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Cohort profile: targeted antenatal screening for haemoglobinopathies in Basel

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ABSTRACT

Purpose The pregnancy cohort was established to examine the prevalence and variety of haemoglobinopathies in a high-risk group of pregnant women.

Participants The pregnancy cohort is located in the Department of Obstetrics and Antenatal Care, University Hospital of Basel. The pregnant women were recruited in the first trimester between June 2015 and May 2019. Family origin questionnaires were used to screen pregnant women for the risk of a haemoglobin variant. Based on the questionnaire, pregnant women were divided into two groups: women with a high risk and women with a low risk of a haemoglobin variant. In women with a high risk, red blood cell indices, iron status and chromatography were conducted.

Findings to date 1785 pregnant women were recruited. Out of the 1785 women, 929 were identified as a part of the high-risk group. Due to the missing data of 74 pregnant women with a high risk, the final analysis was conducted in the remaining 855 women. The prevalence of haemoglobinopathies in the high-risk group was 14.5% (124/855).

Future plans This cohort will be used to: (1) implement the screening in prenatal care in Basel; (2) recommend the screening among pregnant women with a high risk of a haemoglobin variant in Switzerland; (3) improve prenatal and neonatal care in patients with a haemoglobin variant; (4) examine adverse pregnancy outcomes in women with a haemoglobin variant and (5) reduce maternal and neonatal morbidity and mortality in the future.

Trial registration number ClinicalTrials.gov Registry (NCT04029142).

Strengths and limitations of this study

- For the first time, to the best of our knowledge, a prospective study has been conducted to examine the prevalence and the variety of haemoglobinopathies among pregnant women in Switzerland.
- The limitation of our study is the lack of conducting of a high-performance liquid chromatography and molecular analysis in all women. However, conducting a universal screening would not be cost effective in low-risk pregnant women.
- The prevalence of 14.5% in the high-risk group of pregnant women confirms an increasing significance of screening for haemoglobinopathies in this group of patients.
- Our findings provide new insights into the prevalence of haemoglobinopathies and have important implications in the health service within Switzerland.

INTRODUCTION

Haemoglobinopathies are among the most common inherited disorders worldwide. As recommended by the WHO, screening and genetic counselling for haemoglobin (Hb) disorders should be an intrinsic part of healthcare in most countries.[1] Two factors have recently highlighted the need for a more coordinated approach to diagnosis and management of haemoglobinopathies. First, the globalisation of migration flows has increased cultural diversity, bringing to Europe populations from areas with a high prevalence of haemoglobinopathies, and second, there is an increasing number of patients requiring health services.[2–6]

In the publication 'A Roadmap for European Haematology Research',[7] the European Haematology Association in 2016 recommended undertaking detailed epidemiological studies in all countries, particularly in Western Europe, as a prerequisite for the implementation of effective prevention programmes. Since then different policies for the antenatal and neonatal screening for haemoglobinopathies have been adapted in Europe, yet the data covering affected patients are not available to every country.[8] There are a few countries with evidence of increasing numbers of patients; however, planning national strategies of increasing number of patients has not been considered at this time to the best of our knowledge.[8] Although haemoglobinopathies have increased significantly in Switzerland in recent years, there is no routine prenatal and/or neonatal screening for haemoglobinopathies. Therefore, since 2015, we have been conducting targeted prenatal screening at the University Hospital of Basel to investigate the current

prevalence of haemoglobinopathy and improve prenatal care in these patients.

Cohort description

A prospective, cross-sectional study was conducted in the Department of Obstetrics and Antenatal Care, University Hospital of Basel, between June 2015 and May 2019. Pregnant women were recruited in the first trimester from our outpatients' department. Family origin questionnaires were used to screen pregnant women for the risk of haemoglobinopathies in the first trimester (figure 1).

The family origin questionnaire was adopted from the National Health Screening Sickle Cell and Thalassaemia Screening Programme in England figure 1. Based on the questionnaire, pregnant women were divided into two groups: women with a high risk and women with a low risk of haemoglobinopathies.

In women with a high risk, red blood cell (RBC) indices, iron status and high-performance liquid chromatography (HPLC) were conducted. For women identified as carriers, their partner was also tested for

Universitätsspital Basel

Department of Obstetrics

Name of patient and date of birth

„Family origin questionnaire for screening of thalassaemia, sickle cell anaemia and other forms of haemoglobinopathy"

To our pregnant patients

Haemoglobinopathies are among the most common inherited disorders worldwide. As a result of the migration of people from countries with high prevalence of haemoglobin disorders, laboratory diagnosis is of growing importance in Switzerland. Countries C, D, E, F and G (See below; in red) have a high prevalence of haemoglobinopathies. Testing for haemoglobinopathies should be conducted in these women.

