Article

Hereditary spherocytosis: How to optimize its diagnosis

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Keywords

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Abstract

Objectives: Hereditary spherocytosis is the most frequent inherited hemolytic anemia. Through an observational retrospective study of 8 years, this review aims at optimizing hereditary spherocytosis diagnostic approach. Our objectives were to characterize our population, adjust local cut-offs for confirmatory tests and revise our diagnostic algorithm on basis of international guidelines.

Method: Clinical and laboratory data of a Belgian cohort of 33 patients with hereditary spherocytosis were analyzed and compared to 44 non-spherocytosis patients.

Results: Hereditary spherocytosis patients were mostly children (median age of 6 years), jaundice and splenomegaly were rather common. The most discriminating routine tests between hereditary spherocytosis and patients with other hemolytic conditions were red blood cell distribution width and most of the reticulocyte parameters measured ($p \leq 0.01$). While confirmatory tests for hereditary spherocytosis, e.g., cryohemolysis, eosin-5'-maleimide binding test, ektacytometry 0 min and ektacytometry area under the curve were also discriminating between those two populations ($p \leq 0.0001$) with cut-off values for an AUC on ROC curve ≥ 0.8 of 15%, 14%, 17% and -24.5%, respectively. Compared to an international algorithm, no false positive or false negative cases were found with our simplified algorithm and the application of the new cut-off values for confirmatory tests.

Conclusion: The use of reticulocyte parameters is a simple tool as a first step in screening for hereditary spherocytosis in a large number of laboratories. It allows to select patients who need further or not more complex analysis in a context where optimal healthcare costs repartition seems more important than ever.

Introduction

Hereditary spherocytosis (HS) is a worldwide reported pathology. It is an inherited disease due to red blood cell (RBC) membrane protein defect. The highest prevalence is estimated at 1:2000 among Caucasians (1). It is the most common non-immune hemolytic anemia (2) . However, the heterogeneous character of this disease makes its diagnosis complex. Typical presentation comprise anemia, jaundice, splenomegaly and reticulocytosis (3) . The severity of the disease can be defined on basis of the degree of anemia in minor, moderate, moderate to severe or severe HS. (1) Most patients present with minor to moderate form, and up to one third have an isolated compensated hemolysis (3). Diagnosis is then often delayed, especially in the absence of family history. However, in cases of decompensation, anemia may be severe (1). Rarely, HS can be associated with more complex syndromes due to large genomic deletions (2). The challenge is to optimize identification of suspect cases and common hematological parameters could be an effective screening tool.

Current guidelines states that HS diagnosis can be made without any further tests in case of spherocytes on blood smear, compatible red cells indices, negative direct antiglobulin test (DAT) and positive family history (3) . If not clear, at least one screening test for HS should be performed (3). As none of those screening tests has a sensitivity of 100%, a combination of tests is recommended (4). There are various screening tests available for HS, i.e. the osmotic fragility test (OFT), the acid glycerol lysis time test (AGLT), the cryohemolysis (CH) test and the eosin-5'-maleimide binding test flow cytometry based (EMA).

The classic OFT is based on evaluating the degree of hemolysis induced by a hypotonic solution of NaCl. The drawback of this first test is its lack of sensitivity and specificity, often leading to undetermined results. The AGLT is based on measuring the time needed to obtain 50% of hemolysis of a blood sample in a buffered solution of hypotonic saline/glycerol (3).

The CH test is based on the increased susceptibility of spherocytic RBC to hemolysis in cold and hypertonic conditions (5). It has a better sensitivity and specificity than the first two tests cited, varying from 48.5% to 100 % and 77% to 96% respectively according to the cut-off value applied (6). Finally, the EMA test is the most frequently recommended test for HS screening. Dye binding is usually decreased in erythrocyte membrane in HS. It has a higher specificity (93% to 99.1%) than osmotic fragility tests but a lower sensitivity (89% to 99%) (7–10). It can fail to identify some HS cases associated with ankyrin defects and dye binding is also decreased in other conditions like elliptocytosis, pyropoïkylocytosis, and Southeast Asian ovalocytosis (SAO) (7–10). In this context, it might be useful to confirm HS diagnosis with more specific tests but always associated with careful examination of blood films. Osmotic ektacytometry is the analysis of the RBC deformability in changing osmotic conditions with constant shear stress applied. It provides distinct profiles with precise points that enable a good discrimination between different inherited RBC membrane disorders such as HS, hereditary stomatocytosis or elliptocytosis (11). Another test used for HS confirmation is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). It aims to separate RBC membrane proteins on a gel according to their molecular weight and identify quantitative or qualitative alterations of those proteins (11). However it lacks sensitivity in mild HS cases and is usually used in specific situations, as before a splenectomy or if previous tests are equivocal (12). Finally, a molecular analysis is reserved for enigmatic cases.

