Summary/Conclusion: This study presents the results of the first molecular study of a large number of cases of NADH cytochrome b5 reductase deficiency in India Molecular modeling of all the mutations reported in CYB5R3 justifies the association of the mutations with the varying severity of the disease. Knowing the profile of mutations in families with severe neurological disorders allowed us to offer a prenatal diagnosis of RCM Type 2 for the first time in the Indian population. Thus severity and recurrence risk of type II RCM merit could be prevented by prenatal diagnosis in affected families.

PS1202 DIFFERENTIAL DIAGNOSIS OF CONGENITAL HEMOLYTIC ANEMIA OF NEONATES AND INFANTS IN JAPAN

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Background: As a reference center, we analyze erythrocyte membranes, enzymes, and hemoglobin for congenital hemolytic anemia (CHA) in cases that are not diagnosed using ordinary laboratory examinations, and we introduce genetic testing using a hemolytic anemia-related gene panel. We analyzed 136 cases from 2016 to 2017, including 36 patients under 1 year of age and 100 patients aged 1 year and over. Consequently, a definitive diagnosis was obtained for 84% of patients aged 1 and over but for only 72% of patients under 1 year of age.
Aims: We aimed to summarize the final diagnosis for hemolytic anemia in patients less than 1 year of age analyzed over 3 years from 2016 to 2018 and discuss the common clinical features and laboratory data of undiagnosed cases.
Methods: We analyzed 58 infantile CHA casesusing the following laboratory tests: flow-cytometric osmotic fragility (FCM-OF) test, eosin 5'-maleimide binding test, assay of 15 red blood cell (RBC) enzymes, reduced glutathione content, and isopropanol stability test for unstable hemoglobinopathy. For the cases with a difficult diagnosis, genetic analysis with target-captured sequencing (TCS) using a gene panel of 74 genes related to CHA was carried out.
Results: Among 58 infants with CHA, we diagnosed 30 cases (52%); there were 18 cases of hereditary spherocytosis (HS), 2 cases of hereditary elliptocytosis (HE), 1 case of hereditary pyropoikilocytosis, 4 cases of erythro-enzymopathies, and 2 cases of unstable hemoglobinopathies. Among the 28 undiagnosed cases, 14 showed an elevated percentage of residual RBCs in the FCM-OF test. These cases also showed abnormal RBC morphologies such as target cells, stomatocytes, and/or pyknocytes. We performed TCS for 4 of the 14 cases, but none harbored mutations such as *PIEZO1* or *KCNN4*. Of the 7 cases, which could be followed up to at the age of one year, all recovered from CHA within a year.
Summary/Conclusion: Mild hemolytic anemia accompanied by stomatocytes and/or target cells is a typical clinical feature of dehydrated hereditary stomatocytosis (DHSt) in adults. We previously showed that FCM-OF clearly discriminated DHSt from HS or HE (Utsugisawa et al., Blood 2017;130:929). In this study, approximately half of the infants with undiagnosed CHA showed similar RBC morphology and higher osmotic resistance with no causative gene mutations. We noticed that clinical pictures of these infants resembled the description of infantile pyknocytosis (IP), which is a transient neonatal hemolytic anemia with the characteristic RBC morphology. As IP cases follow a relatively benign clinical course, we propose that FCM-OF may be used as a biomarker for the tentative diagnosis of IP.

