RADeep Clinical Study Protocol

Study Title:

A retrospective / prospective, multicenter European Epidemiological Platform for patients diagnosed with Rare Anemia Disorders (RADs) with clinical significance, in concrete Sickle cell disease and Thalassaemia disorders, and other rare defects of the red blood cell and erythropoiesis.

Acronym:

RADeep

Protocol Number/Date:

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Sponsor:

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Governance

Specific parts of governance are described in section 4. Organization and Responsibilities
The overall governance of RADeep is undertaken by a Consortium formed by the Institutions of the Principal Investigators (PIs) of the platform:

- Vall d'hebron Research Institute - Vall d'Hebron Research Institute - University Hospital Vall d'Hebrón (VHIR/HUVH), Barcelona, Spain. PI: Dr Maria del Mar Mañú Pereira
- Hôpital ERASME (ERASME), Brussels, Belgium. PI: Prof Béatrice Gulbis
- Cyprus Institute of Neurology and Genetics (CING), Nicosia, Cyprus. PI: Dr Marina Kleanthous

This consortium is RADeep's decision-making body regarding the following functions:

- To define RADeep's global strategy
- To define RADeep's Policy ensuring General Data Protection Regulation (GDPR) compliance
- To negotiate financial agreements with sponsors
- To develop the IT solution with adequate safeguards for secure data exchanging

VHIR/HUVH is responsible for the scientific protocol of the platform, ERASME is responsible for the medical writing and CING is responsible for the development of the IT platform and processing of data according to the Study protocol. The legal mechanism adopted for RADeep processing of personal data within the Consortium is under discussion by the Consortium's legal departments, based on a first proposal of Joint Controllership (art. 26 GDPR), where the three institutions conforming the Consortium jointly determine the purposes and means of processing and assume equal responsibilities in terms of data protection. Respective roles, rights and relations among parties forming the Consortium as well as responsibilities for compliance with the obligations under GDPR will be defined in the Consortium Agreement.

RADeep Steering Committee (SC) is composed by the Consortium PIs, one hematologist, one pediatrician, one laboratory specialist, one IT and statiscal specialist, one manager and two patients' representatives. RADeep SC is in charge of defining research protocol including research questions and definition of common data elements and their update on time as required, as well as drafting the Policy for data access and publishing.

RADeep Scientific subcommittees (SS) are responsible for the revision of the Research protocol of the platform for the disease specific area, including Data Elements and research questions defined. SS include experts on prevention, diagnosis and clinical care of the specific diseases.

RADeep Data Access Committee (DAC) is in charge of approving and implementing the Policy for data access and publications, ensuring the good use of data assets. The DAC has therefore a key role for reviewing and approving requests for data access received, as well as the anonymised figures published. The DAC is composed by the SC, representatives of the SS, and legal and ethical experts.

Update list of RADeep SC, SS and DAC are published on https://www.radeepnetwork.eu/

Steering Committee

Co-Prinicipal Investigators	**Institution**	**Country**
María del Mar Mañú Pereira	Vall d'Hebron Research Institute - Vall d'Hebron University Hospital	Spain
Béatrice Gulbis,	Hopital ERASME	Belgium
Marina Kleanthous	Cyprus Institute of Neurology and Genetics	Cyprus
Project manager	**Institution**	**Country**
Victoria Gutierrez	Vall d'Hebron Research Institute - Vall d'Hebron University Hospital	Spain
Data Management & Statistics	**Institution**	**Country**
Petros Kountouris	Cyprus Institute of Neurology and Genetics	Cyprus
Hematologist	**Institution**	**Country**
Eduard van Beers	University Medical Center Utrecht	Netherlands
Pediatrician	**Institution**	**Country**
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Laboratory specialist	**Institution**	**Country**
Paola Bianchi	Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico	Italy
Patients representatives	**Institution**	**Country**
Loris Brunetta	Associazione Ligure Thalassemici Onlus	Italy
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Protocol Synopsis

Study Title:

A retrospective / prospective, multicenter European Epidemiological Platform for patients diagnosed with Rare Anemia Disorders (RADs) with clinical significance, in concrete Sickle cell disease and Thalassaemia disorders, and other rare defects of the red blood cell and erythropoiesis.

Study Objectives:

To collect and to describe the demographics, disease-management, and treatment outcomes of patients diagnosed with RAD, ie. Sickle cell disease (SCD), Thalassaemia disorders, including Beta-thalassemia intermedia and major, Hemoglobin H disease and related thalassemic syndromes with clinical significance (THAL), Pyruvate Kinase Deficiency (PKD)[1] or with other RAD (rare defects of the red blood cell and erythropoiesis) according to the ORPHA classification.

To perform observational studies concerning relevant scientific research questions in SCD, THAL, PKD and other RAD using retrospective and prospective clinical data, and to present relevant research outcomes in the fields of health related to organ damage and risk stratification for identification of trial cohorts for newly developed classes of drugs and/or development of research projects.

To disseminate the results of the studies to all stakeholders involved, including patients, health care givers, health care authorities, pharmaceutical companies and health care professionals.

To promote harmonization and best practices in the prevention, diagnosis, treatment and follow-up of SCD, THAL, PKD and other RAD patients by the dissemination of reliable Guidelines and the translation of research results into clinical practice.

Methodology:

Data on patients with SCD, THAL, PKD and other RAD will be collected retrospectively and prospectively at the time of inclusion on the registry and at 12-month intervals for all registered patients. The data will be initially collected in nine European countries and will be combined in one central European Database Platform (Rare Anaemia Disorders European Epidemiological Platform) to be further expand to any EU country.

As defined in GDPR, a Joint Controllership is being established where the three institutions conforming the Consortium (VHIR/HUVH, ERASME and CING) for the jointly determine the purposes and means of processing and assume equal responsibilities in terms of data protection. Data processing and analyses will be conducted in various sub studies, after every 1000 patients included in the European Registry and/or at the end of each interim follow-up period (every 12 months).

Number of Patients & Centres

Data on patients with RADs will be collected at the EU level, starting in nine countries (Belgium, Cyprus, Denmark, France, Germany, Italy, Netherlands, Portugal and Spain) as a pilot to further expand to other EU Member States. Methodological approach will differ depending on the existence of already established national registry for SCD, THAL, PKD and other RAD (although partially implemented) or not.

Population:

The study population will consist of both males and females aged from 0 to 100 year old diagnosed as Rare anaemia disorder according to ORPHANET classification for Rare Anaemia (ORPHA 108997), which includes, among others, the following diseases:

- SCD: Sickle cell anaemia and other related sickle disorders

- THAL: Thalassaemia intermedia or major, including Transfusion dependent Thalassemias (β-thalassaemia major, severe HbE/β-thalassaemia, transfusion dependent HbH disease and surviving HbBart's hydrops) and the Non Transfusion Dependent Thalassemias (β-thalassaemia intermedia, HbE/β-thalassaemia, and HbH disease)

- PKD: Haemolytic anaemia due to pyruvate kinase deficiency
- RAD: Any other rare defects of the red blood cell and erythropoiesis

Study Duration:

An extensive recruitment period and follow-up, as well as geographical coverage, is desirable for long-term sustainability of the European Epidemiological Platform. Accordingly, RADeep has not an expected ending date but continue the pooling and processing of data for an indeterminate period of time.

[1] The abbreviation of SCD, THAL and PKD will cover all subgroups described in the study population, if not mentioned otherwise

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List of abbreviations

CT	Clinical Trial
DAC	Data Access Committee
ERN	European Reference Network
RAD	Rare Anaemia Disorders
RADeep	Rare Anaemia Disorders European Epidemiological Platform
RD	Rare Diseases
THAL	Thalassaemia syndromes
SCA	Sickle cell anaemia
SCD	Sickle cell disorders
SC	Steering Committee
SCT	Sickle cell trait
SS	Scientific Subcommittee

1. Introduction

Rare Anaemia Disorders (RADs) is a group of up to 132 disorders / groups of disorders according to ORPHA classification characterized by anaemia, meaning low levels of haemoglobin concentration, as the main clinical manifestation. As for other rare diseases (RDs), many unmet needs are present in RADs, management of RADs involve several medical and paramedical specialties, creating an impact in the health systems. However, health authorities need reliable epidemiological information for the shaping of policies aiming to ensure the cost effective allocation of resources. In the case of RADs, this is particularly important since expertise cannot be made available in every health unit with very small numbers of patients.

