Letter to the Editor

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Assessment of the lipemia index determined by the Atellica CH 930 analyzer for the detection of monoclonal immunoglobulins

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To the Editor,

Hemolysis, icterus and lipemia (HIL) indices are rapid, fully automated and inexpensive measurements that are widely used to assess sample quality prior to clinical chemistry testing. The lipemia index (L-index), which reflects the turbidity of the sample, has been shown to be clinically relevant in the detection of severe hypertriglyceridemia and glycerol kinase deficiency [1]. In addition, previously published evidence has raised interest in whether the L-index could be used to identify patients with monoclonal gammopathy [2, 3]. Therefore, we aimed to determine the diagnostic accuracy of the L-index for the detection of monoclonal immunoglobulins (M-proteins).

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We retrospectively extracted data from the local laboratory information system (LIS) for all paired serum protein electrophoresis (SPE), serum and plasma L-index tests corresponding to individual patients between 1 March 2022 and 13 July 2022 at the University Hospital of Brest, Brest, France. Only the first recorded SPE was included if there was more than one SPE result per patient. Blood was collected in lithium heparin tubes for plasma (ref. 456083, Vacuette, Greiner Bio-One, Kremsmünster, Austria) or serum (ref. 456071, Vacuette, Greiner Bio-One, Kremsmünster, Austria) according to laboratory recommendations. L-index and total protein were measured on the Atellica CH 930 (Siemens Healthineers, Erlangen, Germany) according to the manufacturer's instructions. Briefly, the L-index assay is based on absorbance measurements (658 and 694 nm) of diluted samples, which are converted into semi-quantitative results within a range of 0–6 AU (arbitrary unit). The SPE method was a capillary zone electrophoresis performed on a CAPILLARYS 3 Tera instrument (Sebia, Issy-les-Moulineaux, France). An M-protein was defined as an abnormal clonal immunoglobulin with a peak detected by SPE and characterized by Sebia's HydraGel 4IF immunofixation or by immunosubtraction performed on the CAPILLARYS 3 Tera instrument. The M-protein concentration was calculated from the area under the peak of the absorbance baseline after measurement of total proteins. Data quality was regularly validated throughout the study by internal quality checks and participation in an external quality assessment scheme. Statistical analysis was performed using Excel 2013 (Microsoft, Redmond, WA) and was based on the assessment of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the L-index ≥1 AU for M-protein detection. Final results were presented as median and interquartile range (IQR) or percentage and 95 % confidence interval (CI). Test results, age and sex of patients were extracted anonymously from the local LIS database, so no informed consent or ethics committee approval was required. The retrospective study was conducted in accordance with the Declaration of Helsinki and local legislation.

A total of 3,795 patients were included in the study, of whom 457 (12 %) were diagnosed with monoclonal gammopathy (Table 1). We found that the L-index had a good specificity of 99.8 % (CI: 99.6–99.9) and 99.7 % (CI: 99.6–99.9), but a low sensitivity of 7.7 % (CI: 6.8–8.5) and 3.1 % (CI 2.5–3.6) for the detection of M-proteins in serum and plasma samples, respectively (Table 2). The accuracy of the L-index differed clearly between M-protein isotypes. The best sensitivity was observed for IgM in serum samples (21.4 %; CI: 20.1–22.7). The concordance between plasma and serum L-index for the detection of M-proteins was low (Table 1). Only 6 (17.1 %) of the patients with monoclonal gammopathy and a serum L-index ≥1 AU also had a plasma L-index ≥1 AU. Of the 14 patients with monoclonal gammopathy and a plasma L-index ≥1 AU, 6 (42.9 %) had a combined plasma and serum L-index >1 AU. Finally, we found no correlation between M-protein and L-index values (Supplementary Material, Figure 1).

The role of the L-index in the diagnostic strategy of medical laboratories for M-protein detection is still unclear due to the lack of data on diagnostic accuracy. Our results are consistent with previous studies showing an association between a high L-index and the presence of M-protein [2, 3]. However, this study provides new information on the diagnostic accuracy of the L-index in the detection of M-protein. We found that the L-index has good specificity but low sensitivity for the detection of M-proteins.