Through an observational retrospective study of 8 years in our reference center, the present study aimed to optimize HS diagnostic approach. We based our work on a cohort of patients with a confirmed diagnosis of HS and a complete medical record. Our objectives were (a) to characterize HS disease in terms of clinical and biological features, (b) to adjust our previously established cut-offs for screening and diagnostic tests and (c) to compare our diagnostic approach with the International Council for Standardization in Haematology (ICSH) algorithm of 2015 and to elaborate our local diagnostic algorithm (3).

Materials and methods

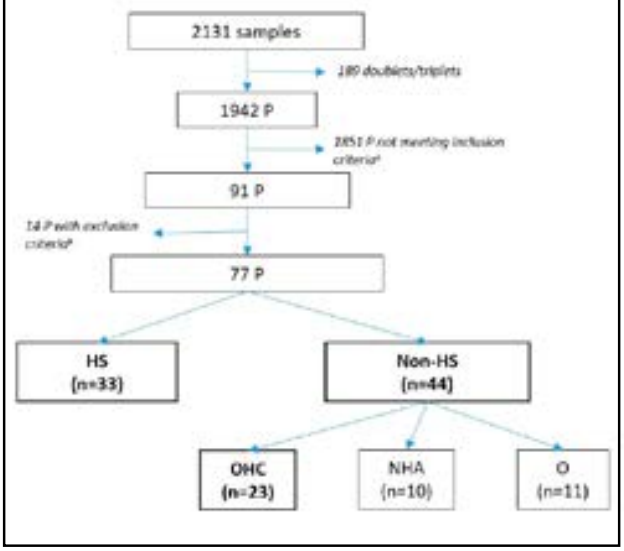
Patients

This study considered patients from 10 different Belgian hospitals located in Brussels and Liege areas: Institut Jules Bordet, CHU Brugmann, CHU de

Liège, CHU Saint-Pierre, HUDERF, CHR Citadelle, CHR Verviers, CUB hôpital Erasme, Esperance and Saint-Joseph (CHC, Liège). Cohort selection process is presented in Figure 1. Patients samples were tested for HS in our laboratory between April 2009 and December 2016. Selection criteria included a complete medical record with follow-up, availability of hematological parameters (at least hemoglobin level and reticulocyte count), and 2 negative screening tests or at least one confirmatory test result in case of a positive screening test. Patients were excluded when splenectomized before sampling (9 patients, 6 with HS) or transfused with RBC within 3 months before sampling (5 patients, none with HS). HS diagnosis was based on family history, clinical and laboratory features of chronic hemolysis, the presence of spherocytes at the peripheral blood smear, and at least one screening test positive, i.e. cryohemolysis or EMA and one confirmation test compatible with HS, i.e. SDS-PAGE or ektacytometry. Other causes of hemolysis were excluded (negative DAT, RBC enzymopathies and hemoglobinopathies).

The final cohort included 77 patients: 33 with a diagnosis of HS and 44 representing non-HS population (Fig. 1). Given the heterogeneous character of the non HS population, subjects were divided into 3 subgroups according to biological parameters 1) other hemolytic conditions (OHC; n=23; decreased haptoglobin and/or an increased lactate dehydrogenase or unconjugated bilirubin; 1 picnocytosis, 1 elliptocytosis, 1 idiopathic thrombocytopenic purpura (ITP), 1 atypical hemolytic uremic syndrome, 2 sickle cell diseases, 1 pernicious anemia, 2 myelodysplastic syndrome, 1 Kaposi sarcoma, 2 autoimmune hemolytic anemia, 1 iso-immunization, 2 cirrhosis, 7 undetermined diagnosis), 2) non-hemolytic anemia (NHA; n=10; 1 infection, 1 myelodysplastic syndrome, 1 acute myeloid leukemia, 3 prematurity, 1 thalassemia and 3 iron deficiency), 3) other subjects (O; n=11; neither anemia nor hemolysis in non-related HS; 7 healthy, 1 transitory idiopathic splenomegaly event, 1 infection and 2 splenomegaly due to onco-hematologic disorders).