In addition, the lack of robust evidence hampers the translation of findings into clinical practice. With only few drugs currently available in clinical practice, there is a need of more comprehensive information on the natural history of the diseases or of specific organ complications in order to engage the research of new drugs that can prevent complications or cure symptoms and diseases.

Regarding diagnosis and patient management, methods for more prevalent RADs are well established and widely implemented in a harmonized way. However, when it comes to less frequent RADs, diagnosis and procedures are not always available even at the national level, and there is a lack of consensus methodology, guidelines and external quality assessment. This leads to a delay in the time of patient diagnosis and increase the number of undiagnosed or misdiagnosed patients.

Numbers of patients are rarely adequate in one centre or one country and, hence, pooling of patients in many countries is necessary. However, the lack of uniform standards for data collection has led to the implementation of a large number of registries through different approaches, gathering fragmented and scattered information on single disorders that have hampered the wide development of collaborative research projects and/or clinical trials.

All in all, a European approach for the standardized collection of data regarding the main clinical complications of RADs is fundamental to establish the need and the priorities in the development of research projects, clinical trials, guidelines and health policies that allow the better provision of healthcare to RADs patients. Research on new treatment options and development of clinical trials could be planned covering several RADs groups, thus increasing the target group and the robustness of evidence base.

Haemoglobinopathies (mainly sickle cell and thalassaemia disorders) are genetic disorders that, in their severe forms, are associated with chronic, life-impairing and -threatening conditions with inherent serious health sequelae that can lead to disability or even death.

Sickle cell disorders (SCD) SCD is consequence of 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. SCD includes a group of conditions in which HbS is the major abnormal protein involved in the clinical disease. SCD is inherited in autosomal recessive pattern, 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, HbSDPunjab, HbSOArab, HbSβ°thalassaemia, HbSβ+thalassaemia. There is a wide range of clinical presentation and severity. Polymerisation is dependent on intra-erythrocytic HbS concentration, the degree of haemoglobin deoxygenation, pH and the intracellular concentration of HbF. The polymer is a rope-like fibre that aligns with others to form a bundle, distorting the red cell into characteristic sickled forms. These deformed sickle red cells can occlude the microvascular circulation producing vascular damage, organ infarcts, painful episodes and other symptoms associated with SCD. In the unscreened population, infants may present with sudden death from pneumococcal sepsis due to splenic hypofunction, or acute splenic sequestration, before a diagnosis is made.

The thalassaemias (THAL) are a group of RAD in which there is quantitative reduction of either of the two globin chains which make up the haemoglobin molecule. In beta thalassaemia (beta-Thal) there is reduced

production of beta globin chains due to mutations on the beta globin gene on chromosome 11. The result is a reduced production of the haemoglobin molecule and consequently anaemia. Beta thalassaemia is the most significant clinically although there is a variation in the severity of the clinical consequences.

Hereditary red blood cell enzyme defects are inherited disorders that disturb red blood cell metabolism. They ultimately may lead to a decreased red cell life span, causing haemolytic anaemia. Some enzyme deficiencies lead to haemolysis 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 haemolysis, a disorder designated hereditary nonspherocytic haemolytic 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. Pyruvate kinase deficiency (PKD) is the most common cause of HNSHA.

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.

Congenital dyserythropoetic anaemia (CDA) is a disease category consisting of a group of hereditary anaemia’s resulting from mutations of different genes. Although related by some common features, clinical appearance and hence methods of diagnosis as well as specific therapeutic measures are different for the different subtypes.

Hereditary erythropoietic failure or aplasia: Diamond Blackfan anaemia (DBA) and Fanconi Anaemia (FA). Diamond Blackfan anaemia (DBA) is a rare bone marrow failure syndrome characterized by severe normochromic macrocytic anaemia and reticulocytopenia, typically presenting in the first year of life. DBA is associated with an increased risk of malignancies, especially haematopoietic 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. DBA is inherited with an autosomal dominant transmission with an incomplete penetrance. Most cases are sporadic. Recently, some cases of mosaicism have been reported. Fanconi anaemia (FA) is a rare inherited syndrome characterized by bone marrow failure (BMF), congenital abnormalities and cancer predisposition. 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.

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. 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.

Hereditary non-sideroblastic anaemias due to iron defects are a group of disorders. Inside this group one can find the following ultra-rare 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 inherited as autosomal recessive genetic diseases.

1.1 Incidence and diagnosis

1.1.1. SCD

In Europe SCD is predominantly a disorder seen in immigrant and minority communities. In the carrier state otherwise known as sickle cell trait (SCT), HbS offers some protection against malaria infection hence it is most commonly seen in people originating from malarial endemic areas, and predominantly people of African origin. The gene however is seen in many other communities, it is present in many groups of Mediterranean, Middle Eastern origin; and Indian groups. Nevertheless, in addition to

immigrants within Europe, some indigenous Southern European people also carry the gene (i.e in Southern and North East Italy and in Greece and Albania). As a result of recent arrivals in Europe; there is increasing number of affected people especially in large urban centres leading to uneven distribution of SCD throughout Europe. These communities are affected by language, socio-cultural and economic barriers and may therefore experience difficulty with the status of their residence in the home country or asylum seekers, all these issues affect the provision of suitable services for adults and children with these conditions. Incidences come from neonatal screening programmes financed by the local or national authorities in public Health and implemented in five countries of the EU: Belgium (Brussels, Liège), England, Holland, France and Spain (Madrid and Catalonia). Table 1.

	Period	SCD
Belgium (2/3) (Brussels)	2013 1994-2013	1:2329 1:1437
England	2005-2013	1:1942
Holland	2007-2013	1:4762
France	1995-2013	1:1881 (selective)
Spain (Madrid)	2003	1:6137
Spain (Catalonia)	2013	1:3909

Table 1 Neonatal/newborn screening for sickle cell disease financed by national authorities within the European Union

When a systematic neonatal or newborn screening programme was implemented (All countries except France), the incidence of SCD ranged from 1:1942 to 1:6137 live births. Registries are the best tools to attend prevalence numbers but very few exist in member states. Prevalence is expected to substantially increase in the near future due to decreased mortality rate; mobility and migration flows can also be suggested as a contributing factor to their increase.

Recent data from Spanish Registry of patients with haemoglobinopathies demonstrates the increase of the affected population and the need to measure indicators for assessing the trends of the disease in the burden of national systems. Global data of SCD neonatal screening programmes and estimated prevalences are shown in figures 1 and 2 from ITHANET project data.

