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RESEARCH ARTICLE

DUAL-METHOD EVALUATION OF ANCA DETECTION: ANALYTICAL CORRELATION BETWEEN IFI AND IMMUNODOT IN CLINICAL PRACTICE

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ABSTRACT

Anti-neutrophil cytoplasmic antibodies, or ANCA, are important bio marker used to help doctors diagnose, predict how a disease might progress, and choose the best treatment for conditions called ANCA-associated vasculitides. These conditions include granulomatosis with polyangiitis, microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis. Finding problems early is important because it helps doctors choose the right treatments, stops organs from getting hurt, and helps predict when a condition might come back. Indirect immunofluorescence (IFI) remains the most effective method for identifying specific patterns, like cytoplasmic (c-ANCA), perinuclear (p-ANCA), and other uncommon staining patterns. But IFI on its own doesn't target specific antigens. Immunodot assays use IFI to detect antibodies against PR3, MPO, BPI, elastase, and cathepsin G, which helps in making more accurate diagnoses and identifying the specific antibodies present. In Morocco, there aren't many set steps for doing ANCA tests, which leads to less uniform understanding in medical settings. This study looked at 249 blood samples from CHU Mohammed VI in Marrakech to check how well IFI and Immunodot tests work, how consistent their results are, and how reliable they are. A new combined diagnostic process that uses IFI, Immunodot, and clinical information was suggested to make results more accurate, reduce unclear outcomes, and create a consistent method for lab standards. Our results show that using these methods improves the accuracy of testing, makes lab work more efficient, and sets up a good starting point for spreading this approach across hospitals in Morocco.

KEYWORDS

ANCA, IFI, Immunodot, PR3, MPO, Vasculitis, Workflow, Autoimmunity

1. INTRODUCTION

ANCA-associated vasculitides (AAV) are types of autoimmune diseases where the body's immune system attacks its own blood vessels, affecting both small and medium-sized ones. These diseases include granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). They can affect the kidneys, lungs, upper part of the respiratory system, and the skin. (Jennette, et al., 2013; Kallenberg, 2020). The body's immune system causes harm to the blood vessel lining, leads to swelling and cell death, and this can lead to organ failure if the condition is not caught early. Finding ANCA early on is very important for managing the disease, deciding which medicines to use, and predicting when the illness might come back. People often use IFI for screening because it can detect common fluorescence patterns, like cytoplasmic (c-ANCA, usually PR3) and perinuclear (p-ANCA, usually MPO) types, as well as unusual patterns. (Falk and Jennette, 2021; Im, et al., 2019).

However, IFI has limitations due to the subjective nature of interpreting these patterns and the lack of specific antigen identification. Immunodot, ELISA, and multiplex assays are examples of tests that look for specific antigens using the immune system's response. Antibodies that target PR3, MPO, and other enzymes found in neutrophils. These tests help make the results more accurate and check the findings from IFI. (Hellmich, et al., 2024 ; Lee et al., 2023). Even with these improvements, there are still some differences. About 20 to 30 percent of the samples show different results when using IFI tests compared to antigen-based tests. (Smith et al., 2022 ; Gao et al., 2023). In Morocco, there are no set standards for how ANCA tests are done in laboratories, which causes differences in how the results are understood. This study wants to look at how well IFI and Immunodot methods work in a group of people from Morocco. It also wants to come up with a better way to test that can make detection more reliable, increase accuracy, and lower differences in results.

2. PHYSIOPATHOLOGY OF ANCA-ASSOCIATED VASCULITIS

The way AAV develops involves the body making antibodies that attack proteins in the cytoplasm of neutrophils, specifically proteinase 3 (PR3) and myeloperoxidase (MPO). These anti-neutrophil cytoplasmic antibodies (ANCA) are important in starting and keeping vascular inflammation going. When ANCA attaches to their matching antigens on the surface of neutrophils that have been activated by cytokines, it starts a series of actions. These actions include the neutrophils releasing their contents, producing harmful oxygen molecules, and forming structures called NETs that trap pathogens. These processes result in damage to the cells lining blood vessels and cause inflammation that destroys tissue in small to medium vessels, leading to reduced blood flow and damage to organs (Kallenberg, 2020 ; Falk and Jennette, 2021).