Figure 1 : *Cohort selection and repartition.*
a complete medical record and follow-up, hemoglobin level and reticulocyte count, 2 negative screening test or at least one confirmatory test result in case of a positive screening test; b red blood cell transfusion within 3 months before sampling or splenectomy before sampling
P, patients; HS, hereditary spherocytosis; OHC, other hemolytic conditions; NHA, non hemolytic anemia; O others



Samples

Peripheral blood samples were collected into tubes containing dipotassium ethylenediaminetetraacetic acid (K2-EDTA). A heparinized tube was added if an SDS-PAGE analysis was requested. All blood samples were processed within 24 hours of sampling and where then stored between 4-7°C within 8 hours after receipt. For samples sent from other laboratories, blood smear and mean corpuscular volume (MCV) were not considered.

Routine biochemical and hematological tests

Serum lactate dehydrogenase activity, haptoglobin, total bilirubin and unconjugated bilirubin concentrations were performed with the Modular P800 (Roche Diagnostics, Vilvoorde, Belgium), hemoglobin level, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW-CV), reticulocyte count, immature reticulocyte fraction (IRF), mean reticulocyte volume (MRV), mean sphered cell volume (MSCV) where obtained through UniCel DxH 800 (Beckman Coulter, Namur, Belgium). The analytical variability was less than 1% for all considered parameters. The operation mode was described previously (13). Given a large heterogeneity of age in our cohort and therefore different reference ranges, parameters were expressed as a percentage of the mean reference value of a test (hemoglobin level, MCV, MCH, MCHC, absolute reticulocyte count) or percentage of the upper reference value of a test (total bilirubin and LDH).

Hereditary spherocytosis specific screening and confirmatory tests

Cryohemolysis and EMA are the two specific screening tests used in our laboratory. Both methods were described previously (11). In order to minimize intra-assay variations in cryohemolysis, patient's sample is compared with a control sample and results are expressed in percentage of hemolysis. Our established performances are a sensitivity of 95% and a specificity of 90% for a cut-off value of 10% (personal data, unpublished). For EMA, results are expressed as the percentage reduction of mean fluorescence intensity (MFI) compared to the mean MFI of 6 healthy donors. Our established performances for a cut-off value of 19% are a sensitivity of 100% and a specificity of 82% (personal data, unpublished).

Ektacytometry and SDS-PAGE tests were described previously (11,12). As there are no standard available for these tests, patient's sample is always processed and compared with a control sample. Our evaluation of ektacytometry sensitivities and specificities were respectively of 42% and 97% for a cut-off value of 21.5% for the osmolality point at the minimal elongation index (O min), and 70% and 95% for a cut-off value of -18.5% for area under the curve (AUC) (12).

Statistical analysis

Data were analyzed using GraphPad Prism 8 software (SanDiego, USA). Descriptive statistics were computed for each parameter. Gaussian distribution was assessed using Shapiro-Wilk normality test.

Concerning HS and non-HS groups comparison, mean and standard deviation (SD) was determined using unpaired Student's t-test for data with Gaussian distribution, while median and interquartile range (IQR) were determined using a Mann-Whitney test for data with non-Gaussian distribution. Multiple comparison tests, i.e. ANOVA and Dunnett for data with Gaussian distribution, Kruskal-Wallis test and Dunn for non-Gaussian distribution data were used to compare HS with non-HS subgroups, with a special attention to OHC, in order to establish which parameter remained discriminant for HS in a context of hemolysis. Data were considered statistically significant if p value ≤ 0.05 (*: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001).

In order to adjust our cut-off values for relevant identified parameters, the area under the receiver-operating characteristic (ROC) curve was calculated and diagnostic values were compared.

