Wild BJ and Stephens AD (1997) Clin Lab Haematol 19:171-176
	Hb electophoresis: - Cellulose acetate membrane or agarose gel electrophoresis (alkaline pH), - Citrate agar or agarose gel electrophoresis (acid pH)	1) International Committee for Standardization in Haematology (1978), Blood 52: 1058-1064 2) International Committee for Standardization in Haematology (1978) 52: 1065-1067
	Iso-electric focusing	1) Righetti PG et al (1986) Methods in Haematology 15: 47-71 2) Basset P et al (1978) Blood 51: 971-982
	Capillary electrophoresis	Van Delft P. et al (2009) Int J Lab Hematol 31:484-495. Various commercial
	Globin chain electrophoresis	Ueda S et al (1969) Blood 34: 230-235
	Globin synthesis in reticulocytes and separation via RP-HPLC	DeSimone et al.(1974) J. Lab. Clin. Med. 84, 517-524 Tegos et al. (1980) Clin. Lab Haemat., 2, 191-197
	Electrospray ionisation mass spectrometry	Wild et al (2001), Blood Cells Mol Dis 27:691-704
	Hb S detection: - Whole blood sickling test, - Sickle solubility test	Wild BJ and Bain BJ (2005) In Lewis SM, Bain BJ and Bates I, eds *Dacie and Lewis Practical Haematology*, 10th ed. Churchill Livingstone
	p50 measurement (oxygen affinity) for altered O2 affinity Hbs	1) Bellingham et al, 1971, J Applied Physiology, 30:903-904 2) Torrance et al (1969), Respiration Physiology, 8:127-136 3) Van Slyke DD and Neill JM (1924) J Biol Chem 61:523-573
	Hb M detection using absorption spectra	Wild BJ and Bain BJ (2005) In Lewis SM, Bain BJ and Bates I, eds *Dacie and Lewis Practical Haematology*, 10th ed. Churchill Livingstone
	2,6 - Dichlorophenolindophenol test for Hb E	Chapple L et al (2005) J Clin Pathol 59: 74-76

Hb Disorders (cont.)

Condition	Specific Laboratory Test	Reference
Haemoglobinopathy	Unstable haemoglobins: - Heat stability test - Isopropanol stability test	Carrell RW (1986) Methods in Haematology 15: 109-124
	DNA analysis (beta, alpha sequence analysis, MLPA) ARMS (Hb S, Hb C, Hb E mutation, beta thalassaemia mutations),Ddel digestion (sickle mutations), Multiplex PCR forcommon alpha thalassaemia deletions, Gamma globin promoter sequence (non-deletional HPFH)	Vulliamy T and Kaeda J (2005) In Lewis SM, Bain BJ and Bates I, eds Dacie and Lewis Practical Haematology, 10th ed. Churchill Livingstone. Traeger-Synodinos and Harteveld (2010) Molecular Diagnosis of Genetic Disease by R.Elles and R. Mountford, 3rd ed Humana Press. Old (2004) Molecular Diagnosis of Genetic Disease by R.Elles and R.Mountford, 2nd ed Humana Press. Harteveld et al. (2005) Journal of Medical Genetics 42:922-931
	HPLC (Hb A2, Hb F, Hb S and other Hb variants)	Wild BJ and Stephens AD (1997) Clin Lab Haemat 19:171-176
	Microcolumn chromatography (Hb A2 and Hb S)	Efremov et al (1974) J Lab Clin Med, 83: 657-664
	Electrophoresis and elution (Hb A2 and Hb S)	Marengo-Rowe (1965) J Clin Pathol 18:790-792
	Alkali denaturation (Hb F)	Betke K et al (1959) Nature 184: 1877-1878
	Kleihauer test (HbF distribution)	Kleihauer E (1974) In Schmidt RM, Huisman THJ, Lehmann H eds, The detection of haemoglobinopathies, CRC Press, Cleveland Ohio.
	Flow cytometry	Hoyer JD et al (2002) Am J Clin Pathol 117: 857-863
	Hb H bodies	Wild BJ and Bain BJ (2005) In Lewis SM, Bain BJ and Bates I, eds *Dacie and Lewis Practical Haematology,* 10th ed. Churchill Livingstone
	Heinz bodies	Wild BJ and Bain BJ (2005) In Lewis SM, Bain BJ and Bates I, eds *Dacie and Lewis Practical Haematology,* 10th ed. Churchill Livingstone

Enzyme deficiencies

Condition	Specific Laboratory Test	Reference
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	NBT spot test	BEUTLER. Blood, 1966, Vol. 28, No. 4, pp. 553-562
	G6PD Fluorescent spot test	JACOB et al. N Engl J Med, 1966, Vol. 274, No. 21, pp. 1162-1167
	Cytochemical demonstration of G6PD deficiency	FAIRBANKS et al. Blood, 1968, Vol. 31, No. 5, pp. 589-603
	Quantitative assay for G6PD activity	1) SMITH et al. Med J Aust, 1975, Vol. 1, No. 18, pp. 558-559 2) MOSCA et al. Haematologica, 1990, Vol. 75, No. 4, pp. 397-399 3) NIE et al. Chin Med (Engl) J, 1999, Vol. 112, No. 4, pp. 349-351 4) CHEN et al. Southeast Asian J Trop Med Public Health, 2003, Vol. 34, Suppl. 3, pp. 143-146 5) FAN et al. J Clin Lab Anal, 2007, Vol. 21, No. 2, pp. 107-113
	Chromate inhibition test	1) Koutras et al. Br J Haematol 1965;11:360-9, and 2) Zurcher et al. Clin Chim Acta 1969; 25: 139-46
	Molecular diagnosis - RFLP - ASO probes - SSCP - Sequencing - RT-PCR-DGGE - HRM	1) D'URSO et al. Am J Hum Genet, 1988, Vol. 42, pp. 735-741 2) FEY et al. Hum Genet, 1990, Vol. 84, No. 5, pp. 471-472 3) HUANG et al. Acta Haematol, 1992, Vol. 88, No. 2-3, pp. 92-95 4) CALABRO' et al. Am J Hum Genet, 1993, Vol. 52, No. 3, pp. 527-536 5) ZUO et al. Int J Hematol, 1992, Vol. 55, No. 1, pp. 39-44 6) CHEN et al. Zhongguo Dang Dai Er Ke Za Zhi, 2009, Vol. 11, No. 8, pp. 613-616 7) JOLY et al. Clin Biochem, 2010, Vol. 43, No. 1-2, pp. 193-197
Pyruvate Kinase deficiency	PK Spot test	BEUTLER. Blood, 1966, Vol. 28, No. 4, pp. 553-562
	Quantitative assay for PK activity	1) TANAKA et al. Blood, 1962, Vol. 19, pp. 267-295 2) BRUNETTI et al. Enzymol Biol Clin, 1964, Vol. 36, pp. 51-57 3) TANPHAICHITR et al. Clin Chim Acta, 1972, Vol. 41, pp. 41-45 4) BEUTLER Clin Chim Acta, 1981, Vol. 116, No. 3, pp. 397-399 5) KASLOW et al. Anal Biochem, 1983, Vol. 134, No. 2, pp. 495-498
	Molecular diagnosis - RFLP - SSCP - Sequencing	1) BARONCIANI et al. J Clin Invest, 1995, Vol. 95, No. 4, pp. 1702-1709 2) ZANELLA et al. Blood, 1997, Vol. 89, No. 10, pp. 3847-3852 3) ZARZA et al. Med Clin (Barc.), 1999, Vol. 112, No. 16, pp. 606-609 4) KANNO et al. Blood, 1994, Vol. 84, No. 10, pp. 3505-3509 5) van Wijk et al. Blood 2003; 101: 1596-1602
	Various Commercial	
Pyrimidine-5'-nucleotidase deficiency	Colorimetric assay	PAGLIA et al. J Biol Chem, 1975, Vol. 250, No. 20, pp. 7973-7979
	Radiometric assay	1) TORRANCE et al. J Lab Clin Med, 1977, Vol. 90, No. 3, pp. 563-568 2) BUC et al. Clin Chim Acta, 1978, Vol. 85, No. 2, pp. 193-196
	31P-NMR	KAGIMOTO et al. Experientia, 1986, Vol. 42, No. 1, pp. 69-72
	HPLC	ADAIR et al. Clin Chim Acta, 1988, Vol. 171, No. 1, pp. 75-83
	Spectrophotometric assay	1) ZEREZ et al. Anal Biochem, 1985, Vol. 151, No. 2, pp. 282-285 2) AMICI et al. Br J Haematol, 1989, Vol. 73, pp. 392-395

Enzyme deficiencies (cont.)

Condition	Specific Laboratory Test	Reference
Other red cell glycolytic enzyme deficiencies	Quantitative assay: HK, GPI, PFK, Aldolase, TPI, PGK, BPGM, GSR, ADA, and AK	1) NYGAARD et al. Scand J Clin Lab Invest, 1969, Vol. 24, No. 4, pp. 399-403 2) KEITT. J Lab Clin Med, 1971, Vol. 77, No. 3, pp. 470-475 3) ATKINSON. Clin Chem, 1972, Vol. 18, No. 9, pp. 1001-1004 4 PEYTON et al. Ann Clin Lab Sci, 1972, Vol. 2, No. 5, pp. 383-388 5) POWELL et al. Clin Chem, 1972, Vol. 18, pp. 1318-1322 6) KÜBLER et al. J Mol Med, 1974, Vol. 52, No. 11, pp. 549-551 7) DETTER et al. Clin Chem 1975, Vol. 21, No. 3, pp. 376-380 8) BEUTLER, E. (1984) Red cell metabolism. A manual of biochemical methods. Grune & Stratton, Orlando
	Molecular diagnosis - PCR & direct sequencing	Hexokinase deficiency: Bianchi and Magnani. Blood Cells Mol Dis 1995; 21: 2-8 van Wijk et al. Blood 2003; 101: 345-347 GPI deficiency: Beutler et al. Blood Cells Mol Dis 1997; 23: 402-409 Phosphofructokinase deficiency: Raben et al. JBC 1993; 268: 4963-4967 Sherman et al. AJHG 1994; 55: 305-313 Aldolase deficiency: Kishi et al. PNAS 1987; 84: 8623-8627 Yao et al. Blood 2003; 2401-2403 TPI deficiency: Valentin et al. Blood 2000; 96: 1130-1135 PGK deficiency: Cohen-Solal et al. Blood 1994; 84: 898-903 6-phosphogluconate dehydrogenase deficiency: Vives Corrons et al. AJH 1996; 53: 221-227 Gamma-glutamylcysteine synthetase deficiency: Beutler et al. Blood 1999; 24:2890-2894 Ristoff et al. Blood 2000; 95:1896-1897 Glutathione synthetase deficiency: Glutathione reductase deficiency: Kamerbeek et al. Blood 2007; 109: 3560-5366 Adenylate kinase deficiency: Bianchi et al. BJH 1999; 105: 75-79
	Reduced glutathione (GSH) assay	1) BHATTACHARYA et al. Biochem J, 1995, Vol. 60, No. 4, pp. 696-702 2) BEUTLER et al. J Lab Clin Invest, 1963, Vol. 61, pp. 882-888 3) LACK et al. Anal Biochem, 1964, Vol. 8, pp. 217-222 4) MORTENSEN. Scand J Clin Lab Invest, 1965, Vol. 17, pp. 93-94 5) STEGHENS et al. J Chromatogr B, 2003, Vol. 798, No. 2, pp. 343-349 6) SAKHI et al. J Chromatogr A, 2006, Vol. 1104, No. 1-2, pp. 179-189 7) GUO et al. Anal Chim Acta, 2009, Vol. 633, No. 1, pp. 71-75 8) IWASAKI et al. J Chromatogr B, 2009, Vol. 877, No. 28, pp. 3393-3317
	Glutathione stability	1) BEUTLER et al. J Lab Clin Med, 1957, Vol. 49, No. 1, pp. 84-95 2) AMOS. Am J Med Technol, 1960, Vol. 26, pp. 333-337 3) 1989, In Chanarin I, ed Laboratory Haematology: an account of laboratory techniques, 1st ed., pp. 90-91, Churchill Livingstone
Methemoglobinemia	Quantitative assay for metHbreductase activity	Hegesh et al., J Lab Clin Med., 1968, Vol. 72, pp339-344
	Molecular diagnosis	Dekker et al. Blood, 2001 Vol. 15, No.97, pp. 1106-1114

