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Diagnostic accuracy of red blood cell distribution width-to-lymphocyte ratio for celiac disease

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Abstract

Background. *Celiac disease (CD) is significantly underdiagnosed, despite significant efforts made in the last decades to increase its diagnostic rate. This has lead to a high need for developing new diagnostic strategies. Our aim was to evaluate the diagnostic performance of two routine hematologic indices for CD.*

Methods. *In a prospective observational study, 34 newly diagnosed CD patients, 34 age-sex matched controls with irritable bowel syndrome (IBS) and 16 treated CD patients were assessed regarding the differences in mean lymphocyte count (LY), red blood cell distribution width (RDW) and their ratio (RDW/LY).* Results. *Elevated RDW (>14) and lymphopenia (<1.5 x 10e9/L) were more frequently seen in newly diagnosed CD patients compared to IBS control group and treated CD patients. Newly diagnosed CD patients had significantly higher mean values of RDW/LY - 10.09, compared to 7.72 in the CD-treated group and 6.79 in IBS controls ($p<0.01$). Subgroup analysis revealed that RDW/LY was higher in patients with destructive histology (Marsh≥3a), 10.54 vs. 7.99. For a value over 7, RDW/LY had a sensitivity of 88.24% (95% CI 72.55-96.70%) and AUROC of 0.785 (95% CI 0.683- 0.887).*

Conclusions. *RDW/LY ratio is a widely available tool which could be used routinely in clinical practice for CD screening. .*

Keywords: *celiac disease, screening, red blood cell distribution width, lymphopenia*

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Introduction

Despite significant efforts made in the last decades by increasing awareness among both medical professionals and non-professionals, improving availability of serological testing, development of point-of-care tests and case-finding strategies (1), celiac disease (CD) remains heavily underdiagnosed (2). Undiagnosed CD is associated with risk of complications and poor quality of life which could be avoided by gluten-free diet (3,4).

In this setting, a need for developing new diagnostic strategies emerged, and attention has been pointed towards the routine workup of patients, aiming at maximizing selection of those with likelihood of CD. This would allow greater number of patients to be correctly selected for further specific testing. Hematologic changes are common in CD patients (5,6), but they are not frequently reported in current guidelines (3,7,8). These changes in routine laboratory workup results can be subtle, but at the same time they can be the only sign of disease in some of the patients.

Among these hematologic indices, red blood cell distribution width (RDW) has been previously reported as a sensitive marker for CD (9,10), and its diagnostic performance as a screening test is improved in association with other markers (11). Low lymphocyte counts have also been described in association with celiac disease (12,13). These two indices are routinely available from the complete blood count (CBC) done by automated hematology analyzers, without any additional cost.

Based on the premise that a combination of two sensitive biomarkers can increase the diagnostic accuracy of each of them taken separately, our aim was to assess the ratio of RDW to lymphocyte count (LY) in detecting CD patients – RDW/LY, who benefit from confirmatory tests.

Material and methods

Study design and patients

For this observational study, we consecutively recruited newly diagnosed adult CD patients (CD-new) admitted to our clinic over a period of five years (2012-2017). Due to the frequent mislabeling as IBS, age-sex matched irritable bowel syndrome (IBS) patients were selected as controls. We also included CD patients on gluten-free diet (CD-treated) who achieved seroconversion during the study period, serving as intra-group control. CD and IBS diagnoses were made based on currently available guidelines (3,14). We excluded from analysis patients with altered white blood cell (WBC) count and differential secondary to acute overt infections, systemic inflammatory states or hematologic malignancy and those with drug-induced WBC count changes. The study was approved by the local ethics committee. All patients signed an informed consent.

Laboratory procedures

Routine hematological parameters were recorded for all patients as part of their routine clinical management. CBC was determined from venous blood samples using a Beckman Coulter Gen S hematology analyzer. A normal RDW was considered at values under 14, whereas the cut-off value for normal lymphocyte count was 1.5 x 10e9/L (1500/mm^3).

