International Journal of Health Sciences and Research
Vol.12; Issue: 9; September 2022
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

The Significance of Cytoplasmic Fluorescence Antinuclear Antibody in Autoimmune Liver Diseases

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DOI: https://doi.org/10.52403/ijhsr.20220902

ABSTRACT

Autoimmune liver disease (AILD) is a rare immune-mediated chronic liver disease with heterogeneous clinical characteristics. There are many cases of hepatitis and cholestasis of unknown etiology that Antinuclear antibody (ANA) are accompanied by specific antibodies for AILD.
We aimed to determine the significance of cytoplasmic antinuclear antibody (ANA) patterns in patients with AILD.
Material and Methods: We retrospectively reviewed all patients were sent to our laboratory from 2016 till 2020 for ANA, Smooth muscle antibody (SMA) and Antimitochondrial antibody (AMA). We examined the diagnoses of the 218 excluded patients. ANA, AMA, SMA were performed by Immunofluorescence test (IFT). Fluorescence intensity was interpreted semi quantitatively
Results: Among 218 cases, 74 (29.4%) resulted as ANA positive and 120 (55%) resulted as cytoplasmic fluorescence positive. ANA resulted positive only in 29 cases. 45 cases resulted as ANA positive and cytoplasmic fluorescence positive. Of interest to study were 75 cases which resulted as ANA negative and cytoplasmic fluorescence positive. Only 69 cases resulted seronegative. All cases with positivity of cytoplasmic fluorescence, regardless of ANA resulted as 82 anti-SMA positive, 3 AMA positive and anti-SMA positive, 35 AMA positive. Furthermore, patients with AILD who exhibited a reticular ANA pattern demonstrated a higher positive rate for AMA 33 (86.8%) and those with the speckled ANA pattern displayed a higher positive rate for SMA 53 (64.6%).
Conclusion: Therefore, cytoplasmic ANA patterns could be used to guide AILD characterization in suspected AILD cases. Thus, it is important to check cytoplasmic ANA patterns for AILD evaluation, even when nuclear ANA patterns are negative.

Keywords: Autoimmune liver disease, Antinuclear antibody, Smooth muscle antibody, Antimitochondrial antibody, Immunofluorescence test.

INTRODUCTION

Autoimmune liver disease is a rare immune-mediated chronic liver disease with heterogeneous clinical characteristics. AILD includes three major disease, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) (1).

There are many cases of hepatitis and cholestasis of unknown etiology that ANA are accompanied by specific antibodies for liver diseases. In unknown hepatitis ANA is accompanied by anti-SMA, anti-liver-kidney-microsomal, anti-soluble liver antigen (2). All together with their positivity help for the diagnosis of autoimmune hepatitis and in its classification. In unknown cholestasis, ANA is required accompanied by AMA, anti-gp120, anti-Lamin-B-receptor, the positivity of which helps in the diagnosis of PBC (3). PSC is an autoimmune cholestatic liver disorder that exhibits a characteristic beaded appearance of the intra- and extra-hepatic bile ducts and

is strongly associated with inflammatory bowel disease and cholangiocarcinoma (4).
Autoimmune serology has been utilized in AILD diagnosis. In particular, the ANA test has been traditionally used as a screening test, followed by secondary tests that identify the presence of specific autoantibodies (5). ANA has been reported to occur in 50–70% of AIH patients (6). In PBC, the positivity rate of ANA is about 50%, whereas that of AMA is 90%, thus showing specificity (7). ANA prevalence in PSC varies widely from 8% to 77% (8).
Of interest in AILD is the fluorescent pattern of the nucleus and the cytoplasmic fluorescence that we identify when we are evaluating ANA. In our laboratory and in Europe, it is generally preferred that autoantibody screening be performed with (IFT). IFT has advantages not only in assessing the antibody titer, but also looking at the fluorescent patterns of the nucleus and the fluorescence pattern in the cytoplasm when it is present. The 2015 International Consensus on ANA Patterns (ICAP) workshop classified ANA patterns into three major groups, nuclear, cytoplasmic, and mitotic patterns. Nuclear patterns include the staining pattern of the nucleoplasm - speckled or homogeneous - and patterns attributed to specific nuclear subcomponents, centromere, nuclear dots, nucleolar, or nuclear envelope. Cytoplasmic patterns represent staining of the cytoplasm and are subdivided into five different patterns, fibrillar, speckled, reticular, polar/Golgi-like, and rods and rings (9). These ANA patterns may provide clinically relevant insights into AILD, such as the suspected disease entity and further recommended diagnostic measures (10, 11, 12). Although ANA positivity plays an important role in AILD diagnosis. In assessing the positivity of ANA we look at the intensity of fluorescence and its pattern.
Aim of the study: Determination of ANA and their cytoplasmic fluorescence positivity. The correlation of cytoplasmic fluorescence positivity with AMA and SMA positivity

MATERIALS AND METHODS

We retrospectively reviewed all patients were sent to our laboratory from 2016 till 2020 for ANA, SMA and AMA. We examined the diagnoses of the 218 excluded patients. ANA immunofluorescence test was performed on HEp-2 cells (aesku diagnostics, Germany) according to the manufacturer's specifications. The sera of the patients were diluted 1:100 for screening. The screening dilution for AMA and SMA was 1:20. All test slides for ANA, AMA and SMA tests were manually analyzed by a laboratory medicine specialist using a fluorescence microscope. The slides were evaluated under the fluorescence microscope using 40X objectives. Fluorescence intensity was interpreted semi quantitatively based on negative control (0) and positive control (+4).