What are your family origins?

	Mother-to-be	Father-to-be
A. *Swiss*	☐	☐
B. *Northern European*		
Germany, France, Austria, Belgium, Netherlands, Scandinavia, Ireland, United Kingdom and so on	☐	☐
Any other European family origins (white) (Please fill in) (Russia, Australia, North America, South Africa, Canada and so on)	▭	▭
South-East Europa		
Kosovo, Macedonia, Serbia, Albania, Bosnia-Herzegovina, Croatia, Slovenia, Romania, Bulgaria, Hungary, Poland, Slovakia, Czech Republic	☐☐☐	☐☐☐
C. *Southern & Other European (white)*		
Cyprus	☐	☐
Greece, Turkey	☐	☐
Italy, Portugal, Spain	☐	☐
Any other Mediterranean country (Please fill in)	▭	▭
D. *South Asian (Asian)*		
India, Sri Lanka	☐	☐
Pakistan, Afghanistan	☐	☐
Bangladesh	☐	☐
E. *African or African-Caribbean (black)*		
Caribbean Islands or Central America	☐	☐
Africa (excluding North Africa) Eritrea, Ethiopia, Congo and so on	☐	☐
Any other African or African-Caribbean family origins (Please fill in)	▭	▭
F. *South East Asian (Asian)*		
China	☐	☐
Thailand	☐	☐
Malaysia, Vietnam, Philippines and so on	☐	☐
Any other Asian family origins (Please fill in)	▭	▭
G. *Other Non-European (Other)*		
North Africa, South America and so on	☐	☐
Middle East (Saudi Arabia, Iran, Libya, Israel, Jordan and so on)	☐	☐
H. *Decline to answer*	☐	☐
Date of blood withdrawal		

Gesamtleiterin/Chefärztin Frauenklinik des Universitätsspitals Basel: Prof. Dr. med. Viola Heinzelmann-Schwarz
Gynäkologie und Gyn. Onkologie: Chefärztin: Prof. Dr. med. Viola Heinzelmann-Schwarz, Chefarzt Senologie: PD Dr. med. Christian Kurzeder
Stv. Chefarzt: Dr. med. André Kind, MPH (Gyn), Leitender Arzt: Dr. med. Bernhard Fellmann-Fischer, MBA
Geburtshilfe und Schwangerschaftsmedizin: Chefärztin: Prof. Dr. med. Irene Hösli, Stv. Chefarzt: Prof. Dr. med. Olav Lapaire
Gyn. Endokrinologie und Reproduktionsmedizin: Chefarzt: Prof. Dr. med. Christian De Geyter
Gyn. Sozialmedizin und Psychosomatik: Leitende Ärztin: PD Dr. med. Sibil Tschudin
Gyn. Sonographie und Pränataldiagnostik: Leitende Ärztin PD Dr. med. Gwendolin Manegold-Brauer, Stv. Prof. Dr. med. Olav Lapaire
Poliklinik: Leitender Arzt Dr. med. André Kind, MPH

Accredited by EBCOG and ESGO

«Anerkennung als Baby-Friendly» Hospital durch UNICEF/WHO

Qualitätslabel der Krebsliga Schweiz und der Schweizerischen Gesellschaft für Senologie

Zertifiziertes Brustzentrum Gyn. Tumorzentrum

Figure 1 The family origin questionnaire.

Table 1 Haematological data and serum iron status (n=855)

Hb (g/L)	121±12.7 (68–174)
RBC (×10^6/µL)	4.18±0.45 (2.2–6.2)
MCV (fL)	84.5±7.4 (59–122.2)
MCH (pg)	24.2±3.7 (19.2–32.9)
HRC (%)	0.8 (0–58.2)
RDW (%)	14.2±1.9 (11.7–32.1)
Reticulocytes (%)	18.5±5.1 (7–38)
Ferritin (µg/L)	40 (4–5607)
CRP (mg/L)	4.1 (0.3–89.9)

CRP: C- reactive protein
Hb, haemoglobin; HRC, hypochromic red blood cells ; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; RBC, red blood cell; RDW, RBC distribution width.