Results

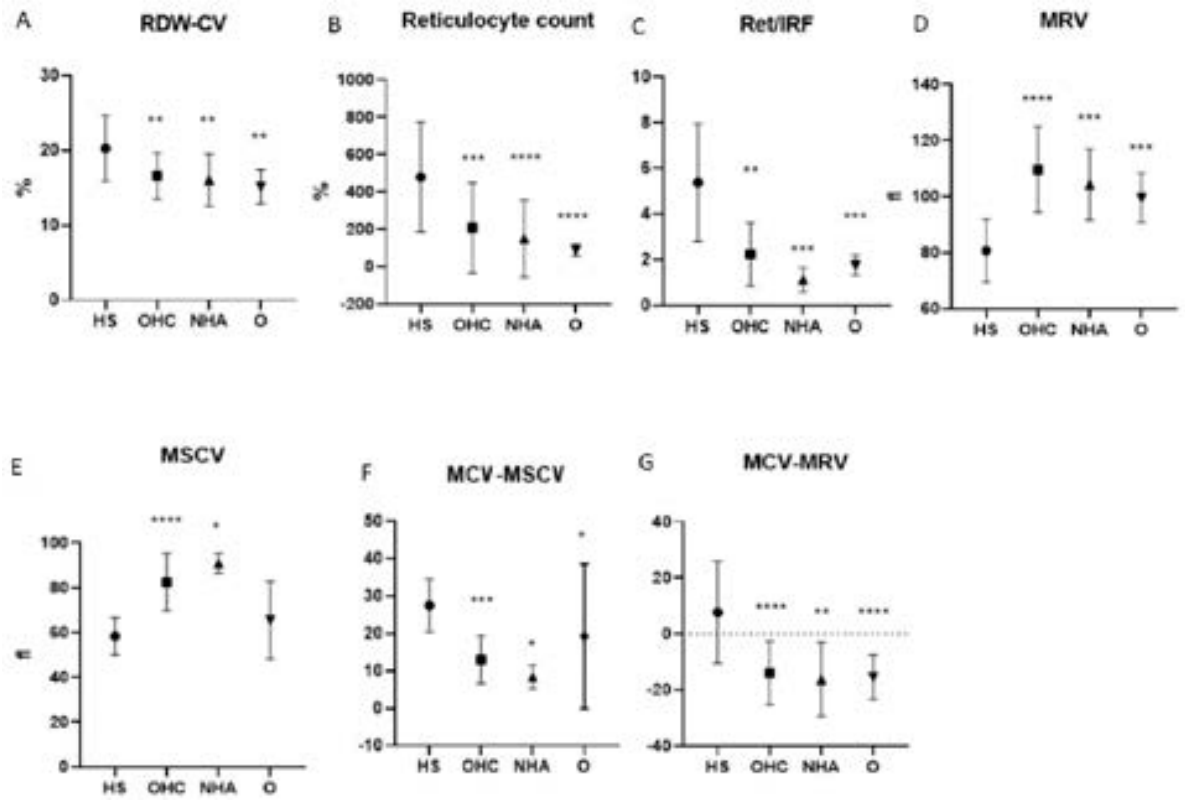
Cohort characterization

Clinical and laboratory characteristics are depicted in Table 1. HS patients were significantly younger than non-HS patients with no significant difference of gender repartitions between the two groups. Jaundice and splenomegaly, a positive family history of HS and transfusion requirement were significantly more frequent in HS patients (p≤0.01). Decrease in haptoglobin and increase in total bilirubin, MCHC and RDW-CV were significantly more often observed in HS patients (p<0.05).

Fig. 2 shows RDW-CV and parameters provided by the reticulocyte channel that are statistically significantly different between HS and non-HS different subgroups.

Cryohemolysis and EMA were strongly discriminating between HS and the

Figure 1 : *Discriminant hematological parameters between HS and non-HS subgroups.*
*(A) RDW-CV was significantly higher in HS compared to all non-HS subgroups. (B) Reticulocyte count was significantly higher in HS compared to all non-HS subgroups. (C) Ret to IRF ratio was significantly higher in HS compared to all non-HS subgroups. (D) MRV was significantly lower in HS compared to all non-HS subgroups. (E) MSCV was significantly lower in HS patients compared to OHC and NHA subgroups. No statistically significant difference was observed between HS and O subgroup. (F) Delta MCV-MSCV was significantly higher in HS compared to all non-HS subgroups. (G) Delta MCV-MRV was significantly higher in HS compared to all non-HS subgroups. HS, hereditary spherocytosis; MCV, mean cell volume; MRV, mean reticulocyte volume; MSCV, mean sphered cell volume; NHA, non-hemolytic anemia; O, others; OHC, other hemolytic conditions; RDW-CV, red blood cell distribution width; Ret/IRF, ratio between reticulocyte count and immature reticulocyte fraction; *, p≤0.05; **, p≤0.01; ***, p≤0.001; ****, p≤0.0001.*



different subgroups, as shown in Fig. 3 (p<0.0001). Regarding ektacytometry, O min appeared particularly useful for HS differential diagnosis with other hemolytic conditions (p<0.0001). AUC and EI max were only discriminating between HS and "others" group (p<0.01). There was no osmoscan data for patients from the NHA group.

