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Research Article

Re-Evaluating the Current ANCA Screening Method: Multiplex Indirect Immunofluorescence Assay in ANCA Testing

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Abstract

Objective: The Indirect Immunofluorescent Test (IIFT) method is a crucial component of Anti-Neutrophil Cytoplasmic Antibodies (ANCA) diagnostics as it is the only method that can be used to detect not only small vessel vasculitis related ANCA, but also ANCAs in Chronic Inflammatory Bowel Disease (CIBD) and other disease conditions. The conventional IIFT method only uses ethanol fixed granulocytes for initial testing, which results in a lack of specificity. The purpose of this study is to outline an improvement in the screening and interpretation of ANCA by IIFT, using a 3-chip combination per incubation well. With the inclusion of an ethanol fixed granulocyte chip, formalin fixed granulocyte chip, and mixed cell chip containing both ethanol fixed granulocytes and HEp-2 cells, we aim to demonstrate a reduction in the number of false positives and false negatives, increase ANCA testing specificity without the loss of sensitivity, as well as simplify the reporting.

Design/Method: 261 serum samples were obtained from the serum bank of Euroimmun Canada. Each sample was tested for both Anti-Nuclear Antibodies (ANA) and ANCA (3-chip combination) using IIFT, and was also tested using Anti-MPO/PR3 ELISA. ANCA profile (including six antigens) ELISA was used as well. All reagents were from Euroimmun.

Results: Distinct ANCA patterns were identified according to our simplified scheme, including: ANCA (c- or p-ANCA), atypical ANCA (atypical c- or p-ANCA) and atypical inconclusive ANCA. The use of a 3-chip combination eliminated ANA interference and resulted in a significant reduction of 75.6% in the unsure "positive" ANCA results that were initially determined from using ethanol fixed granulocytes only. The ethanol fixed granulocyte chip and formalin fixed granulocyte chip are important for pattern classification, while the mixed cell chip is crucial for differentiating true positive ANCA from ANA interference. Noticeably, this IIFT 3-chip combination testing revealed 5% more true ANCA positive samples, which were ANCA negative when using ethanol fixed granulocytes only as the initial screening.

Conclusion: The use of a 3-chip combination multiplex IIFT approach is necessary for an accurate interpretation and analysis of patient serum for ANCA screening. This proposed inclusion of additional substrates in the IIFT ANCA diagnostic procedure has shown to result in increasing specificity of the IIFT, while maintaining a high sensitivity. The simplified reporting also makes the ANCA result standardization a reality. ANCA IIFT method continues to be very useful in routine testing for different groups of diseases.

Keywords: ANCA; Autoantibodies; ANA Interference; ANCA Sensitivity; ANCA Specificity; IIFT Method