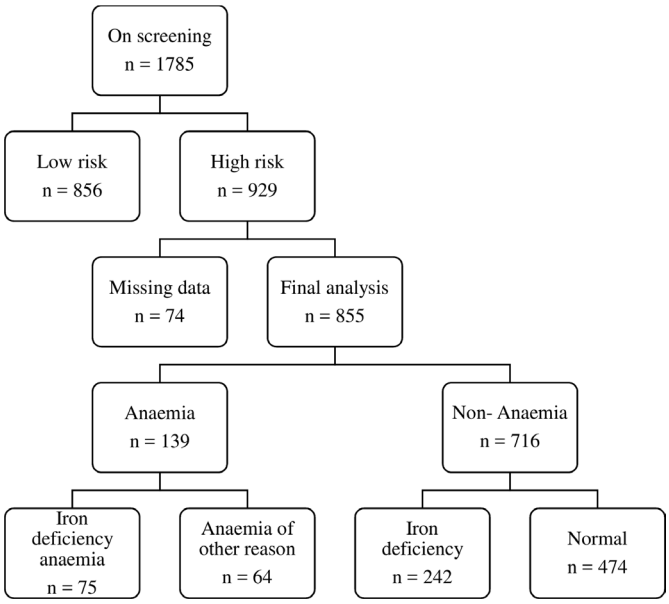

Figure 2 Pregnant women on screening, on testing and completion. Allocation of pregnant women with a high risk of haemoglobinopathy according to haemoglobin and serum ferritin.

haemoglobinopathy, irrespective of family origin. In cases where alpha thalassaemia was suspected based on haematological parameters (mean corpuscular haemoglobin (MCH) <27 pg regardless of iron status),[9] a molecular analysis was performed. If both were carriers of haemoglobinopathies, genetic counselling was recommended and an antenatal genetic testing via choriovillous sampling or amniocentesis was discussed with the patient.

Inclusion criteria were patients aged ≥18 years and having a gestational age at recruitment between 11 and 14 weeks. The primary outcome was the prevalence of haemoglobinopathies in pregnant women. Secondary outcome measures included a variety of haemoglobinopathies, the prevalence of anaemia, iron deficiency anaemia and iron deficiency.

Haematological assessment

Screening for anaemia in pregnancy is generally recommended in Switzerland, including the RBC count and ferritin at the end of the first trimester.[10] Therefore, blood samples were collected by venepuncture. All blood measurements (blood count, CRP (C- reactive protein), ferritin and HPLC) were conducted in the Department of Laboratory Medicine, University Hospital of Basel.

Hb, RBC count, haematocrit, mean corpuscular volume (MCV), MCH, hypochromic RBCs (HRC) and RBC distribution width (RDW) were measured using a haematology analyser. The MCH was automatically calculated from Hb and RBC counts. Haematological parameters were measured using an ADVIA haematology analyser system (Bayer Diagnostics, Leverkusen, Germany).

Serum ferritin was assessed by chemiluminescence immunoassay and CRP was assessed by immunoturbidimetry. The Hbs were separated and processed by an HPLC using a model-II machine from the company Bio-Rad.

Study criteria

Based on the guidelines from the Centre for Disease Control (CDC, USA), anaemia in pregnancy was defined as an Hb of less than 110 g/L in the first trimester.[11] Iron deficiency (ID) was defined as a serum ferritin of less than 30 µg/L. Iron deficiency anaemia (IDA) was defined as an Hb of less than 110 g/L and serum ferritin of less than 30 µg/L. Anaemia of other aetiology was defined as an Hb of less than 110 g/L and serum ferritin 30 µg/L or more.

The determination of haemoglobin A_2 ($HbA_{2)}$ ≥3.5% was indicative of beta thalassaemia.[9 12] In some cases of

Table 2 The haematological data of the four groups (n=855)

	Group 1		Group 2		Group 3		Group 4	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Hb (g/L)	125 (8.1)	110–155	124 (8.5)	110–148	100 (8.1)	75–109	100 (8.4)	68–109
	Median		Median Mean (SD)		Median Mean (SD)		Median	
CRP (mg/L)	3.9	0.3–89.9	4	0.3–141	5.4	1–27.6	5.1	1.2–19.4
Ferritin (µg/L)	62	30–436	19 (6.6)	5–29	14 (6.5)	4–27	73.5	(30–827)

Group 1: normal (474); group 2: iron deficiency (242); group 3: iron deficiency anaemia (75) and group 4: anaemia of other causes (64).
CRP, C- reactive protein; Hb, haemoglobin; ID, iron deficiency; IDA, iron deficiency anaemia.