Table 1 - Characteristics of HS and non-HS patients.

Characteristic	HS (n=33)	Non-HS (n=44)	*P* value
Age (years; median (IQR))	6 (1-31)	28 (4-45)	0.0274
Gender (% males)	67	52	ns
Typical clinical and laboratory features (n; median (IQR))[a]	3 (2-4)	2 (1-3)	0.0004
Positive family history (%)	79	5	<0.0001
Transfusion requirement (%)	42	14	0.0044

[a]neonatal jaundice, anemia, gallbladder stone, hemolysis, splenomegaly, jaundice.
n, number of typical features that are present within an individual patient; IQR, interquartile range; HS, hereditary spherocytosis; ns, non-significant

Cut-off values

Cut-off values were established on basis of ROC curve analysis results. Parameters with an AUC ≥ 0.8 were considered. Chosen cut-off values as well as those previously published are depicted in Table 2.

Working algorithm evaluation

In order to evaluate our diagnostic approach, the algorithm proposed by the ICSH in 2015 was applied to the entire cohort. Based on these patients' diagnoses of HS, neither false negative nor false positive cases of HS were found with the old nor the new cut-off values. However, it appeared that several tests could have been avoided. In fact, ektacytometry and SDS-PAGE analysis were both performed in 6 patients despite the absence of family history and negative results for cryohemolysis and EMA. All these cases were finally negative for HS. Also, cryohemolysis, EMA and at least one confirmation test were performed in 15 patients who had a positive family history and spherocytes seen on blood smear. They were eventually all positive for HS.

Based on this experience, we elaborated a local diagnostic algorithm inspired by the one published by ICSH in 2015 (Fig. 4). Step 1: in case of HS suspicion facing typical HS features and/or positive family history, in order to confirm hemolysis, RDW-CV and parameters from the reticulocyte channel are required and a blood smear is performed for the detection of spherocytes or other abnormalities in red blood cells morphology. If relevant, frequent other hemolytic conditions have to be excluded. Step 2: if a diagnosis of HS is still retained, cryohemolysis and EMA tests are performed. If both of them are negative, there is no need for further exploration. In contrast, if results are positive or doubtful, or if spherocytes are seen on the blood smear without any other explanation, we go to step 3. Step 3: an ektacytometry is conducted. If the profile is typical for HS, the diagnosis can be retained. If the profile is doubtful or if a splenectomy is planned, SDS-PAGE is performed in order to exclude CDA II, to confirm the diagnosis of HS and to determine the type of protein defect. Molecular analysis is only performed in specific cases of chronic transfusion or discrepancies between clinical picture and analytical results.

Table 2 – Cut-off values based on ROC curve analysis of the entire cohort.

	AUC	95% CI	New cut-off value			Old cut-off value		
			Value	Ss (%)	Sp (%)	Value	Ss (%)	Sp (%)
Delta (MCV-MSCV)	0,9	0,8-1,02	**>18**	100	80	>18[c]	92	94
MRV (fl)	0,9	0,9-1	**<96**	88	70	<92[c]	92	94
Ret /IRF	0,9	0,8-1	**>2.2**	92	81	>2.6[c]	92	89
MSCV (fl)	0,9	0,8-1	**<71**	87	70	<70.2[c]	92	90
CH[a]	0,9	0,9-1	**>15**	94	70	>10	95	90
EMA[a]	1	0,9-1	**>14**	88	100	>19	100	82
Ektacytometry O min[b]	0,9	0,8-1	**>17**	67	100	>21.5[d]	42	97
Ektacytometry AUC[b]	0,8	0,6-1	**<-24.5**	71	80	<-18.5[d]	70	95

a patient/control (%), b patient/control ratio, c proposed cut-off for clinical use by Lazarova et al in 2014 (13), d proposed cut-off for clinical use by Lazarova et al in 2017 (12) ROC, receiver operating characteristics; AUC, area under the curve; CI, confidence interval; Ss, sensitivity; Sp, sensitivity; MCV, mean cell volume; MSCV, mean sphered cell volume; MRV, mean reticulocyte volume; Ret/IRF, ratio between reticulocytes count and immature reticulocyte fraction; CH, cryohemolysis; EMA, eosin-5'-maleimide flow cytometric test; ektacytometry O min, ektacytomatry osmolality point at the minimal elongation index; ektacytometry AUC, ektacytometry area under the curve

