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Neonatal haemoglobinopathy screening in Belgium

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ABSTRACT

Background: A neonatal haemoglobinopathy screening programme was implemented in Brussels more than a decade ago and in Liège 5 years ago; the programme was adapted to the local situation.

Methods: Neonatal screening for haemoglobinopathies was universal, performed using liquid cord blood and an isoelectric focusing technique. All samples with abnormalities underwent confirmatory testing. Major and minor haemoglobinopathies were reported. Affected children were referred to a specialist centre. A central database in which all screening results were stored was available and accessible to local care workers.
A central clinical database to monitor follow-up is under construction.

Results: A total of 191 783 newborns were screened. One hundred and twenty-three (1:1559) newborns were diagnosed with sickle cell disease, seven (1:27 398) with β thalassaemia major, five (1:38 357) with haemoglobin H disease, and seven (1:27 398) with haemoglobin C disease. All major haemoglobinopathies were confirmed, and follow-up of the infants was undertaken except for three infants who did not attend the first medical consultation despite all efforts.

Conclusions: The universal neonatal screening programme was effective because no case of major haemoglobinopathy was identified after the neonatal period. The affected children received dedicated medical care from birth. The screening programme, and specifically the reporting of minor haemoglobinopathies, has been an excellent health education tool in Belgium for more than 12 years.

Migration has brought haemoglobinopathies to north Europe and, within the European Union, Belgium is one of the countries that is most involved. Belgium now has around 10 million inhabitants and an annual growth rate of around 0.54%. In 2005, the percentage of migrants coming from countries with a high prevalence of haemoglobinopathy was 3.9% of the total Belgian population. To this number, one should add an average number of 20 000 immigrants or children born to immigrant parents per year (0.2% of the total population per year) who acquired Belgian nationality during the last decade.[1] There are no official data on the prevalence of haemoglobinopathies and no annual registration of new cases, but an estimation of the prevalence and demographic picture of the major haemoglobinopathies in Belgium has been recently approached through a confidential inquiry.[2] From a response rate of 42% from all the Belgian adult internal medicine and paediatric departments, 417 patients have been reported, including 83% with sickle cell disease (SCD), 13% with β thalassaemia major, 2% with β thalassaemia intermedia, and 1% with haemoglobin H disease.[2]

In 1994 the immigration context was known, but neither the Belgian Authorities nor health practitioners were aware of the incidence of haemoglobinopathies and thus of the services that needed to be implemented to reduce the burden of those disorders. To follow English and French programmes,[3–6] it was decided to determine the birth incidence of haemoglobinopathies in Brussels at the end of 1994. That epidemiological approach was soon transformed into the implementation of a neonatal screening programme in Brussels and, from 2002, also in Liège. The Belgian Authorities have not yet approved its extension to the entire country.

The aim of the present article is to report our experience with neonatal haemoglobinopathy screening of more than 150 000 newborns and the birth incidence of haemoglobinopathies in Brussels and Liège, and to try to outline the screening approaches that have been chosen and implemented within the Belgian neonatal haemoglobinopathy screening programme.

METHODS

Universal screening

A universal neonatal screening for haemoglobinopathies has been in operation since December 1994 and September 2002 in Brussels and Liège, respectively. Becoming part of a national programme "Convention INAMI relative au dépistage néonatal des hémoglobinopathies en Région de Bruxelles-capitale" in 2003, the screening included all the neonates born under the care of the Brussels maternity services from that year. However it included only one maternity service in Liège (Hôpital de la Citadelle). The programme was quite separate from other mandatory testing at birth, such as screening for phenylketonuria or hypothyroidism. For each test, around €4.5 were given to the reference centre and €0.5 to the maternity service. The programme included all the procedures as well as the integration of the results into a central database.

Since 2006, a specific label entitled "Haemoglobinopathies screening" has been attached to the baby's medical record to certify that the screening had been performed.

Patient data

Ethnicity and birth weight of the neonates as well as a transfusion prior to the test were recorded. The screening was approved by each local ethical committee.

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Sample type and laboratory technique

Liquid cord blood samples in EDTA were analysed by an isoelectric focusing (IEF) technique on agar gels, as previously described (PerkinElmer Life Sciences, Zaventem, Belgium).[7]

Confirmation of results

All samples with suspected abnormal haemoglobin fractions on IEF were further analysed by high-performance liquid chromatography (HPLC) using the β-thalassaemia short programme kit on a BioRad Variant (BioRad, Hercules, California, USA), as previously described.[7]

A repeat sample was requested, before the family left the maternity service, for newborns in whom no haemoglobin (Hb)A or a very low level of HbA was detected, who had high levels of Hb Bart's or in whom there was a higher percentage of a variant Hb than HbA.

Reporting results

In each maternity service, a local official neonatal screening specialist was chosen. He or she was in charge of the local implementation of the screening programme as well as of its follow-up.

Major and minor haemoglobinopathies were reported. Moreover, a central database in which all results were stored was readily accessible to local healthcare workers.