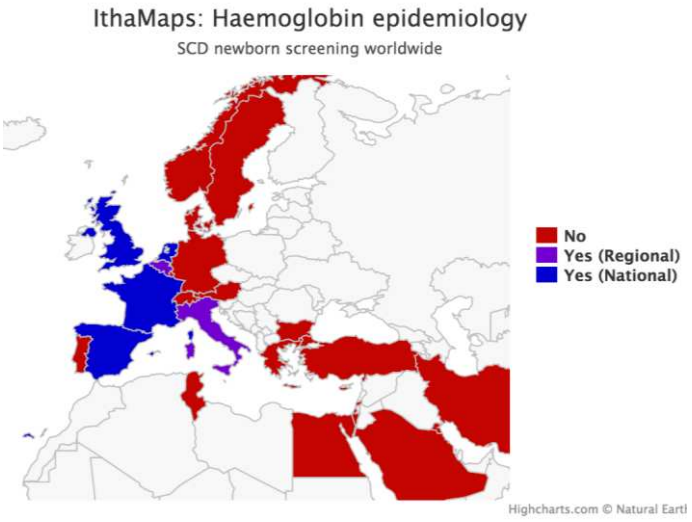

Figure 1 Map from IthaMaps showing status of NBS for SCD (worldwide map also available: (http://www.ithanet.eu/db/ithamaps?hb=2)

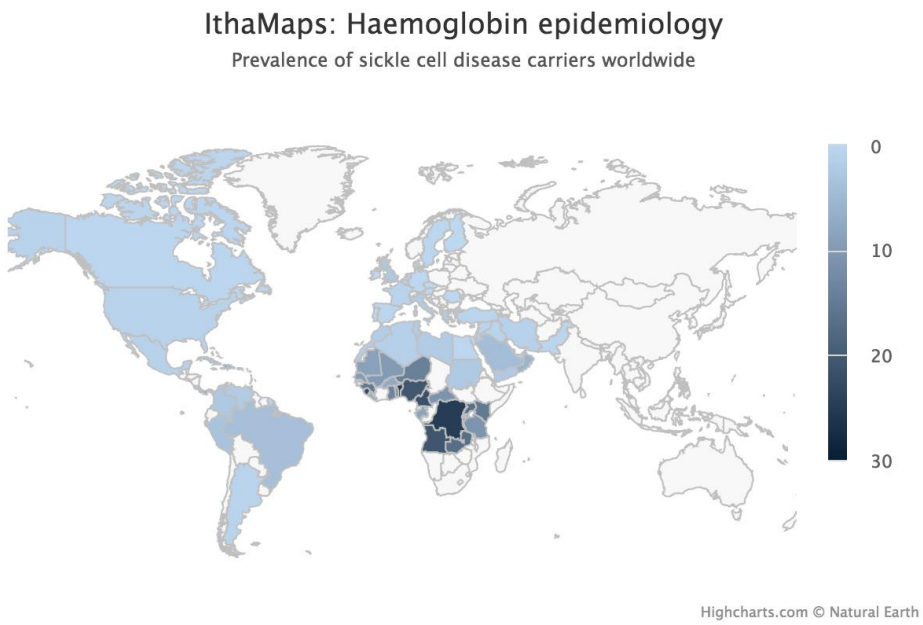

Figure 2. From IthaMaps (http://www.ithanet.eu/db/ithamaps?hb=2)

SCD Diagnosis and prevention: SCD is consequence of 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. SCD includes a group of conditions in which HbS is the major abnormal protein involved in the clinical disease. SCD is inherited in autosomal recessive pattern, 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, HbSDPunjab, HbSOArab, HbSβ°thalassaemia, HbSβ+thalassaemia. A full blood cell count, a separation of the haemoglobin fractions and quantification of abnormal haemoglobin fractions, Hb A2 and Hb F are the key parameters in screening for haemoglobinopathies. Antenatal and neonatal screening programmes are the best tools for the institution of early interventions including prevention and the delivery of suitable care for families and patients. Antenatal screening with a view to identify at-risk pregnancies is feasible; as evidenced by the national screening programme in England. Neonatal screening ensures early treatment to prevent premature mortality in infants secondary to pneumococcal septicaemia. They also allow early entry to other preventative programmes aiming to detect early organ complications of SCD, the most established of which is the use of transcranial Doppler technology to assess stroke risk.

1.1.2. THAL

The THAL genes are indigenous in many parts of the world, from the countries of the Mediterranean basin, the Middle East, Asia including the Indian subcontinent, southern China and South East Asia. Migrations have carried the mutated genes to non-endemic areas so the presence of these disorders is now almost universal. From the known carrier frequencies it is calculated that around 60,000 new affected births of major and intermedia thalassaemia occur every year, although in some high prevalence areas prevention programmes limit these births. Most births (around 90%) are in Asia where poor healthcare development results in early death of many affected individuals. Global epidemiology for beta-thalassaemia is shown in Table 2. For Europe the data are shown in Table 3

WHO Region	Carrier Range	Annual Affected Births
Europe	0.1%-15%	1.636
East Mediterranean Region	1.5%- 6%	8.128
South Asia	2.2% - 16% (up to 30% HbE)	41.366
Asia Pacific Region	0.4% - 6.8% (up to 30% HbE)	5.945
Americas	0.4% - 1.3%	614
Africa (Algeria only)	3%	123
Total		57.812

Table 2. Global Epidemiology of Beta – thalassemia

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

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)

Europe is a continent where beta thalassaemia has a very variable prevalence since in the southern Mediterranean coastal area the thalassaemia 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 (Figure 3). 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.

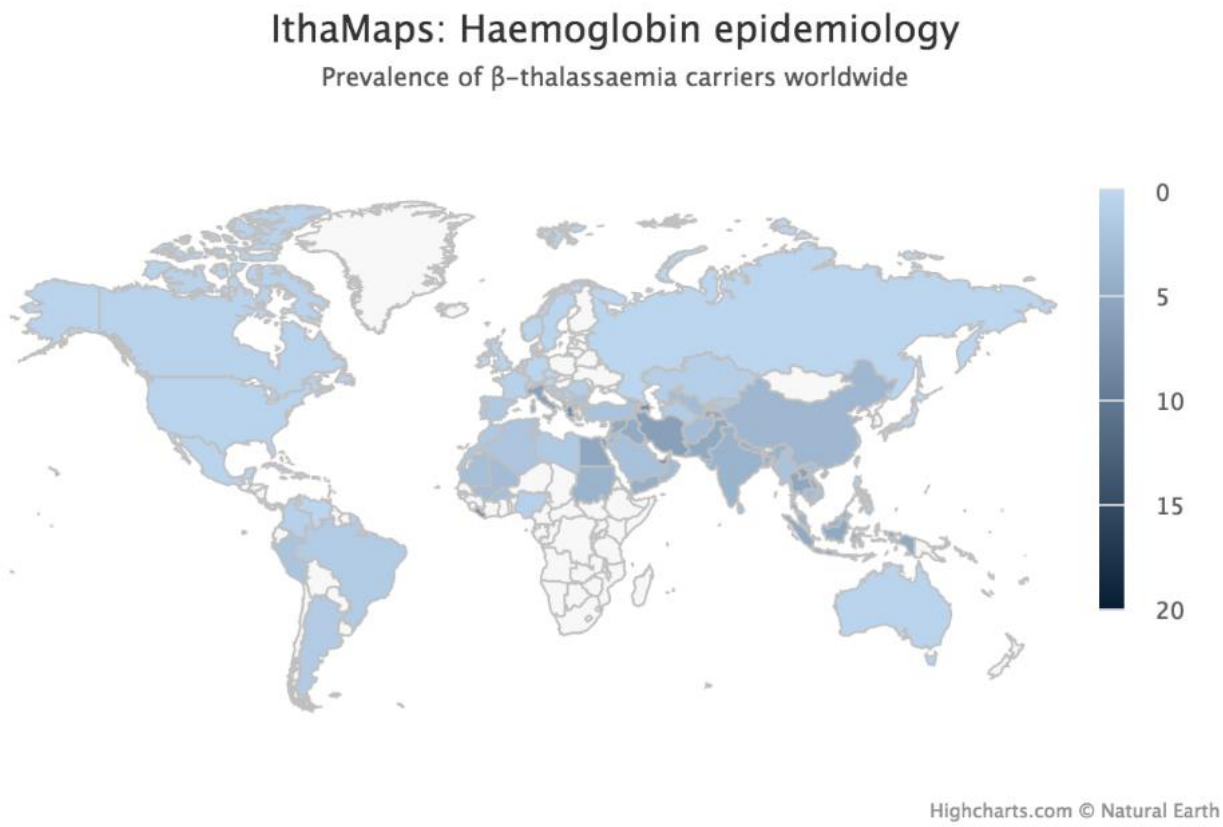

Figure 3. From IthaMaps (http://www.ithanet.eu/db/ithamaps?hb=1)

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

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

Prevention: A comprehensive programme of health education can achieve reduction of affected births. (Screening to identify carriers, genetic counselling).