Figure 3 : *Results distribution of the most efficient tests for HS diagnosis. (A) Cryohemolysis was significantly increased in HS patients (p<0.0001); (B) MFI reduction of Band 3 protein was significantly more important in HS patients (p<0.0001); (C) On the osmoscan curve, O min point was the only discriminant point between HS and OHC, moving to the right (p=0.0303). Ektacytometry was not performed in NHA group. HS, hereditary spherocytosis; OHC, other hemolytic conditions; NHA, non-hemolytic anemia; O, others; EMA, decrease eosin-5'-maleimide binding test; *, p≤0.05; **, p≤0.01; ***, p≤0.001; ****, p≤0.0001.t*

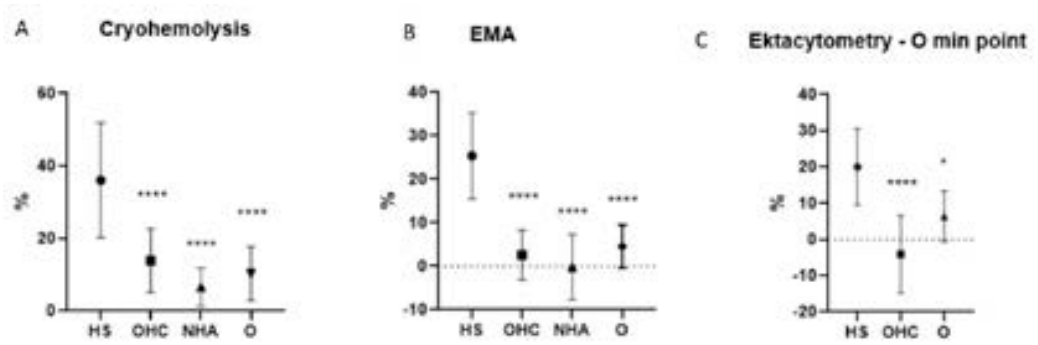
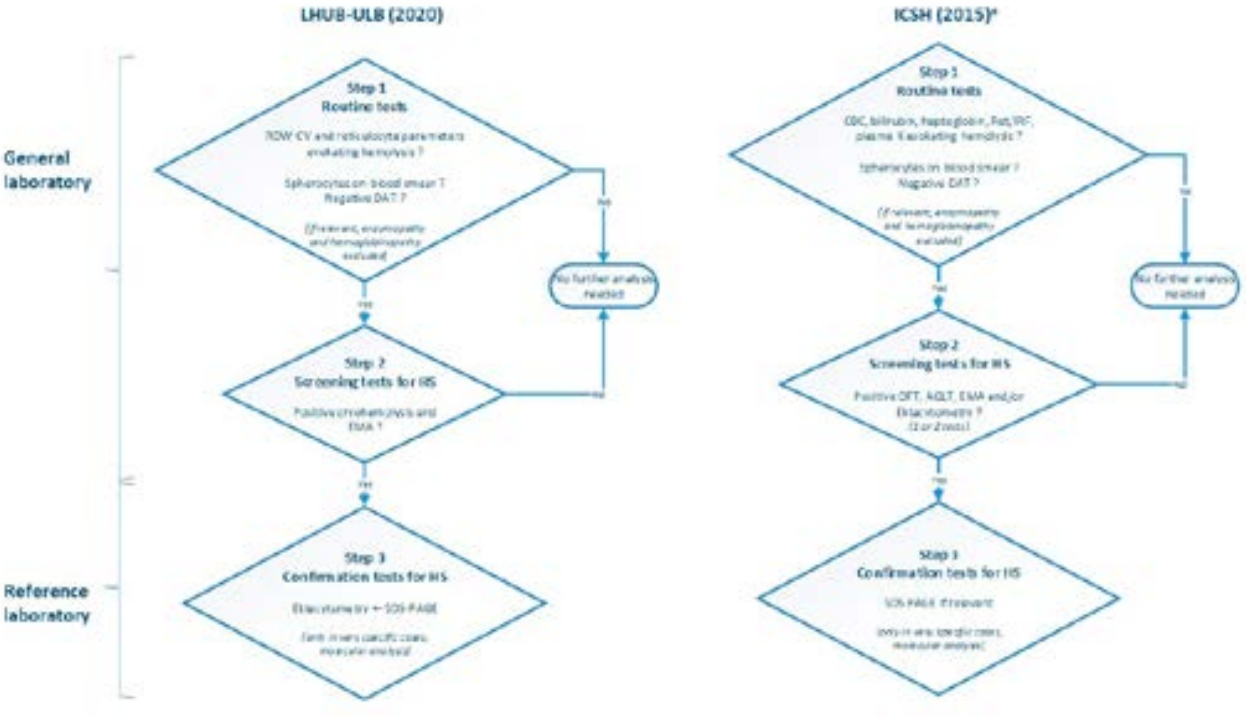