Membrane defects

Condition	Specific Laboratory Test	Reference
All membrane defects	Peripheral blood film (membrane defects in general)	1) LECOMPTE et al. C R Acad Sci III, 1988, Vol. 306, No. 2, pp. 43-46 2) PALEK et al. Semin Hematol, 1993, Vol. 30, No. 4, pp. 249-283
	Molecular diagnosis (SSCP, RFLP, direct sequencing) (membrane defects in general)	1) SAHR et al. J Clin Invest, 1989, Vol. 84, No. 4, pp. 1243-1253 2) SPINARDI et al. Nucleic Acids res, 1991, Vol. 19, No. 14, pp. 4009 3) BECKER et al. J Clin Invest, 1993, Vol. 92, No. 3, pp. 612-616 4) MIRAGLIA DEL GIUDICE et al. Haematologica, 1994, Vol. 79, No. 5, pp. 400-405 5) MATSUDA et al. Hum Mol Genet, 1995, Vol. 4, No. 7, pp. 1187-1191 6) BEETON et al. Hum Genet, 1995, Vol. 95, No. 3, pp. 365-366 7) QUALTIERI et al. Br J Haematol, 1997, Vol. 97, No. 2, pp. 273-278 8) MAILLET et al. Hum Mutat, 1998, Vol. 11, No. 4, pp. 342-343 9) AKANISHI et al. Int J hematol, Vol. 73, No. 1, pp. 54- 63
Hereditary spherocytosis, Hereditary elliptocytosis, Stomatocytosis	Demonstration of red cell membrane proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (HS, HE, Stomatocytoses)	1) LAEMMLI. Nature, 1970, Vol. 227, No. 5259, pp. 680-685 2) FAIRBANKS et al. Biochem, 1971, Vol. 10, No. 13, pp. 2606-2617
	Osmotic gradient ektacytometry (HS, HE, Stomatocytosis)	1) MOHANDAS et al. Blood, 1982, Vol. 59, No. 4, pp. 768-74 2) CLARK et al. Blood, 1983, Vol. 61, No. 5, pp. 899-910
Hereditary elliptocytosis	Proportion of spectrin dimers and tetramers in red cell membranes (HE)	1) RALSTON et al. Biochim Biophys Acta, 1977, Vol. 491, No. 1, pp. 345-348 2) UGEWICKELL. Eur J Biochem, 1978, Vol. 88, No. 2, pp. 379-385 3) COETZER et al. Blood, 1982, Vol. 59, No. 5, pp. 900-905
	Tryptic digestion of spectrin (HE)	1) ANDERSON. J Biol Chem, 1979, Vol. 254, No. 3, pp. 939-944 2) COETZER et al. J Clin Invest, 1981, Vol. 67, No. 5, pp. 1241-1248 3) MARCHESI et al. J Clin Invest, 1987, Vol. 80, No. 1, pp. 191-198 4) PETERSON et al. Am J Clin Pathol, 1987, Vol. 88, No. 1, pp. 58-65 5) LECOMPTE et al. Br J Haematol, 1990, Vol. 76, No. 3, pp. 406-413

Membrane defects (cont.)

Condition	Specific Laboratory Test	Reference
Hereditary spherocytosis	Osmotic fragility test (OFT) (especially HS)	1) PARPART et al. J Clin Invest, 1947, Vol. 26, No. 4, pp. 636-640 2) YOUNG et al. Blood, 1951, Vol. 6, No. 11, pp. 1073-1098
	Autohaemolysis test (HS)	1) SELWYN et al. Blood, 1954, Vol. 9, No. 5, pp. 414-438 2) YOUNG et al. Blood, 1956, Vol. 11, No.11, pp. 977-997 3) GRIMES et al. Br J Haematol, 1968, Vol. 14, No. 3, pp. 309-322
	Acidified glycerol lysis test (AGLT) (HS)	1) GOTTFRIED et al. J Lab Clin Med, 1974, Vol. 83, No. 2, pp. 323-333 2) ZANELLA et al. Br J Haematol, 1980, Vol. 45, No. 3, pp. 481-486 3) RUTHERFORD et al. Br J Haematol, 1986, Vol. 63, No. 1, pp. 119-121 4) ACHARYA et al. Br J Haematol, 1987, Vol. 65, No. 3, pp. 343-345
	Pink test (HS)	1) VETTORE et al. Acta Haematol, 1984, Vol. 72, No. 4, pp. 258-263 2) JUDKIEWICZ et al. Am J Haematol, 1987, Vol. 26, No. 1, pp. 89-91 3) FERNÁNDEZ-FUERTES et al. Sangre (Barc.), 1989, Vol. 34, No. 6, pp. 509-513
	Spectrin in red cells (espescially HS)	1) Zwieten et al. NTvG, 1995, Vol. 4, No.44, pp. 2256-2261. Dutch
Hereditary spherocytosis South Asian ovalocytosis	Cryohaemolysis test (CHT) (HS, positive results also for SAO)	1) STREICHMAN et al. Am J Haematol, 1990, Vol. 35, No. 2, pp. 104-109 2) ROMERO et al. Arch Med Res, 1997, Vol. 28, No. 2, pp. 247-251 3) STREICHMAN et al. Am J Hematol, 1998, Vol. 58, No. 3, pp. 206-212
Hereditary spherocytosis South Asian ovalocytosis	Eosin-5-maleimide (EMA) binding test (HS, positive results also for SAO)	1) KING et al. Br J Haematol, 2000, Vol. 111, No. 3, pp. 924-933 2) KING et al. Br J Haematol, 2004, Vol. 124, No. 1, pp. 106-113 3) KING et al. Cytometry B Clin Cytom, 2008, Vol. 74, No. 4, pp. 244-250

PNH

Condition	Specific Laboratory Test	Reference
PNH	Ham test (acidified serum lysis test)	1) Ham TH. New Engl J Med 1937;217:915-7.
	Sucrose lysis test (sugar-water test)	1) Hartman RC, Jenkins Dej. New Eng J Med 1966;275:155-7. 2) Hartman RC et al. Blood 1970;35:462-75.
	Flow cytometry for CD55 and CD59	1) Hall SE, Rosse WF. Blood, 1996;87(12):5332-40. 2) Richards et al. Cytometry 2000;42(4):223-33.
	FLAER assay (fluorescently labeled aerolysin test)	1) Brodsky RA, et al. Am J Clin Pathol 2000;114:459-66. 2) Sutherland DR, et al. Cytometry B Clin Cytom 2007;728:167-77.

Miscellaneous

CONDITION	SPECIFIC LABORATORY TEST
Congenital Diserythropoietic anaemia	Sequencing SEC23B PAGE Band3 low mw HEMPAS
Diamond-Blackfan anaemia	

Iron assessment

Condition	Specific Laboratory Test	Reference
Iron assessment	Serum ferritin - IRMA RIA ELISA IFT LIA - Nephelometric immunoassay - Immunoturbidimetric assay	1) CAZZOLA et al. Br J Haematol, 1983, Vol. 53, No. 4, pp. 659-665 2) FINCH et al. West J Med, 1986, Vol. 145, No. 5, pp. 657-663 3) ADDISON et al. J Clin Path, 1972, Vol. 25, No. 4, pp. 326-329 4) WORWOOD. CBC Crit Rev Clin Lab Sci, 1979, Vol. 10, No. 2, pp. 171-204 5) CONRADIE et al. S Afr Med J, 1980, Vol. 57, No. 8, pp. 282-287 6) PALENCIA-DOMINGUEZ et al. Eur J Clin Chem Clin Biochem, 1997, Vol. 35, No. 2, pp. 117-120 7) BORQUE et al. Clin Chem Lab Med, 1999, Vol. 37, No. 9, pp. 899-905
	Transferrin - Radial immunodiffusion - Immunonephelometric assay - Immunoassay - Immunoturbidimetric assay	1) MANCINI et al. Immunochemistry, 1965, Vol. 2, No. 3, pp. 235-243 2) KREUTZER. J Clin Chem Clin Biochem, 1976, Vol. 14, No. 8, pp. 401-406 3) EL GUINDI et al. Am J Clin Nutr, 1988, Vol. 47, No. 1, pp. 37-41
	Serum transferrin receptor - Immunoassay - RIA - IEMA - Immunoturbidimetric assay - Immunonephelometric assay	1) KOHGO et al. Br J Haematol, 1986, Vol. 64, No. 2, pp. 277-281 2) KOHGO et al. Blood, 1887, Vol. 70, No. 6, pp. 1955-1958 3) SOUMINEN et al. Clin Chem, 1997, Vol. 43, No. 9, pp. 1641-1646 4) ALLEN et al. Clin Chem, 1998, Vol. 43, No. 9, pp. 1641-1646 5) AKESSON et al. Scan J Clin Lab Invest, 1999, Vol. 59, No. 2, pp. 77-82 6) COTTON et al. Clin Biochem, 2000, Vol. 33, No. 4, pp. 263-267
	Serum iron and total iron binding capacity (TIBC)	1) RAMSAY. Clin Chim Acta, 1957, Vol. 2, No. 3, pp. 221-226 2) International Committee for Standardization in Haematology, Br J Haematol, 1978, Vol. 38, No. 2, pp. 281-287 3) YAMANISHI et al. Clin Chem, 1997, Vol. 43, No. 12, pp. 2413-2417 4) SIEK et al. Clin Chem, 2002, Vol. 48, No. , pp. 161-166 5) KASVOSVE et al. Clin Chem Lab Med, 2002, Vol. 40, No. 10, pp. 1014-1018

Iron assessment (cont.)

Condition	Specific Laboratory Test	Reference
Iron assessment	Liver iron concentration (SQID or MRT); Myocardial iron concentration (MRT)	1989, In Chanarin I, ed *Laboratory Haematology: an account of laboratory techniques,* 1st ed., pp. 90-91, Churchill Livingstone (reference covers liver biopsy iron only).
	Urine ferrioxamine iron (especially for iron overload)	1) FIELDING. J Clin Path,1965, Vol. 18, pp. 88-97 2) SINGH. J Clin Pathol, 1967, Vol.20, No. 3, pp. 257-259 3) ARTS et al. Clin Chem, 1984, Vol. 30, No. 1, pp. 155
	Serum haptoglobin	1) OWEN et al. J Clin Pathol, 1960, Vol. 13, pp. 163-164 2) OWEN et al. J Clin Pathol, 1960, Vol. 13, No. 6, pp. 478-482 3) ROWE. J Clin Pathol, 1961, Vol. 14, pp. 205-206 4) RATCLIFF et al. J Clin Path, 1964, Vol. 17, pp. 676-679 5) BERNIER. Clin Chim Acta, 1967, Vol. 18, No. 2, pp. 309-312
	Zinc-protoporphyrin	1) BLUMBERG et al. J Lab Clin Med, 1977, Vol. 89, No. 4, pp. 712-723 2) INOUE. Osaka Med Coll, 1989, Vol. 35, No. 1-2, pp. 49-60 (reference not available in most libraries)
	Bone marrow iron stain	
	Diagnostic tests for HSA	
	HFE mutations	

General use tests

Test Name
Complete blood count
Blood film morphology
Reticulocyte count
Unconjugated bilirubin
Conjugated bilirubin
Total bilirubin
Serum haptoglobin
LDL levels
Serum LDH
Folates
Cobalamin
Urine hemosiderin
Haemoglobinuria
Ultrasound of abdomen
Spleen examination

Annex 3. ENERCA Working group on Rare Anaemias (EGRA)

Institution	Acronym	Country	Contact person
Hôpital Erasme- Université Libre de Bruxelles	ERASME	Belgium	Béatrice Gulbis
Thalassaemia International Federation	TIF	Cyprus	Androulla Eleftheriou
			Michael Angastiniotis
Centre Hospitalier Universitaire de Montpellier	CHU-MTP	France	Patricia Aguilar Martinez
Universität of Ulm	UULM	Germany	Hermann Heimpel
Università degli Estudi di Milano	UNIMILANO	Italy	Andrea Mosca
Fondazione IRCCS Ospedale Maggiore Policlinico	IRCCS	Italy	Paola Bianchi
Hospital Clínic de Barcelona	CLINIC	Spain	Juan Lluis Vives Corrons
			María del Mar Mañú Pereira
Universidad de Deusto	UD	Spain	Carlos Romeo
			Pilar Nicolás
Universitair Medisch Centrum Utrecht	UMCU	The Netherlands	Richard van Wijk
UK National External Quality Assessment Service	UKNEQAS	United Kingdom	Barbara De la Salle

Annex 4

Cover Letter – Questionnaire on the legal and ethical problems existing in each MS (ENQUE- LEGAL -I)

Dear Mr. / Ms.,

We are contacting you on behalf of the ENERCA Project, supported by the European Commission's Seventh Framework Programme. The main objective of the ENERCA Project is to establish a European Reference Network of Expert Centres in Rare Anaemias.