Introduction

Anti-Neutrophil Cytoplasmic Antibodies (ANCA) are a group of autoantibodies that react with various proteins within neutrophils. Screening patient serum for these antibodies holds clinical significance and gives insight into various autoimmune disorders, including ANCA-Associated Vasculitis (AAV), Chronic Inflammatory Bowel Disease (CIBD), autoimmune liver disease, collagenosis, and more [1-8]. There are three common ANCA patterns: Cytoplasmic ANCA (cANCA), Perinuclear ANCA (pAN-CA), and atypical ANCA [1,2,5,4,9,10]. cANCA shows fluorescence patterns of the cytoplasm on ethanol fixed granulocytes, which is typically a result of the presence of autoantibodies that react with the major target antigen, Proteinase 3 (PR3). pANCA demonstrates fluorescence of the outer edge of ethanol fixed granulocyte cell nuclei, resulting from antibodies that react with various target antigens, the major antigen being Myeloperoxidase (MPO) [4,5,9]. Atypical ANCA includes patterns not incorporated by the cANCA and pANCA patterns [2,5,11,12]. The atypical pattern is not clearly described in the literature and varies between laboratories. However, the target antigens for the described atypical pattern seem to include elastase, lactoferrin, cathepsin 3, Bactericidal Permeability Increasing Protein (BPI) and other specificities [5,7,8,13]. Diagnostic testing for ANCA is conducted using the Indirect Immunofluorescent Test (IIFT) and monospecific antibody testing. Currently the standard diagnostic algorithm for the testing and reporting of ANCA involves both the IIFT, using ethanol fixed granulocytes, and MPO and PR3 specific assays [2,14]. When patient sera are tested for ANCA, it is routinely tested using ethanol fixed granulocytes, displaying fluorescent patterns that illustrate the types of autoantibodies present in the sera and give insight into the specific autoimmune disease [15]. Monospecific ANCA testing is typically used to detect anti-PR3 and anti-MPO antibodies in AAV [1]. However, additional ANCA profile can be run on patient samples to determine whether alternative antigen specific autoantibodies are present in other disease groups, including CIBD, autoimmune liver disease, and other systemic rheumatic diseases, etc. Although monospecific assays are used in the diagnostic process of ANCA, the IIFT remains important since many antigens have yet to be identified. Oudkerk Pool M, Ellerbroek P, Ridwan B, Goldschmeding R, von Blomberg B, et al. [16] showed that combining both ELISA and IIFT in the screening algorithm increases the specificity and sensitivity of ANCA diagnostic testing as a whole. The IIFT is able to identify almost all ANCA positive sera, as it also detects the presence of antibodies for antigens aside from MPO and PR3, which are the only two included in the standard ANCA monospecific test. A study by Lin M, Silvestrini R, Culican S, Campbell D, Fulcher D [17] demonstrated that many ANCA positive patient samples are negative for PR3 and MPO antigens, which would have been identified as false negatives if IIFT was not used in addition to monospecific testing. The problem with the conventional IIFT is that the method is not standardized and lacks specificity as a result of using inadequate substrates. Due to the subjective nature of interpreting IIFT ANCA results, there is a low specificity of the test, resulting in a significant number of false positives. Studies have attributed false positives in IIFT testing for ANCA to the potential influence of ANA on ANCA results, as ANA patterns appear when using ethanol fixation [1,5,9,14]. Therefore, false positives can occur due to ANA positive results being mistaken for ANCA positive results. Since ANA can interfere with the results of ANCA when strictly using ethanol fixation, research investigating additional fixations is crucial. Hagen E, Daha M, Hermans J, Andrassy K, Csernok E, et al. [14] exemplified this requirement by establishing a sensitivity range of 81%-85%, for various forms of AAVs, and a specificity of 76%, for diseased controls. This study strictly used IIFT with only the ethanol fixation and demonstrated a significant amount of false positives. Stone J, Talor M, Stebbing J, Uhlfelder M, Rose N, et al. [1] explored the validity of the IIFT for ANCA diagnostics, and included both ethanol fixed granulocytes and formalin fixed granulocytes. The inclusion of the formalin fixation allowed for an increase in specificity from 76%, as seen in the study by Hagen, et al. [14], to 93% [1]. The inclusion of formalin fixed granulocytes has been shown in a number of studies to increase the specificity and result in more accurate ANCA testing. Additionally, ANA presence can interfere with ANCA results. A number of laboratories are including HEp-2 cells to differentiate ANA and ANCA which could subsequently further reduce the number of false positives present in the current ANCA IIFT algorithm [18]. However, it is important to have a clear and standard procedure that combines findings from the literature. Therefore, it is obvious that a multiplex approach is the best choice for ANCA testing, including the IIFT. This study aims to use a multiplex substrate approach in the IIFT, developed by Euroimmun, to standardize the IIFT for ANCA testing, that is easy to comprehend and reduces the subjectivity of analysing results. We intend to illustrate the reduction of false positives with the inclusion of formalin fixed granulocytes and a mixture of HEp-2 cells with ethanol fixed granulocytes, alongside an ethanol fixed chip. This study is focused on quality diagnostics from a laboratory point of view.

