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Article

Application Research of Immunoassay in Clinical Diagnosis of Autoimmune Diseases

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Abstract: Autoimmune diseases present significant clinical challenges due to their complex pathogenesis and diverse manifestations, making early and accurate diagnosis crucial for effective patient management. This study aimed to comprehensively examine the impact and diagnostic utility of various immunoassays in the clinical evaluation of autoimmune diseases. A total of 128 patients suspected of having autoimmune disorders, admitted to a tertiary hospital between January 2022 and December 2024, were enrolled as the observation group. Concurrently, 64 healthy individuals undergoing routine physical examinations were selected as the control group. Both cohorts underwent extensive serological testing, including the detection of antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-extractable nuclear antigens (anti-ENA), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, immunoglobulins, and complement components C3 and C4. The results demonstrated that the positivity rates of ANA, anti-dsDNA, anti-ENA, RF, and anti-CCP in the observation cohort were significantly higher than those in the control group ($P < 0.05$). Furthermore, the combined detection of these multiple immunological indicators yielded a remarkable diagnostic sensitivity of 92.19% and an overall accuracy of 90.10%, substantially outperforming single-item detection methods. In conclusion, comprehensive immunoassay profiling provides robust laboratory evidence for the effective screening, classification, and definitive diagnosis of autoimmune conditions. Implementing this multifaceted diagnostic tool can significantly accelerate detection speed, optimize clinical workflows, and substantially reduce the risk of missed or delayed diagnoses.

Keywords: immunoassay; autoimmune diseases; autoantibodies; clinical diagnosis; combined detection

1. Introduction

Autoimmune diseases are characterized by the body's immune system failing to maintain proper tolerance and mistakenly targeting its own tissues, cells, or blood components. These conditions encompass a range of disorders, including systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis, and mixed connective tissue diseases. The initial symptoms often manifest unexpectedly and may include fever, fatigue, joint pain, skin rashes, dryness of the mouth and eyes, proteinuria, or reductions in blood cell counts. These symptoms can be easily misinterpreted as signs of infections, metabolic disorders, or other chronic inflammatory conditions, complicating early diagnosis. Laboratory markers, particularly autoantibodies, play a pivotal role in identifying autoimmune diseases, assessing risk, and aiding in clinical classification. Among these markers, antinuclear antibodies (ANA) are frequently utilized in clinical settings as a preliminary screening tool for systemic autoimmune diseases [1, 2]. The observation of fluorescent patterns in HEp-2 cells through direct immunofluorescence serves as a valuable reference for identifying specific antibody types and their associated diseases [3, 4]. Standardized reporting practices for ANA, including fluorescence patterns, titers, and clinical interpretations, have been recommended to minimize discrepancies in laboratory results across different facilities. In addition to ANA, other indicators such as

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anti-dsDNA, anti-Sm, anti-SSA/Ro, anti-SSB/La, anti-Scl-70, rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibodies are closely linked to specific autoimmune conditions. Recent advancements in diagnostic technologies, such as multiparameter autoantibody analysis, chemiluminescent immunoassays, and multiplex detection platforms, have enhanced the efficiency and accuracy of antibody screening by enabling simultaneous detection of multiple markers within a short timeframe.

Despite these advancements, clinical testing for autoimmune diseases continues to face significant challenges. One major issue is the inability of single antibody tests to account for the heterogeneity of these diseases, which often involve complex and overlapping symptoms. Another challenge lies in the variability of sensitivity, specificity, and reporting standards across different testing methods, which can lead to biased interpretations if results are not analyzed comprehensively [1, 5]. To address these limitations, this study aims to evaluate the diagnostic value of various immune detection indicators and testing methodologies by analyzing clinical case data. Patients presenting with suspected autoimmune diseases at a specific hospital were selected as the sample population for this investigation [3]. The study focuses on the application of widely recognized immune detection techniques, providing insights into their practical utility in clinical settings. By examining real-world laboratory procedures and outcomes, the findings are intended to serve as a reference for both academic publications and routine clinical practices. Furthermore, the study underscores the importance of integrating multiple diagnostic approaches to improve the accuracy and reliability of autoimmune disease detection, thereby facilitating better patient management and treatment strategies.