The activities of our Work Package in this project are related to the legal and ethical issues involved in creating this network.

We are sending you this questionnaire in order to gain as much insight as possible into the common practices in this area. We would be very grateful if you would read the attached protocol and answer the questions related to your activities.

Based on the results of this questionnaire we will identify the items that should be studied from the legal and ethical perspectives and we will make a comparative analysis of the criteria followed by different centres. These conclusions will be sent to the legal experts of ten Member States, who will use them as a basis for a legal study that will have a homogeneous scheme.

These national reports will be studied by the WP 1 coordinator and the final result will be a comparative analysis.

Conclusions will be communicated to all partners and will be discussed in order to include them in the final white paper on the network.

We would be very grateful if you could send the questionnaire back before 28 February.

We look forward to hearing from you soon,

Protocol for answering the questionnaire (ENQUE- LEGAL-I)

Purpose of the study

One of the goals of ENERCA'S Work Package 1 is to analyse the legal and ethical issues of creating an international network of centres in the area of medical diagnosis.

This analysis will be an important tool for identifying problems and also for suggesting solutions. Finally, we will try to establish several criteria that a centre should follow in order to participate in the ENERCA network.

To achieve this goal we are going to prepare a report with the collaboration of several national legal experts, but it is essential that we have an accurate vision of the situation as basis of this work, and this is the aim of the questionnaire we would like you to answer.

Methodology

As WP 1 leader, the Interuniversity Chair in Law and the Human Genome has developed a list of items to be included in the questionnaire that have been reviewed by the other WP leaders.

With this collaboration, we have tried to work in a multidisciplinary way, including ethical, legal and biomedical perspectives. Since 1993, the Chair has been a centre specialising in studying, teaching and disseminating legal and ethical issues of biomedicine in general and genetics in particular. The other partners involved in developing the questionnaire also have a biomedical profile.

The questionnaire was designed to be answered quickly. You only have to tick the boxes and include a brief comment if you think it is necessary or appropriate.

Please take into account that we need the answers from the daily practice perspective.

Variables

the content of the questionnaire is structured in seven groups of items that refer to the following matters:

- Checking quality standards in the international circulation of samples or data;
- Organisation issues involved in data transfer and patient mobility.
- Personal Data Issues
- Obtaining, use, transfer, storage and destruction of biological samples
- Referring patients
- Genetic Counselling
- Legislation knowledge

Further analysis of the data

The staff of the Chair in Law and the Human Genome participating in Enerca, will study the answers given on the questionnaire, in order to identify the items that should be studied from the legal and ethical perspectives and to have a comparative idea of the criteria followed by different centres.

These conclusions will be sent to the national legal experts of ten Member States, who will use them as a basis for a legal study that should have a similar outline.

These national reports will be analysed by the WP 1 coordinator. The final result will be a comparative analysis.

Neither the name of the institution nor the identification of any person is necessary at any stage of the process for our objective. Once we have received the completed questionnaire, we will destroy any link between the person or institution sending it and the answers themselves.

Enque-Legal-I

Enerca Project. Wp1 Questionnaire

Information about your centre

– Name and type of institution
  Private ☐
  Public ☐
– Type of structure
  Clinical department ☐
  Diagnosis laboratory ☐
  Research laboratory ☐
  Other (please specify) _____
– Country _____
– Professional profile of the person answering this questionnaire _____

1. Quality Standards

1.1. Do you send patient samples or personal data only after checking if the centres that will receive them follow quality standards?

Yes ☐
No ☐

1.2. Do you accept samples or data only after checking that the centres that have sent them follow quality standards?

Yes ☐
No ☐

2. Organisation Issues

2.1. Is your institution aware of the data transfer and patient mobility from your centre to institutions in other countries?

Yes ☐
No ☐

2.2. Is there any specific procedure established in your centre describing this type of circulation?

Yes ☐
No ☐

2.3 Is there an Ethical Committee in your institution?

Yes ☐
No ☐

3. Personal Data Issues

3.1. Do you send personal data of patients to other national or international institutions?

Yes ☐
To institutions that participate in the diagnosis and therapy of the individual patient ☐
To institutions that do not participate in the diagnosis and therapy of the individual patient ☐
No ☐

3.2. Do you send codified data of patients?

Yes ☐
No ☐

3.3. Do you store personal data of patients that you have received from other centres?

Yes ☐
Paper ☐
Electronic format ☐
No ☐

3.4. For how long do you store this type of data? Please describe the criteria followed to establish this period, if they exist

☐ Years

3.5. Do you apply any security measures in these cases?

Yes ☐
(Please describe)
No ☐

3.6. Do you use the data for research studies?

Yes ☐
We use codified data ☐
We keep the codes ☐
We destroy the codes ☐
We use the data of identified patients ☐
No ☐

Do you ask for the patient consent to transfer the data to other countries?

Yes ☐
No ☐

4. Samples

4.1. Do you ask for patient consent to analyse the samples with diagnostic purposes, or do you check that the consent has been obtained by the physician or other health professional?

Yes ☐

If yes, Is there any standard information and consent form?

Yes ☐

No ☐

Who signs it?

Patient or patient's representative ☐

Physician/institution providing the sample ☐

Other: _____

(Please attach the form used by your institution) Yes ☐ No ☐

No ☐

4.2. If you receive samples for diagnosis that you are also going to use for research purposes, do you ask for patient consent to this secondary use?

Yes ☐

If yes, is there any standard information and consent form?

Yes ☐

No ☐

Who signs it?

Patient or patient's representative ☐

Physician/institution providing the sample ☐

Others: _____

(Please attach the form used by your institution) Yes ☐ No ☐

No ☐

4.3. Do you ask for the patient consent to send his/her samples to other centres for diagnostic purposes?

Yes ☐

No ☐

4.4. Do you ask for the patient consent from the centre from which you have received the sample?

Yes ☐

No ☐

4.5. If yes, what information do you give to the patient when requesting a patient's consent to use the sample for diagnostic purposes?

☐ 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) The patient's right to choose whether or not to receive communication regarding the data obtained in the research

☐ f) Information regarding the implication of the results for the patient's family members

☐ g) The possibility that, when appropriate, this information can be communicated to the family members, in case it is relevant for their health

- ☐ h) Indicating the possibility to get in contact with the patient, and the way to do so
- ☐ i) Information regarding the data will be stored
- ☐ j) dentification and contact details of the person responsible for the storage of data
- ☐ k) Procedure the patient has to follow to exercise rights regarding data storage
- ☐ l) Provisions about international circulation of data
- ☐ m) Provisions about international circulation of samples
- ☐ Other items

_____

_____

4.6. What information do you give to the patient when asking for consent to use the sample for research purposes?

- ☐ a) Purpose of the research, especially if DNA or RNA data are going to be obtained.
- ☐ b) Expected benefits
- ☐ c) Possible inconveniences linked to donating and obtaining the sample
- ☐ d) Identity and contact details of the person responsible for the research
- ☐ e) The patient's right with regard to revoking 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 right to gain access to the obtained data
- ☐ h) Guarantee of confidentiality of the information obtained
- ☐ i) Information regarding the implications of genetic analysis
- ☐ j) Patient's right to decide whether or not to receive communications regarding the data obtained from the research
- ☐ k) Information regarding the implication of the results for the patient's family members
- ☐ l) The right that the person has to, where appropriate, transmit this information to family members, in case it would be relevant for their health
- ☐ m) Indicating the possibility to get in contact with the patient, 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 the patient has to follow to exercise rights regarding data storage
- ☐ q) Provisions about international circulation of data
- ☐ r) Provisions about international circulation of samples
- ☐ Other items

_____

_____

4.7. Where do you store documents of consent?

In our department ☐

In the patients clinical record stored in the centre ☐

Other _____

4.8. For how long do you store the samples? Please describe the criteria followed to establish this period, if it exists

☐ Years_____

4.9. Do you have protocols regarding the quality process of obtaining, storing and transferring samples?

Yes ☐

No ☐

5. Patients

5.1. Are patients referred to your centre from other centres?

Yes ☐

No ☐

5.2. Do you refer patients to other centres?

Yes ☐

No ☐

6. Genetic Counselling

6.1. Is genetic counselling available in your centre?

Yes ☐

No ☐

6.2. Is this service accredited?

Yes ☐

No ☐

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

Yes ☐

No ☐

6.4. Are the professionals in the centre/s accredited as geneticists?

Yes ☐

No ☐

6.5. In case of an inherited disease, do you offer the possibility of genetic counselling?

Yes ☐

No ☐

7. Legislation

7.1. Do you know if there is any regulation in your country concerning the use of samples for research or diagnostic purposes? If so, please specify

Yes ☐

No ☐

7.1. Do you know if there is any international or national legislation in your country regarding the international circulation of samples for diagnosis? If so, please specify.

Yes ☐

No ☐

7.2. Do you know if there is any international or national legislation in your country regarding the international circulation of patients? If so, please specify.

Yes ☐

No ☐

7.3. Do you know if there is any international or national legislation in your country regarding the international circulation of data? If so, please specify.

Yes ☐

No ☐

Annex 5

Cover Letter – Legal and ethical survey for the analysis of current transnational regulations regarding exchanges of patients, biological samples and data between different Member States (MS)

Dear xxxxx,

We are contacting you on behalf of the ENERCA Project, supported by the European Commission's Seventh Framework Programme. The main objective of the ENERCA Project is to establish a European Reference Network of Expert Centres in Rare Anaemias.

The activities of our Work Package in this project are related to the legal and ethical issues involved in creating this Network.

We have sent a questionnaire to several centres in Europe in order to gain as much as possible insight into the common practices in this area.

Based on the results of this questionnaire we will identify the items that should be studied from the legal and ethical perspectives and we will make a comparative analysis of the criteria followed by different centres. These conclusions will be sent to national legal experts of some Member States, who will use them as the basis for a legal study that will have a homogeneous scheme.

The purpose of this letter is to invite you to take part in the legal study as an expert in ethical and legal issues relating to bio-medicine.

At present, we are compiling responses to the initial questionnaire. Once we have analysed the questionnaires, we will prepare a structure for the reports. The reports will need to be prepared during May, June and July 2010.

We will compile all this material and draw general conclusions. During this stage we may well need your help in resolving doubts and making small revisions.

If you are interested, we will send you further details of the work involved.

We look forward to receiving your reply.

Regards

Dear colleague,

I am contacting you again in order to give you further details of your participation in the Enerca project.

First of all, I would like to explain that **the main objective of ENERCA 3 is the establishment of a European Reference Network (ERN) of Expert Centres (EC) in Rare Anaemias (RA).** Identifying appropriate centres of expertise (Expert Centres) in each national territory involved in ENERCA 3 Project is one of its most important goals. The ERN will establish a link between the ECs and serve as a platform to foster their participation, respecting national competences and regulations regarding their authorisation or recognition.

Once established, the ERN will be responsible for organising care pathways for patients suffering from rare anaemias by promoting cooperation between relevant experts and professionals so that they can share expertise, whether they are located within the same country or abroad. The ECs will include in their plans or strategies the conditions required to disseminate and mobilise expertise and knowledge in order to facilitate the treatment of patients located near them. One of the most important tools for ENERCA activities will be its web site (www.enerca.org), established by ENERCA 1 and consolidated by ENERCA 2. The web site will also be very useful as it will provide support for the use of information and communication technologies, such as telemedicine, when necessary to ensure remote access to the specific health-care required.

It is clear that the success of a network dedicated to improving the diagnosis and treatment of patients with Rare Diseases (RD) depends of the feasibility of easy communication between the involved MS, not only by Internet but also other means, such as translational referral procedures. It is well known that clear differences in legislation exist between the different MS which sometimes make this practice very difficult.

We will try to find the best way to overcome the existing legal barriers and to facilitate cross border healthcare for patients with rare anaemias. To accomplish this WP1 will first define the current state of European Community and national regulations for cross border healthcare and in a second phase, will seek to find a consensus on the criteria needed to be considered an expert centre within the ERN.