Statistics

For statistical analysis, the results of ANA, SMA, AMA were recorded as continuous variables and categorical data. Statistical test analysis was carried out using the SPSS software version 18.

RESULTS

The study included 218 individuals. 136 (62.4%) of them were females. 133 (61%) and 85 (39%) were diagnosed with hepatitis and cholestasis of unknown etiology respectively. The mean age of them was 42.5±17.0. Among 218 cases, 74 (29.4%) resulted as ANA positive and 120 (55%) resulted as cytoplasmic fluorescence positive. For all patients ANA was requested accompanied by AMA, anti-SMA. ANA resulted positive only in 29 cases. 45 cases resulted as ANA positive and cytoplasmic fluorescence positive. Of interest to study were 75 cases which resulted as ANA negative and cytoplasmic fluorescence positive. The proportion of speckled cytoplasmic ANA pattern was higher in ANA positive than in ANA negative (p=0.044). 69 (36.2%) of 218 were seronegative. All cases with positivity of cytoplasmic fluorescence, regardless of

ANA resulted as 82 anti-SMA positive, 3 AMA positive and anti-SMA positive, 35 AMA positive. Based on AMA and SMA serology resulted that reticular cytoplasmic pattern was higher in AMA positive than SMA positive (86.8%) and speckled cytoplasmic pattern was higher in SMA positive than AMA positive (64.6%).

Table.1 General characteristics, ANA and cytoplasmic fluorescence seropositivity rates in 218 patients studied.

Data of patients	Number (%)
Age in years (mean ± standard deviation)	42.5 ± 17.0
Unknown hepatitis patients	133 61%
Unknown kolestasis patients	85 39%
female patients	136 62.4%
Nuclear pattern	
Positive 1:100	74 33.9%
Negative 1:100	144 66.1%
Cytoplasmic fluorescence	
Positive	120 55%
Negative	98 45%

Table.2 Results of ANA and their cytoplasmic fluorescence

Subgroups of ANA	Number %
ANA positive	29 13.3%
ANA positive / CF positive	45 16.1%
ANA negative / CF positive	75 34.4%
ANA negative / CF negative	69 36.2%
Total	218 100

Table 3. Cytoplasmic ANA pattern of patients with or without Nuclear pattern

Cytoplasmic pattern	ANA positive (N=45)	ANA negative (N=75)	p-value
Reticular	28 (62.2%)	26 (34.7%)	**0.044**
Speckled	17 (37.8%)	49 (65.3%)	

Data are presented as n (%); p-values were calculated using Fisher's exact test.

Table 4. The contribution of Anti-SMA and AMA in cytoplasmic fluorescence positivity

Antibodies	No %
Anti-SMA positive	82 68.3%
Anti-SMA positive & AMA positive	3 2.5%
AMA positive	35 29.2%
Total	120 100%

Table 5. Cytoplasmic ANA pattern based on SMA and AMA serology

Cytoplasmic pattern	SMA positive (N=82)	AMA positive (N=38)
Reticular	29 (35.4%)	33 (86.8%)
Speckled	53 (64.6%)	15 (13.2%)

DISCUSSION

Nuclear ANA patterns have been utilized to characterize AILD based on its clinical and prognostic relevance (6). Approximately 75% of the AIH-1 patients display a homogeneous pattern, and the remainder display a speckled pattern of nuclear ANA (13, 14). PBC also is associated with multiple nuclear dots or rim-like membranous nuclear pattern of ANA. These two staining patterns provide strong evidence of PBC, which is especially useful for diagnosing 5 –10% of the patients with PBC who are negative for AMA (15, 16). These staining patterns can be relevant for distinguishing AIH from PBC, because patients with PBC will very rarely present a homogeneous pattern, and patients with AIH alone will not typically display PBC-specific staining patterns (13). In contrast to nuclear ANA patterns, cytoplasmic staining patterns of ANA have been recognition recently. In this study, among the patients clinically suspected with AILD who exhibited a positive cytoplasmic ANA pattern, 55% were diagnosed with AILD. Therefore, AILD should be suspected in patients with positive cytoplasmic ANA staining pattern, especially when the clinical presentation aligns with AILD. It should be useful to check the cytoplasmic pattern of ANA - in addition to nuclear ANA patterns - to establish a diagnosis. In the current study, 34.7% of AILD patients with the reticular cytoplasmic ANA pattern showed negative nuclear ANA pattern. Moreover, our study showed that 86.8% of the patients with the reticular cytoplasmic ANA pattern tested positive for AMA. Among patients with AILD who exhibited a speckled cytoplasmic ANA pattern, 64.6% patients, tested positive for SMA. We can conclude that having negative nuclear ANA does not preclude the patient from having positive cytoplasmic ANA patterns. Clinicians should consider assessing patients with suspected AILD for positive cytoplasmic patterns, even when they have negative nuclear ANA.

CONCLUSION

During the evaluation of ANA by IFI we notice the presence of fluorescence in the cytoplasm or perinuclear fluorescence that

we describe in the ANA response. This positivity can be associated with AMA and SMA. So cytoplasmic staining should be assessed and reported for patients suspected of having AIH and PBC. A negative ANA should not be used to exclude this diagnosis. IFT is the screening tool of choice in patients with suspected autoimmune liver disease and it may be helpful to diagnose AIH and PBC.

Source of Funding: None

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How to cite this article: Margarita Kurti. The significance of cytoplasmic fluorescence antinuclear antibody in autoimmune liver diseases. *Int J Health Sci Res.* 2022; 12(9):8-11. DOI: *https://doi.org/10.52403/ijhsr.20220902*

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