PS1203 HEREDITARY SPHEROCYTOSIS: SCREENING AND DIAGNOSTIC APPROACH IN A BELGIAN LABORATORY

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Background: Hereditary spherocytosis (HS) is a worldwide reported pathology, but the highest prevalence is found among the northern European population (estimated at 1:2000 births). However, its diagnosis is still complex. As a Belgian reference laboratory for this condition, we provide a review of our 8 years' experience.
Aims: Our objectives were (a) to characterize a cohort of confirmed HS patients in term of clinical and biological parameters, (b) to evaluate and eventually adjust our previously established cutoffs for screening and diagnostic tests , and (c) to evaluate our diagnostic approach and compare it with the last ICSH algorithm .
Methods: This retrospective study focused on patients from 10 different Belgian hospitals explored in our laboratory for HS between April 2009 and December 2016. Patients previously splenectomized, with red blood cell transfusion received within 3 months prior to sampling or without a complete medical record were excluded. We divided the cohort (77 patients) in 2 groups: HS (n = 33) and non-HS (n = 44). Non-HS patients were then divided into 3 groups: other hemolytic conditions (OHC; n = 23), non-hemolytic anemia (NHA; n = 10) and others (O; n = 11). HS diagnosis was based on the following criteria: family history, chronic hemolytic anemia features, spherocytes on blood smear, positive screening test i.e. cryohemolysis (CH) or eosin-5-maleimid (EMA), and at least one positive diagnostic test (ektacytometry or erythrocytes membrane protein electrophoresis). Hematological parameters were achieved using the UniCel DxH800 (Beckman Coulter) hematology analyzer.
Results: The median age of HS and non-HS groups were respectively 6 and 28 years. Jaundice and splenomegaly were the most discriminating clinical signs. Between HS and non-HS as well as between HS and OHA groups, reticulocytes count, mean reticulocyte volume (MRV), immature reticulocyte fraction (IRF), mean sphered cell volume (MSCV), screening tests i.e. CH and EMA and ektacytometrywere the most statistically significant biological parameters ($p < 0.001$). New cutoffs have been established for most discriminating reticulocytes parameters, CH, EMA and ektacytometry (Table 1). Application of the last ICSH algorithm did not reveal diagnostic discordance. However, some tests were realized while not recommended.

Table 1 – Cutoff update for reticulocytes parameters, screening and diagnostic tests.

	New cutoff					Old cutoff			
	AUC	95% CI	Value	Ss (%)	Sp (%)	Value	Ss (%)	Sp (%)	References
Delta (MCV-MSCV)	0,9	0,8-1,02	>18.2	100	80	>18	92	94	*Lazarova E et al., 2014*
MRV (fl)	0,9	0,86-1	<98	92	68	<92	92	94	*Lazarova E et al., 2014*
Ret/IRF	0,9	0,74-0,98	>2.25	92	81	>2.6	92	89	*Lazarova E et al., 2014*
MSCV (fl)	0,9	0,78-1	<73	96	65	<70.2	92	90	*Lazarova E et al., 2014*
CH*	0,9	0,89-0,99	>15	94	70	>10	95	90	/
EMA*	1	0,89-1,02	>14	88	100	>19	100	82	/
Ektacytometry O min*	0,9	0,8-1	>17.1	67	100	>21.5	42	97	*Lazarova E et al., 2017*
Ektacytometry Area*	0,8	0,63-1,03	<-24.35	71	80	<-18.5	70	95	*Lazarova E et al., 2017*

AUC area under the curve, *CI* confidence interval, *Ss* sensitivity, *Sp* specificity
*: Percentage of change compared to a control sample

Summary/Conclusion: This study confirms published results regarding the major clinical features of HS and the most discriminant reticulocytes parameters available with the DxH800 analyzer for suspicion of HS. Cutoffs for those parameters, for screening tests and ektacytometry values were adjusted on the basis of an extended study period on a restricted cohort of patients with a confirmed diagnosis of HS.
In a general laboratory, a higher number of patients could be screened for HS using the automated reticulocytes parameters on a DxH800. Moreover, in a reference laboratory, those parameters could be integrated in the first steps of the HS diagnosis algorithm.

PS1204 SEVERE HEREDITARY HAEMOLYTIC ANAEMIA DUE TO THREE NOVEL SPTA1 MUTATIONS IN TWO COMPOUND HETEROZYGOUS UNRELATED PATIENTS

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