As a first step, a questionnaire has been sent to centres in order to detect problems in practice and with this information we have prepared a list of items to be analysed from a legal perspective. T**his legal analysis is going to be carried out by several legal experts in the framework of their own national legislation.** With the information they provide, the Interuniversity Chair will prepare a comparative study. This will be the basis to establish a protocol for every centre in the net.

We are very grateful for your participation in this stage, and we hope our work will help create a tool that helps improve the care of patients with rare anaemias. We also hope this project will be a model to develop other networks related to other rare diseases.

We are sending you **the model to be followed in the national reports.** As you will see, in some cases we just ask for a very short answer (yes/no) and in other cases we ask for a brief description. Please, give concrete and brief answers /analyses, in order to facilitate a comparative study. **Please, return your report before 31 July 2010.**

Once we received your reports, we will **contact you again** in case it is necessary to clarify any point.

National Ethical / Legal Report

COUNTRY:
AUTHOR:
INSTITUTION:

1. Introduction. Legal framework

- Specific or general regulations on patient circulation in Europe (legal references and a brief summary)
- Specific or general regulations on data or biological sample exchange (legal references and a brief summary)
- Specific regulation on electronic clinical records
- Specific regulation on genetic counselling (legal references and a brief summary)
- Does accreditation on genetic counsellors exist in your country?
- Specific or general provisions about quality standards for clinical departments/ laboratories
- Is there regulation on Ethical Committees? (Legal references and a brief summary)
- Is there any legal distinction in this area between private or public centres?
- Are the legal provisions for the centres, the professionals, the clinical department, the diagnosis laboratory, the research laboratory, others?

2. Personal Data Issues

- Definitions: personal data / codified data/ anonymous data
- Period of storage for clinical data
- Security measures for the storage of health data (paper or electronic format)
- Provisions on the transfer of clinical data: national or international
- Conditions for using the personal / codified / anonymised data for research studies: consent, intervention of an ethics committees.....

3. Samples

- Conditions to obtain and analyse samples for diagnostic purposes (information, consent....)
- Period of storage of samples for diagnostic purposes
- Who is in charge?
- Does the laboratory have to check that consent has been obtained by the physician or other health professional?
- Is consent necessary to send samples to other centres for diagnostic purposes?
- What information should be provided to the patient when taking samples for diagnostic purposes?

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) Where the analysis will take place and the destination of the sample after analysis is completed: storage for diagnostic purposes codification, destruction, use for research purposes
d) Guarantee of confidentiality of the information obtained
e) The patient's right to decide if they will receive information on the data obtained from the research
f) Information regarding the implication of the results for the patient's family members
g) The right of the patient to, where appropriate, transmit this information to family members, in case it is relevant to their health
h) Indicating the possibility of contacting the patient, and the way to do so
i) Information regarding the data that will be stored
j) I dentification and contact details of the person in charge of storing the data
k) Procedure for the patient to exercise rights regarding data storage
l) Provisions about international circulation of data
m) Provisions about international circulation of samples

Other items

- Conditions to obtain and analyse samples for research purposes
- Is it necessary to obtain specific consent to use a sample that had been obtained for diagnosis for research ?
- What information should be given to the patient when asking for consent to use the sample for research purposes?

a) Purpose of the research, especially if DNA or RNA data are going to be obtained.
b) Expected benefits
c) Possible inconveniences involved with donating and obtaining the sample
d) Identity and contact details of the person responsible for the research.
e) The patient's right to revoke the consent, and the effects this may have
f) Location where the analysis will be carried out and the destination of the sample at the end of the research: codification, destruction or other research
g) The patient's right to gain access to the obtained data
h) Guarantee of confidentiality of the information obtained
i) Information regarding the implications of genetic analysis
j) The patient's right to decide whether or not to be informed about the data obtained in the research
k) nformation regarding the implication of the results for the patient's family members
l) The right the patient has to , where appropriate, transmit this information to family members, when it is relevant to their health
m) Indicating the possibility of contacting the patient and the way to do so.

n) Information regarding the data that will be stored
o) Identification and contact details of the person responsible for the storage of data
p) Procedure the patient must follow to exercise rights regarding data storage
q) Provisions about international circulation of data
r) Provisions about international circulation of samples

Other items

- Where should the document/s of consent be stored? In the department, in the patients clinical record stored in the centre, other..
- For how long should the samples be stored?

4. The Proposal for a Directive of the European Parliament and of the Council on the application of patients' rights in cross-border healthcare

- http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri= COM:2008:0414:FIN:EN:PDF
- Implications for the national health system and for patients
- Implications for international diagnosis networks

5. Other points

Annex 6

Cover Letter – Survey on general laboratory requirements for basic diagnosis and clinical management of patients with RA (ENQUE-TECHNICAL-I)

TO BE TRANSLATED INTO THE LANGUAGE OF THE COUNTRY

In name of ENERCA http://www.enerca.org, a network co-funded by the European Commission through its Public Health and Consumer Protection Directorate (DG SANCO), PHEA programme, we would like to invite you to participate in a European survey.

In view of empowering patients with rare anaemias, our objectives include creating a map of centres that take care of patients with rare anaemias in Europe and building a directory of facilities available per centre for patients with rare anaemias.

There are no questions regarding trials but rather the idea is to create an overview of the current situation.

This inventory will allow the creation of new networks, facilitating exchange between centres not only within a country but also located in different European countries. This will be extremely helpful, as a rare disease in one European country may be more common in another and shared expertise will be greatly appreciated and prompt collaboration can occur via the network.

All participants will receive a report on this work.

The ENERCA website will list the centres that are identified. Information published on the ENERCA website or via any other medium will only be made with your consent.

In name of ENERCA

...

Name of contact person who will distribute the questionnaire in his country (region)

Enque-Technical-I

European survey: facilities for patients with rare and very rare anaemias

Name:
Job Title:
Institution:
Department :
Address:
Zip code:
City:
Country:
Phone Number:
Fax No.:
Website:

Section 1 – facilities for sickle cell disorders and thalassaemias

This section of the questionnaire does not apply to my centre

With regard to sickle cell disorders and thalassaemias, do you consider your centre to be:

☐ A reference centre
☐ A general centre

In your country, do reference centres exist (either within a framework of a national policy on rare diseases, or outside national policy)?

☐ Yes
☐ No

Is your centre part of a rare disease national network?

☐ Yes
☐ No

Type of patients treated (please tick all that apply):

☐ Children under age 18
☐ Adults
☐ Children and adults
☐ Out-patients
☐ In-patients
☐ Out- and in-patients
☐ Patients from your city or region only
☐ Patients from your country
☐ Patients from other countries (please specify)

Clinic: Average number of patients treated annually:

children _____ / year

adults _____ / year

Total Registered patients: Sickle Cell Syndromes _____

Thalassaemia Syndromes: _____

Diagnosis centres: Average number of specimens received:

For care _____ / year

For advice _____ / year (confirmation or final diagnosis after first line tests are obtained in another centre)

Phenotype _____ / year

Genotype _____ / year

The expertise covered in your centre

(Please tick all that apply)

- ☐ **The areas of expertise not covered in our centre are available in other expert centres**
- ☐ **A close collaboration exists with those centres**
- ☐ **No collaboration exists with other centres**

1. Diagnosis – prevention

- ☐ Phenotypic diagnosis of Haemoglobin Disorders
- ☐ Genotypic diagnosis of Haemoglobin Disorders
- ☐ Neonatal screening
  - ☐ Local
  - ☐ National
- ☐ Antenatal screening
- ☐ Pre-marital screening
  - ☐ Local
  - ☐ National
- ☐ Genetic counselling
- ☐ Pre-natal diagnosis
- ☐ Pre-implantation diagnosis
- ☐ Subject to external quality control (QC)
  - ☐ Phenotype, name of the external QC: …….
  - ☐ Genotype, name of the external QC: …….

2. Follow-up/case management

Acute events - Special services in your centre

Does the centre have staff with specific expertise in dealing with acute events of Haemoglobin disorders (e.g., pain control, stroke)?

- ☐ Yes
- ☐ No

Allocated beds:

- ☐ Day unit
- ☐ Hospital
- ☐ Day unit and hospital

Trained, dedicated staff:

- ☐ Nurses
- ☐ Medical doctors
- ☐ Psychologist
- ☐ Neurosurgical unit
- ☐ Imaging staff

Blood transfusions:

Do you perform an extended immuno-phenotype (beyond ABO and D)?

- ☐ Yes
- ☐ No

Do you have access to donor red blood cell units with rare phenotypes?

- ☐ Yes
- ☐ No

Do you have:

- ☐ Access to exchange blood transfusion (trained staff)
- ☐ 24-hour service

3. Follow-up/case management

Chronic events – special services in your centre

Does the centre have staff experienced in monitoring, preventing and dealing with chronic complications of Haemoglobin disorders?

- ☐ Yes
- ☐ No

Please tick the areas of case monitoring/follow-up that are offered by your centre:

- ☐ Chronic pain
- ☐ Stroke (prevention), please give an example _____
- ☐ Stroke (care)
- ☐ Leg ulcer
- ☐ Osteonecrosis
- ☐ Bone disease (e.g. osteoporosis)
- ☐ Chronic renal disease
- ☐ Pulmonary disease, please give an example _____
- ☐ Cardiac disease
- ☐ Eye complications
- ☐ Hearing complications
- ☐ Liver complications
- ☐ Growth
- ☐ Endocrine complications
- ☐ Pregnancy
- ☐ Contraception - fertility
- ☐ Iron overload, please give an example _____
- ☐ Psychological support

Are patients monitored by specialists in:

- ☐ Cardiology?
- ☐ Liver disorders?
- ☐ Endocrinology?

Criteria of expertise in your centre

1. Availability of specialised services, able to deal with SCD/thalassaemia complications (please tick all that apply):

- ☐ Intensive care unit
  - ☐ Trained in sickle-related organ damage and multiple organ damage
- ☐ Transcranial echo-doppler
- ☐ MRI
- ☐ Angio MRI
- ☐ CT
- ☐ Angiofluorography
- ☐ Audiometry
- ☐ Assessment of cardiac iron by T2* MRI
- ☐ Measurement of liver iron concentration:
  - ☐ Biopsy
  - ☐ MRI
  - ☐ SQUID

2. Availability of treatments:

- ☐ Stem cell transplantation
- ☐ Hydroxyurea
- ☐ Iron chelation
- ☐ Transfusions
  - ☐ With rare blood phenotype
- ☐ Blood bank
- ☐ Exchange transfusion
  - ☐ Automated
  - ☐ Manual

3. Availability of patient services:

- ☐ Psychology services
- ☐ Link with education services (i.e., coordinating with school for educational problems)
- ☐ Link with other social services (e.g., employment, social security)
- ☐ Link with patients' associations

4. Availability of decision support (guidelines)

  - ☐ SCD
  - ☐ thalassaemia
  - ☐ Available electronically
  - ☐ Available in booklet form
- ☐ Standard follow-up of patients with SCD
  - ☐ Children

- ☐ Adults
- ☐ Management of chronic pain
- ☐ Management of chronic complications
- ☐ Acute blood transfusion management
- ☐ Chronic blood transfusion management
- ☐ Management of pregnancy
- ☐ Pre- and peri-operative surgery management
- ☐ Criteria for hospitalisation
- ☐ Diagnosis of complications
- ☐ Treatment of complications
- ☐ Pain management
- ☐ Blood transfusions management
- ☐ Pre- and peri-operative surgery management

5. Availability of registries:

- ☐ Epidemiological surveillance
- ☐ Clinical research

6. Link with research:

- ☐ Yes
- ☐ No

7. High level of expertise and experience documented:

- ☐ Through publications
- ☐ Grants
- ☐ Teaching and training activities

Section 2 – facilities for very rare anaemias:

This section of the questionnaire does not apply to my centre

Or:

- Red blood cell membrane disorders
- Red blood cell enzyme disorders
- Congenital Dyserythropoietic Anaemia (CDA)
- Diamond Blackfan Anaemia (DBA)
- Paroxysmal Nocturnal Haemoglobinuria (PNH)
- Hereditary Sideroblastic Anaemia (HSA)
- Very rare anaemias due to defective iron utilization

With regard to very rare anaemias, do you consider your centre to be:

- ☐ A reference centre
- ☐ A general centre

In your country, do reference centres exist (either within a framework of a national policy on rare diseases, or outside national policy)?