2. Materials and Methods

2.1. General Information

The observation group consisted of 128 patients diagnosed with various autoimmune diseases who were admitted to the hospital between January 2022 and December 2024. This group included 32 men and 96 women, with ages ranging from 21 to 72 years and an average age of 45.38 years, accompanied by a standard deviation of 10.27 years. The autoimmune conditions observed in this group included systemic lupus erythematosus, which accounted for 38 cases, rheumatoid arthritis with 42 cases, Sjögren's syndrome observed in 24 cases, systemic sclerosis in 10 cases, and mixed connective tissue disease in 14 cases. To provide a comparative analysis, a control group was established, comprising 64 individuals who underwent routine physical examinations during the same period [1, 6]. This control group included 18 men and 46 women, with ages ranging from 22 to 70 years and an average age of 44.91 years, with a standard deviation of 9.86 years. Statistical analysis revealed no significant differences in age or gender distribution between the observation and control groups, ensuring comparability and minimizing potential biases in the study outcomes. The selection criteria for both groups were carefully designed to ensure the reliability of the data, focusing on the absence of confounding variables that could influence the results. The study aimed to explore the underlying mechanisms and clinical manifestations of autoimmune diseases, as well as to identify potential differences in physiological markers between affected individuals and healthy controls. By maintaining a balanced demographic profile across both groups, the research sought to provide robust insights into the pathophysiology of autoimmune conditions while adhering to rigorous scientific standards.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria for this study were carefully defined to ensure the selection of participants who could provide meaningful and reliable data for the research objectives. Participants were required to exhibit symptoms indicative of autoimmune diseases, which could include manifestations such as joint pain, skin rashes, recurrent episodes of fever, dryness in the mouth and eyes, proteinuria, or a reduction in blood cell counts. These symptoms are commonly associated with autoimmune conditions and serve as critical indicators for identifying potential cases. Additionally, it was mandatory for participants

to have comprehensive clinical and laboratory data available, as this information is essential for accurate diagnosis and analysis. Another key inclusion criterion was the absence of long-term intensive immunosuppressive therapy prior to testing, as such treatments could potentially alter the clinical presentation and laboratory findings, thereby confounding the study results [7, 8]. On the other hand, specific exclusion criteria were established to eliminate confounding factors that could compromise the validity of the findings. Individuals with severe comorbid conditions, such as significant infections, malignancies, or advanced liver or kidney failure, were excluded due to the potential impact of these conditions on the study outcomes. Furthermore, samples that exhibited hemolysis, lipemia, or insufficient blood volume were deemed unsuitable for analysis, as these factors could interfere with the accuracy of laboratory tests. Lastly, participants with incomplete diagnostic histories, which would render a definitive clinical judgment unattainable, were also excluded to maintain the integrity and reliability of the study's conclusions.

2.3. Detection Indicators

The test items utilized in the study included a comprehensive range of immunological and biochemical markers aimed at diagnosing and monitoring autoimmune diseases and related conditions. These markers encompassed antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-extractable nuclear antigen (anti-ENA) antibody profiles, rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), immunoglobulins such as IgG, IgA, and IgM, as well as complement components C3 and C4. The anti-ENA antibody profiles were further categorized into specific subtypes, including anti-Sm, anti-SSA/Ro, anti-SSB/La, anti-U1-RNP, anti-Scl-70, and anti-Jo-1, each serving distinct diagnostic purposes. For instance, RF and anti-CCP are particularly significant in the context of rheumatoid arthritis, as anti-CCP antibodies are instrumental in facilitating early diagnosis and aiding clinical decision-making regarding rheumatism. Similarly, the detection of anti-SSA/Ro or anti-SSB/La antibodies is pivotal in diagnosing Sjögren's syndrome, a condition characterized by autoimmune-mediated damage to salivary and lacrimal glands. Moreover, advancements in immunological research have led to the identification of novel autoantibodies that enhance diagnostic accuracy, particularly for patients who test negative for traditional markers such as SSA. These developments underscore the dynamic nature of autoimmune diagnostics, where emerging biomarkers continually refine the ability to identify and manage complex conditions. The inclusion of complement components C3 and C4 further broadens the diagnostic scope, as these proteins play a critical role in immune system regulation and are often altered in autoimmune disorders, providing additional insights into disease activity and progression [9–11].