Major haemoglobinopathies (eg, SCD) were reported without delay by phone and by mail to those specialists who were responsible for obtaining a repeat sample.

Minor haemoglobinopathies (eg, sickle cell trait) were reported by regular mail within 2 weeks.

Follow-up

Tracking newborns that might have been missed by the programme was implemented in two ways. If the specific label entitled ''Haemoglobinopathies screening'' was not found in the newborn record, a liquid heel prick sample at day 3 post-delivery was used for screening. Moreover, with the database, it was easy for each local neonatal screening specialist to identify newborns who had been missed and who needed to be recalled for testing.

Before leaving the maternity service, the families of an affected newborn received counselling and were referred to a specialised healthcare centre where an initial consultation was scheduled. The reference centre ensured monitoring of affected children. To do this, a form to be completed by the recipient, incorporating questions regarding prenatal monitoring for the couple, whether antenatal diagnosis had been offered and the possible reason for its refusal as well as the name of the paediatrician who would take care of the child, was sent to the local official responsible for screening. From the beginning of the screening, a centralised database that includes all the results of screening has been accessible to local official neonatal screening specialists. Since 2007, a centralised patient registry has allowed the monitoring of outcome of affected children. Diagnosis, significant events and treatment are retrospectively registered. An annual follow-up is recorded.

Concerning the minor haemoglobinopathies, counselling the families and the identification of previously unknown at-risk couples was done in specialised healthcare centres wherever there was a significant prevalence of haemoglobinopathies.

RESULTS

Patients

During the study period, 191 783 newborns were screened in Brussels and Liège. This represented annually around 13.5% of all births in Belgium. Geographical origins of the neonates screened in 2007 showed that 45% of the neonates had both parents not of an ancestry at risk for a haemoglobinopathy (table 1). The screening test revealed mainly two minor and one major haemoglobinopathies (ie, 2929 (1:65) newborns were found to have a sickle cell trait, 385 HbC trait, and 123 SCD) (table 2). The most frequent haemoglobin variant encountered in populations not at risk for a haemoglobinopathy was HbD-Punjab. Out of 80 reported cases, 20 newborns were identified on their name and the self-reported ethnicity of both their parents of north European ancestry.

The other presumptive positive newborns for a major haemoglobinopathy were seven newborns with β thalassaemia major, five with HbH disease, and five with HbC disease. Of the 142 newborns found at screening to have a major haemoglobinopathy, all were retested and the presumptive diagnosis was confirmed. As infants were still within the maternity service, it was unnecessary to recall the families.

No newborns were misdiagnosed. No infant was reported to have missed screening at birth and subsequently found to be positive for a major haemoglobinopathy.

The origin registered for parents of SCD infants was sub-Saharan Africa in 117 cases, North Africa in two cases, and a mixed origin in four cases (Mediterranean basin and sub-Saharan Africa or North Africa in three and one cases, respectively). For infants with β thalassaemia major, the parents' origin was North Africa in four cases, the Middle East in two cases, and a mixed origin in one case (Middle East and Mediterranean basin). All parents originated from Asia in cases of HbH disease.

Follow-up

After leaving the maternity hospital, and despite our efforts, three families did not attend the first medical visit to a specialised reference care centre.

Despite follow-up, death of two SCD newborns was reported: one died of septicaemia when antibiotic prophylaxis had been interrupted by the family, and one died from respiratory distress not related to SCD.

All infants with β thalassaemia major or HbH disease are alive. From the follow-up data in the retrospective centralised database, serious complications and treatment will be known for the screened newborns and comparison can be made with those who did not benefit from screening.

Table 1 Geographical origin of the neonates screened in Brussels maternity service in 2007

Geographical origin	Number, %
North Europe	45
North Africa*	25
Mediterranean basin*†	12
Sub-Saharan Africa*	9
East Europe*	5
Asia*	2
South America*	2

*At least one parent of ancestry at risk for a haemoglobinopathy.
†Excluding North Africa.

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Table 2 Incidence of haemoglobinopathies in Brussels and Liège

Laboratory diagnosis	Live births 12/94–12/07 Brussels: number (incidence)	Live births 9/02–12/07 Liège: number (incidence)
Disease		
SS	92 (1:1954)	7 (1:1714)
SC	15 (1:11 986)	2 (1:5998)
Sβ Thalassaemia	6 (1:29 965)	1 (1:11 995)
β Thalassaemia major	7 (1:25 684)	–
H	5 (1:35 958)	–
CC	7 (1:25 684)	–
Trait		
AS	2745 (1:65)	184 (1:65)
AC	354 (1:508)	31 (1:387)
AD-Punjab	80 (1:2247)	5 (1:2399)
AE	80 (1:2247)	12 (1:1000)
AO-Arab	40 (1:4495)	–
Other variants	214 (1:840)	7 (1:1714)
Total newborns screened	179 788	11 995

Genetic counselling and prenatal diagnosis in the SCD newborns families

For 88 newborns with a major haemoglobinopathy, information regarding antenatal screening, genetic counselling and discussion of prenatal diagnosis with the parents was recorded. This represented 84 couples (two couples had twins, and two had two SDC infants): six newborns had β thalassaemia major, and 78 had SCD. The first antenatal visit was later than the first trimester in 22 cases (26%). No screening of the mother was done in 20 cases (24%) of which three presented during the third trimester and two had been screened during an earlier pregnancy. Of the 65 mothers screened, 19 partners were not available or refused to be tested. The genetic counselling was carried out by a specialist in 28 cases. Prenatal diagnosis was accepted in 21 cases. In two cases, an obstetrician misinterpreted tests results and genetic counselling was not offered (two cases of sickle cell/β thalassaemia compound heterozygosity).