- ☐ Yes
- ☐ No

Is your centre part of a rare disease national network?

☐ Yes
☐ No

Type of patients treated (please tick all that apply):

☐ Children under age 18
☐ Adults
☐ Children and adults
☐ Out-patients
☐ In-patients
☐ Out- and in-patients
☐ Patients from your city or region only
☐ Patients from your country
☐ Patients from other countries (please specify)

Clinic: Average number of patients treated annually:

children _____ / year
adults _____ / year

Total registered patients with:
Red blood cell membrane disorders: _____
Red blood cell enzyme disorders: _____
CDA: _____
DBA: _____
PNH: _____
HSA: _____
Very rare anaemias due to defective iron utilisation: _____
Other anaemias (please specify): _____

Diagnosis centres: Average number of specimens received:

For care _____ / year
For advice _____ / year (confirmation or final diagnosis after first line tests are obtained in another centre)
Phenotype –_____ / year
Genotype –_____ / year

The Expertise Covered In Your Centre

(Please tick all that apply)

☐ **The areas of expertise not covered in our centre are available in other expert centres**
☐ **A close collaboration exists with those centres**
☐ **No collaboration exists with other centres**

4. Diagnosis – prevention

☐ Genetic counselling, please specify
☐ Pre-natal diagnosis, please specify
☐ Pre-implantation diagnosis, please specify

- ☐ Subject to external quality control (QC)
  - ☐ Phenotype, name of the external QC: _____
  - ☐ Genotype, name of the external QC: _____

5. Follow-up/case management

Acute events - Special services in your centre

Does the centre have staff with specific expertise in dealing with acute events of very rare anaemias (e.g., consequences of iron overload, hemolytic crises, aplastic crises)?

- ☐ Yes
- ☐ No

Allocated beds:

- ☐ Day unit
- ☐ Hospital
- ☐ Day unit and hospital

Trained, dedicated staff:

- ☐ Nurses
- ☐ Medical doctors
- ☐ Psychologist
- ☐ Neurosurgical unit
- ☐ Imaging staff

Blood transfusions:

Do you perform an extended immuno-phenotype (beyond ABO and D)?

- ☐ Yes
- ☐ No

Do you have access to donor red blood cell units with rare phenotypes?

- ☐ Yes
- ☐ No

Do you have:

- ☐ Access to exchange blood transfusion (trained staff)
- ☐ 24-hour service

6. Follow-up/case management

Chronic events – special services in your centre

Does the centre have staff experienced in monitoring, preventing and dealing with chronic complications of very rare anaemias?

- ☐ Yes
- ☐ No

Please tick the areas of case monitoring/follow-up that are offered by your centre:

- ☐ Leg ulcer
- ☐ Osteonecrosis
- ☐ Bone disease (e.g., osteoporosis)

- ☐ Chronic renal disease
- ☐ Pulmonary disease
- ☐ Cardiac disease
- ☐ Eye complications
- ☐ Hearing complications
- ☐ Liver complications
- ☐ Growth
- ☐ Endocrine complications
- ☐ Pregnancy
- ☐ Contraception - fertility
- ☐ Iron overload, please give an example _____
- ☐ Psychological support
- ☐ Other, please specify

Are patients monitored by specialists in:

- ☐ Cardiology
- ☐ Liver disorders
- ☐ Endocrinology
- ☐ Other (please specify)

Criteria of expertise in your centre

8. Availability of specialised services, able to deal with complications of very rare anaemias (please tick all that apply):

- ☐ Intensive care unit
- ☐ MRI
- ☐ CT
- ☐ Audiometry
- ☐ Assessment of cardiac iron by T2* MRI
- ☐ Measurement of liver iron concentration:
  - ☐ Biopsy
  - ☐ MRI
  - ☐ SQUID

9. Availability of treatments:

- ☐ Stem cell transplantation
- ☐ Iron chelation
- ☐ Phlebotomy
- ☐ Transfusions
  - ☐ With rare blood phenotype
  - ☐ Exchange transfusion
    - ☐ Automated
    - ☐ Manual

10. Availability of patient services:

- ☐ Psychology services
- ☐ Link with education services (i.e., coordinating with school for educational problems)
- ☐ Link with other social services (e.g., employment, social security)
- ☐ Link with patients' associations

11. Availability of decision support (guidelines)

- ☐ Available electronically for patients
- ☐ Available electronically for health professionals
- ☐ Available in booklet form for patients
- ☐ Available in booklet form for health professionals

- ☐ Standard follow-up of patients with very rare anaemias
  - ☐ Children
  - ☐ Adults
- ☐ Management of chronic pain
- ☐ Management of chronic complications
- ☐ Acute blood transfusion management
- ☐ Chronic blood transfusion management
- ☐ Management of pregnancy
- ☐ Pre- and peri-operative surgery management
- ☐ Criteria for hospitalisation
- ☐ Diagnosis of complications
- ☐ Treatment of complications
- ☐ Pain management
- ☐ Blood transfusions management
- ☐ Pre- and peri-operative surgery management

12. Availability of registries:

- ☐ Epidemiological surveillance
- ☐ Long term follow up for clinical care and decision making
- ☐ Collection of material for research and teaching
- ☐ Clinical research

13. Link with research:

- ☐ Yes
- ☐ No

14. High level of expertise and experience documented:

- ☐ Through publications
- ☐ Grants
- ☐ Teaching and training activities

Annex 7

Cover letter - Survey on specific laboratory tests for Red Cell Blood Enzyme and Membrane Disorders for rare microcytic anaemias

Dear colleagues,

As you probably know, ENERCA is an EU-funded project that involves the creation and maintenance of a European Network for Rare and Congenital Anaemias. One of the goals of the 3rd phase of this project involves the creation of an overview of the Expert Centres in very rare anaemias in Europe, and the facilities available in those centres.

On behalf of the "Very rare anaemias due to red cell membrane defects and metabolism disorders" Working Group we ask your cooperation with regard to this matter. To this purpose, we would like to perform an inventory of all red cell osmotic fragility tests or other diagnostic tests that are performed in Expert Centres in Europe with the ultimate goal of establishing a common guideline for the diagnosis of these diseases.

If you are interested, please complete the attached questionnaire and return it to us by mail or fax. (div_emat@policlinico.mi.it Fax +390255033439).

Furthermore, we would be very thankful if you would forward this questionnaire to other centres in your country involved in the diagnosis of these diseases. We will keep you updated on the results of the survey.

Thank you in advance for your cooperation.

Best regards,

Alberto Zanella
Prof. Alberto Zanella
Foundation IRCCS Ca' Granda Ospedale Maggiore of Milan
Hematology 2 Unit
Via Francesco Sforza, 35
20122 Milan
Italy
FAX +390255033439
Phone +39 0255033471

Survey on specific laboratory tests for Red Cell Blood Enzyme and Membrane Disorders for rare microcytic anaemias

ORGANISATION: .....
DEPARTMENT: .....
CONTACT PERSON: NAME ..... **SURNAME** .....
CITY/TOWN ..... **ADDRESS** .....
ZIP CODE ..... **COUNTRY** .....
PHONE (including country and area code) .....
FAX ..... **e-MAIL** .....
PROFESSION (e.g., physician, researcher)..

Red cell disorders	Mean number of new laboratory diagnoses per year	Number of patients in regular follow-up
Hereditary spherocytosis		
Hereditary elliptocytosis		
Pyropoikilocytosis		
Stomatocytosis		
Congenital dyserytropoietic anemias type II		
Glucose-6-phosphate dehydrogenase deficiency		
Pyruvate kinase deficiency		
Other red blood cell enzyme deficiencies		
Other red blood cell membrane disorders		

Red cell membrane disorders

Diagnostic tests performed (please indicate if performed in your lab (A) or elsewhere (B)*

A/B		When they are used		
		In all cases	Only when…	Never used
	Red cell Morphology on peripheral blood smears	☐		☐
	Availability of "image bank" yes ☐ no ☐			

A/B		When they are used		
		In all cases	Only when…	Never used
	Osmotic Fragility tests - on fresh blood - on pre-incubated (37°C) blood - two different NaCl concentrations - Curve (no …. of different NaCl concentration)	☐ ☐ ☐ ☐		☐ ☐ ☐ ☐
	Glycerol Lysis Test (GLT)	☐		☐
	Acidified Glycerol Lysis Test (AGLT)	☐		☐
	Cryohemolysis tests	☐		☐
	Pink test	☐		☐
	Flow cytometric EMA-binding assay	☐		☐
	Other commercial tests	☐		☐
	SDS-PAGE of red blood cell membrane proteins	☐		☐
	Ektacytometer	☐		☐
	Laser-assisted Optical Rotational Cell Analyzer (LORRCA)	☐		☐
	RBC/Reticulocytes automated parameters(MCV, MCHC, IRF..)	☐		☐
	Molecular characterization at the DNA/RNA level			
Method with best specificity and sensitivity in your experience				

Red cell enzyme disorders

	Total number of deficiencies detected	Enzymatic assay method used**)	Molecular characterization
Enzymes of glycolysis			
Hexokinase (HK)			☐ yes
Glucosephosphate isomerase (GPI)			☐ yes
Phosphofructokinase (PFK)			☐ yes
Glyceraldehyde-3-phosphate dehydrogenase			☐ yes
Phosphoglycerate kinase (PGK)			☐ yes
Pyruvate kinase (PK)			☐ yes
Triosephosphate isomerase (TPI)			☐ yes
Lactate dehydrogenase (LDH)			☐ yes
Aldolase			☐ yes
Enolase			☐ yes
Bisphosphoglycerate mutase (BPGM)			☐ yes
Monophosphoglycerate mutase (MPGM)			☐ yes
Phosphogluconate mutase (PGM)			☐ yes
Enzymes of hexose-monophosphate shunt and glutathione metabolism			
Glucose-6-phosphate dehydrogenase (G6PD)			☐ yes
6-phosphogluconate dehydrogenase (6-PGD)			☐ yes
Gamma-glutamylcysteine synthetase (GCS)			☐ yes
Glutathione synthetase			☐ yes
Glutathione reductase (GR)			☐ yes
Glutathione peroxidase (GSH-Px)			☐ yes
Glutathione S-transferase (GST)			☐ yes
Enzymes of nucleotide metabolism			
Adenylate kinase (AK)			☐ yes
Pyrimidine-5' nucleotidase			☐ yes
purine/pyrimidine nucleotides ratio			☐ yes
Other red blood cell enzyme activities			
NADH diaphorase			☐ yes
NADPH diaphorase			☐ yes
Superoxide dismutase (SOD)			☐ yes
Catalase			☐ yes
Other….			☐ yes
Glycolysis intermediates		**Method used****	
Glucose-6-phosphate (G6P)	☐ yes		
Fructose-6-phosphate (F6P)	☐ yes		
Fructose- bi-phosphate (FBP)	☐ yes		
Dihydroxyacetone-phosphate (DHAP)	☐ yes		
Glyceraldehyde-3- phosphate (GAP)	☐ yes		
2.3-diphosphoglycerate (2.3 DPG)	☐ yes		
3-phoshoglyceric acid (3PGA)	☐ yes		
2-phosphoglyceric acid (2PGA)	☐ yes		
Phosphoenolpyruvate (PEP)	☐ yes		
Adenosinemonophosphate (AMP)	☐ yes		
Adenosine biphosphate (ADP)	☐ yes		
Adenosintriphosphate (ATP)	☐ yes		

	Total number of deficiencies detected	Enzymatic assay method used**)	Molecular characterization
Total glutathione (GSSG+GSH)	☐ yes		
Pyruvate (PYR)	☐ yes		
Lactate (LACT)	☐ yes		
☐ What percentage of patients remain undiagnosed after testing and exclusion of other causes of hemolytic anaemias?			
☐ Do you maintain a register of patients with red cell membrane and/or enzyme disorder?			
Please add any additional comments you wish to make regarding the diagnosis of red cell membrane or enzyme disorders:			

* Name of the centre
**spectrophotometric method, commercial kits, …..