Figure 4 : *Diagnostic approach facing biological and clinical features and family history evoking HS: A summary of our "step-by-step" approach in comparison with the 2015 ICSH guidelines (3).*
a Adapted from 2015 ICSH guidelines (3). RDW-CV, red blood cell distribution width; DAT, direct antiglobulin test; HS, hereditary spherocytosis; EMA, eosin-5'-maleimide flow cytometry; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; CBC, complete blood count; Ret/IRF, ratio between reticulocyte count and immature reticulocyte fraction; K: potassium; OFT, osmotic fragility test; AGLT, acidified glycerol lysis test



Discussion

In order to optimize HS diagnosis in our reference center, the first objective of this work was to characterize a confirmed cohort of HS patients in terms of clinical and biological data. HS patients were mostly children at time of exploration, as it is an inherited condition. The higher rate of positive family history in this population and the most discriminant characteristics were consistent with those reported in the literature (1,14). The reticulocyte count was significantly higher in HS and remained a discriminating parameter while compared only to other hemolytic conditions. One explanation is a real increase of the reticulocyte count in HS, due to a stimulation of erythropoiesis by decreased oxygenation conditions (15)a hypoxia-inducible cytokine, is required for survival, proliferation, and differentiation of erythroid progenitor cells. EPO can also stimulate proliferation and angiogenesis of endothelial cells that express EPO receptors (EPORs. Another hypothesis is related to a prolonged retention of the endoplasmic reticulum (ER) in HS. It is well known that altered vertical membrane interactions in HS lead to migration disorders (16). As reticulocytes are distinguished from mature erythrocytes by the presence of their ER, this phenomenon could lead to an increase of the total amount measured. Immature reticulocyte fraction (IRF) appeared only discriminating between HS patients and those without anemia and hemolysis. The reticulocyte count showed a higher proportional increase than IRF in HS and this explain that the ratio reticulocyte count/IRF is a sensitive screening test for HS, as described previously (17–19)leading to the release of microparticles. All the reference tests suffer from specific limitations. The aim of this study was to develop easy to use diagnostic tool for screening of hereditary spherocytosis based on routinely acquired haematological parameters like percentage of microcytes, percentage of hypochromic cells, reticulocyte counts, and percentage of immature reticulocytes. The levels of haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, reticulocytes (Ret. Indeed, potential retention of the ER could lead to a bigger proportion of « old » reticulocytes (16). Since IRF represents the proportion of highly fluorescent reticulocytes, this would result in an under estimation of this parameter. MRV and MSCV were significantly lower in HS compared to non-HS subgroups, and especially compared to other hemolytic conditions. This is due to the increased fragility of spherocytes, as these volumes are measured after spherization of RBC. Moreover, as membrane loss mainly occurs during erythroblast maturation in HS, it is not surprising to get a lower MRV than in other hemolytic conditions (16). Finally, delta MCV-MSCV and MCV-MRV were significantly higher in HS and were still discriminating in a context of hemolysis, as attested in the literature (20–22)conductivity and scatter technology. It has been observed that the difference between mean corpuscular volume (MCV. For cryohemolysis, there was a slight recovery between HS and other hemolytic conditions, as this test is often positive in AIHA. However, EMA is most of the time negative in the latter, which enables the distinction in case of positive DAT (23,24). This emphasizes the interest to combine cryohemolysis and EMA when HS is suspected, as none of the specialized tests available is able to identify all cases, in particular mild and moderate forms of this condition (12). Using our updated cut-offs, cryohemolysis provides a sensitive test for detecting slight membrane protein deficiency while EMA is more specific but might lead to false negative (3). Ektacytometry, O min and AUC parameters were significantly different between HS and non-HS patients, as described previously (12). However, sole the O min appeared helpful to make a differential diagnosis when presence of hemolysis.