2.4. Detection Methods

All patients provided 5 mL of venous blood on an empty stomach, which was then processed to separate serum through centrifugation at 3000 rpm for 10 minutes. The detection of antinuclear antibodies (ANA) was carried out using indirect immunofluorescence, with a titer threshold of less than 1:100 considered positive. Advanced techniques such as enzyme-linked immunosorbent assay and chemiluminescent immunoassay were employed to identify anti-dsDNA and anti-CCP antibodies, ensuring high sensitivity and specificity. Additionally, anti-nucleosome antibody profiles were analyzed using immunoblotting methods, which allow for the identification of specific protein interactions. Rheumatoid factor (RF), immunoglobulins, and complement levels were measured using autoturbidimetric assays, which are widely recognized for their precision in quantifying these biomarkers. The selection of these methodologies was guided by the instructions provided with the diagnostic kits, adherence to internal quality control standards, and established laboratory practices to ensure reliability and reproducibility of results. Furthermore, the automated HEp-2 immunofluorescence interpretation system was utilized to enhance standardization and quality control in ANA detection. This system offers significant advantages, including

consistent interpretation of fluorescence patterns and improved accuracy in determining positive thresholds and karyotypes. Manual validation was also incorporated as a supplementary measure to ensure the robustness of the automated system. The integration of these diverse diagnostic approaches reflects a comprehensive strategy aimed at achieving accurate and reliable detection of biomarkers relevant to the study. By combining automation with manual oversight, the methodology ensures both efficiency and precision, addressing potential variability in diagnostic outcomes and supporting the overall integrity of the research findings.

2.5. Observation Indicators and Statistical Methods

The study focused on observing several critical indicators to evaluate the effectiveness and reliability of the detection methods employed. These indicators included the relative prevalence of autoantibodies in the two groups under investigation, as well as the comparative levels of major antibodies associated with specific disease types. Additionally, the sensitivity, specificity, and accuracy of the detection methods were key parameters assessed to determine their diagnostic utility. The study also examined the relative efficacy of single versus joint detection approaches, providing a comprehensive analysis of their respective contributions to diagnostic accuracy. The final clinical diagnosis served as the benchmark or default result against which all observations and measurements were compared, ensuring that the findings were grounded in clinically validated outcomes. Statistical analysis was conducted using SPSS 26.0, a widely recognized software for data analysis in medical research. Quantitative data were expressed as the mean ± standard deviation, allowing for a clear representation of central tendencies and variability within the data. To compare these quantitative measures between groups, t-tests were employed, which are standard statistical tools for assessing differences in means. For categorical data, which were represented as the number of cases and their corresponding percentages, $\chi 2$ tests were utilized to evaluate differences among the groups. This dual approach ensured that both continuous and categorical variables were analyzed appropriately. Statistical significance was rigorously defined as $P < 0.05$, a threshold commonly accepted in scientific research to indicate meaningful differences or associations. This methodological rigor underscores the reliability and validity of the study's findings.

3. Results

3.1. Comparison of the Positive Rates of Autoantibodies between the Two Groups

The positive rates of ANA, anti-dsDNA, anti-aspartate, RF, and anti-CCP in the observation group were observed to be higher compared to those in the control group, with the differences being statistically significant. This indicates a potential correlation between these autoantibodies and the conditions being studied. Among these, the positivity rate of ANA was the highest, suggesting its potential utility as a primary marker for the screening of systemic autoimmune diseases. However, it is important to note that while ANA positivity is a significant indicator, it is not exclusive to specific diseases and may also be present in the general population. This variability in ANA positivity can be influenced by factors such as population demographics and testing conditions. Therefore, clinical interpretation of ANA results should always be contextualized with the patient's symptoms and other diagnostic signs to ensure accurate diagnosis and management [12, 13]. Additionally, while the positivity rates for other autoantibodies such as anti-dsDNA, anti-ENA, RF, and anti-CCP were lower than that of ANA, they still demonstrated relevance to specific disease types. This highlights the importance of a comprehensive approach in autoantibody testing, where multiple markers are considered collectively rather than in isolation. Such an approach can enhance diagnostic accuracy and provide a more nuanced understanding of the underlying autoimmune conditions. The findings underscore the necessity of integrating laboratory results with clinical evaluations to avoid over-reliance on singular markers, which might lead to misinterpretation. The detailed comparison of autoantibody positivity rates between the observation and control

groups is presented in Table 1, providing a clear quantitative representation of the observed differences and supporting the conclusions drawn from the analysis.