Please return the questionnaire by 30 November 2011 to Paola Bianchi: div_emat@policlinico.mi.it or by fax at +39 02 55033439

Annex 8

EQALM Haematology WG: Outline proposal for new work item

European survey of existing EQAS for rare diseases diagnostic tests

Title

The provision of External Quality Assessment Schemes (EQAS) for the diagnostic tests associated with rare anaemias in Europe

Aims and objectives

1. To survey EQA providers within European Union member states to determine the provision of EQAS for rare and congenital anaemias.
2. To discuss with EQA providers the use of reference methods for haematological parameters within EQAS and how IVDD companies use reference methods in the calibration and development of instruments and kits.
3. To work in collaboration with the European Network for Rare and Congenital Anaemias (ENERCA) to produce a collated catalogue of relevant EQAS.
4. To present the outcome of the survey at the EQALM annual meeting, 2011.

Background

The ENERCA project (www.enerca.org) has operated for nearly 10 years, as 3 work projects, with the support of the European Parliament. The aims of the project are to improve the provision of care for individuals with rare and congenital anaemias, including the haemoglobinopathies, thalassaemia syndromes, red cell enzymopathies, red cell membrane disorders, erythropoietic disorders, metabolic disorders of iron and acquired haemolytic disease. For many of these conditions, the number of patients in any one member state is very small with only a few laboratories providing diagnostic testing. In these cases, the development of pan-European EQA may be the only means by which standardisation of methods and results can be achieved.

Method

ENERCA has established a list of core laboratory tests that are used in the diagnosis of rare and congenital anaemias. This project will survey EQAS providers within Europe and members of EQALM to identify the EQAS provision for tests that are included in this core list. EQAS providers will also be asked to provide details of the EQAS, such as the nature of the survey material, the performance criteria, the region covered etc. In addition, the survey will determine whether the EQAS provider is willing or able to

accept participants from other member states or regions, and whether they are willing to work with other EQAS providers to establish pan-European services. The use of higher order reference methods, where available, to determine target values in EQA and to calibrate IVDDs will also be examined as part of the survey.

The results of this survey will be used to collate a catalogue of available EQAS within Europe, in collaboration with the ENERCA project. This catalogue will be an information resource for providers of diagnostic testing for rare anaemias in Europe and will identify the most important areas for EQA collaboration and development.

Timescale

This survey will be undertaken in the early part of 2011, and the report will be available for initial consultation and comment in June 2011. The work item will be reported at the EQALM 2011 meeting.

Barbara De la Salle
On behalf of the EQALM Haematology WG
November 2010

Questionnaire to european eqa providers

ENQUE-Harmonisation-2

Dear Colleague,

The European Network for Rare and Congenital Anaemias (ENERCA) is undertaking a questionnaire on the provision of external quality assessment for investigations used in the diagnosis and monitoring of patients with rare anaemias.

ENERCA has created a core list of laboratory tests that are used in the diagnosis of haemoglobin disorders, thalassaemia disorders, red cell membrane disorders, enzymopathies and other rare anaemias. We are now keen to establish a comprehensive database of EQA provision in these areas to improve the quality of laboratory performance and to identify where additional EQA provision is required.

We would like to invite you to complete a questionnaire on the services that you provide in this field. The questionnaire can be accessed from the following link (*to be completed once set up*). If you have any queries or problems, please contact Barbara De la Salle (haem@ukneqas.org.uk) or the ENERCA office (enerca@enerca.org).

This initiative is supported by EQALM and UK NEQAS.

Thank you for your time,

Barbara De la Salle, UK NEQAS General Haematology
Professor Andrea Mosca, University of Milan
On behalf of the ENERCA Executive Committee
November 2010

For information about ENERCA: www.enerca.org

ENERCA III –Questionnaire ENQUE-Harmonisation-2 To all European EQAS providers

(note this questionnaire will be delivered on-line and linked to the core laboratory tests database used in questionnaire)

Q1

a. Do you provide EQAS for any of the tests listed?
b. Would you agree to your scheme being listed as a provider of EQA for this test on the ENERCA website?
c. Do you think that there is a need for EQAS for this test?
    a. If yes - Would you be prepared to offer EQAS for this test in collaboration with another European EQA provider?
    b. If no – Why do you think there is no need?

Q2

For each test that you provide an EQAS:

a. How frequently do you distribute specimens?
b. What is the nature of the survey material?
c. What is the nature of the performance assessment?
d. How many participants take part in this survey?
e. Do you accept laboratories from other countries?
f. Is the programme accredited?
g. If it is accredited, give the name of the accreditation body.

Q3

About your EQA scheme:

a. Organisation name
b. Nature of organisation – private company, government organisation, charity
c. Scheme organiser
d. Contact details
    a. Address
    b. Phone
    c. Fax
    d. Email
    e. Website
    f. Name of person completing this questionnaire

Core list of tests

PLEASE COPY THE Q1 SHEET AND COMPLETE FOR EACH ANALYTE/MEASURAND FOR WHICH YOU PROVIDE AN EQA SCHEME

General Laboratory Tests	Hb disorders	Red cell enzyme disorders	RBC membrane disorders	PNH
Blood film morphology	Hb variant detection	Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Demonstration of red cell membrane proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (HS, HE, Stomatocytosis)	Ham test (acidified serum lysis test)
CBC (complete blood count)	HPLC	NBT spot test	Osmotic gradient ektacytometry (HS, HE, Stomatocytosis)	Sucrose lysis test (sugar-water test)
Reticulocyte count	Hb electrophoresis	Fluorescent spot test	Proportion of spectrin dimers and tetramers in red cell membranes (HE)	Flow cytometry for CD55 and CD59
Bilirubin	Iso-electric focusing	Cytochemical demonstration of G6PD deficiency	Tryptic digestion of spectrin (HE)	FLAER assay (fluorescently labeled aerolysin test)
Serum haptoglobin	Capillary electrophoresis	Quantitative assay for G6PD activity	Osmotic fragility test (OFT) (especially HS)	
LDL levels	Globin chain electrophoresis	Chromium inhibition test	Autohaemolysis test (HE)	
Serum LDH	Hb S screening tests - sickle brand soluing test, sickle solubility test	Molecular diagnosis - RFLP - ASO probes - SSCP - sequencing - RT-PCR-DGGE - HRM	Acidified glycerol lysis test (AGLT) (HS)	
Urine hemosiderin	Hb A2, Hb F, Hb S quantification	Pyruvate Kinase deficiency	Pink test (HS)	
Haemoglobinuria	HPLC Microcolumn chromatography Electrophoresis and elution Alkali denaturation	Spot test	Cryohaemolysis test (CHT) (HS, positive results also for TAO)	
Urine hemosiderin iron	Unstable haemoglobins: heat stability test isopropanol stability test	Quantitative assay for PK activity	Eosin-5-maleimide (EMA) binding test (HS, positive results also for SAO)	
Serum folate	Hb H bodies	Molecular diagnosis - RFLP - SSCP - sequencing	Molecular diagnosis (SSCP, RFLP, direct sequencing) (membrane defects in general)	
Red cell folate	Heinz bodies	Pyrimidine-5'-nucleotidase deficiency Radiometric assay Spectrophotometric assay HPLC		
Cobalamin	Kleihauer test (HbF distribution)			
Serum ferritin *IRMA, RIA, sLISA, IFT, LIA, nephelometric immunoassay, immunoturbidimetric assay,*	Flow cytometry for Hb F cells	Other red cell glycolytic enzyme deficiencies Quantitative assay: HK, GPI, PFK, Aldolase, TPI, PGK, BPGM, GDR, ADA, and AK		
Transferrin *radial immunodiffusion, immunonephelometric assay, immunoassay, immunoturbidimetric assay*	p50 measurement (oxygen affinity) for altered O2 affinity Hbs	Molecular diagnosis - PCR & direct sequencing		
Serum transferrin receptor *Immunoassay, RIA, IEMA, immunoturbidimetric assay , immunonephelometric assay*	2,6 - Dichlorophenolindophenol test for Hb E	Reduced glutathione (GSH) assay		
Serum iron and total iron binding capacity (TIBC)	Hb M detection using absorption spectra	Glutathione stability		
Liver iron concentration (SQID or MRT); Myocardial iron concentration (MRT)	Electrospray ionisation mass spectrometry	Methemoglobinemia		
Zinc-protoporphyrin	Globin synthesis in reticulocytes	Quantitative assay for met-fluoridase activity Molecular diagnosis		
Bone marrow iron stain	DNA analysis Alpha thalassaemia mutations Beta thalassaemia mutations Structural variants			

PLEASE LIST ANY ADDITIONAL TESTS HERE - APPLICABLE TO RARE AND CONGENITAL ANAEMIA DIAGNOSIS

General Laboratory Tests	Hb disorders	Red cell enzyme disorders	RBC membrane disorders	PNH

Questionnaire Part 1 – completed for each test provided

1,1 What is the name of the EQA programme or survey provided?

1,2 What tests from the 'core list of tests' are covered?

1,3 How frequently do you distribute specimens?

1,4 How many specimens are distributed each year?

1,5 What is the type of survey material provided?

1,6 How many participants are registered?

1,7 Do you accept participants from outside your own country?

Yes ☐

No ☐

1,8 Is there a higher order reference method for this test?

Yes ☐

No ☐

If yes, please give the reference if you can

Do you use the higher order reference method in your laboratory?

Yes ☐

No ☐

Do you know if IVDD manufacturers use this reference method to calibrate their kits or equipment?

Yes ☐

No ☐

1.9 How do you establish your target value?

Higher order reference method

Consensus of selected expert laboratories

Consensus mean or median of participants' results

Other

1.10 Do you provide performance assessment?

Yes ☐

No ☐

1.11 Do you have document that describes how this is done?

Yes ☐

No ☐

1.12 Is the programme accredited?

Yes ☐

No ☐

1,13 If yes, please give the name of the accreditation body

1.14 Would you agree to your scheme being listed on the European Network for Rare and Congenital Anaemias website (www.enerca.org)?

Yes ☐

No ☐

Questionnaire Part 2 – Completed by each EQA provider

Future EQAS development

2,1 Of the tests that you do NOT provide EQAS for, list the 5 that you think would benefit most from EQAS provision.

2,2 Would you be prepared to offer EQAS for rare anaemias in collaboration with another EQAS provider?

Yes ☐

No ☐

2,3 Are there any other tests that should be included in the core tests list?

About your EQA scheme

2,4 Organisation name

2,5 Nature of the organisation

- *Private company* ☐
- *Government organisation* ☐
- *Charity* ☐
- *Other* ☐

2,6 Name of the Scheme Organiser/Director

2,7 Address

2,8 Telephone number

2,9 Fax number

2.10 Email

2.11 Website

2.12 Name of the person completing this form

Annex 9

LIST OF PARTICIPANTS - Survey on general laboratory requirements for basic diagnosis and clinical management of patients with RA

Centre	Department	City	Country
CHR de la Citadelle	Pediatrie	Liège	Belgium
CHU Liège	Haematology	Liège	Belgium
Hôpital de Jolimont- Lobbes	PaediatricHaematology	La Louvière	Belgium
HUDERF	PaediatricHemato- Oncology	Brussels	Belgium
KUL-UZ Leuven	Haematology (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	Paediatric	Varna	Bulgaria
Nat. Spec. Hosp. Active Treatm. ofHemat.Diseases	Thalassaemia Department	Sofia	Bulgaria
Specialized ChildrensOncoHaematology 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 Paediatrics, Department of blood transfusion	Ulm	Germany
Charité-Universitätsmedizin Berlin	KlinikfürPädiatriem. S.Onkologie/ Hämatol./KMT	Berlin	Germany
University Düsseldorf	PaediatricHaematology / Oncology	Düsseldorf	Germany
University of Würzburg and Internal Medicine II (Red cell Lab)	Dep of Paediatrics	Wurzburg	Germany
Universitatsmedizin Gottingen (UMG)	Paediatrics	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. ofPaediatrics, Auth.	Thessaloniki	Greece
General Hospital of Athens "Laiko"	Thalassaemia and Haemoglobinopathies Centre	Ampelokipi, Athens	Greece

LIST OF PARTICIPANTS - Survey on general laboratory requirements for basic diagnosis and clinical management of patients with RA (Cont.)