1.1.3. Other RADs

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. It was concluded that in The Netherlands and Italy, 2 countries with a large and well-characterized database of patients with PK deficiency the true frequency was, in fact, about 10 times lower than predicted.

Diagnosis of the causative enzyme disorder underlying HNSHA is best achieved by determining red cell enzyme activity with a quantitative assay or a screening test. Molecular characterization of the defect confirms the diagnosis and is necessary for genetic counseling. It may also be helpful in recommendations for treatment, since patients with some enzyme deficiencies tend to respond more favorably to splenectomy than do others. In HNSHA the main clinical symptom is anaemia of variable degree, ranging from severe transfusion-dependent haemolytic anaemia to compensated haemolysis with a normal steady-state haemoglobin concentration. Chronic jaundice is a common finding, and splenomegaly is often present. Gallstones are common and ankle ulcers may be present.

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. A survey conducted by ENERCA 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. 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. In severe/atypical HS and HE cases, or when HSt is suspected, the diagnostic workout is more complex requiring specific diagnostic tools. This “second level” diagnostic step is usually performed only in a few Expert Centres (or in EC networks) where these latter tools are available.

Worldwide reports on CDA were collated in the German Registry on CDA data bank. An attempt to estimate prevalence was made in Europe, covering all EU-MS and Switzerland using the period prevalence of 50 years, including 1968 (first reports on CDA) up to 2008, and limited the data on CDA I and II. Results showed that prevalence of CDA II is about two three times higher as compared to CDA I with large differences in the various countries. 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). 40 years period prevalences for CDA I vary between 0 and 0.59 /per million inhabitants with an average of 0.24 and for CDA II vary between 0.04 and 2.46 /per million inhabitants with an average of 0.71. The common clinical features allow suspecting the diagnosis of CDA, to be followed of special tests specific for the subtypes. It is recommended to do and to interpret these tests in the few expert centres for CDA . 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.

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. The implementation of accurate patient registries and regular update of the locus specific DBA Mutation Database (www.dbagenes.unito.it) will certainly allow to better define the genotype/phenotype correlations in DBA. In Europe DBA patient Registries have been started in France (Faivre 2006), Germany (Faivre 2006), Italy (Boria 2010), Czeck Republic (Pospisilova 2012), UK (Orfali 2004). 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. It is performed only in a few Expert Centres (or in EC networks).

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

FANCC and FANCA respectively. 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. The final diagnostic confirmation of FA fully relies on an excess of chromosome fragility. Complementary diagnostic assays are the analysis of an excess of cell death or cell cycle arrest.

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. 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 should be excluded first. Molecular diagnosis in expert centres might give the final diagnosis.

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. Different genes are involved and particular diagnosis should be considered on the basis of biochemical findings (level of iron, hepcidin, transferrin, transferrin saturation or ceruloplasmin), clinical presentation and age of onset. Most causative genetic mutations variations are private, requiring full sequence analysis of the relevant gene that requires contacting a genetic diagnostic expert centre.

1.2 Classification

Rare hematological disorders in the scope of RADeep includes any type of Rare Anaemia Disorder (RADs). ORPHANET defines in its classification the entities under the code ORPHA 108997, which includes among others, the following diseases:

Orpha_Code	Disease	Orpha_Code	Disease
68364	**Hemoglobinopathy**		
275752	**Sickle cell disease and related diseases**		
		232	Sickle cell anemia
		251355	Sickle cell disease associated with an other hemoglobin anomaly
		251359	Sickle cell-beta-thalassemia disease syndrome
		251365	Sickle cell-hemoglobin C disease syndrome
		251370	Sickle cell-hemoglobin D disease syndrome
		251375	Sickle cell-hemoglobin E disease syndrome
		251380	Hereditary persistence of fetal hemoglobin-sickle cell disease syndrome
848	**Beta-thalassemia**		
		231214	Beta-thalassemia major
		231222	Beta-thalassemia intermedia
		231226	Dominant beta-thalassemia
		231230	Beta-thalassemia associated with another hemoglobin anomaly
		46532	Hereditary persistence of fetal hemoglobin-beta-thalassemia syndrome
		231237	Delta-beta-thalassemia
		231242	Hemoglobin C-beta-thalassemia syndrome
		231249	Hemoglobin E-beta-thalassemia syndrome
		330032	Hemoglobin Lepore-beta-thalassemia syndrome
846	**Alpha-thalassemia**		
		93616	Hemoglobin H disease
		163596	Hb Bart's hydrops fetalis
98363	**Rare hemolytic anemia**		
98369	**Rare constitutional hemolytic anemia due to an enzyme disorder**		
		79277	Congenital erythropoietic porphyria
		32	Glutathione synthetase deficiency
		33574	Gamma-glutamylcysteine synthetase deficiency
		90030	Hemolytic anemia due to glutathione reductase deficiency
		99135	6-phosphogluconate dehydrogenase deficiency
		371	Glycogen storage disease due to muscle phosphofructokinase deficiency
		868	Triose phosphate-isomerase deficiency
		57	Glycogen storage disease due to aldolase A deficiency
		713	Glycogen storage disease due to phosphoglycerate kinase 1 deficiency
		766	**Hemolytic anemia due to red cell pyruvate kinase deficiency**
		712	Hemolytic anemia due to glucophosphate isomerase deficiency
		714	Hemolytic anemia due to diphosphoglycerate mutase deficiency
		90031	Non-spherocytic hemolytic anemia due to hexokinase deficiency
		248305	Hemolytic anemia due to glyceraldehyde-3-phosphate dehydrogenase deficiency
		466026	Class I glucose-6-phosphate dehydrogenase deficiency
		35120	Hemolytic anemia due to pyrimidine 5' nucleotidase deficiency
		86817	Hemolytic anemia due to adenylate kinase deficiency
		99138	Hemolytic anemia due to erythrocyte adenosine deaminase overproduction
98364	**Rare constitutional hemolytic anemia due to a red cell membrane anomaly**		
		822	Hereditary spherocytosis
		288	Hereditary elliptocytosis
		98365	Hereditary stomatocytosis

Orpha_Code	Disease	Orpha_Code	Disease
293830	**Constitutional dyserythropoietic anemia**		
		98869	Congenital dyserythropoietic anemia type I
		98870	Congenital dyserythropoietic anemia type III
		98873	Congenital dyserythropoietic anemia type II
		293825	Congenital dyserythropoietic anemia type IV
1047	**Sideroblastic anemia**		
98360	**Constitutional anemia due to iron metabolism disorder**		
		1195	Congenital atransferrinemia
		48818	Aceruloplasminemia
		83642	Microcytic anemia with liver iron overload
		209981	IRIDA syndrome
		300298	Severe congenital hypochromic anemia with ringed sideroblasts
182040	**Aplastic anemia**		
68383	**Rare constitutional aplastic anemia**		
		124	Blackfan-Diamond anemia
		1775	Dyskeratosis congenita
		84	Fanconi anemia
		3088	Revesz syndrome
		3322	Hoyeraal-Hreidarsson syndrome
		3466	WT limb-blood syndrome
		3319	Congenital amegakaryocytic thrombocytopenia
		811	Shwachman-Diamond syndrome
		314399	Autosomal dominant aplasia and myelodysplasia
		397692	Hereditary isolated aplastic anemia
		401764	Pancytopenia-developmental delay syndrome
164823	**Rare acquired aplastic anemia**		
		447	Paroxysmal nocturnal hemoglobinuria
		824	Primary myelofibrosis
		88	Idiopathic aplastic anemia
		98421	Red cell aplasia
248296	**Constitutional deficiency anemia**		
		98396	Constitutional megaloblastic anemia due to vitamin B12 metabolism disorder
		98408	Constitutional megaloblastic anemia due to folate metabolism disorder
		98415	Vitamin B12- and folate-independent constitutional megaloblastic anemia

Table 4. Example of RADs included under the scope of RADeep (ORPHA 108997)

1.3 Treatment

1.3.1. SCD

Neonatal screening and enrolment in comprehensive care programmes

Neonatal screening of SCD reduces mortality in infants, through education of parents and early implementation of daily prophylactic penicillin [Vichinsky, 1988. Newborns diagnosed with a major SCD syndrome have to be addressed to an expertise centre [Consensus Conference. Newborn screening for sickle cell disease and other haemoglobinopathies. JAMA,258 (9),1205-1209 (1987)], where the parents will be informed that their child has SCD by an expert physician who will organize the care of the baby.