Table 3 Types of haemoglobin variants

Haemoglobin variant	Number of patients (%)
Beta thalassaemia	42 (33.9)
Alpha thalassaemia trait	39 (31.5)
Heterozygous alpha^{+} thalassaemia	23
Homozygous alpha^{+} thalassaemia	12
Heterozygous alpha^{0} thalassaemia	4
Sickle cell anaemia	23 (18.5)
Heterozygous	18
Homozygous	5
Other haemoglobins	12 (9.7)
Heterozygous delta thalassaemia	3
Haemoglobin E	6
Heterozygous haemoglobin E	5
Homozygous haemoglobin E	1
Heterozygous haemoglobin C	1
Heterozygous haemoglobin D	2
Compound haemoglobins	8 (6.4)
Heterozygous sickle cell anaemia/ heterozygous alpha thalassaemia	4
Heterozygous sickle cell anaemia/homozygous alpha thalassaemia	1
Homozygous sickle cell anaemia/heterozygous alpha thalassaemia	1
Heterozygous haemoglobin C/homozygous alpha thalassaemia	1
Heterozygous haemoglobin E/heterozygous alpha thalassaemia	1

beta thalassaemia (borderline elevated HbA_2 and in cases where both partners are carriers for beta thalassaemia), confirmation by molecular analysis was conducted. Hb variants (C, D, E, F and S) were identified using the HPLC technique. A sickle solubility test was performed whenever Hb S was detected by HPLC.

A genetic test was performed in women with MCH <27 pg to confirm alpha thalassaemia.[9] Two forms of alpha thalassaemia trait were described; the homozygous alpha trait if the missing genes were on opposite chromosomes and the heterozygous alpha trait if both missing genes were on the same chromosomes.

Anaemia of other aetiologies was found to be primarily caused by haemoglobinopathies, diseases of the liver or kidney, HIV infection, antiphospholipid syndrome and so on.

Statistical analysis was conducted using STATA V.12.0 (Stata Corporation, College Station, Texas, USA). Blood indices, CRP and serum ferritin were expressed as mean±SD and range, or median and range.

Dissemination

The findings of this study will be published in a peer-reviewed journal and presented at national scientific conferences to disseminate the results to academic and health professional audiences. In addition, they will be made available to the participants and to the wider public on our website at the time of publication.

Patient and public involvement

The patients and the public were neither involved in developing the hypothesis, the specific aims or the research question, nor were they involved in developing the plan for design or implementation of the study.

Findings to date

In brief, 1785 pregnant women were recruited. Out of 1785 women, 929 were identified as a part of the high-risk group. Due to the missing data of 74 pregnant women within the high-risk group, the analysis was conducted in 855 women (figure 2). The mean gestational age at the time of screening was 12.3±2 weeks. The mean of Hb was 121±13 g/L (68–174 g/L) and the median of ferritin was 40 µg/L (4–5607 µg/L) (table 1).

There were 139 anaemic women (139/855; 16.3%); namely, iron deficiency anaemia was identified in 75 women (75/855; 8.8%) and anaemia of other aetiology in 64 women (64/855; 7.5%) (figure 2). There were 242 women with iron deficiency (242/855; 28.3%). The mean of Hb, serum ferritin and CRP of each group is presented in table 2.

Most of the pregnant women in the screening originated from Africa (primarily Eritrea), Turkey, India and the Middle East (primarily Syria). The prevalence of haemoglobinopathies was 14.5% in the high-risk group (124/855) and 6.95% for all patients (124/1785). There were 5 women with sickle cell anaemia, 18 with the sickle cell trait, 39 with the alpha thalassaemia (4 women with heterozygous alpha^{0} thalassaemia trait, 23 with heterozygous alpha^{+} thalassaemia and 12 with homozygous alpha^{+} thalassaemia), 42 with heterozygous beta thalassaemia, 12

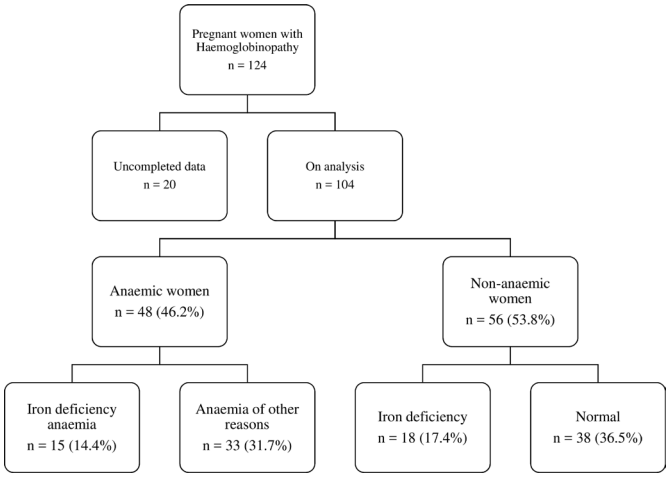

Figure 3 Allocation of pregnant women with haemoglobin (Hb) variants according to Hb and serum ferritin.

with other Hb variants and 8 women with an Hb variant and alpha thalassaemia (table 3).