Centre	Department	City	Country
University of Athens	First Dept of Paediatrics	Athens	Greece
U.O. di Pediatria ad indirizzo oncoematologico	Dipart. Integrato materno infantile Policlinico Modena	Modena	Italy
Meyer Paediatric Hospital	PaediatricOnco-Haematology department	Firenze	Italy
University of Catania	PaediatricHaematology and Oncology	Catania	Italy
Azienda Ospedaliero Universitaria Consorziale Policlinico di Bari	Division of Haematology and Oncology "F. Vecchio"	Bari	Italy
Azienda Ospedaliera-University of Padova	Clin. Of Pediatr. Hematol.- Oncol Departimento Pediatria	Padova	Italy
San Gerardo Hospital	Paediatric Haematology Department	MONZA	Italy
Agostino Gemelli Hospital	Paediatric Haematology 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 Paediatric	Brescia	Italy
Fond. IRCCS Cà Granda Osp. Maggiore Policlinico	UO Ematologia – UOS Patofisiologia dell'anemia	Milan	Italy
Catholic University of Sacred Heart	Haematology	Rome	Italy
Universita' degli studi di Cagliari		Cagliari	Italy
Ospedale Microcitemico-Istituto Superiore di Sanita'	Dep. Haematology 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	Haematology Division	Luxembourg	Luxembourg
Belfast City Hospital	Haematology	Belfast	Northern Ireland
Warsaw Medical University	Department of Paediatrics, Haematology and Oncology	Warsaw	Poland
Hospital InfantilUniversitario Nino Jesus	Servicio de Hemato-OncologiaPaediatrica	Madrid	Spain
Hospital Virgen del Puerto		Plasencia	Spain
H.U. "Puerta del Mar"	Haematology	Cadiz	Spain
Hospital de Cruces	Haematology	Baracaldo.Bizkaia	Spain
Hospital de Tortosa Verge DelaCinta	Haematology / Pathology	Tortosat	Spain
Huca	Haematology	Oviedo	Spain
Hospital de Sabadell	Paediatrics	Sabadell	Spain
K. General Yagüe (Burgos)		Burgos	Spain
Hospital La Candelaria Tenerife	Haematology	S/ Cruz de Tenerife	Spain
Hospital Universitario Central de Asturias	Haematology	OUJEDO	Spain
Hospital Valld'Hebron	PaediatricHaematology and oncology	Barcelona	Spain
Hospital Germans Trias. Badalona	Haematology	Badalona	Spain

LIST OF PARTICIPANTS - Survey on general laboratory requirements for basic diagnosis and clinical management of patients with RA (Cont.)

Centre	Department	City	Country
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	Haematolgy	LasPalmasde GranCanaria	Spain
Hospital de Tortosa Verge de la Cinta	Haematology	Tortosa	Spain
IMPPC-Instit. ofPredict.and Pers. Med of Cancer	Genetics and Epigenetics	Badalona. Barcelona	Spain
Hospital Sant Pau	PaediatricHaematology and Oncology, Ped. BMT Unit	Barcelona	Spain
Hospital Virgen de la Salud	Haematology	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	Paediatrics / Haematology	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	Haematology	Utrecht	The Netherlands
LUMC	Haematology	Leiden	The Netherlands
Sanquin	Blood Cell Diagnostics	Amsterdam	The Netherlands
UMC Utrecht	Haematology	Utrecht	The Netherlands
Haga Teaching Hospital	Haematology	The Hague	The Netherlands
University Medical Centre Groningen	Haematology (adult patients only)	Groningen	The Netherlands
AMC	Haematology	Amsterdam	The Netherlands
Erasmus MC	Haematology	Rotterdam	The Netherlands
Emma Children's Hospital AMC	Paediatrics 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

Annex 10

LIST OF PARTICIPANTS - Survey on red cell membrane disorders and enzyme defects

Centre	City	Country
CHU Liege, Human Genetics	Liege	Belgium
Hôpital Erasme, Clinical Chemistry	Brussels	Belgium
Specialized Hospital for Active Treatment of Haematologic Diseases, Laboratory of Cytogenetics and Molecular Biology	Sofia	Bulgaria
AP-HP, Hopital Bicêtre, Hematology Lab	Le Kremlin Bicetre	France
Centre Hospitalier Universitaire (CHU), Laboratoire de génétique moléculaire et biochimie	Paris	France
CHU of Montpellier, Laboratory of Haematology	Montpellier	France
AP-HP, GH Henri Mondor, Biochemistry and Génétique	Creteil	France
Robert Debré Hospital, AP-HP, Haematology laboratory	Paris	France
Universitätsmedizin Göttingen (UMG), Pädiatrie I	Göttingen	Germany
University of Würzburg (Universitätsklinikum Würzburg),Department of Paediatrics and Internal Medicine II (Red Cell Laboratory)	Munich- Würzburg	Germany
Zentrum für Kinder- und Jugendmedizin, Kinderheilkunde III, Universtitätsklinikum Heidelberg	Heidelberg	Germany
E.O. Ospedali Galliera, Ematologia, Centro della Microcitemia e Anemie Congenite	Genova	Italy
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, UO Ematologia, UOS Patofisiologia dell'Anemia	Milano	Italy
Istituto Superiore di Sanità, Dip. Ematologia, Oncologia e Medicina Molecolare	Roma	Italy
Ospedali Riuniti Villa Sofia Cervello, UOC Ematologia per le Malattie Rare del Sangue e degli Organi Ematopoietici	Palermo	Italy
Università di Napoli, Dipartimento di Pediatria, Seconda Università	Napoli	Italy
Università Cattolica del Sacro Cuore, Dipartimento di Ematologia	Roma	Italy
Università degli Studi Cagliari-Ospedale Microcitemico, 2a Clinica Pediatric	Cagliari	Italy
Università Federico II Napoli, CEINGE srl	Napoli	Italy
Medical University of Warsaw, Pediatric Hematology/Oncology	Warsaw	Poland
Centro Hospitalar de Coimbra - Hematology	Coimbra	Portugal
Hospital Clinic, University of Barcelona, Red Cell Pathology Unit	Barcelona	Spain
Hospital Clinico San Carlos – Servicio de Hematologia y Hemoterapia	Madrid	Spain
Sanquin Diagnostic Services, Red Cell Diagnostics	Amsterdam	The Netherlands
University Medical Center Utrecht, Clinical Chemistry and Haematology	Utrecht	The Netherlands
International Blood Group Reference laboratories, NHS Blood and Transplant, Membrane Biochemistry	Bristol	United Kingdom
King College Hospital, Red Cell Laboratory	London	United Kingdom

Annex 11

Cover letter- Patient Expectations Questionnaire

Enerca questionnaire into xxx- for expert medical centres

Dear Friends,

We are writing to you in order to respectfully request that you to take part in the ENERCA questionnaire that has been attached to this email. The questionnaire has been translated into xxx for your convenience.

We would really appreciate if you would take some time to thoroughly complete the questionnaire since it will benefit all patients with Thalassaemia and Sickle Cell Disease.

Could you please send the completed questionnaire to the TIF office by 19 July, 2 weeks from today?

As you are aware, TIF focuses considerable attention and effort on improving the quality of healthcare provided to patients with haemoglobin disorders all over the world and more recently in every European country. Last year we became partners in a European project called the "European Network for Rare Congenital Anaemias" (ENERCA), within which haemoglobin disorders constitute a significant part.

TIF represents the perspective of patients in this important project, whose main purpose is to identify the criteria and requirements that are necessary to recognise a medical centre as expert in the treatment and healthcare of patients with haemoglobin disorders. Your invaluable help is essential in achieving this goal. The attached questionnaire is the instrument that we are using to ensure that the perspective of patients is well represented in this project. Our ultimate purpose and objective is to be able to create in the near future, centres that are truly expert in the care of patients with haemoglobin disorders.

Thank you in advance for your feedback.

Kindest regards,

ENERCA Patient Questionnaire

"Patients' Needs and Expectations of Expert Centres in Haemoglobin Disorders"

This questionnaire should be answered by patients over 15 years old, or parents of patients under the age of 15. Please read the accompanying letter before answering. All information will be treated as confidential.

Please note: Section 3 (including 3a and 3b) is obligatory. Sections 1, 2 and 4 are optional, but it will be helpful it you complete them also.

Section 1 – About the patient (optional section)

Q1. Questionnaire completed by:

Patient ☐
Parent ☐
Other (e.g. relative, helper, patient association representative) Please specify:

Q2. Patient's age:

Q3. Patient's gender ☐ *Male* ☐ *Female*

Q4. Patient's marital status ☐ *Married* ☐ *Single* ☐ *Cohabiting*
☐ *Divorced* ☐ *Children (number: ___)*

Q5. Patient's occupation / education

University graduate	☐	*Student / school*	☐
Playschool / kindergarten	☐	*Infant (not yet in kindergarten)*	☐
Working part-time	☐	*Working full-time*	☐
Not working	☐	*If not working: through choice*	☐
		or inability to find a job	☐

Q6. Patient's ethnic origin:

Note: this information is important epidemiogical data. It is confidential and anonymous.

Q7. Patient's country of residence:

Section 2 – Your (the patient's) medical information (optional section)

Q8. Diagnosis

Thalassaemia major ☐
Thalassaemia intermedia ☐
HbH disease ☐
Sickle cell disease ☐
Sickle cell/b-thal ☐
Other ☐ *(specify: _____)*

Q9. Current transfusion regime

(a) Not transfused ☐

(b) Transfused: Frequency regular ☐ *occasional* ☐

(c) If regularly transfused, what is the usual pre-transfusion level? _____

Q10. Current chelation regime

Age chelation started? _____

Desferrioxamine (Desferal) ☐

Deferiprone (Ferriprox / L1) ☐

Deferasirox (Exjade) ☐

Combination ☐

I take it regularly as prescribed ☐

I do not take it regularly ☐

Any other comments:

For sickle cell patients only. If you are not a sickle cell patient, please continue to Section 3.

Q11. You were diagnosed:

Through newborn screening ☐ First 2 years of life ☐ Later ☐

Q12. Infection control (tick all that apply):

☐ Penicillin by mouth — Started at age: _____
Stopped at age: _____

☐ Pneumococcal vaccine — First dose given at age: _____
Last does given at age: _____

☐ H. influenzae vaccine — First dose given at age: _____
Last does given at age: _____

☐ I have been advised that fever above 38.5°C is a reason to seek urgent medical attention.

Q13. I have been instructed (tick all that apply):

☐ How to control pain at home
☐ When to go to hospital for pain control
☐ To drink a lot of fluids during a painful crisis

Q14. My treatment centre can provide the following services (tick all that apply):

☐ Transcranial Doppler
☐ MRI
☐ Tests for hearing
☐ Measurement of liver iron concentration
☐ Exchange transfusion

Section 3 – The medical services you (the patient) are currently using

Q15. Where do you receive medical treatment for your condition? (tick all that apply)

Specialised haemoglobinopathy centre ☐

General haematology department at a hospital ☐

General paediatric department of a hospital ☐

Private clinic/centre (non-specialist) ☐

Other (describe) ☐

Q16. How long do you usually wait for a transfusion to be set up?

Under 30 min ☐ 30-60 min ☐
1-2 hours ☐ 2-3 hours ☐ Longer ☐

Q17. Where are you transfused?

Haematology day unit ☐ Adult Haematology ward ☐
Children's ward ☐ Accident & Emergency ☐
Specialised Haemoglobinopathy unit ☐ Home ☐

Q18. When are you usually transfused?

Morning ☐ Afternoon ☐ Evening ☐ Overnight ☐ Weekend ☐

Q19. The treatment centre where you go for treatment is located:

Local/near where I live ☐
Another region / city ☐
Another country ☐ *If so, why do you go to another country treatment?*

Q20. Is access to the treatment centre (in terms of distance, cost etc.):

Very easy? ☐ *Easy?* ☐
Difficult? ☐ *Very difficult?* ☐
Not available? ☐ *Available but too expensive* ☐

Q21. Who pays for your treatment? (Tick all that apply)

Myself/my family ☐
Health insurance (private):Mine ☐ *My employer's* ☐
Health insurance (state) ☐
State-provided free healthcare ☐ *State-provided, partly free* ☐
Other model of payment ☐
Please describe:

Q22. What specialist(s) do you visit, in addition to your main treating doctor?

Internal medicine ☐ *Frequency of visits:*
Haematologist ☐ *Frequency of visits:*
Paediatrician ☐ *Frequency of visits:*
Heart specialist ☐ *Frequency of visits:*
Endocrinologist ☐ *Frequency of visits:*

(for checking growth and development, fertility, bone disease)

Psychologist ☐ *Frequency of visits:*
Liver specialist ☐ *Frequency of visits:*

Q23. Where do you see the specialist(s)? (Tick all that apply)

Same hospital ☐
Other hospital ☐

Q24. Who pays for your specialist referrals?