Prevention of infections

Fulminant infections related to encapsulated bacteria and explained by the functional asplenia were until these very recent years the first cause of death in SCD children aged less than 5 years [Leikin, 1989]. A randomised study published in 1986 showed that prophylaxis with penicillin twice a day in SCD children younger than 3 years at study inclusion was associated with an 84% reduction in the incidence of infection, compared to placebo therapy [Gaston, 1986]. Penicillin is therefore recommended twice daily starting at 2 months of age, but further research is needed to determine the age at which penicillin prophylaxis can be stopped safely [Hirst, 2002]. Given the risk of poor adherence to daily prophylaxis and the development of penicillin resistant Streptococcus pneumoniae strains, pneumococcal immunisation as well as prophylactic penicillin is recommended [Davies, 2004]. The recommended immunisation schedule for previously unvaccinated children with SCD consists of three doses of conjugated vaccine six to eight weeks apart, followed by a booster dose one year later, then by a polysaccharide vaccine after age 2 years, with additional doses every three to five years. Transition from pediatric to adult clinics must be carefully presented and prepared.

Prevention of strokes

Up to these very last years, it was observed that 11% of patients with SCD will have an apparent clinical stroke by age twenty [Ohene-Frempong, 1998]. Silent infarcts are also evidenced in up to 35% of children, with possible impairment of cognitive functions. Adams demonstrated in 1992 that it was possible to screen early the children the more at risk to develop an overt stroke using a transcranial Doppler, showing that 40% of the children with an increased blood flow velocity in the internal carotid or middle cerebral artery will have an overt stroke in the next 3 years. [Adams, 1992].

Six years later, Adams demonstrated that a first stroke could be prevented by monthly transfusions in children with abnormal TCD findings, evidencing in a randomized study a 92% difference in the risk 22 of stroke between the transfused and non -transfused arms [Adams, 1998].

Lastly, he randomized discontinuation of transfusion in children undergoing chronic transfusion for an abnormal transcranial Doppler, during which time the transcranial Doppler ultrasonography became normal. Stopping the transfusions was followed by a high rate of stroke or reversion to abnormal velocities of cerebral blood flow [Adams, 2005]. These well designed studies led to the recommendation that transcranial Doppler ultrasonography be performed annually in children aged 2-16 years with SCD and that regular blood transfusions should be strongly considered in those with abnormal findings on transcranial Doppler ultrasonography [National Institutes of Health. The management of sickle cell disease. 4th ed. 2002. (NIH publication No 02-2117) www.nhlbi.nih.gov/health/prof/blood/sickle/index.htm].

Education and psychological support

Patients and families should be educated about the factors that increase the risk of vasoocclusive episodes, such as exposure to cold, fever, dehydration, stress and tobacco. They are taught to manage mild pain with rest, hydration, and weak opioids (such as codeine or propoxyphene) and to recognize the signs that require

an immediate visit to the emergency room, such as pallor, asthenia, fever, respiratory distress. SCD is a chronic, painful and distressing disease. Most parents are despaired when diagnosis is given, and some of them experience thereafter repetition of life-threatening complications in their children. Children feel their parents' continuous fear, and some of them are victims of repeated painful events and hospitalizations, sometimes in Intensive Care Units. They may be unable to attend school regularly and fail to perform. Furthermore, silent microinfarcts may be responsible to learning difficulties. Proposing specific individual and family psychological interventions could very likely help to disrupt the vicious circle of pain and fear of pain in SCD children. Early detection of school difficulties may help to organize school support. Adolescents and their families should be informed and reassured about frequently delayed sexual development and growth but with normal final height in most of them.

Annual follow-up investigations

Adult patients with SCD may suffer from several organ damages which can be, for some of them, detected in older children and adolescents and treated early. This justifies the organization of yearly check-ups assessing any chronic organ deficiency [Haute Autorité de Santé. Prise en charge de la drépanocytose chez l'enfant et l'adolescent [Clinical practice guidelines in French]. 2005. www.has-sante.fr/portail/display.jsp?id=c_272479].

Table 1. RECOMMENDED EXAMS TO BE PERFORMED ANNUALLY

	0 – 1 Year	2 years	3 - 5 years	6 - 9 years	10 – 15 years	16 -18 years
Physical examination	■	■	■	■	■	■
Transcutaneous O_2 saturation	■	■	■	■	■	■
Biological tests*	■	■	■	■	■	■
Pulmonary function tests	■	■	■	■	■	■
School success	■	■	■	■	■	■
Adherence (treatments, appointments)	■	■	■	■	■	■
TCD		■	■	■	■	
Hepatic US			■	■	■	■
Hip X-Ray				■	■	■
Electrocardiography				■	■	■
Ophtalmologic evaluation				**	■	■

* Complete blood count, liver profile, electrolytes, BUN, creatinine, γalbuminuria, ferritin if transfused, calcium metabolism including vitamin D and PTH, Parvovirus B19 serology until positive.

** Since the age of 6 y.o. if Hb SC disease

Figure 4. Recommended exams to be performed annually

Preoperative preparation The complications of sickle cell disease often require surgical procedures such as cholecystectomy, hip replacement, and splenectomy. However, patients with the disease are at high risk of perioperative complications, chiefly acute chest syndrome and pain. Transfusion or exchange transfusion are therefore recommended for surgeries requiring a prolonged time of anesthesia [Wayne, 1993].

TREATMENT INTENSIFICATION

A considerable number of SCD children have a severe form either because they have repeated painful episodes or acute chest syndromes, or because they have a risk of cerebral vasculopathy or severe baseline anaemia.

Several disease modifying treatments can be propose to these children, hydroxyurea, chronic transfusion, or bone marrow transplant when they have a HLA identical sibling. For more information consult the ENERCA

Recommendations for sickle cell disease https://www.enerca.org/activities/training/enerca-recommendations-2013.html

1.3.2. THAL

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,.

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. Haemopoietic stem cell transplantation may be curative and is offered to patients when a compatible donor is available, particularly if the donor is brother or sister.

Non-transfusion dependent thalassaemia syndromes have a wide spectrum of severity. Over the years, complications arise such as hypersplenism, iron overload, 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.

1.3.3. Other RADs

The main features of red cell enzyme defects and 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. Gallstones are common; iron overload may occasionally develop even in the absence of transfusions. Most patients with chronic HNSHA do not require therapy, other than blood transfusion during haemolytic periods. Splenectomy is beneficial but not in all enzymes deficiencies. Stem cell transplantation is the only curative treatment for severe cases.

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's for physicians and for patients on these very rare disorders are available from ENERCA and/or Expert centres. Assurance of physician's expertise and respectful consideration of the patient's perceptions are mandatory for the patient's compliance, the cornerstone to maintain quality of live and improve life expectancy.

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. Patients who do not respond to steroids undergo chronic blood transfusions and need iron chelation to avoid secondary haemochromatosis. SCT is the only life-saving procedure for FA patients with available donor using clinical protocols specifically designed for FA patients. 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.

All HSA patients require a multidisciplinary approach and 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.

