Diagnostic Significance Of Predictive Biomarkers In Forecasting The Development Of Autoimmune Hepatitis

Khushbokova G.U Tashkent State Medical University

Kurbonova Z.Ch. Tashkent State Medical University

ABSTRACT

This study investigated the diagnostic and prognostic significance of immunological panels and non-invasive biochemical indices in predicting the development of autoimmune hepatitis (AIH) in patients with autoimmune hemolytic anemia (AIHA). The prognostic potential of Liver-9-Line immunological systems, as well as APRI, FIB-4, King's score, and the De Ritis index, was evaluated at the early and late stages of the disease. The analysis demonstrated that immunological panels possess higher diagnostic and prognostic efficiency compared to conventional indices. In particular, Liver-9-Line and Liver-3-Line were identified as the most reliable markers for the early detection of AIH development. The obtained results confirm that immunological profiling plays an important role in the early identification and monitoring of patients belonging to high-risk groups.

Keywords: autoimmune hemolytic anemia, autoimmune hepatitis, Liver-9-Line, Liver-3-Line, APRI, FIB-4, King's score, De Ritis index, ROC analysis, AUC, OR, HR, biomarker, prognosis, immunological panel.

Autoimmune hemolytic anemia (AIHA) is a hematological disorder characterized by immune-mediated destruction of red blood cells caused by autoantibodies directed against erythrocyte antigens. The disease may occur as a primary idiopathic condition or develop secondarily under the influence of infections, autoimmune diseases, lymphoproliferative disorders, and other factors [5].

AIHA is classified according to the direct antiglobulin test (DAT) and the thermal activity of autoantibodies. Warm AIHA is the most common form and is mainly associated with IgG autoantibodies. These antibodies activate splenic macrophages, leading to extravascular hemolysis [4]. Cold AIHA is more commonly associated with IgM antibodies, which activate the complement system and result in complement-mediated hemolysis. In mixed AIHA, both warm and cold antibodies are involved simultaneously. This condition leads to combined mechanisms of hemolysis, and its clinical course may be relatively severe. Mixed AIHA is characterized by the coexistence of warm IgG and cold IgM autoantibodies. Accurate diagnosis requires DAT together with additional immunohematological investigations [3,5,7].

Disruption of immune tolerance, alterations in B- and T-lymphocyte activity, excessive production of autoantibodies, and activation of the complement system play an important role in the pathogenesis of AIHA. Hemolysis may occur through various immunological mechanisms either intravascularly or via the extravascular pathway [2].

AIHA is a rare but heterogeneous disease. Its development is associated with genetic predisposition, immune dysregulation, infections, autoimmune diseases, medications, and environmental factors [3]. The main pathogenetic mechanisms include autoantibody

production, macrophage activation, erythrophagocytosis, and immune regulatory disturbances [2].

In some cases of severe AIHA, uncontrolled hemolysis may develop, resulting in severe anemia and life-threatening complications. The severity of the disease course is associated with immunological characteristics, underlying diseases, and the degree of hemolysis. Therefore, early identification of high-risk patients and assessment of immunological markers are of great importance [2,4,8].

The rarity of AIHA and the complexity of its diagnosis make the study of its immunological features, clinical course, and diagnostic biomarkers a highly relevant issue [3,5,7].

To assess the probability of subsequent development of autoimmune hepatitis (AIH) in patients with AIHA, the prognostic value of several immunological panels and non-invasive fibrosis indices was investigated, while the diagnostic significance of biomarkers was evaluated using odds ratio (OR) and hazard ratio (HR). In addition, the independent predictive potential of the specialized immunological Liver-3-Line panel, which includes autoantibodies directed against cytoskeletal structures such as F-actin, Actinin, and Tropomyosin, was further analyzed. This approach was aimed at identifying early immunological alterations leading to autoimmune liver injury in AIHA [1,2].

The study results demonstrated that immunological panels possess significantly higher predictive value compared to other indices. In particular, the Liver-9-Line panel showed the strongest statistical association with the development of AIH. Patients with positive results for this panel had a significantly increased probability of developing autoimmune hepatitis, while OR and HR indicators confirmed the high reliability of this association. These findings suggest that this immunological panel may serve as an effective tool for the early detection of autoimmune liver damage in the setting of AIHA [1,3].

The high OR and HR values of the Liver-9-Line panel indicated that this combination of autoantibodies is strongly associated with the risk of AIH development. This suggests that the immune response directed against cytoskeletal antigens represents an important immunological marker of the initiation of liver involvement in the pathogenesis of AIHA [4].

Among the non-immunological parameters, the APRI index retained relatively high diagnostic significance. Statistically significant OR and HR values for this index demonstrated that the relationship between aspartate aminotransferase (AST) levels and platelet count is associated with the development of fibrotic and inflammatory processes in the liver. Other fibrosis indices, including FIB-4 and King's score, showed positive associations with AIH in certain cases; however, their time-dependent predictive value did not reach a reliable level. This finding suggests that these indices may primarily reflect accumulated morphofunctional liver damage [4,5,6]. The De Ritis coefficient did not demonstrate a statistically significant association with the risk of AIH development. The low OR and HR values indicate that this index has limited potential as an independent prognostic biomarker [2,7].

