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LETTER TO THE EDITOR

Screening for hereditary spherocytosis in routine practice: evaluation of a diagnostic algorithm with focus on non-splenectomised patients

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Dear Editor,

We have read with interest the recent paper of Mullier et al. [1] about the development of the hereditary spherocytosis (HS) diagnostic tool. The authors state that this diagnostic tool has a sensitivity of 100%, specificity of 99.3%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 100% and could be used routinely as an excellent screening method for the diagnosis of HS. As a university hospital, we have a large population of patients diagnosed with HS. Using our laboratory database, we evaluated retrospectively the value of the HS diagnostic tool. Complete data (reticulocytes and research parameters) were obtained from in total 2,593 individuals (including 25 patients with clinical diagnosis of HS) during the period July 2010 till December 2010. All measurements were performed on the XE-5000 (Sysmex, Kobe, Japan) as necessary for the tool.

Using the diagnostic tool of Mullier et al. (Table 1), we obtained a sensitivity of 76% (95% CI=54.9–90.6%), specificity of 98% (97.4–98.5%), PPV of 26.8% (17.0–38.6%) and NPV of 99.8% (99.5–99.9%). This performance is much lower than stated in the original paper. We missed 6/25 patients with known HS: 3 due to lower reticulocyte counts (range=50.6–69.2×10^9/L), possibly because they all were splenectomised, and 3 due to lower percentage of microcytic erythrocytes (MicroR, range=2.6–3.4%). The higher amount of false positives, and hence lower PPV, is probably due to more severe pathologies in our university hospital population including a lot of transplant patients (10/52 false positives with the criteria of Mullier et al.) and patients with hemoglobinopathy/thalassemia (7/52 false positives). Therefore, we propose to adapt the original diagnostic tool in order to improve the performance characteristics for our clinical setting (Table 2). By decreasing the percentage of MicroR in the severity rule from ≥3.5% to ≥2.6%, sensitivity is strongly improved without important loss of specificity and PPV. As our primary goal is to identify undiagnosed patients and as splenectomy evokes a decrease in reticulocyte count [2], we omitted the splenectomised patients (5/25). Consequently, the reticulocyte count precondition can be increased, as all non-splenectomised patients had reticulocyte counts of >140×10^9/L (range=142–1,010×10^9/L).

Based on these observations, we adapted the HS diagnostic tool: reticulocytes ≥100×10^9/L instead of ≥80×10^9/L and MicroR ≥2.6% instead of ≥3.5%. The new approach leads to a sensitivity of 100% (95% CI=83.0–100%) for non-splenectomised HS patients and 84% (63.9–95.4%) for all HS patients with respectively a PPV of 42.6% (28.3–57.8%) and 43.8% (29.5–58.8%). When the screening rule is positive, the presence of spherocytes is

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Table 1 Hereditary spherocytosis diagnostic tool (according to Mullier et al. [1])

Rule		Parameters		
Rule 1	Precondition	Ret ≥80 and Ret/IRF ≥7.7		
Rule 2	Severity	Trait or mild HS Hb >12 g/dl	Moderate HS 8 g/dl≤Hb≤12 g/dl	Severe HS Hb <8 g/dl
		Ret/IRF ≥19.9	MicroR ≥3.5% and MicroR/ Hypo-He ≥2.5	MicroR ≥3.5% and MicroR/ Hypo-He ≥2

Ret reticulocytes (10^9/L), *IRF* immature reticulocytes fraction (%), *HS* hereditary spherocytosis, *Hb* haemoglobin, *MicroR* microcytic erythrocytes (%), *Hypo-He* hypochromic erythrocytes (%)

Table 2 Performance characteristics of original and adapted HS diagnostic tool on all HS patients and non-splenectomised HS patients

	MicroR ≥3.5% and reticulocytes (10^9/L)				MicroR ≥2.6% and reticulocytes (10^9/L)			
	≥140	≥100	≥80	≥65	≥140	≥100	≥80	≥65
Sens (%)	68.0 (85.0)	72.0 (85.0)	76.0 (85.0)	84.0 (85.0)	80.0 (100.0)	84.0 (100.0)	88.0 (100.0)	96.0 (100.0)
Spec (%)	99.6 (99.6)	99.2 (99.2)	98.0 (98.0)	97.2 (97.2)	99.5 (99.5)	99.0 (99.0)	97.9 (97.9)	97.0 (97.0)
PPV (%)	60.7 (60.7)	47.4 (46.0)	26.8 (24.6)	22.6 (19.1)	58.8 (58.8)	43.8 (42.6)	29.3 (27.4)	24.0 (20.8)
NPV (%)	99.7 (99.9)	99.7 (99.9)	99.8 (99.9)	100.0 (100.0)	99.8 (100.0)	99.8 (100.0)	99.9 (100.0)	100.0 (100.0)

Results for non-splenectomised patients between parentheses

HS hereditary spherocytosis, *MicroR* microcytic erythrocytes (%), *Sens* sensitivity, *Spec* specificity, *PPV* positive predictive value, *NPV* negative predictive value

evaluated on a blood smear. If clinically suspected for HS or without clear diagnosis, the flow cytometric eosin-5-maleimide (EMA) test is performed [3–4].

In our routine practice, the adapted HS diagnostic tool is performed on all samples with reticulocyte counts requested by the clinician or by XE-5000 flagging for optical platelet counting in the reticulocyte channel. One month after implementation, 9/731 individuals were flagged positive. Four individuals were suspected of HS of which three had a positive EMA test.

In summary, we evaluated the HS diagnostic tool, as published by Mullier et al. for our hospital setting. Furthermore, we optimised the algorithm for use as a HS screening tool. Laboratories having this sophisticated hematology equipment available can easily implement flagging of positive matches for further definitive HS evaluation. In this way it will enable HS screening of a much larger population with no additional cost.

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