1.4 RADeep

The Rare Anaemia Disorders European Epidemiological Platform – RADeep, https://www.radeepnetwork.eu/, is an initiative endorsed by ERN-EuroBloodNet, https://www.eurobloodnet.eu/, the European Reference Network on rare haematological disorders as an umbrella for both new and already existing European patients' registries in rare anaemias disorders (RAD). RADeep is built in line with the EU Rare Disease Platform recommendations for patients' registries on rare disorders and with the European Rare Blood Disorders Platform (ENROL). ENROL is officially endorsed by the European Hematology Association (EHA)). RADeep contributes to ENROL by sharing pseudonymised data of patients affected by a RAD.

In agreement with RADeep Principle: "to maximize public benefit from data on RAs opened-up through the platform with the only restriction needed to guarantee patient's rights and confidentially in agreement with EU regulations for cross-border sharing of clinical data", a legal frame for secure sharing and re-use of data on patients affected by RAD has been established for enabling both entering certified medical data from available sources and re-use of data with third parties.

The following diagram summarizes the pathways for RADeep data entry, data processing and data request:

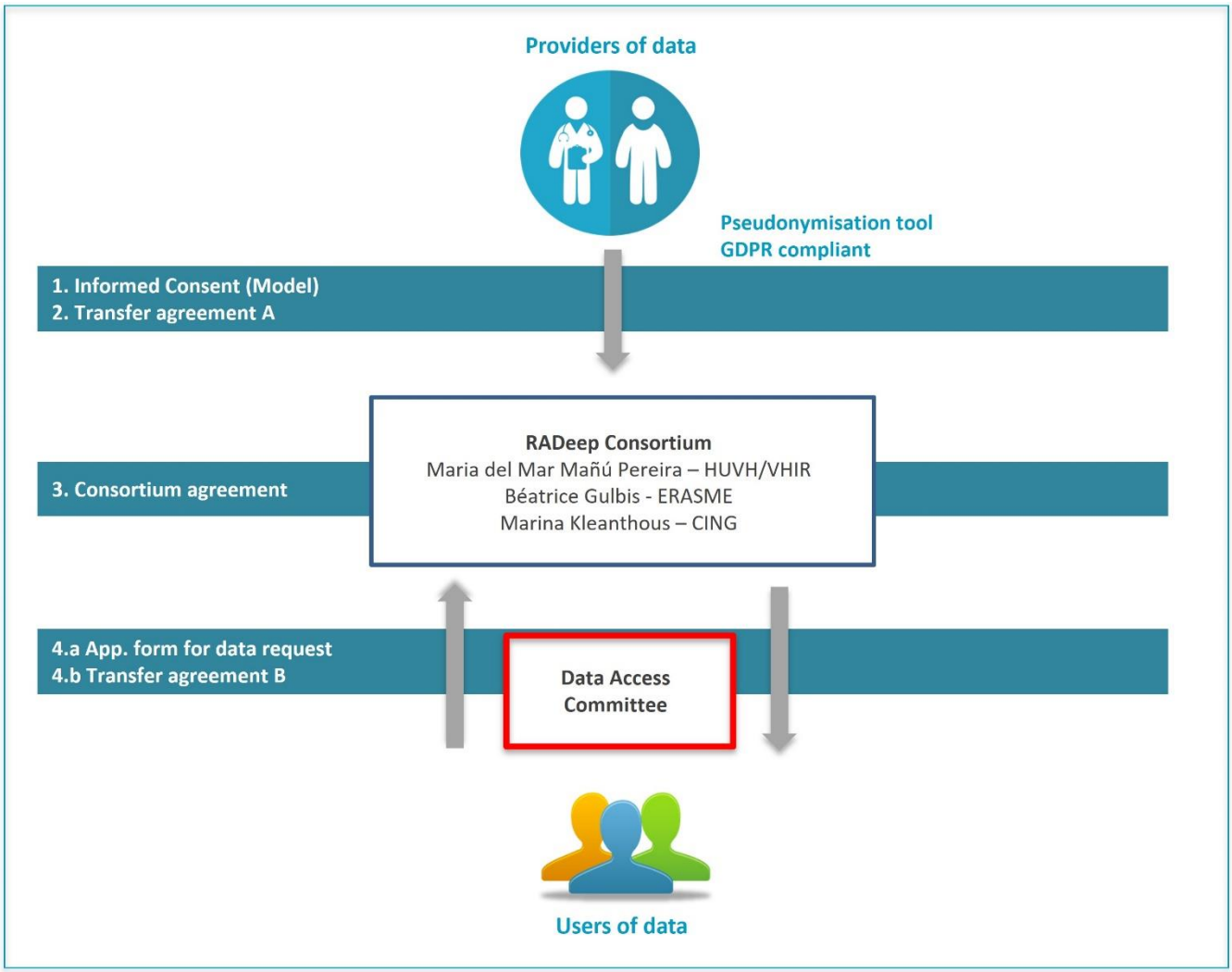

Figure 5. RADeep data flow

Where:

Providers of data

Any legal entity providing data to RADeep, including:

- Healthcare providers
- European/national/regional registries
- Scientific networks registries

Users of data

Any stakeholder with interest on RADs, including:

- Healthcare providers
- Researchers from both public and private institutions
- Patients associations
- Health authorities (policy makers)

Legal and ethical documents:

1- Informed consent
2- Transfer agreement A (from Provider of data to RADeep)
3- Consortium Agreement
4- Application form for data request
5- Transfer agreement B (from RADeep to User of data)

RADeep Policy enables participating stakeholders to comply with all legal and ethical considerations that apply to the processing and use of sensitive, personal information and health data.

In addition to the above general principle, RADeep is being developed having reviewed all the relevant recommendations particularly concerning interoperability, in order to receive data from existing registries and assist networking.

Although there are some examples of well-established national registries on RADs, data on prevalence, survival, main clinical manifestations or treatments are not available in most of the European countries. A European approach for the standardised collection of data regarding the main clinical complications of RADs is fundamental to establish the need and the priorities in the development of research projects and clinical trials, and to answer in the short and long term specific, clinical or research questions.

2. Study objectives

2.1 Primary objective

To collect and to describe demographics and epidemiological data of any type of RADs according to ORPHA classification for Rare Anaemia (ORPHA 108997), which includes, among others, the following diseases: Sickle cell disease (SCD), Thalassaemia disorders (THAL), Pyruvate Kinase Deficiency (PKD) or with other RAD (rare defects of the red blood cell and erythropoiesis), in order to assess the incidence and prevalence of RADs patients in Europe.

2.2 Secondary objectives

1. To collect and to describe clinical manifestations and treatments of RADs (ie. SCD, THAL, PKD and other RADs) patients in order to:
    a. Assess survival of RADs patients in Europe
    b. Evaluate and follow-up clinical outcomes
    c. Assess organ damage in children and adults
2. Investigate the relationship between:
    a. Clinical characteristics at inclusion and during follow-up
    b. Treatments received and
    c. Responses to treatment as defined in the treatment section
3. Perform observational studies concerning relevant scientific research questions in RADs (SCD, THAL, PKD and other RADs) using clinical data and to present relevant research outcomes in the fields of diagnosis & prognosis, and risk stratification for newly developed classes of drugs.
4. To disseminate the results of the studies to all stakeholders involved.
5. To promote best practices and guidelines sharing and implementation.

2.3 Endpoints

1. Estimation of the prevalence and incidence of RADs (ie. SCD, THAL, PKD and other RADs) at the European level, by EU-Member State and by centre through statistical analysis based on study sample data
2. Mean survival age of RADs registered patients
3. Number of main clinical complications events and severity of the disease expressed by number of hospitalizations and their evolution for the registered patients
4. Determine specific markers of a single organ damage

3. Investigational Plan

3.1 Overall Study Design

The registry is designed to collect information about a large cohort of patients diagnosed with RADs (ie. SCD, THAL, PKD and other RADs) from clinical centres or national registries within the participating European countries.