Overall, the obtained results confirmed that immunological panels, particularly Liver-9-Line and Liver-3-Line, possess significantly higher accuracy in predicting the development of autoimmune hepatitis in patients with AIHA compared to conventional non-invasive indices. This finding indicates that immunological profiling plays an important role in the early identification and monitoring of patients belonging to high-risk groups [3].

Materials and Methods. The present study was conducted at Tashkent State Medical University from 2020 to 2024. A total of 64 patients voluntarily participated in the main study group, while the control group consisted of 20 healthy individuals matched by age and sex with the patients of the main group. According to the duration of AIHA, patients in the main group were divided into three groups: Group 1 included 25 patients with AIHA duration up to 6 months, Group 2 included 21 patients with disease duration from 6 months to 2 years, and Group 3 consisted of 18 patients with AIHA duration from 2 to 4 years. In addition, each group was subdivided according to the presence or absence of autoimmune hepatitis (AIH):

patients with AIH (AIH+) and patients without AIH (AIH−). AIH was identified in 4 patients of Group 1, 6 patients of Group 2, and 8 patients of Group 3.

Modern comprehensive clinical and laboratory approaches were used for the diagnosis of AIHA and AIH. Particular attention was paid to clinical symptoms and medical history during patient selection. Strict exclusion criteria included viral hepatitis and toxic liver injury (alcoholic or drug-induced), which were not included in the main study group. Most patients were between 18 and 44 years of age, while the mean age in the control group was 44.3 ± 2.64 years. Statistical analysis revealed no significant differences according to age and sex.

Hematological parameters were analyzed using the Mindray BS-20 analyzer (China), while biochemical investigations were performed using the BC 200 automated biochemical analyzer (China) with reagents produced by the German company HUMAN. The following parameters were evaluated: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), indirect bilirubin (IB), total protein, albumin, and urea levels.

ROC analysis was performed in order to assess the predictive capability for AIH in patients with different durations of AIHA. This analysis made it possible to evaluate how the diagnostic significance of various biomarkers and indices changed during disease progression. Diagnostic efficiency was assessed using the AUC indicator, where higher values reflected greater reliability of the test.

In patients at the early stage of the disease, immunological panels demonstrated the highest diagnostic accuracy. Liver-9-Line panels showed high AUC values and were found to be highly effective in the early detection of AIH. This finding suggests that immunological alterations can be detected before complete clinical and morphological liver abnormalities become fully established.

The APRI index also demonstrated good diagnostic value at the early stage, showing high sensitivity and specificity at the established cut-off value. This indicates that the AST-to-platelet ratio may reflect the earliest signs of autoimmune liver injury. The De Ritis coefficient showed moderate diagnostic significance, whereas the FIB-4 and King's indices demonstrated relatively low or satisfactory diagnostic performance at this stage.

In patients with a relatively prolonged disease course, the diagnostic profile changed to some extent. At this stage, the APRI index demonstrated the highest diagnostic accuracy and enabled reliable differentiation of autoimmune liver damage. King's score also showed high AUC values and considerable significance in detecting AIH. The FIB-4 index also demonstrated high diagnostic value, indicating that its significance increases with the progression of fibrotic and parenchymal liver changes.

The ROC analysis results demonstrated that immunological and biochemical parameters possess different levels of diagnostic significance in the assessment of liver pathologies. Among the investigated markers, the Liver-9-Line and Liver-3-Line panels showed the highest efficiency. These test systems demonstrated an almost perfect discriminatory ability in disease detection, while overall accuracy indicators were assessed within the highest range.

The balance between sensitivity and specificity for these panels was highly stable, and false-positive or false-negative results were observed only rarely. At the same time, certain statistical parameters indicated the presence of limitations when evaluating them as independent markers, suggesting that their maximum effectiveness is achieved only within a complecsive diagnostic approach.

On the other hand, the De Ritis index demonstrated moderate diagnostic capability in reflecting changes in hepatic enzymatic activity. Although its ROC performance was not high, it retained value as an additional source of information in certain clinical situations. Furthermore, despite relatively high sensitivity around the cut-off point, its overall prognostic value remained limited.

In prolonged forms of AIHA, the diagnostic strength of biomarkers increased significantly. At this stage, immunological panels operated

with very high accuracy, particularly Liver-9-Line and Liver-3-Line, which demonstrated an almost ideal discriminatory capacity. Their ROC indicators approached maximal values, enabling reliable confirmation of disease presence.

The APRI index also demonstrated high diagnostic significance at this stage, sensitively reflecting changes associated with liver fibrosis. At its optimal cut-off point, sensitivity and specificity remained highly balanced, supporting its application as a useful instrument in practical clinical settings.

The FIB-4 index showed relatively stable but lower diagnostic power compared to immunological panels. It plays an additional role in differentiating parenchymal and fibrotic changes but has limited value as an independent marker.

The results for King's score were mixed in nature. On the one hand, sensitivity was high, while on the other hand, statistical significance was unstable, reducing its reliability. Therefore, it is considered more appropriate as an additional clinical index.