CD patients and controls were checked for serum CD autoantibodies, including IgA anti-tissue transglutaminase 2 and anti-endomysial antibodies. They also underwent upper GI endoscopy with multiple biopsies, both from the bulb and distal duodenum, using high-definition scopes (Pentax, Tokyo, Japan; and Olympus, Tokyo, Japan). Duodenal lesions were reported according to the Marsh-Oberhuber classification.

We evaluated the differences in the aforementioned hematologic indices and their ratio

among the three groups (CD-new, CD-treated, and IBS) and assessed the diagnostic accuracy of RDW/LY in detecting CD.

Statistical analysis

Data analysis was performed using SPSS Statistics version 20 (SPSS Inc., Chicago, IL) and Epi Info 7.1.5 (CDC, Atlanta, Georgia, USA). Results are expressed as mean ± standard deviation (SD) or proportion (%). Comparison of means between the groups was carried out using ANOVA. Diagnostic accuracy was assessed by sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios. Receiver operating characteristic (ROC) curves were generated for ratios of hematologic indices and the area under the receiver operating characteristic curve (AUROC) was used to compare the diagnostic performance of different ratios. Statistical significance was set at α=0.05.

Results

Altogether 34 newly diagnosed CD patients and 34 IBS-controls were included in the analysis. Also, 16 GFD-treated CD patients served as intra-group control. All 34 controls had negative serum CD autoantibodies and were normal on duodenal biopsy. Of the 34 newly diagnosed CD patients, 70.59% were female, with a mean age of 40 ± 12 years. Of these, almost two thirds had anemia (64.70%). Mean RDW was highest in CD-new, and mean lymphocyte count was lowest in this group also (Table 1). An elevated RDW (>14) was seen in 79.41% of patients in the CD-new group, compared to 37.5% in CD-treated and 17.65% in the IBS group. Similarly, lymphopenia (<1.5 x 10e9/L) was most prevalent in the CD group (38.24%), compared to 18.75% and 11.76% respectively, indicating some persisting low lymphocyte counts in GFD-treated CD patients.

Newly diagnosed CD patients had the highest RDW to lymphocyte ratio (RDW/LY), with a mean value of 10.09, compared to 7.72 and 6.79 respectively (p<0.01) (Table 2). When analyzing its diagnostic performance, RDW/LY had a sensitivity of 88.24% (95% CI 72.55-96.70%) and specificity of 68.00% (95% CI 53.30-80.48%) for a value over 7, with a PPV of 65.22% (95% CI 55.14-74.09%), NPV 89.47% (95% CI 76.85-95.61%), positive likelihood ratio 2.76 (95% CI 1.81-4.21), negative likelihood ratio 0.17 (95% CI 0.07-0.44) and an AUROC of 0.785 (95% CI 0.683-0.887); its overall diagnostic accuracy was 76.19% (95% CI 65.65%-84.81%) and diagnostic odds ratio of 15.94 (95% CI 4.79-52.95).

Further analysis looking at the severity of histologic lesions in the CD-new group revealed that RDW/LY was higher in patients with destructive histology (Marsh≥3a, n=28), 10.54 ±

Table 1. Mean RDW and lymphocyte count in the study groups

(mean ± SD)	*CD-new (n=34)*	*CD-treated (n=16)*	*IBS (n=34)*	*P value*
RDW	15.58 ± 2.51	14.11 ± 1.53	13.69 ± 1.38	0.0004
Lymphocyte count	1.760 ± 0.592	1.900 ± 0.441	2.211 ± 0.598	0.0059

Table 2. Hematologic indices ratios in the study groups

	CD-new (n=34)	*CD-treated (n=16)*	*IBS (n=34)*	*P value*
RDW/LY	10.09 ± 6.02	7.72 ± 1.61	6.79 ± 2.64	0.007
NEU/LY	2.96 ± 3.19	2.01 ± 0.47	2.11 ± 0.73	0.16
PLT/LY	192.53 ± 86.72	144.47 ± 32.94	122.96 ± 41.55	<0.001

6.54 vs. 7.99 ± 1.32, but the difference did not reach statistical significance (p=0.09).