Table 1. Comparison of Autoantibody Positivity Rates between the Two Groups [N (%)]

Group	Number of examples	ANA positive	anti-dsDNA positive	Anti-ENA positive	RF positive	Anti-CCP positive
Observation group	128	101 (78.91)	42 (32.81)	76 (59.38)	48 (37.50)	39 (30.47)
control group	64	8 (12.50)	1 (1.56)	4 (6.25)	5 (7.81)	2 (3.13)
χ^2 value	—	75.42	24.31	49.87	19.26	18.77
p-value	—	<0.001	<0.001	<0.001	<0.001	<0.001

3.2. Distribution of Major Immune Indicators in Different Disease Types

The antibody profiles observed in patients with various autoimmune diseases exhibit distinct patterns, which are critical for accurate diagnosis and classification. For instance, individuals diagnosed with systemic lupus erythematosus often display elevated positive rates for specific antibodies such as ANA, anti-dsDNA, and anti-Sm. These markers are indicative of the disease's underlying immune dysregulation, although they may sometimes be associated with reduced complement levels, reflecting disease activity. In contrast, patients with rheumatoid arthritis are characterized by high positive rates for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies, which are considered hallmark indicators of this condition. Similarly, Sjögren's syndrome is frequently associated with elevated positive rates of anti-SSA/Ro and anti-SSB/La antibodies, which are instrumental in identifying this autoimmune disorder. Systemic sclerosis, another autoimmune condition, is often linked to the presence of anti-Scl-70 antibodies or related markers, which provide valuable insights into the disease's pathophysiology. Additionally, the presence of anti-U1-RNP antibodies is particularly significant in the context of mixed connective tissue diseases, where it serves as a key diagnostic marker. The use of multiparameter antibody profile analysis has proven to be an effective approach for enhancing the classification and diagnostic accuracy of connective tissue diseases [14, 15]. By examining a comprehensive range of antibody markers, clinicians can gain a more nuanced understanding of the immune responses associated with these conditions, thereby facilitating tailored treatment strategies. This analytical method underscores the importance of integrating immunological data into clinical decision-making processes, as it enables the identification of overlapping features and distinctions among various autoimmune diseases. Such insights are essential for improving patient outcomes and advancing the field of autoimmune disease research (As shown in Table 2).

Table 2. Positive Status of Major Immune Indicators in Different Disease Types [N (%)]

Disease type	Number of examples	ANA positive	Characteristic antibody positive	Complement reduction
Systemic lupus erythematosus	38	36 (94.74)	28 (73.68)	24 (63.16)
Rheumatoid Arthritis	42	25 (59.52)	31 (73.81)	6 (14.29)
Sjögren's syndrome	24	21 (87.50)	17 (70.83)	8 (33.33)
Systemic sclerosis	10	8 (80.00)	6 (60.00)	2 (20.00)
Mixed connective tissue disease	14	11 (78.57)	10 (71.43)	4 (28.57)

Note: Characteristic antibodies include anti-dsDNA, anti-Sm, anti-SSA/Ro, anti-SSB/La, anti-CCP, anti-Scl-70, and anti-U1-RNP.