The De Ritis index maintained moderate diagnostic significance throughout the study. Although it reflected changes in hepatic cytolysis and enzymatic activity, its overall prognostic value remained limited.

The overall comparative analysis across different groups demonstrated that immunologically oriented test panels had clear superiority over all biochemical indices. In particular, Liver-9-Line and Liver-3-Line maintained high diagnostic stability at all stages and played the leading role in disease detection. Biochemical indices may serve as supportive diagnostic tools and can improve overall accuracy when interpreted together with immunological data. Their main significance becomes evident within a comprehensive evaluation system.

Analysis of individual autoantibodies revealed that F-actin and actinin possess relatively high diagnostic potential. Although they demonstrated a certain degree of reliability when used separately, their full clinical value became evident only as components of a comprehensive panel.

The remaining autoantibodies were considered ineffective as individual markers because of their low sensitivity and limited diagnostic value.

In conclusion, the highest diagnostic efficiency belongs to immunological panels, particularly Liver-9-Line and Liver-3-Line. These panels are significantly superior to conventional biochemical indices and are recommended as the principal tools for the early and reliable detection of autoimmune hepatitis in the setting of AIHA.

Conclusion. The conducted analyses demonstrated that immunological panels possess the highest diagnostic and prognostic value in predicting the development of autoimmune hepatitis in patients with AIHA. In particular, the Liver-9-Line test enabled highly accurate detection of AIH both at the early stages of the disease and in prolonged cases. Although conventional non-invasive indices such as APRI, FIB-4, and King's score have additional diagnostic significance, their predictive capabilities were found to be lower compared to immunological panels. The De Ritis index demonstrated limited value as an independent prognostic marker. Overall, immunologically oriented test systems represent the most effective tools for the early detection of AIHA-associated autoimmune hepatitis, risk assessment, and patient stratification.

References.

1. Fattizzo B, Barcellini W. Autoimmune hemolytic anemia: causes and consequences. Expert Rev Clin Immunol. 2022 Jul;18(7):731-745. doi: 10.1080/1744666X.2022.2089115. Epub 2022 Jun 14. PMID: 35702053.
2. Jacobs JW, Raza S, Clark LM, Stephens LD, Allen ES, Woo JS, Walden RL, Figueroa Villalba CA, Tormey CA, Stanek CG, Adkins BD, Bloch EM, Booth GS. Mixed Autoimmune Hemolytic Anemia: A Systematic Review of Epidemiology, Clinical Characteristics, Therapies, and Outcomes. Am J Hematol. 2025 Aug;100(8):1397-1407. doi:

10.1002/ajh.27721. Epub 2025 May 20. PMID: 40392014; PMCID: PMC12232602.
3. Kar S, Kumar CGD, Kar R, Basavarajegowda A. Autoimmune Hemolytic Anemia in Children: Clinical Profile and Outcome. Indian J Pediatr. 2024 Feb;91(2):143-148. doi: 10.1007/s12098-022-04469-6. Epub 2023 Feb 14. PMID: 36787020.
4. Loriamini M, Cserti-Gazdewich C, Branch DR. Autoimmune Hemolytic Anemias: Classifications, Pathophysiology, Diagnoses and Management. Int J Mol Sci. 2024 Apr 12;25(8):4296. doi: 10.3390/ijms25084296. PMID: 38673882; PMCID: PMC11049952.
5. Mulder FVM, Evers D, de Haas M, Cruijsen MJ, Bernelot Moens SJ, Barcellini W, Fattizzo B, Vos JMI. Severe autoimmune hemolytic anemia; epidemiology, clinical management, outcomes and knowledge gaps. Front Immunol. 2023 Sep 18;14:1228142. doi: 10.3389/fimmu.2023.1228142. PMID: 37795092; PMCID: PMC10545865.
6. Parasiliti-Caprino M, Bioletto F, Frigerio T, D'Angelo V, Ceccato F, Ferraù F, Ferrigno R, Minnetti M, Scaroni C, Cannavò S, Pivonello R, Isidori A, Broglio F, Giordano R, Spinello M, Grottoli S, Arvat E. A New Clinical Model to Estimate the Pre-Test Probability of Cushing's Syndrome: The Cushing Score. Front Endocrinol (Lausanne). 2021 Oct 5;12:747549. doi: 10.3389/fendo.2021.747549. PMID: 34675882; PMCID: PMC8524092.
7. Pinto LP, Marroni CA, Czermainski J, Dahlem MLF, Carteri RB, Fernandes SA. Role of the phase angle in the prognosis of the cirrhotic patient: 15 years of follow-up. World J Methodol. 2023 Sep 20;13(4):238-247. doi: 10.5662/wjm.v13.i4.238. PMID: 37771877; PMCID: PMC10523241.
8. Thielecke F, Blannin A. Omega-3 Fatty Acids for Sport Performance-Are They Equally Beneficial for Athletes and Amateurs? A Narrative Review. Nutrients. 2020 Nov 30;12(12):3712. doi: 10.3390/nu12123712. PMID: 33266318; PMCID: PMC7760705.