The task of developing the RADeep registry, which is interoperable, means that we are facilitating, if existing, each local registry to share their data in a pan-European pool. This would serve the primary purpose of the registry, which is epidemiological mapping of RADs (SCD, THAL, PKD and other RADs) in the EU. But interoperability is also a requirement for ensuring the re-use of data opened-up by RADeep Registry engaging research community and industry to promote basic research and development of new treatments and clinical trials. Accordingly, internationally established codification standards will be utilised for the definition of diseases (e.g. ICD-10 or ICD-11), clinical trials (e.g. SNOMED), observed phenotypes (e.g. HPO), genes and variations (e.g. OMIM and ClinVar).

Accordingly, RADeep is foreseen as the European epidemiological platform open to any country and/or medical center willing to actively collaborate as data providers, starting with a pilot in 9 European countries: (Belgium, Cyprus, Denmark, France, Germany, Italy, Netherlands, Portugal and Spain).

Based on the existing registry, UK will also act as an advisor; it will ensure a comprehensive approach resulting in a European picture of SCD and THAL.

In addition, during the first 9 months of the study, an on-line form will be developed within the RADeep dedicated webpage https://www.radeepnetwork.eu/ in order to map at the European levels medical centres dealing with patients diagnosed with RADs (ie. SCD, THAL, PKD and other RADs) including their activity based on total number of patients in follow-up for pediatrics and adults.

In this study, no clinical, instrumental, laboratory assessments, or therapeutic intervention will be performed other than those required for disease management according to local best practice.

3.2 Study Population

The RADeep registry will be limited to patients diagnosed as RADs according to ORPHANET classification (ORPHA 108997). For the avoidance of any doubt, trait condition for recessive disorders is excluded.

3.2.1 Study sample size

Study sample size for SCD and THAL has been determined based on direct query to Steering Committee members. The recruitment target is 21,149 for SCD patients and 11,415 for THAL patients. This sample size is intended to be a broad representation of the European SCD and THAL patients and sufficiently large for meaningful analysis of SCD and THAL subgroups.

EU-MS	Number of SCD patients registered*	Number of estimated SCD patients in the country**	Number of THAL patients registered*	Number of estimated THAL patients in the country**
Belgium (BE)	500	1.200		60
Cyprus (CY)	49	49	600	650
France (FR)		15.000	650	750
Germany (DE)				680
Italy (IT)		2.000		9.000
Netherlands (NL)	1.500	2.000		200
Spain (ES)	826	900	62	75
TOTAL	**2.875**	**21.149**	**1.312**	**11.415**

Table 5. Sample size estimation for SCD and THAL

*Number of SCD/THAL patients that are already registered in currently existing national registries for SCD/THAL

**Estimation of the number of total patients affected by SCD/THAL in the country

3.2.2 Inclusion Criteria

Patients must meet all of the following criteria to be included in the RADeep Registry:

- Age from 0-100, both female and male
- Diagnosed as RADs (SCD, THAL, PKD and other RADs THAL according to ORPHANET classification in Chapter 1.2)
- Able and willing to provide the written informed consent (patient or legal representative for minors)

3.2.3 Exclusion Criteria

- Patient or legal representative for minors unwilling or unable to give consent
- Patients diagnosed as SCD or THAL (alpha-thalassaemia and beta-thalassaemia) traits, or trait condition for other recessive RADs

3.2.4 Follow-up & withdrawal from the Study

Patients will be followed until termination of follow-up (i.e. death, withdrawal, loss to follow-up, or termination of follow-up period). Patients will be withdrawn from the study in case of:

- Withdrawal of consent. A patient may withdraw consent at any time, without providing a reason.

3.3 Visits and Assessments

3.3.1 Visit Schedule and Assessments

3.3.1.1 At inclusion

The following data will be collected at inclusion of the patient:

- Inclusion and Exclusion Criteria
- Date of patient inclusion
- Data Set:

  Information about the status of the patient and geographical location for prevalence studies and survival (Date of birth, gender, patient status, country, medical centre)
  Information to appreciate methods used for diagnosis and to calculate diagnosis delay and diagnosis wavering (Clinical and genetic diagnosis, onset, first symptoms)
  Hematological parameters
  Serological data: Rate of infections
  Splenomegaly and splenectomy information
  Assessment of SCD, THAL, PKD and other RADs severity
  Blood transfusion requirement
  HSCT transplant information and outcomes
  Assessment of use of specific treatments for SCD, THAL, PKD and other RADs
  Assessment of inclusion in CT protocols

3.3.1.2 At each follow-up visit, including end of study:

Follow-up data will be reported at approximately 12-monthly intervals for all registered patients.

- Date of last visit prior to report
- Data defined as update/longitudinal in the Data set

3.3.2 Laboratory Tests

Laboratory tests will be performed as judged appropriate by the treating physician. This study does not require additional laboratory tests to be performed. The laboratory test results of interest will be registered if available.

4. Organization and Responsibilities

4.1 Overall organization

The registry is built as a central international platform for registration of data collected by centres (referral sites) sometimes in the context of their local/national registries.

4.2 Steering Committee

The Steering Committee (SC) is responsible for the general design (i.e. study protocol, common data elements), conduct, and overall progress of the Registry, including its revision over time by updating when required (ie. Revision of common data elements). Proposed research questions (sub studies) as well as statistical analysis plans have to be approved by the SC. Finally, is in charge of drafting the Policy for data access and publishing.

SC is composed by the coordinators, one hematologist, one pediatrician, one laboratory specialist, one IT and statiscal specialist, one manager and two patients' representatives

During the inclusion period, the SC will meet at least once per year in a plenary session or/and by teleconference if necessary. The project manager will be responsible for drafting the minutes of each meeting and circulating this document after approval.

4.3 Scientific Subcommittees

Scientific subcommittees (SS) are responsible for the revision of the Research protocol of the platform for the disease specific area, including Data Elements and research questions defined.

SS are formed by experts on prevention, diagnosis and clinical care of the specific diseases.

4.4 Data Access Committee

Data Access Committee (DAC) has the overall aim to promote the re-use of the data collected in RADeep while ensuring protection of data subjects, and specifically is in charge of approving and implementing the Policy for data access and publishing, ensuring the good use of data assets.

DAC is formed by SC, Legal and ethics experts and representatives of the Scientific subcommittees.

4.5 Project management

Project management (PM) is responsible for the general day-to-day coordination and execution of general tasks of the Registry (i.e. administrative, financial, contractual, newsletters). It is also responsible for the day-to-day management of the Registry, and to advice / prepare proposals for the SC. The PM consist of SC co-Chairs and project managers in the related sites.

The PM is responsible for the overall coordination of the project in the participating countries. This includes the arrangement of support for the contract duties, the distribution of sites metrics, such as the number of patients included, coordination of the referral sites and the organization of Site Training. The project managers organize all meetings, prepare and distribute the agenda and minutes. Finally, the project managers support the preparation of publications.

The PM is responsible for the design and maintenance of the different sections of RADeep network website.

The PM will meet every 12 months in a plenary session (during and after completion of recruitment) and TC will be scheduled on demand. The project manager is responsible for the preparation of the minutes of each meeting and circulation of this document. It is the duty of the project manager to report the important issues to the members of the SC.

4.6 Platform development and Statistic Unit

The Central Data Management and Statistics Unit is responsible for the design and maintenance of the core database, data transfer algorithms. The data management centre prepares working instructions related to the data entry and cleaning, executes the data cleaning and provides a database lock. The statistics centre prepares and executes the statistical analysis. It provides statistical support during the preparation of publications and provides metrics by site.

5. Statistics

The Central Statistical Unit is responsible for the development of the details of the statistical analysis plan. The detailed statistical analysis plan has to be approved by the SC. This also applies whenever changes in the analysis plan are being considered.

5.1 Sample size

This study is exploratory in nature. Thus, the estimated sample size is not based on a statistical hypothesis, but on an estimation of the number of patients who are diagnosed with SCD, THAL, PKD and other RADs per centre in an observation period and sufficiently large to perform some subgroup analyses.

5.2 Collection of clinical variables

All data collected for each patient are displayed in the patient data listings. Unless otherwise stated, *baseline* is defined as the first observation at the time of diagnosis. Each value is classified as falling above, below or within normal limit. It is impossible to use a single central laboratory for all parameters and all patients. However, to avoid the issue of collecting hundreds of normal ranges, standard normal ranges will be defined and applied for the purpose of statistical analysis.