3.3. Comparison of Results from Single-Item Testing and Combined Testing

The single-indicator combined detection rate was observed to be 92.19%, which represents a significant improvement compared to the detection rates achieved through individual tests such as ANA single detection, anti-ENA antibody profile detection, and RF+anti-CCP detection. This finding underscores the inherent complexity and heterogeneity of autoimmune diseases, where the absence of a detectable antibody does not necessarily rule out the presence of the disease. The use of combined detection methodologies offers a more comprehensive approach, enabling early screening, precise subtyping, and more accurate disease-level assessments. This approach is particularly advantageous for patients presenting with severe symptoms, overlapping clinical manifestations, or atypical early-stage indicators that might otherwise be overlooked in single-item testing. Furthermore, advancements in diagnostic technologies have highlighted the potential of integrating multi-omics approaches, multi-antibody panels, and high-throughput systems to enhance diagnostic accuracy and efficiency [16]. These innovations are expected to play a pivotal role in addressing the diagnostic challenges posed by the diverse and often unpredictable nature of autoimmune disorders. By leveraging these advanced methodologies, clinicians can achieve a more nuanced understanding of disease mechanisms, leading to improved patient outcomes. Table 3 provides a detailed comparison of the positive detection rates between single-item and combined-item tests, illustrating the superior performance of the latter in identifying autoimmune conditions. This evidence supports the growing consensus that a multi-faceted diagnostic strategy is essential for effectively managing the complexities associated with autoimmune diseases. The integration of such approaches into routine clinical practice could significantly enhance the early detection and management of these conditions, ultimately contributing to better prognostic outcomes for affected individuals.

Table 3. Comparison of Positive Detection Rates between Single-Item and Combined-Item Tests [N (%)]

Testing Plan	Number of examples	Number of positive cases	Positive rate
ANA single test	128	101	78.91
Anti-ENA antibody profile detection	128	76	59.38
RF+ Anti-CCP Detection	128	53	41.41
ANA + Anti-ENA Combined Detection	128	112	87.50
Multi-indicator joint detection	128	118	92.19

4. Conclusion

Immunoassays play a central role in the clinical evaluation and diagnosis of autoimmune diseases and remain essential tools for both initial screening and disease classification. Antinuclear antibody testing is an important entry point in the assessment of suspected systemic autoimmune disorders, particularly when patients present with nonspecific manifestations such as fatigue, rash, arthralgia, serositis, or multisystem involvement. As an initial screening approach, ANA testing can support the early recognition of systemic disease and may help reduce delays in diagnosis when interpreted together with the broader clinical picture. However, ANA positivity alone is not sufficient for a definitive diagnosis, and its value is greatest when followed by more specific serological investigations.

Disease-associated autoantibodies, including anti-dsDNA, anti-Sm, anti-SSA/Ro, anti-SSB/La, anti-Scl-70, and anti-U1-RNP, provide important information for subtype identification and differential diagnosis among connective tissue and other autoimmune diseases. In rheumatoid arthritis, rheumatoid factor and anti-cyclic citrullinated peptide antibodies are valuable complementary markers that can improve diagnostic confidence, especially when clinical findings are compatible with inflammatory joint disease. The

combined assessment of multiple antibodies is generally more informative than reliance on a single marker because autoimmune diseases are heterogeneous, overlapping, and often characterized by variable serological patterns.

Different analytical platforms offer distinct clinical advantages. Indirect immunofluorescence is useful for broad screening and pattern recognition, immunoblotting supports antibody profiling across multiple targets, and chemiluminescent immunoassays provide automated, quantitative, and high-throughput detection. The rational integration of these methods can improve laboratory efficiency and strengthen diagnostic performance. Compared with single-analyte testing, multi-indicator detection strategies may increase positive detection rates, enhance diagnostic accuracy, and reduce the likelihood of missed or incomplete diagnoses.

In clinical practice, immunological test results should always be interpreted in conjunction with symptoms, physical signs, imaging findings, routine laboratory parameters, and disease history. A diagnosis should not be established solely on the basis of one antibody result, because false-positive findings, low-titer reactivity, and clinically insignificant seropositivity may occur. Future research should include larger and more diverse patient cohorts, incorporate disease activity indices and longitudinal follow-up, and further evaluate the value of immunoassay indicators in treatment monitoring, prognosis assessment, and risk stratification. Given the complexity of autoimmune diseases and their underlying immune mechanisms, future testing systems should continue to improve in standardization, automation, quantitative consistency, and multi-indicator integration to better support precise and clinically meaningful decision-making.

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