The second objective of our work was to revise our cut-off values. As RBC parameters constitute a first line screening tool in the overall population, cut-off values were chosen in order to ensure a sensitivity > 85%, leading to a specificity from 70 to 81%. A cut-off value of 15% was determined for cryohemolysis, ensuring high sensitivity (94%) but rather low specificity (70%). The resulting performance values were similar to those found in the literature, and seem appropriate for a screening test (6). EMA (cut-off value >14%) revealed a lower sensitivity (88%) but a maximal specificity (100%), in accordance with other centers as well (7–10). Finally, regarding ektacytomatry (osmoscan) profiles, cut-off values were selected for O min (>17) and AUC (<-24.5) with rather low sensitivity (67 and 71%, respectively), but a good specificity (100 and 80%, respectively), given that this last test is mainly used as a confirmatory test, to distinguish HS from other hereditary membrane disorders (12).

The last objective of this study was to evaluate our diagnostic algorithm. Our approach appears similar to what was proposed in the latest published guidelines (3). All patients with typical clinical picture, positive family history and spherocytes on blood smear were positive for HS. This supports the assessment of British guidelines suggesting to avoid any further analysis when the diagnosis is evident (14). Also, should we give more importance to RDW-CV and reticulocyte parameters, which provide a quick and inexpensive first screening step. According to our experience, specific diagnostic tests are performed too frequently. Such practice incurs a significant cost, generates false positive results, and complicates the differential diagnosis with other RBC disorders. Caution is therefore required when a positive result does not fit with clinical presentation, biological parameters and blood smear. If results interpretation is equivocal, a family study should be performed.

According to 2011 British guidelines, SDS-PAGE is the method of choice when a confirmation test is needed. In particular, it enables to exclude CDA II (14). In 2015, ICSH recommendations were updated and improved the awareness for the differential diagnosis between HS and hereditary stomatocytosis. Indeed, the latter is associated with an increased risk of thromboembolic events after splenectomy. This is why ektacytometry, which is currently the only simple and reliable diagnostic test for hereditary stomatocytosis in addition to the blood smear, takes part of these new guidelines. In our laboratory, ektacytometry is currently our confirmation method of choice, and SDS-PAGE is eventually performed if relevant. Molecular analysis should be restricted to specific cases, after a multidisciplinary discussion.

An important limitation of our study is the restricted character of our cohort. As a reference center, we receive a lot of external samples and it was decided to use very strict inclusion and exclusion criteria in order to insure a reliable cohort, with known final diagnoses. In a future and ideally prospective study, it would be interesting to confirm our results on a larger cohort of patients. A second limitation of our study is the heterogeneity of the control group. Given the retrospective nature of our study, healthy control subjects were rare since HS exploration is mainly performed in case of hemolysis and/or anemia. However, comparison of HS patients with a control group including a large panel of differential diagnoses of these two features enabled to evaluate our diagnostic approach in real life conditions, and to highlight the most discriminant biological parameters and specific diagnostic tests in this context.

Conclusion

This work supports previously published results regarding the major clinical features and the most discriminant biological factors for HS exploration. It highlights the importance of blood smear, RDW-CV and reticulocyte channel parameters in all anemia or hemolysis exploration. Some parameters are specific to the DxH800, others being available on most modern devices. Cut-off values for the most relevant variables were defined on basis of an extended study period and on a restricted cohort of patients with a confirmed diagnosis of HS and a complete medical record. Application of these cut-offs could provide a simple tool for HS screening in a large number of laboratories. Moreover, in a reference laboratory, they could be integrated in the first steps of HS diagnosis.

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Declaration of interests: There is no conflict of interest to disclose.

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