DISCUSSION

In the European Union, four countries have adopted a neonatal screening programme financed by the authorities in public health: England, France, Belgium and recently The Netherlands.[3–7]

It is interesting to know that estimated European prevalence of conceptions with sickle cell disorders was the highest (ie, higher than 0.20 per 1000) in England and Wales, The Netherlands, Belgium, France, Portugal, Greece, Albania and Cyprus.[1]

The screening policy adopted in Belgium varies in some aspects compared with that of the other European countries. First, the financed neonatal screening is not national but largely limited to the capital city. It took 8 years to obtain funding by the national health authorities, and it was restricted to Brussels. Ongoing discussions with health authorities are focused on the feasibility of antenatal screening, rather than on expanding national neonatal screening. Such a project, not linked to neonatal screening, was implemented in Portugal.[8] As for neonatal screening, a reference laboratory carried out the haemoglobinopathy screening tests (quantification of HbA_2 and HbF; detection and quantification of Hb variants) while the red blood cell parameters were measured in a peripheral laboratory. Thus it could serve as a model.

A different policy (ie, linkage of newborn screening with an antenatal screening programme) has been adopted in England. In fact there are two strategies: one for high prevalence areas where all women are offered screening. and one for low prevalence areas where a more complex algorithm has been developed that is related to the red blood cell indices results and the ethnic origin of the woman or her partner.[3 4] This could be an alternative model to restrictive policies offering only antenatal screening (ie, Portugal) or only neonatal screening (ie, France and The Netherlands).

Dried blood spots on filter paper from a heel prick have been the specimen of choice for neonatal screening except in Belgium where liquid cord blood is used. Heel prick on filter paper cards is the standard specimen collection used in a reference laboratory for screening for congenital hypothyroidism and phenylketonuria.[3–5] Naturally in most countries when the neonatal screening was extended to haemoglobinopathies the same specimen type was used. Liquid cord blood has been chosen in Belgium since it has been implemented in a reference laboratory for haemoglobinopathies and not in a reference laboratory for screening for congenital hypothyroidism and phenylketonuria. Moreover, the primary study had an epidemiological purpose. It was very important that samples for neonatal screening should not be confused with those of the study. This gave us the opportunity to evaluate that type of specimen collection. Liquid cord blood was very stable and provided a high amount of sample for confirmation techniques or DNA tests. When an affected newborn was identified, there was another advantage: an immediate repeat sample could be obtained in order to give the result of the screening to the parents before discharge of the baby.

IEF and HPLC are the main screening techniques used. New methods applicable to screening are now available (ie, capillary electrophoresis, tandem mass spectrometry) or are under development (ie, immunoassay).[9–11] A capillary electrophoresis system (Sebia Benelux, Brussels, Belgium) is under evaluation and we will soon evaluate an immunoassay (Perkin Elmer, Zaventem, Belgium).

In European countries, apart from the ethics, equity and discrimination issues that may arise if universal screening is not applied, it has been demonstrated that universal screening identifies more newborns with disease and prevents more deaths.[12] The choice of the screening method is based on cost-effectiveness and it has been demonstrated that at a prevalence of at least 16 sickle cell trait/1000 and 0.5 SCD/1000 there is no significant cost difference between universal and targeted screening programmes.[12] In Brussels and Liège, these numbers are applicable and universal screening was therefore chosen.

A neonatal haemoglobinopathy screening programme is an opportunity to provide information to healthcare practitioners and families, and withholding information from parents does not seem justified. Nevertheless, others have highlighted the potential risk of neonatal identification of carriers such as inadvertent exposure of non-paternity, social stigma for the individual and family, and adverse psychological effects for the individual and family.[13] In our experience, these aspects can be taken into account and it was decided to report all the results. However, the delivery of these results must be done by specifically trained healthcare staff.

Follow-up of all the affected newborns should remain a major goal of neonatal screening. To ensure monitoring of outcome, a centralised patient registry was implemented in 2007. All SCD patients living in Belgium will be included. The aims of this database will be to evaluate the natural history of the disease

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and to characterise our population in terms of demography, medical history, family history and physical and laboratory findings. Since neonatal screening has not been extended to the whole country, it will be interesting to compare the outcomes of SCD patients diagnosed at birth and those diagnosed later.

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