Materials and Methodology

Clinical Samples

All patient samples were obtained through the serum bank of Euroimmun Medical Diagnostics Canada. No patient records were required for the purposes of the present study. 261 serum samples were included in the study. Most samples were previously tested as ANA positive and 55 samples were previously tested as ANCA positive. All samples were re-tested for ANA using IIFT and tested for ANCA using IIFT and Anti-PR3/Anti-MPO ELISA. Addition-

ally, an ANCA profile (ELISA) was completed for 101 samples.

IIFT - ANA

Each well on the slide consists of a HEp-2 epithelial cell chip (Euroimmun, Germany). Samples were diluted 1:80 for testing. All testing for IIFT was done via the automated IF Sprinter (Euroimmun). The instrument automatically pipetted 30ul of the positive control, negative control, and the diluted sample, respectively, onto each BIOCHIP well. After this step, the slides were incubated at room temperature for 30 minutes. After the incubation period, the IF Sprinter brought the slides to the washing station to complete the washing procedure using PBS Tween 20 (PBST). After washing, the IF Sprinter pipetted 25 µl of the conjugate (FITC-labelled goat anti-human IgG) to each of the BIOCHIP wells, which were then incubated. The wells were then washed again as previously described. After the automated processing, each of the BIOCHIP slides was removed from the trays. The coverslips were placed on each slide with the embedding medium. The slides were then read through EUROStar LED microscope (Euroimmun) and results were recorded.

IIFT - ANCA

Each 3-chip BIOCHIP well on the slide consists of ethanol fixed granulocytes, formalin fixed granulocytes, and a mixture of HEp-2 cells and ethanol fixed granulocytes (mixed cell chip) (Euroimmun, see Figure 1). Patient sera were diluted to 1:10. All testing for ANCA was processed via the automated IF Sprinter, following the same procedure explained previously for ANA testing.

Figure 1: BIOCHIP (3-chip per well) slide illustration with a zoomed in image of the first well.

Analysis of IIFT Results

For both ANA and ANCA, the pattern and intensity level was determined and recorded. Specifically, for ANA, the identified patterns were nuclear patterns (homogenous, speckled, nuclear dots, centromeres, nucleolus, nuclear membrane, mitotic), cytoplasmic patterns (fine granular, coarse granular, droplets and filamentous) and combination of these. Through visual analysis under EURO-Star LED microscope, ANA intensity was categorized into 5 levels: 0= (negative), 1= (weak positive), 2= (positive), 3= (strong positive), 4= (very strong positive). With ANCA IIFT, the ethanol fixed granulocytes and formalin fixed granulocytes were used to determine the pattern. The mixed cell chip was used to check ANA interference and antibody specificity. In most cases, ANCA exists when the granulocytes in the mixed cell chip have greater fluorescence intensity than the HEp-2 cells; and the comparison can be used to differentiate ANA and ANCA coexistence from ANA interference. The ANCA patterns were analyzed and categorized as following:

- ANCA (c- or p-ANCA) with a positive reaction on the ethanol fixed granulocyte chip, the formalin fixed granulocyte chip and the granulocytes on the mixed cell chip.

- Atypical ANCA (atypical c- or atypical p-ANCA) with a positive reaction on the ethanol fixed granulocyte chip and the granulocytes on the mixed cell chip, and a negative reaction on the formalin fixed granulocyte chip.

- Atypical inconclusive ANCA with a negative reaction on the ethanol fixed granulocyte chip and a positive reaction on the formalin fixed granulocyte chip and a positive or negative reaction of the granulocytes on the mixed cell chip.

ANA intensity at sample dilution of 1:10 was categorized using the mixed cell chip into 5 distinct levels, as described for ANA intensity.

Monospecific Confirmation ELISA Testing for ANCA

All samples were tested using anti-MPO and anti-PR3 antibody ELISA kits (Euroimmun) and 101 samples were tested additionally for anti-elastase, anti-lactoferrin, anti-cathepsin 3 and anti-BPI, using ANCA profile ELISA kit (Euroimmun).