Pro-inflammatory chemicals like tumor necrosis factor-alpha (TNF-), interleukin-8 (IL-8), and interferon-gamma (IFN-) help make neutrophils more active and encourage them to move to the blood vessel walls, which makes the inflammation process worse. Also, T cells help keep

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inflammation going by releasing chemicals called cytokines and activating macrophages, which makes the damage to blood vessels worse. Long-term activation of the immune system can cause scarring and ongoing problems with organs. (Brouwer, 2021). Knowing these immune processes is important for understanding blood tests and linking lab results to actual symptoms (Brouwer, 2021). shows the steps involved in ANCA-related damage to blood vessels. It explains how the antibodies stick to cells, causing white blood cells to become active, creating harmful free radicals, and damaging blood vessel walls, which leads to ongoing inflammation and harm to organs.

3. Materials and Methods

3.1 Study Design and Population

This study looked at 249 patients who were sent for ANCA testing at CHU Mohammed VI in Marrakech. Information about patients' age, gender, and symptoms was taken from the hospital records. The inclusion criteria included all patients who were referred for ANCA testing. The exclusion criteria were samples that were hemolyzed or lipemic, had not enough volume, or lacked complete clinical information.

3.2 Sample Handling

The serum samples were spun in a centrifuge within two hours, divided into smaller parts, and kept in a freezer at –20°C. Tests were performed within one week. Internal and external quality checks were done according to ISO 15189 standards (Bossuyt, 2023).

3.2 Indirect Immunofluorescence (IFI)

Human neutrophil slides that were fixed with ethanol and formalin were used. These slides were obtained from EUROIMMUN in Germany. The Sera were diluted in a 1:20 ratio, left to sit for 30 minutes, then washed and stained using FITC-conjugated anti-human IgG. Slides were evaluated by two independent observers. Patterns were grouped into c-ANCA, p-ANCA, and atypical types. Discrepancies were resolved by consensus (Rout and Verma, 2019).

3.3 Immunodot Assay

The EUROLINE Autoimmune Vasculitis Profile was used to find antibodies against PR3, MPO, BPI, elastase, and cathepsin G. Densitometry measured the signal strength as being either weak, moderate, or strong. Positive and negative controls were included (de Weck, 1996 ; Falk, 2021).

3.4 Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's kappa coefficient were computed using SPSS version 26. Clinical diagnosis served as the reference standard. A p-value of 0.05 or less was considered statistically significant.

4. Results

4.1 Demographics

Of the 249 patients, 161 (64.7%) were female and 88 (35.3%) were male. The mean age was 38.7 years and its standard deviation was 19.4 years (Table 1). The primary reasons for specifically treating patients were renal in 42.1%, respiratory in 24.5%, ears and throat: (10.5%) or dermal symptoms at a rate of (18.7%) (Rout and Verma, 2019).

Table 1: Sex distribution of patients included in the study.

Sex	Count	Percentage
Female	161	64.7
Male	88	35.3

Table 2: Distribution of patients according to clinical service (n=249).

Service	Number of Patients (n)
Internal Medecine	88
Nephrology	54
Others	41
Pediatrics	34
outpatient	32
Total	249

Most cases occurred in the Internal Medicine department, at a rate of 35.44% (n = 88) patients. Those from the Nephrology department followed with 21.58% of the total Some 13.65% (n = 34) of pediatric cases and 12.9% of outpatient cases suggest significant contribution from ambulatory settings **(Table 2) .** This distribution in organs of involvement portrays the systemic and renal predominance that drives the well-established clinical phenotypes seen with autoimmune/inflammatory diseases.

4.2 IFI Results

C-ANCA was the most dominant pattern by indirect immunofluorescence (IFI) analysis, which were found in 109 patients (43.77%). ANCA-negative was found in 45 samples (18.07%) and P-ANCA, 95 patients (38.15%). These are consistent with higher prevalence of C-ANCA in the studied population and supports diagnostic relevance of IFI to detect ANCA-associated vasculitides.(Figure 1)

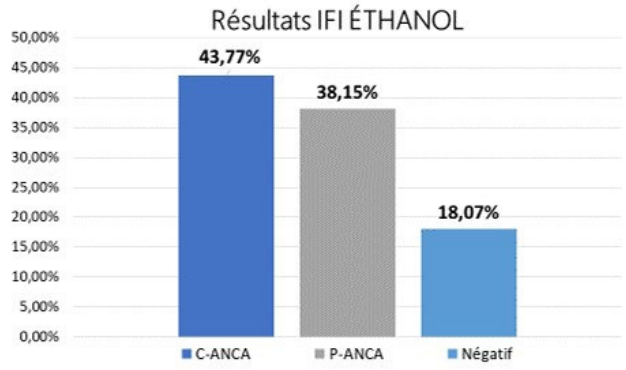

Figure 1: IFI on ethanol-fixed neutrophils showing c-ANCA.