Myself/my family ☐
Health insurance (private): Mine ☐ *My employer's* ☐
State-provided free healthcare ☐ *State-provided, partly free* ☐
Other model of payment ☐
Please describe:

Any other comments:

Q25. If you are a sickle cell patient: Have you been given clear information about pain control at home?

Yes ☐ No ☐

Q26. How many days per year do you lose from education or work because of having thalassaemia or sickle cell disease?

None ☐ 1-5 days ☐ 6-10 days ☐ 11-15 days ☐ 16 or more days ☐

Q27. How easy do you find it to talk to friends and colleagues about having thalassaemia or sickle cell?

Very easy ☐ Easy ☐ Difficult ☐ Very difficult/impossible ☐

Q28. How do you find out what the correct treatment for your condition is?

From my doctor ☐ Other doctor ☐ Reading the protocol ☐
The association ☐ Other patients ☐ Other (e.g. internet) ☐

Q29. Do you think the treatment is correct and complete?

Yes ☐ No ☐ Not sure ☐

Any other comments?

Section 3a – Assessment of current services *

Please indicate to what extent the following statements are true. Please answer all the questions, even where they seem to repeat the same thing.

Q ≠	I am…	Never	Sometimes	Most of the time	Always
Q30	Given choices about treatment to think about?	☐	☐	☐	☐
Q31	Asked to talk about any problems with medication?	☐	☐	☐	☐
Q32	Given a written copy of my treatment plan?	☐	☐	☐	☐
Q33	Satisfied that care is well organised?	☐	☐	☐	☐
Q34	Asked to talk about my goals in caring for my condition?	☐	☐	☐	☐
Q35	Sure that my doctor considered my beliefs, habits, education and employment needs when recommending treatment?	☐	☐	☐	☐
Q36	Doctor helped to make a treatment plan I can carry out in may daily life?	☐	☐	☐	☐
Q37	Discussed how thalassaemia/SCD affects my daily life?	☐	☐	☐	☐
Q38	Contacted after a visit to the clinic to see how I am?	☐	☐	☐	☐
Q39	Encouraged to join a patients' association and other community activities?	☐	☐	☐	☐

Any other comments?

** Questions based on the PACIC (Patients' Assessment of Care for Chronic Conditions) questionnaire*

Section 3b – What would you like to see in a specialised Haemoglobinopathy centre?

Please indicate how important and/or useful you would find the following factors. Please answer all the questions, even where they seem to repeat the same thing.

Q ≠		Not necessary	Little use	Useful	Essential
Q40	Experience and technical support to diagnose and assess complications of thalassaemia & sickle cell?	☐	☐	☐	☐
Q41	A separate unit from other hospital departments?	☐	☐	☐	☐
Q42	Follow good clinical practice guidelines?	☐	☐	☐	☐
Q43	A coordinated team with an experienced doctor in charge?	☐	☐	☐	☐
Q44	A team including specialists in internal medicine, cardiology and endocrinology within the centre?	☐	☐	☐	☐
Q45	A doctor who understands my needs well	☐	☐	☐	☐
Q46	The presence of a psychologist/social worker in the centre?	☐	☐	☐	☐
Q47	Doctors and nurses stay the same over the years (no high turnover of staff)?	☐	☐	☐	☐
Q48	The centre is involved in research?	☐	☐	☐	☐
Q49	Enough staff to give me time with my doctor and reduce waiting time?	☐	☐	☐	☐
Q50	Staff to pay attention to me and my concerns?	☐	☐	☐	☐
Q51	Staff to take time to inform me and teach me how to take care of myself?	☐	☐	☐	☐
Q52	Doctors discuss treatment plans and gives me choices?	☐	☐	☐	☐
Q53	Staff informs me about my rights as a patient?	☐	☐	☐	☐
Q54	Staff guides me towards any social services, educational or leisure activities or support groups that may be useful to me?	☐	☐	☐	☐
Q55	The centre is in touch with and gives guidance to my general practitioner/local health services?	☐	☐	☐	☐
Q56	The centre communicates and collaborates with other specialised centres in the country?	☐	☐	☐	☐
Q57	The centre communicates and collaborates with other similar centres abroad?	☐	☐	☐	☐
Q58	The centre maintains close links with my support association?	☐	☐	☐	☐
Q59	Patients to have a voice (representation) in advisory committees of the centre/health authorities?	☐	☐	☐	☐

Any other comments?

Section 4 – What would you change? (Optional section)

Q60. What more (if anything) could your hospital do to help your treatment?

Q61.What more (if anything) could your doctor do to help your treatment?

Q56. What (if anything) would you change about your treatment?

Q62. What is the most helpful thing that nurse could do to help your treatment?

Q63. What would you like to see your association or support group to do for you?

Thank you very much for your input!

Please return this questionnaire to the Thalassaemia International Federation
PO Box 2880, 2083 Strovolos, Cyprus – Tel: +357 22 310 120 / Fax: +357 22 314 552
Email: thalassaemia@cytanet.com.cy

Annex 12. List of participants-Patient Expectations questionnaire

LIST OF PARTICIPANTS - Questionnaire on Patient Expectations

Patients' associations	City	Country
ALBANIAN THALASSAEMICS ASSOCIATION (ATA)	Tirana	Albania
ASSOCIATION BELGE DE THALASSEMIE ASBL	Villers-la-Ville	Belgium
THALASSAEMICS' ORGANIZATION IN BULGARIA	Sofia	Bulgaria
PANCYPRIAN ANTIANEMIC ASSOCIATION		Cyprus
ASSOCIATION FRANCAISE DE LUTTE CONTRE LES THALASSAEMIES (AFLT)	Ajaccio	France
THALASSAEMIEVEREIN ULM E.V.	Eislingen	Germany
GREEK THALASSAEMIA FEDERATION (EOTHA)	Athens	Greece
ASSOCIAZIONE LIGURE THALASSEMICI ONLUS (ALT)	Genova	Italy
ASSOCIAZIONE TALASSEMICI E DREPANOCITICI LOMBARDI ONLUS (ATDL)	Milano	Italy
ASSOCIAZIONE VENETA PER LA LOTTA ALLA TALASSEMIA (AVLT)	Rovigo	Italy
FONDAZIONE ITALIANA "L. GIAMBRONE" PER LA GUARIGIONE DALLA THALASSEMIA	Castelvolturno	Italy
THALASSAEMIA AWARENESS MALTESE ASSOCIATION (TAMA)	Qawra	Malta
ASSOCIACAO PORTUGUESA DE PAIS E DOENTES COM HEMOGLOBINOPATIAS	Almada	Portugal
ASOCIATIA PERSOANELOR CU THALASEMIE MAJORA	Bucuresti	Romania
ALHETA (ASOCIACION ESPANOLA DE LUCHA CONTRA LAS HEMOGLOBINOPATIAS Y TALASEMIAS)	Badajoz	Spain
AKDENIZ TALASEMI DERNEGI	Antalya	Turkey
TADAD - TALASSEMI DAYANISMA DERNEGI	Istanbul	Turkey
TALASEMI FEDERASYONU (THALASSEMIA FEDERATION OF TURKEY)	Antalya	Turkey
UNITED KINGDOM THALASSAEMIA SOCIETY (UKTS)	London	United Kingdom

Annex 13. Situation of expert centres in Rare Anaemias in France

1. Situation of expert centres in rare anaemias in France

In 2005, the French government launched a national plan for rare diseases, which considered rare diseases one of the five major priorities of the 2004 Law regarding public health policy. The main objective of this plan was "to ensure equal access to diagnosis, treatment and 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 partnerships1. 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. The reference laboratories were asked to form a national network in order to develop harmonised diagnostic steps, propose recommendations, organise training, set quality controls, give expert advice on atypical cases and ensure scientific and technology monitoring. The six laboratories selected were mainly specialised in the diagnosis of haemoglobin disorders. In order to cover all rare red cell disorders, laboratories working in other fields of red cell disorders that are not officially labelled as reference laboratories have progressively become involved in the network .

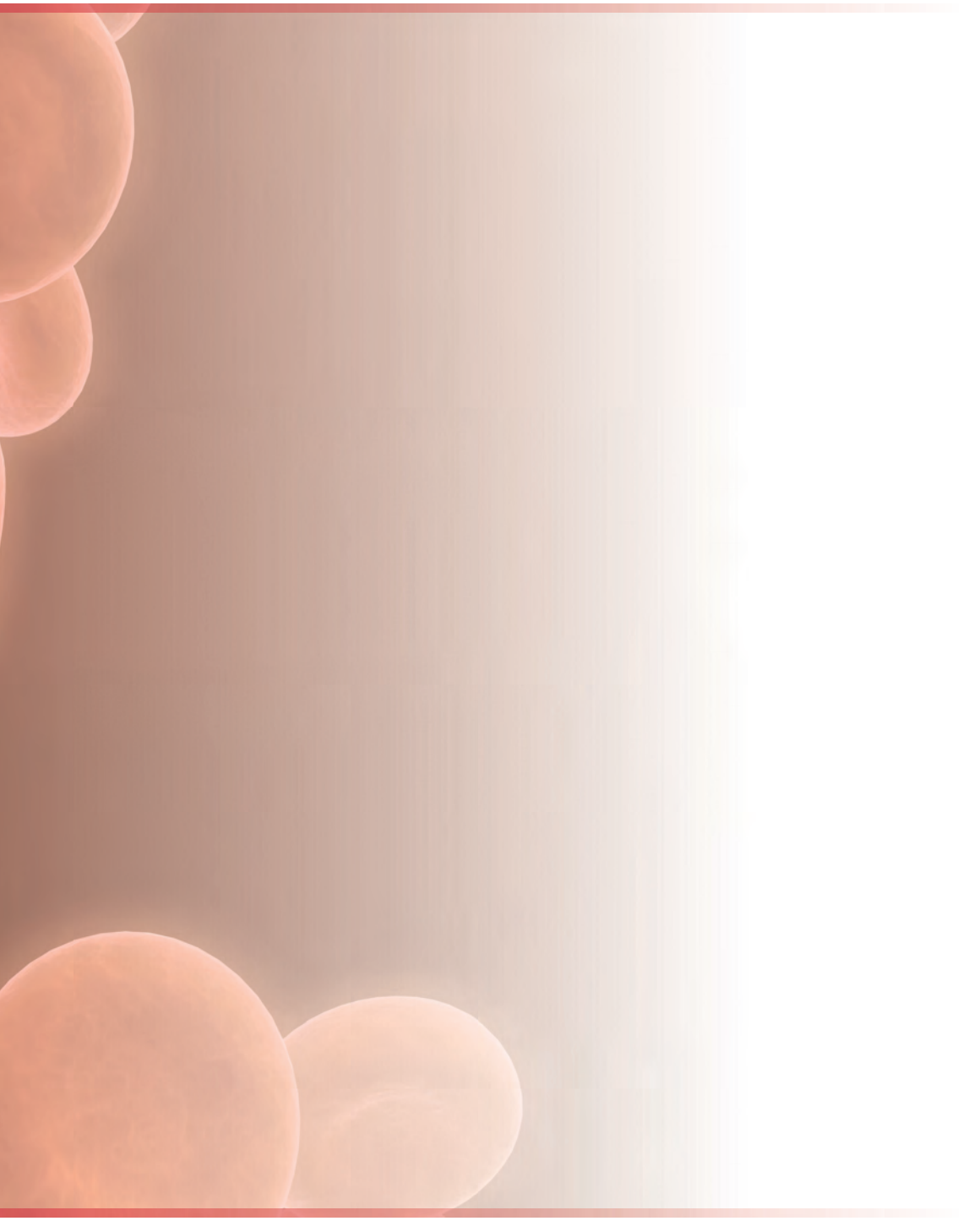
In the clinical field, the policy was to label two types of expert centres: only a few reference centres and then, in order to ensure a comprehensive healthcare coverage all over the country, to build a network of "competence centres" at the regional level. In the field of rare anaemias, two national "reference" centres on sickle cell disease and one on thalassaemia were named in 2004 and 2006. Fourteen centres of "competence" were later 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 officially nominated 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 organised in a network with periodical meetings.

Annex 14. List of official reference laboratories, centres of reference and centres of competence in France

Reference diagnosis laboratories*	
1.	Laboratoire d'hématologie, Hôpital St-Eloi, CHU de Montpellier, Pr 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.
* other diagnosis laboratories are specialised in specific rare anaemias	
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
Centres of competence on rare red cell disorders	
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

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



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