Anti-MPO and Anti-PR3 ELISA

All Anti-MPO and anti-PR3 antibodies were tested using the fully automated Euroimmun Analyzer 1. In short, patient samples were diluted 1:201 in sample buffer and placed into wells. 100µL of calibrators, positive controls, negative controls and diluted samples were pipetted into the ELISA plate wells, respectively, and incubated for 30 minutes. The wells were then washed with wash buffer in the automated washing station. After washing, 100µL of enzyme conjugate (peroxidase-labelled rabbit anti-human IgG) was added to each plate well and incubated for 30 minutes. The wells were then washed as previously described. After washing, 100µL of substrate solution (TMB/H_2O_2) was pipetted into each well and incubated for 15 minutes. 100µL of stop solution (0.5 M sulphuric acid) was added to each microplate well. The complete plate was then read on the wavelength of 450nm, with a reference
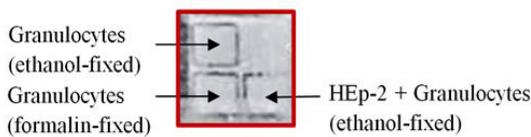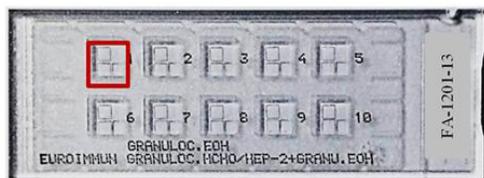
wavelength of 620nm.

ANCA Profile

101 of the samples were tested using ANCA profile, to determine alternative antigen specificity in addition to MPO and PR3, including: lactoferrin, elastase, cathepsin 3 and BPI. The ELISA testing for ANCA profile was completed using the Euroimmun Analyzer 1, following the same procedure explained previously for anti-MPO and anti-PR3 ELISA.

Analysis of ELISA and ANCA Profile

For the anti-MPO and anti-PR3 ELISA test, a quantitative value was calculated according to a standard calibrator curve. Results that are equal to or greater than 20.0 (RU/mL) are considered positive for either anti-MPO or anti-PR3 antibodies. For the ANCA profile ELISA test, semi-quantitative value was obtained using a cut off calibrator. Results that are equal to or above 1.0 (ratio) are positive for antibody presence.

Statistics

A chi-square test and a sign-test were conducted for the statistical analysis. A p-value less than 0.05 indicated a significant difference.

Results

Categorizing the ANCA Patterns

Positive ANCA samples were identified and categorized into distinct ANCA patterns, including:

- ANCA (c- or p-ANCA).
- Atypical ANCA (atypical c- or atypical p-ANCA).
- Atypical inconclusive ANCA.

Identification of the specific pattern, pANCA or cANCA, of the sample is dependent on whether the ethanol fixed granulocyte chip is positive. A cANCA or pANCA pattern is presented when the formalin fixed granulocyte chip, the ethanol fixed granulocyte chip and the granulocytes on the mixed cell chip are positive, as seen in Figure 2a and Figure 2b, respectively. A sample is defined as atypical pANCA or atypical cANCA, when the ethanol fixed granulocyte chip is positive, the formalin fixed granulocyte chip is negative, and the granulocytes on the mixed cell chip are positive, as seen in Figure 2c (shown as atypical pANCA). There were 6 samples with an atypical cANCA-like pattern; however, the atypical cANCA pattern was excluded for these cases due to non-ANCA related cytoplasmic autoantibody interference, identified through the use of the HEp-2 and granulocyte mixed chip.

The last type of ANCA pattern is termed as atypical inconclusive ANCA, which occurs when the ethanol fixed granulocyte chip is negative, the formalin fixed granulocyte chip is positive,