In formalin-fixed indirect immunofluorescence (IFI), we identified a predominant pattern of C-ANCA in 81 patients (85.26%) while atypical P-ANCA alone were identified in 14 patients (14.73%) **(Figure 2).** This distribution emphasizes the dominant role of C-ANCA in ANCA-associated vasculitis and confirms the effectiveness of IFI in differentiating antibody patterns.

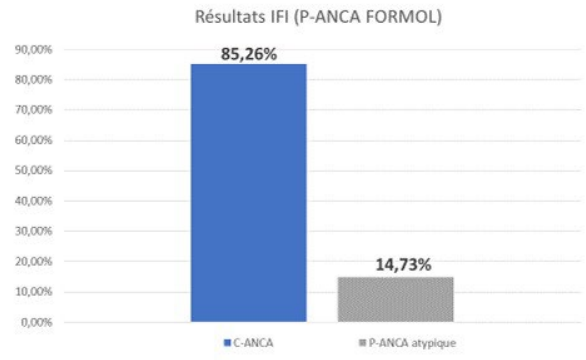

Figure 2: IFI on formalin-fixed neutrophils showing p-ANCA.

4.3 Immunodot Results

Immunodot analysis revealed a predominance of PR3 antibodies, detected in 57 patients (52.29%), followed by MPO antibodies in 28 patients

(25.68%). The remaining 24 patients (22.01%) exhibited reactivity to other antigens, including anti-mitochondrial antibodies.**(Figure 3)**

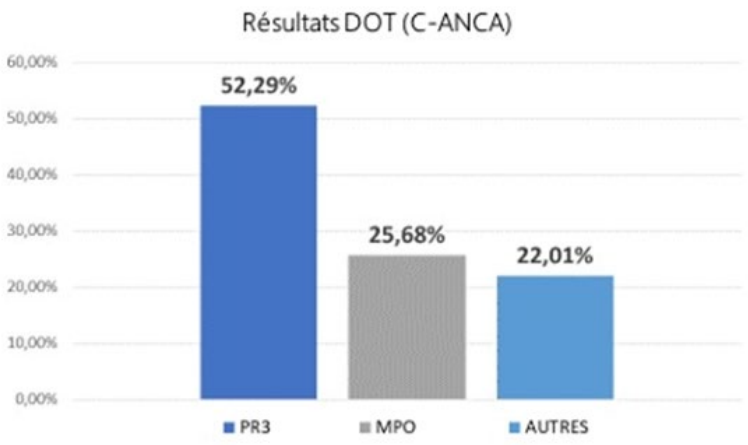

Figure 3: Immunodot assay for PR3 (c-ANCA).

Immunodot confirms antigen-specific profiles, complementing IFI results, and allows semi-quantitative assessment of antibody levels for clinical interpretation.

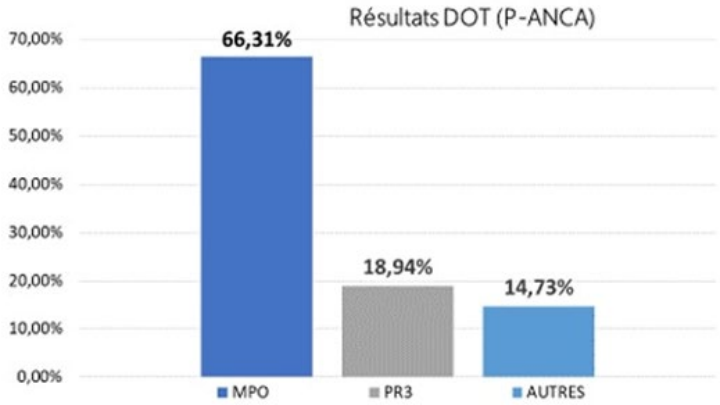

Figure 4: Immunodot assay for MPO (p-ANCA).