In pregnant women with an Hb variant, the mean of Hb was 111±14 g/L (79–162 g/L) and the median of ferritin was 54 µg/L (5–5607 µg/L). There were 48 anaemic women with an Hb variant (48/104; 46.2%) and 56 were non-anaemic (56/104; 53.8%) (figure 3). Of the identified women with an Hb variant, 15 showed iron deficiency anaemia in the first trimester (15/104; 14.4%) (figure 3). There were 33 women with an Hb variant and concomitant iron deficiency. In 71 pregnant women with an Hb variant, the ferritin was ≥30 µg/L. In 12 women, the ferritin was ≥150 µg/L (12/104; 11.5%), 2 women presented alpha thalassaemia, 6 beta thalassaemia and 4 homozygous sickle cell anaemia. There was a very low mean of MCV and MCH in the group of women with alpha and beta thalassaemia (mean MCV 70±7 fL (59–88) and MCH 23±3 pg (16.3–31.6)). In 23 pregnant women (23/124), an Hb variant was previously diagnosed and 101 women were diagnosed based on our screening (101/124).

DISCUSSION

Population movements affect the distribution of inherited disorders of Hb within countries, with previously isolated populations increasingly interacting and large numbers of migrants moving from rural to urban areas; the complexity of genotypes can be observed as newly introduced variants may interact with local ones to create more or less severe phenotypes.[13]

The prevalence of haemoglobinopathies of 6.95% (124/1785) in total in our study group corresponds to an overall average of 8%.[14] In the high-risk group of women, the prevalence of haemoglobinopathies was twice as high (124/855; 14.5%). Using the family origin questionnaire, we identified a group of pregnant women with haemoglobinopathies, which might have otherwise been overlooked. Half of the pregnant women with haemoglobinopathies were non-anaemic and two-thirds of them had normal iron status in the first trimester. One-third of the women with haemoglobinopathies showed anaemia with normal iron status in the first trimester. The majority of pregnant women in the screening originated from Africa, Turkey, the Middle East and India. Due to the political changes significant more women were reported from Syria and Eritrea in the last few years.

In the UK, where there is a well-established, linked neonatal and antenatal screening programme for haemoglobinopathies, a downward trend in reported screen-positive results is discernible in some areas.[8] In contrast, Germany, Italy and France have recently been accepting large numbers of refugees and have faced a dramatic increase in their patient numbers since 2014.[8] With the exception of Belgium, the UK, Cyprus, Greece, Germany and Spain, no national registry exists for haemoglobinopathies in European countries.[8] According to the Organisation for Economic Co-operation and Development, the percentage of foreign-born populations within the European Union in 2008 ranged from 4% in Finland to 37% in Luxembourg.[14] Switzerland has one of the highest proportions of foreigners in its midst among all nations: 24.6% in 2016.[14]

European countries with a high prevalence of haemoglobinopathies have adopted a neonatal screening programme (France, Belgium, the Netherlands and Spain), an early antenatal screening programme (Sweden and Italy), a linked neonatal and antenatal screening programme (the UK) or a preconceptional, premarital screening programme (Cyprus, Greece and Turkey). Antenatal screening programmes are generally assessed by the uptake of prenatal diagnosis, optimal care management and/or the allowance to terminate affected pregnancies.

The limitation of our study is the lack of HPLC in all women. However, conducting universal screening would not be cost effective in low-risk pregnant women. 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 traits/1000, there is no significant cost difference between universal and targeted screening programmes. Therefore, targeted antenatal screening is recommended in Switzerland. On the other hand, it would be impossible to detect women with a sickle cell trait by using the full blood count alone for screening due to normal RBC parameters in these patients.

Our findings will be used to further implement the screening in prenatal care in Basel and will be recommended among all pregnant women with a high risk of haemoglobinopathies in Switzerland. Early recognition of Hb variants in women enables early testing of partners and provides the opportunity for further testing where required. Thereby, the ability to improve prenatal and neonatal care in these patients and to reduce the number of children with severe clinically relevant Hb variants can be offered.

Contributors GAB is the principal investigator who designed the study, carried out the quantitative analysis and drafted the article. FG collected the data. IH reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study received ethical approval from the local ethics committee in Basel (ID 2019–01065). All members of the research team were aware of the guidelines for good clinical practice.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. After publication of the study individual, anonymous participant data, including variable keys, will be available from the corresponding author on request. Researchers may request data to repeat the analyses or use the data for secondary analyses (eg, systematic review and meta-analysis).

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