and the granulocytes on the mixed cell chip are either negative (shown in the Figure) or positive (not shown in the Figure), as illustrated in Figure 2d.
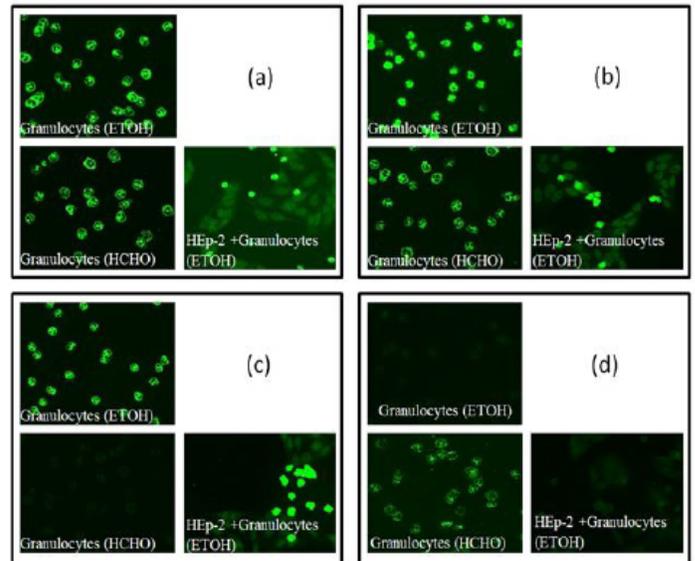

Figure 2: The characterized ANCA immunofluorescence patterns: (a) cANCA, (b) pANCA, (c) atypical pANCA, (d) atypical inconclusive ANCA. Ethanol fixed substrate is represented as ETOH, while formalin fixed substrate is represented as HCHO.

ANA Fluorescence Intensity at a Sample Dilution of 1:10 vs. 1:80

As previously described, all 261 samples were tested using IIFT for ANA, using HEp-2 cells, at a 1:80 sample dilution. Additionally, all samples were tested for ANCA, using HEp-2 cells (mixed with ethanol fixed granulocytes), at a 1:10 dilution. The intensity level of the HEp-2 cells at both dilutions were analyzed and recorded as described in the methods section. The intensity levels between the two test results were compared, illustrating significantly higher intensity level when using the 1:10 dilution than the 1:80. All statistical analyses were completed on the 200 samples that tested positive for ANA (see Table 1).

Table 1: ANA Fluorescence Intensity Levels at a Sample Dilution of 1:10 vs 1:80.

	Sample Dilution	**ANA Intensity***			
		1	2	3	4
Number of Samples	1:10**	48	63	40	49
	1:80**	96	88	9	7
*1=weak positive, 2=positive, 3=strong positive, 4=very strong positive					
**Chi-Square Test: $x2(3df) = 71.3$ ($p<0.001$); Phi Association of 0.422 ($p<0.001$); Sign-Test: $p<0.001$					

ANCA Results: Ethanol Fixed Granulocytes Alone vs. 3-Chip BIOCHIP

200 samples that tested positive for ANA were selected for the comparison of results when using only ethanol fixed granulocytes and those when using the 3-chip BIOCHIP. 135 of the ANA positive samples were determined to be unsure ANCA "positive", as they were based solely on the ethanol fixed granulocytes. With the additional analysis of the formalin fixed granulocyte chip and the mixed cell chip, only 33 samples were identified as true ANCA positives. With the 3-chip BIOCHIP, the number of false positives caused by ANA interference were significantly reduced by 75.6% (see Figure 3).
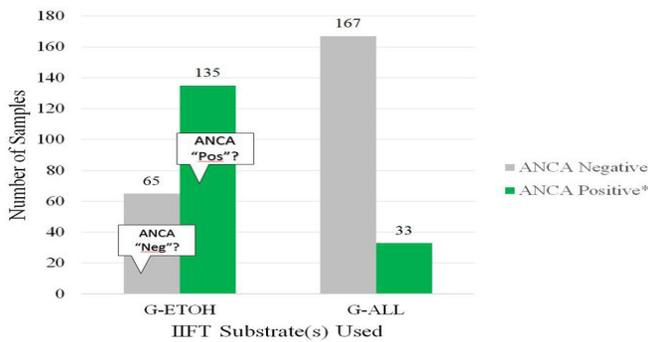

Figure 3: The graph illustrates the difference in positive and negative results between the G-ALL and G-ETOH. G-ALL represents the IIFT results when using all three substrates (ethanol fixed granulocyte chip, formalin fixed granulocyte chip, and the mixed cell chip), while G-ETOH represents the results using only ethanol fixed granulocytes. *Chi-Square Test: $x2(1df) = 106.8$ ($p<0.001$); Phi Association of 0.517 ($p<0.001$).