The Immunodot test showed that the highest number of antibodies was directed against MPO (66.31%), then against PR3 (18.94%). Other antibodies like elastase and lactoferrin have been found in 14.73% of patients. This diversity in antibodies indicates that there is a variety in the autoimmune reaction in this group of patients**(Figure 4).** The presence of MPO through Immunodot helps in confirming antibody specificity and eliminating IFI false positives.

4.4 Statistical Analysis and Results

Diagnostic performance of IFI and Immunodot was evaluated using sensitivity, specificity, PPV, NPV, and Cohen's kappa , with the clinical diagnosis as reference. Analyses were performed in SPSS v26, and a p-value < 0.05 was considered significant. IFI showed predominance of C-ANCA (43.8%) and P-ANCA (38.2%), with 18.0% negative. Immunodot confirmed antigen specificity: PR3 (52.3%), MPO (25.7%), and other antibodies (22.0%). For P-ANCA, MPO predominated (66.3%). Overall agreement between IFI and Immunodot was substantial (k = 0 .75), highlighting that combining methods provides sensitive and specific ANCA detection while reducing discordances (Table 3).The combined workflow, starting with IFI screening and Immunodot confirmation,

enhances accuracy, reduces subjective interpretation, and supports reproducible, standardized testing.

Table 3: Diagnostic Performance of IFI and Immunodot Assays.

Test Method	C-ANCA/ PR3	P-ANCA/ MPO	Other/ negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa (k)
IFI	109 (43.8%)	95 (38.2%)	45 (18.0%)	89.0	91.5	92.0	88	0.75
immunodot	57 (52.3%)	28 (25.7%)	24 (22.0%)	86.5	95.0	94.5	87	0.75

5. Discussion

Using both IFI and Immunodot together offers a way that is both sensitive and specific for detecting ANCA. IFI is important for the first check and spotting patterns, while Immunodot helps confirm the type of antibody and cuts down on disagreements. Ethanol IFI mainly finds c-ANCA linked to GPA, while formalin IFI shows p-ANCA commonly seen in MPA and EGPA. Using these methods in a set process makes the lab work faster, lessens guesswork, and follows global guidelines (Hellmich et al., 2024 ; Smith et al., 2022).

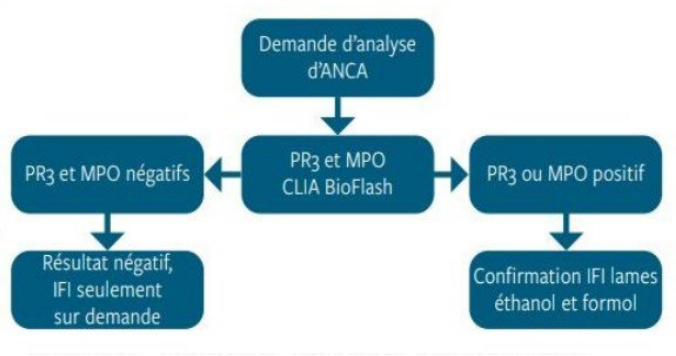

Figure 5: Proposed ANCA diagnostic workflow integrating IFI, Immunodot, and clinical evaluation.

This figure (Figure 5**)** shows the **new diagnostic method we suggest**. Samples are tested for IFI, and any positive or unclear results are checked again using Immunodot. Clinical correlation is included at each step to help ensure accuracy. This process helps reduce unclear situations, lowers the chances of incorrect results, and creates a reliable method that can be used nationwide. The process can be adjusted for use with automation and possible help from AI, which allows for quicker and more accurate outcomes in labs that handle a lot of tests. Implementing this approach could help make ANCA testing the same in all labs across Morocco, lower differences between labs, and lead to better care for patients.

6. Conclusion

Combining IFI and Immunodot methods into a clear diagnostic process greatly enhances the accuracy of ANCA testing. The new method improves the ability to detect true positives and minimizes false positives, makes results easier to understand across different people, and gives reliable outcomes that match what is found in real patient cases. This method is especially useful in Moroccan labs, where there aren't many standard procedures in place. Future looks to include using automated image analysis, AI help for finding patterns, and high-throughput Immunodot tools to improve diagnostic accuracy even more. Using this workflow could set a standard for labs across the country and region, which would help improve how patients are cared for and the results they get in cases of ANCA-associated vasculitis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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