Discussions

In the current study we evaluated changes in hematologic indices, focusing on RDW, lymphocyte count and their ratio, in newly diagnosed and treated CD patients and IBS patients as controls.

As previously shown in the literature (15), our study revealed a significant reduction in peripheral lymphocyte counts in newly-diagnosed CD patients compared to controls and GFD-treated CD patients. Possible explanations for the low lymphocyte counts could be migration of lymphocytes in the intestinal mucosa, enteric loss and apoptosis of lymphocytes in the setting of the immune alterations of CD, and not least malnutrition (12,15,16). This has also been seen in other small bowel disorders such as protein-losing enteropathies, where fecal loss of lymphocytes and alterations in lymphocyte subpopulations have been documented (17,18). In accordance with the findings of others (19) who reported lymphocyte counts returning to normal on GFD, we have also seen a significant increase in lymphocyte counts in GFD-treated patients; this is probably due to a decrease in lymphocyte leakage through the healed mucosa (Table 1). However, mean lymphocyte counts in GFD treated CD patients do not reach the values of healthy controls; in fact, treated CD patients do not complete reverse the mucosal alterations as to a healthy person, and this has been reinforced by two studies reporting persistent duodenal intraepithelial lymphocytosis even in long-term strictly adherent patients (20,21).

Regarding RDW, its value has been studied in various conditions, from cardiovascular disease to oncology (22,23). In our cohort, significantly more patients in the newly diagnosed CD group had elevated RDW compared to the other two groups (79% vs. 37% and 17% respectively). Our findings are consistent with those of previous studies, which also reported high rates of elevated RDW in newly diagnosed CD patients (53.7% and 67% respectively) (9,11,24). RDW is known to be an early indicator of iron deficiency and altered erythropoiesis in CD patients.

As shown in our study cohort, alterations in these two parameters (RDW and lymphocyte count) are frequent in newly diagnosed CD and they could be thought of as "hematologic diagnostic clues", especially where a high index of suspicion exists.

High statistical significance was achieved for the difference in the ratio of the two parameters, RDW/LY, in the three groups (Table 2). Interestingly, in treated CD patients, the RDW/LY ratio decreased to values similar to those of IBS controls. This is an additional argument that alterations in the two parameters leading to a high ratio are due to gluten-intolerance.

Other hematologic ratios (neutrophil-to-lymphocyte ratio NEU/LY, platelet-to-lymphocyte

Table 3. Diagnostic performance of hematologic ratios for CD

	Sarikaya et al. 2014, 2015 (27,28)	*Our results*
NEU/LY	Cut-off 2.32: Sn 80%, Sp 41% AUROC 0.607	AUROC 0.573
PLT/LY	Cut-off 143.7: Sn 80.20%, Sp 53.90% AUROC 0.708	AUROC 0.754
RDW/LY	Not reported previously	Cut-off 7: Sn 88.2%, Sp 68.0% AUROC 0.785

ratio PLT/LY) previously reported in the literature in various conditions such as bladder cancer or myocardial infarction (25,26), have also been studied in CD (27,28); they had good diagnostic performance in our cohort also, but RDW/LY ratio proved to be better (Table 3). To the best of our knowledge, this is the first study to investigate the diagnostic accuracy of RDW/LY ratio to diagnose CD. Changes in leukocytic formula in active CD leading to alterations in these hematologic ratios may be related to the lymphocytic infiltration of the gastrointestinal tract, the inflammation and cytokines involved in the pathogenesis of CD (28).

The main limitation of the present study is the small sample size. If reproduced in larger studies, the ratio could have great implication for practice and serve as a valuable screening tool for CD. Screening using this ratio could be done even in primary care or ambulatory settings, where rapid hematology analyzers are available.

Conclusion

Hematologic indices routinely measured by CBC may be useful in selecting patients with likelihood of CD, who benefit from further testing. We report for the first time the good diagnostic performance of RDW/LY ratio for CD. This is a simple, widely available and cheap tool which could be used routinely in clinical practice for CD screening.

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