ANCA Pattern Distribution

A total of 82 true positive ANCA samples were identified and an ANCA pattern distribution was produced as shown in Figure 4.

ANCA Positive Samples with Multiple Antigen Specificities

Monospecific confirmation demonstrated that a total of 8 out of the 82 true positive ANCA samples had reaction to multiple antigens (see Table 3).
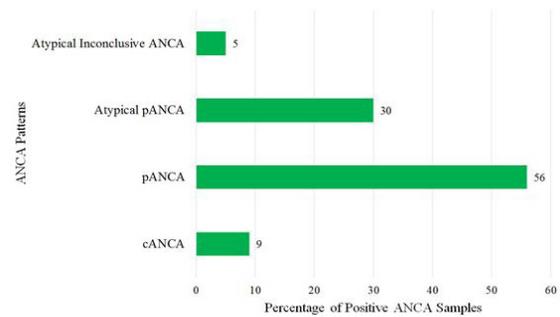

Figure 4: Illustrates the percentage of true positive samples for each type of the ANCA patterns: atypical inconclusive ANCA, atypical pANCA, pANCA and cANCA.

Atypical Inconclusive ANCA

This ANCA pattern is defined as a pattern that shows the ethanol fixed granulocytes as negative and the formalin fixed granulocytes as positive. Four of the 82 true positive ANCA samples were identified as atypical inconclusive ANCA. Monospecific confirmation showed these samples to have either anti-MPO and anti-PR3 antibodies (see Table 2).

Table 2: The Monospecific ANCA Confirmation of the Atypical Inconclusive ANCA Pattern.

	Autoantibodies Against*					
Sample	**MPO**	**PR3**	**Lacto-ferrin**	**Elastase**	**Cathe-psin 3**	**BPI**
1	<0.1	3.2	<0.1	<0.1	<0.1	<0.1
2	3.1	0.1	0.1	0.2	<0.1	<0.1
3	<0.1	5.2	<0.1	<0.1	<0.1	0.3
4	<0.1	1.4	<0.1	<0.1	<0.1	<0.1
*cut-off value (ratio) 1.0						

Table 3: Eight of the True ANCA Positive Samples that Exhibited 2 or 3 Monospecific Antibodies through ANCA Profile Testing.

		Autoantibodies Against*					
Sample	Pattern	MPO	PR3	Lactoferrin	Elastase	Cathepsin 3	BPI
1	cANCA	<0.1	1.1	1.2	0.3	0.2	0.1
2	pANCA	6.4	<0.1	2.4	<0.1	<0.1	1.2
3	pANCA	<0.1	2	1.5	0.7	0.2	0.3
4	pANCA	10.4	0.3	0.6	0.5	1.6	0.4
5	pANCA	0.8	<0.1	1.9	5.4	0.2	<0.1
6	pANCA	0.5	1.6	0.1	2.4	0.3	<0.1
7	pANCA	5.2	1.2	<0.1	<0.1	<0.1	<0.1
8	Atypical pANCA	0.3	<0.1	1.7	3.9	0.1	<0.1
*cut-off value (ratio) 1.0							

Discussion

Simplifying ANCA Patterns

The complexity of ANCA pattern recognition described in previous consensus articles [2, 11] is difficult to follow in a routine laboratory setting. Using the multiplex 3-chip approach, ANCA patterns can be simplified according to the positivity of different substrates used. In this study, 3 ANCA pattern categories were identified, as described in the results (see Figure 2). This multiplex approach allows for results to be confidently analyzed with certainty of classification, providing reliable and consistent results.