5.3 Demographics and disease management

Descriptive analyses will be undertaken at the end of the follow-up period using standard statistical methods to examine the subjects' demographics, disease characteristics and management of these disorders. Interim analyses are described in 5.5.

Time-to-event analyses, namely Kaplan-Meier and Cox proportional hazard regression will be used to estimate overall survival:

- The proportion (with 95% CI) of patients that has died during follow-up. The median, range and 95% CI for survival will be calculated. Overall survival is calculated for all patients from the date of SCD, THAL, PKD and other RADs diagnosis to the date of death from any cause. Patients with no documented death are censored at the last date they were known to be alive.
- The proportion (with 95% CI) of patients that experiences an event (e.g. acute chest syndrome, vaso-occlusive crises).
- The median, range and 95% CI for time to development of an event.
- The proportion (with 95% CI) of patients treated with any treatment for SCD, THAL, PKD and other RADs recorded in the registry.

5.4 Correlation between patient characteristics and prognosis

Multivariate Cox proportional hazards regression models will be used to identify variables that are important in predicting variables that predict are applied to correlate survival. These include clinical variables, but also the impact of various treatments received during the course of the disease.

5.5 Interim analysis

Interim (descriptive) analyses will be conducted when requested for the various sub studies, and at specific time points as decided or requested by the SC, but at least once a year. These analyses will report the patient and disease characteristics, treatment pathways and examine recruitment level across the different centres and countries. These analyses will allow an accurate statistical analytical plan to be developed including formal power calculation to determine the sample size necessary to examine important secondary endpoints, including the impact of the various therapeutic interventions reported in the Registry study.

6. Data recording and data management

6.1 Data recording

Data are recorded and entered through the web-based e-CRF at each national registry site and at clinical sites within each country or uploaded from National Registries by means of tailor made data transfer algorithms *(if (re-)consent is adequate)*. A screening log is maintained at each site to ensure consecutive patient enrolment. Dedicated resources are available for collecting data by a specialized nurse, data manager or equivalent for each national registry site. This person co-ordinates data entry with the clinical sites and is responsible for validation of data from all clinical sites prior to upload into the central study Database. All data collected for each patient are displayed in the patient data listings. History and clinical conditions are assessed from routine documentation and clinical evaluation performed in the context of inclusion and follow-up visits. The data management centre is responsible for generation of queries.

6.2 Data Management

The platform development and statistical unit is responsible for the Data Management. Thus, it is responsible for the import of data from the national registry sites and for the merging of all data in a central database. Procedures concerning data export, cleaning and database merging will be described in the Data Management Manual. Training is provided for each site and a dedicated helpdesk is available.

The EU general Data Protection Regulation provides every EU citizen with the ‘Right to be forgotten. This might have implications for the data management. Procedures concerning the ‘Right to be forgotten’ will be described in the Manual of Procedures.

7. Quality Control and Quality Assurance

The European Registry is a non-interventional study. Therefore, it is not considered necessary to conduct close monitoring activities with 100% source data verification for all patients. Instead, the quality of the data provided by the referral sites are evaluated on a sample of patients. This evaluation is conducted by the PM and the statistical Unit.

In order to ensure source data verification, the participating centres must provide access to all relevant clinical records. Information concerning the identity of the patient does not leave the premises of the centre.

8. Ethics, GCP Compliance and pharmacovigilance

8.1 Subject identification and protection

Patients are cared for according to their treating physician's best judgement. They are not being subjected to any experimental treatment or examination for the purposes of this study.

Personal data allowing patients' direct identification, as name and surname, national identity number or home address will not be received by RADeep. Instead, medical information will be received by RADeep along with a pseudonym generated by their medical doctors (at local level). The use of a pseudonymisation tool offered by the EU-RD Platform in the context of rare disease registries (https://eu-rd-platform.jrc.ec.europa.eu/_en) GDPR compliant is envisaged for this aim. The pseudonym is created at the local site and the link among the pseudonym and patients' identity is never transferred to RADeep. To avoid any doubt, the link among pseudonym and patients identity remains at local level and is not transferred to RADeep.

Pseudonymised medical information to be included in RADeep is the information gathered in the routine of medical care: date of birth, gender, diagnosis including genetics, blood parameters, clinical manifestations and therapeutic interventions. This information is the strictly necessary for the achievement of RADeep aims, corresponding to a set of parameters defined at the European level as "Common data elements" to all Rare diseases registries, and a consensus of experts involved in RADeep team. As the registry evolves, data elements will be reviewed by the Steering Committee for assessing if an update shall be required. Part of the pseudonymised data processed and stored in RADeep will be shared with the European Rare Blood Disorders Platform (ENROL), which applies the same level of safeguards to patients' data.

RADeep will share patients' pseudonymised data with third parties under the implementation of the following appropriate safeguards to protect the data during and after the study:

Pseudonymised data held in RADeep will be shared to third parties (researchers, patients' associations, policy makers, industry) in order to contribute to projects whose objectives are directly connected to improve healthcare provision for RADs, thus connected to RADeep's aims. As previously indicated, RADeep gathers pseudonymized data, which means that information allowing the direct identification of the patient has not been transferred to the platform. Thus, the risk of re-identification is residual. Accordingly, and for the avoidance of any doubt, these third parties will never have access to the information that may directly lead to patients identification.

Third parties interested in accessing data held by RADeep will be required to submit an application form that details the scientific purposes of the project for which the data is needed for its revision by RADeep Data Access Committee. Researchers may come from both public and private institutions in any country, including non-EU countries. All third parties will be required to sign legal agreements respecting the EU legislation and committing them to (i) use the data only for the purpose intended and authorized; (ii) not attempt to re-identification, including merging RADeep's data to other sources of data; and (iii) not contact the patient directly. In addition, in cases that data is transferred to non-EU countries, the same level of protection and commitments with regard to data protection will be imposed according to the GDPR.

This version of the protocol will be reviewed by the Local, Regional or National Ethics Committees.

8.2 Informed Consent

In the frame of RADeep, a policy has been established from the onset of the project aiming at establishing the legal frame for the setting up of a European Epidemiological platform ensuring safe sharing of patients' data in agreement with the Regulation (EU) 2016/679 of the European Parliament and the Council 27 April

2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation).

The General Data Protection Regulation supports the processing of personal data in the frame of registries in order to obtain high quality knowledge for the improvement of the quality of life for a number of people and improve the efficiency of health and social care services, providing that appropriate conditions and safeguards are set out in Union or Member State law.

RADs patients data will be collected from any EU Member State combining different sources of data, including a) existing registries and b) Healthcare providers hospital records. Accordingly, patients will be recruited at the national level in the patients' reference centers. The informed consent will be obtained by the physician in charge of the patient at the local level, and its obtention will be the responsibility of the local centre.

Documented informed consent will be obtained for all patients before they are registered, if it is required by national regulation. If applies, all patients who are eligible for inclusion are informed of the aims and nature of the study. They are informed that all their clinical data will be treated confidentially, but that their medical records may be reviewed by authorized persons other than their treating physician for study purposes. All patients will be informed that participation is voluntary and that they can refuse participation at any time, without consequences for their further treatment.

The informed consent procedure will be conforming to the Regulation (EU) 2016/679 of the European Parliament and the Council 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation) and will be in accordance with national and local regulatory requirements.

8.3 Pharmacovigilance

In case that Regulations require that Adverse Events (AEs) are collected in the context of the Study, the RADeep coordinators shall have an obligation to collect and report pharmacovigilance information to related pharmaceutical companies if the Institution identifies in the conduct of the Study an Adverse Event, Pregnancy or Special Situation relevant to pharmaceutical companies Product(s).

Updated information on pharmacovigilance responsibilities will be published in https://www.radeepnetwork.eu/

9. Financing

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