As early as 1966, Franglen et al. adapted a classic test, the Sia water test, for use as a screening test for Waldenström's macroglobulinemia [4]. Based on the property of IgM to precipitate at neutral pH, the addition of a drop of serum containing an IgM M-protein to distilled water can produce a white precipitate. The interference caused by M-proteins is generally attributed to their ability to precipitate under certain conditions, such as the isoelectric point of the M-protein, pH, ionic strength and chemical composition of the diluent or reagent solution, resulting in an increase in sample turbidity and an apparent increase in light absorbance, including at the wavelengths used in clinical chemistry assays [5]. Although any M-protein can cause precipitation, IgM has been reported to be frequently involved [5], explaining the predominance of this isotype detected in this and previous studies [2]. However, Fisher et al. report that approximately one third of the M-proteins with a high L-index value measured on the Dimension RXL Max and Dimension Vista analyzers (Siemens Healthineers, Erlangen, Germany) were of the IgG isotype [3]. This suggests that the diagnostic accuracy of the L-index may vary between analyzers, probably due to the lack of standardization of HIL methods [6]. Interestingly, the sensitivity of the serum L-index (7.7 %, CI: 6.8–8.5 %) was twice that of the plasma L-index (3.1 %, CI: 2.5–3.6 %), suggesting that the presence of either fibrinogen or lithium heparinate may partially increase the solubility of the M-protein under L-index assay conditions.

This relatively inexpensive and readily available test is a potential tool for identifying IgM plasma cell disorders and could guide appropriate investigations and reduce the time to diagnosis. Even if asymptomatic, diagnosis of these patients may be useful, particularly in the case of smoldering Waldenström's macroglobulinemia, as follow-up will ensure that they do not develop symptomatic disease [7]. We therefore recommend that an elevated L-index be reported to the clinician as a possible manifestation of the presence of the M-protein and that an SPE be recommended to confirm or refute this possibility.

Table 1: Patient characteristics of the study population.

	Patients (n=3,795)	Patients without M-protein (n=3,338)	Patients with M-protein (n=457)	Patients with M-protein and	
				Serum L-index ≥1 (n=35)	Plasma L-index ≥1 (n=14)
Demographics					
Age, in years	68 (53–78)	66 (52–76)	75 (66–82)	76 (71.5–84.5)	77 (66.8–87.3)
Gender, male	1,887 (49.7)	1,639 (49.1)	249 (54.5)	20 (57.1)	9 (64.3)
Laboratory results					
Serum L-index ≥1, UA	43 (1.1)	8 (0.2)	35 (7.7)	35 (100)	6 (42.9)
Plasma L-index ≥1, UA	26 (0.7)	12 (0.4)	14 (3.1)	6 (17.1)	14 (100)
M-protein value, g/L	5.3 (3–10)	0 (0–0)	5.3 (3–10)	7.6 (4.7–11.7)	5.7 (3.8–7.7)
IgG M-protein	225 (5.9)	0 (0)	225 (49.2)	1 (2.9)	1 (7.1)
IgM M-protein	159 (4.2)	0 (0)	159 (34.8)	34 (97.1)	11 (78.6)
IgA M-protein	42 (1.1)	0 (0)	42 (9.2)	0 (0)	0 (0)
IgD M-protein	2 (0.1)	0 (0)	2 (0.4)	0 (0)	0 (0)
FLC	3 (0.1)	0 (0)	3 (0.7)	0 (0)	0 (0)
Biclonal gammopathy	26 (0.7)	0 (0)	26 (5.7)	0 (0)	2 (14.3)

Data presented as median (interquartile range) or number (percentage), where appropriate. UA, arbitrary unit; FLC, free light chain; L-index, lipemia index; M-protein, monoclonal immunoglobulin.