ANA Interference

ANA (both anti-nuclear and anti-cytoplasmic) interference often accounts for false ANCA positives when using ethanol fixed granulocytes as the only IIFT substrate [1,5,9,14]. Anti-nuclear antigens could contribute to the pANCA-like pattern, while anti-cytoplasmic antigens could cause the cANCA-like pattern [1,14]. When either of these situations occurs, the ANCA results are truly negative when the coexistence of ANA and ANCA is excluded. Therefore, it is important to evaluate the ANA interference to determine whether the ANCA-like patterns are caused by either ANA alone or the coexistence of ANA and ANCA. The use of the mixed cell chip (HEp-2 and granulocytes) is crucial to further aid in ANCA analysis, by excluding ANA interference and revealing the coexistence of ANA and ANCA.

This mixed cell chip is very helpful when ethanol fixed granulocytes and formalin fixed granulocytes cannot give a clear result of ANCA. In the mixed chip, if the HEp-2 cells display stronger fluorescence intensity than the granulocytes, or the same intensity, then there is ANA interference and the ANCA result is considered as negative if the substrate of the formalin fixed granulocytes (chip) shows negative as well. When the granulocytes in the mixed cell chip are of greater fluorescence intensity than the HEp-2 cells, positive ANCA and the specific pattern can be recorded with the combined use of the other 2 chips. During ANA routine testing, ANA results are based on a sample dilution of 1:80 (some laboratories use different screening dilutions). In comparison, ANCA routine testing uses a 1:10 dilution, which is at a lower dilution than that of the ANA testing procedure. Since the dilutions differ between the routine ANA testing at a 1:80 dilution and the ANCA testing at a 1:10 dilution, the predetermined ANA results from routine testing (1:80 dilution) cannot be used to exclude ANA interference. The significant difference between ANA results at a sample dilution of 1:10 and 1:80 can be seen in Table 1. Therefore, it is important to test patient sera on HEp-2 cells at the same dilution as the granulocytes to differentiate ANA interference on ANCA results.

Ethanol Fixed Granulocytes Alone vs. 3-Chip BIOCHIP

The results demonstrate that with the use of multiple substrates and fixations, the IIFT becomes a more accurate screening assessment. Conventional ANCA diagnosis of patient sera strictly includes the analysis of ethanol fixed granulocytes. Some laboratories may use separate slides containing formalin fixed granulocytes to confirm ANCA positivity either after obtaining positive results from ethanol fixed granulocytes, or in parallel. This additional step is time consuming and impractical. As seen in a previous study, the specificity and sensitivity of the IIFT with only ethanol fixed granulocytes has been identified as 76% and 81%-85%, respectively. The low specificity was attributed to a high number of false positive pANCA results in the disease control group, which was thought to be attributed to potential ANA interference [1,14,19,20]. ANA and atypical ANCA patterns appear when using alcohol fixation, which could explain why strictly testing sera on ethanol fixed granulocytes results in many false positives and misdiagnoses [9, 21]. Baslund, et al. [22] determined that 9% of the positive ANCA samples identified in their study were false positives when using ethanol fixation alone, which can be minimized by using additional substrates. In the present study (see Figure 3), a higher number of indeterminate ANCA positives were identified when a single substrate (ethanol fixed granulocytes only) was used for ANCA testing. These unsure ANCA results were due to ANA interference since the majority of the samples used in this study were ANA positive. A small number of false ANCA negatives were also found when using a single substrate (ethanol fixed granulocytes only) due to the lack of a formalin fixed granulocyte substrate being used as well (discussed under the atypical inconclusive ANCA). The addition of a formalin fixed granulocyte chip and mixed cell chip resulted in the identification of a significant number of false positives attributed to ANA interference and ensured reliable ANCA results.

Atypical Inconclusive ANCA

A recent study by Lin, et al. [17] identified the following distinct positive IIFT ANCA patterns, pANCA, cANCA, atypical pANCA, and atypical cANCA, with the use of ethanol and formalin fixations. Furthermore, the study outlined a potential method for analysis and interpretation of ANCA results that is clear and concise. In the present study, an additional pattern was identified with the use of a 3-chip BIOCHIP: "Atypical Inconclusive ANCA".

The atypical inconclusive ANCA pattern is not described in the literature. Four of the 82 (5%, see Figure 4) ANCA positive samples that were identified in this study were categorized as atypical inconclusive ANCA. Interestingly, the monospecific confirmation of these 4 samples were either anti-MPO or anti-PR3

positive, without multiple specificities (see Table 2). This indicates that this pattern will be missed if ethanol fixed granulocytes are used alone for ANCA screening, resulting in false negatives. Using ethanol and formalin fixed granulocytes together increases the sensitivity of ANCA IIFT testing.

ANCA with Multiple Monospecificities

It is well documented and is common knowledge that the major target antigen of the cANCA pattern is PR3, while MPO is the major target antigen of the pANCA pattern [4,5,9]. However, exceptions do exist. In this study, of the 82 ANCA positive samples, 3 of the pANCA samples demonstrated anti-PR3 positivity. Moreover, 8 of the positive ANCA samples exhibited 2 or 3 monospecific antibodies (see Table 3). This indicates that monospecific confirmation for ANCA should include more target antigens than just MPO and PR3. Therefore, only testing anti-MPO and anti-PR3 cannot conclude single monospecificity. ANCAs that have multiple monospecificities may indicate different clinical relevance [23].

Conclusion

The use of a 3-chip BIOCHIP in the IIFT is necessary for an accurate interpretation and analysis of patient serum for ANCA screening. This proposed inclusion of additional substrates in the IIFT ANCA diagnostic procedure has shown to result in an increased specificity of the IIFT, while maintaining a high sensitivity. Figure 5 illustrates the proposed testing strategies for ANCA by using this 3-chip BIOCHIP. In the present study, the IIFT ANCA test did not fail to detect any anti-MPO or anti-PR3 positive samples. However, due to the relatively small sample volume used, it is still cautiously recommended to confirm the ANCA IIFT negative samples with the use of monospecific assays until more studies with a larger sample size are done. The simplified reporting makes the ANCA result standardization a reality. The ANCA IIFT method continues to be very useful in routine testing for different groups of diseases.
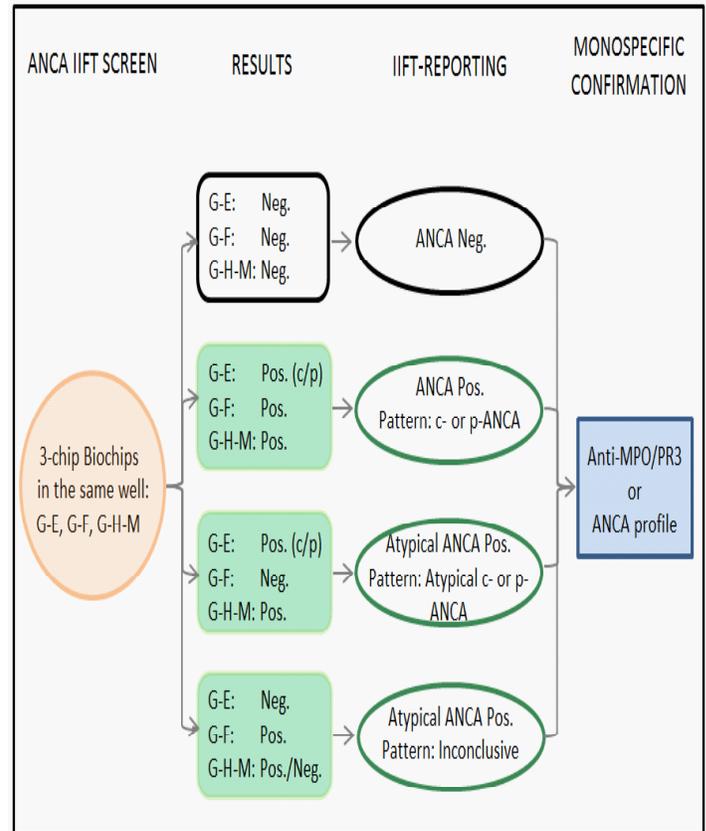

Figure 5: Schematic representation of the analysis of IIFT ANCA results. Ethanol fixed Granulocytes= G-E, Formalin fixed Granulocytes= G-F, Granulocytes & HEp-2 Mix= G-H-M.

Acknowledgement

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