Table 2: Method performance characteristics of the L-index for the detection of monoclonal immunoglobulins IgG, IgM, and IgA in a serum and plasma sample compared to serum protein electrophoresis as the standard.

	M-protein		IgM M-protein		IgG M-protein		IgA M-protein		Biclonal gammopathy	
	Serum	Plasma	Serum	Plasma	Serum	Plasma	Serum	Plasma	Serum	Plasma
Sens	7.7 (6.8–8.5)	3.1 (2.5–3.6)	21.4 (20.1–22.7)	8.1 (7.2–8.9)	0.4 (0.2–0.7)	0.4 (0.2–0.7)	0 (0–0)	0 (0–0)	0 (0–0)	7.7 (6.8–8.5)
Spec	99.8 (99.6–99.9)	99.7 (99.6–99.9)	99.8 (99.6–99.9)	99.7 (99.6–99.9)	98.8 (98.5–99.2)	99.4 (99.1–99.6)	98.9 (98.5–99.2)	99.4 (99.1–99.6)	98.9 (98.5–99.2)	99.4 (99.1–99.6)
PPV	81.4 (80.2–82.6)	60.9 (59.3–62.4)	79.1 (77.8–80.4)	56.5 (54.9–58.1)	2.3 (1.8–2.8)	4.3 (3.7–5)	0 (0–0)	0 (0–0)	0 (0–0)	8.7 (7.8–9.6)
NPV	88.8 (87.7–89.8)	88.3 (87.2–89.3)	96.7 (96.1–97.2)	96.7 (95.5–96.7)	94 (93.3–94.8)	94.1 (93.3–94.8)	98.9 (98.5–99.2)	98.9 (98.5–99.2)	99.3 (99–99.6)	99.4 (99.1–99.6)
TN	3,328 (87.7)	3,329 (87.7)	3,627 (95.6)	3,624 (95.5)	3,528 (93)	3,548 (93.5)	3,710 (97.8)	3,730 (98.3)	3,726 (98.2)	3,748 (98.2)
FP	8 (0.2)	9 (0.2)	9 (0.2)	10 (0.3)	42 (1.1)	22 (0.6)	43 (1.1)	23 (0.6)	43 (1.1)	21 (0.6)
TP	35 (0.9)	14 (0.4)	34 (0.9)	13 (0.3)	1 (<0.1)	1 (<0.1)	0 (0)	0 (0)	0 (0)	2 (0.1)
FN	422 (11.1)	443 (11.7)	125 (3.3)	148 (3.9)	224 (5.9)	224 (5.9)	42 (1.1)	42 (1.1)	26 (0.7)	24 (0.6)

Data presented as percentage (95 % confidence interval) or number (percentage), where appropriate. There were 457 patients with monoclonal gammopathy and 3,338 patients without monoclonal gammopathy. Monoclonal gammopathy prevalence was 12 %. Sens, sensitivity; spec, specificity; PPV, positive predictive value; NPV, negative predictive value; TN, true negative; FP, false positive; TP, true positive; FN, false negative; L-index, lipemia index; M-protein, monoclonal immunoglobulin.

This study has several limitations. First, the introduction of a bias in the predictive value due to the local distribution of the M-protein cannot be excluded. As reported in our study, the M-protein distribution showed an IgM isotype proportion of 34.8 %, which is classically reported in Western France but higher than reported in other areas [8]. Finally, triglyceride values were not recorded in this retrospective study. A high L-index without M-protein occurred in 8 (0.2 %) and 9 (0.2 %) patients in serum and plasma samples, respectively, possibly reflecting hypertriglyceridemia. This means that the diagnostic performance obtained in this study could be improved by performing a triglyceride measurement or visual assessment of the blood sample prior to SPE determination. Therefore, future studies are needed to improve the strategy of medical laboratories in the presence of a high L-index, given the variety of available methods and potential clinical applications.

Increasing the L-index values on the Atellica CH 930 analyzer was found to be specific but not sensitive for the detection of IgM M-proteins. Therefore, this relatively inexpensive and readily available test is a potential tool for the identification of M-proteins.

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