INTERFERENCE OF ANTINUCLEAR ANTIBODIES IN THE INTERPRETATION OF ANCA

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INTRODUCTION

ANCA testing by Indirect immunofluorescence assay (IFA) is commonly used for the diagnosis of auto immune vasculitis. A retrospective study was done to investigate the interference of positive antinuclear antibodies (ANA) in the interpretation of ANCA.

METHODS

- Blood Samples with request for ANA and ANCA were collected from 64 patients (*Sex ratio*:1.13 M/F, *Mean age* 56ys) admitted to our hospital between 2018 and 2023.
- ANA were sought by IFA on HEp-2 (Biosystem®), ANCA by IFA on ethanol fixed granulocytes (Euroimmun® or Biosystem®)and /or on ethanol and formol granulocytes (Mosaics by Euroimmun®)and by ELISA (ANCA-Pro AESKU®)
- Statistical analysis was performed using SPSS v.26.0

RESULTS

- 64 patients were positive for ANA-IFA testing.
- 22 (34.4%) of them were positive for ANCA by IFA. Based on clinical information and availability of different techniques, ANCA typing by ELISA was realized for 11 patients, 4 of them were positive which meant the coexistence of true positive ANCA and ANA. The remaining were negative and thus considered false positive for ANCA.
- ANCA mosaic was also realized for 7 patients, 2 were positive and 5 were negative.

A total of 12 cases of false positive for ANCA were found in the study. (Figure 1)

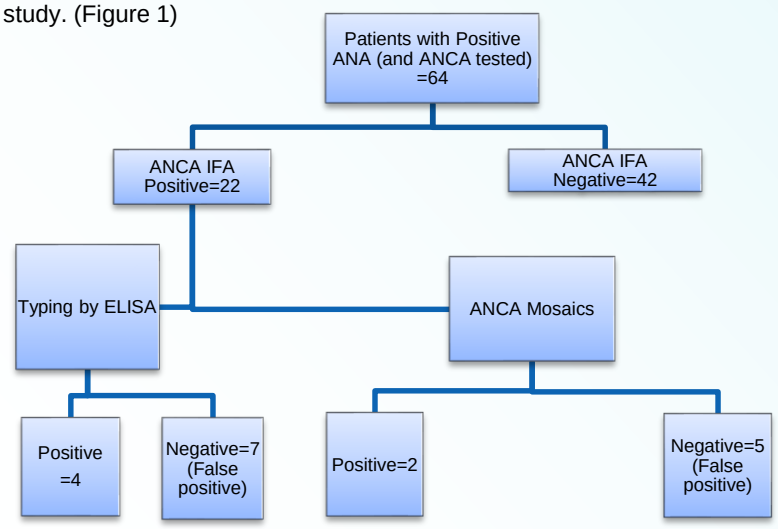

Figure 1

Patients were also divided into subgroups based on their ANA pattern: homogenous, speckled and cytoplasmic.
We found a significant correlation between the homogenous ANA pattern and false positivity for ANCA (p=0.033). Samples with a homogenous ANA also displayed a significant correlation with the P-ANCA pattern.

DISCUSSION

Patients with high ANA titers were more likely to yield a positive ANCA pattern than those with lower titers (50% vs 32.8%).In our study, 34.4% of patients with positive ANA were also positive for ANCA. In this particular subgroup where patients were both positive for ANA and ANCA by IFA technique, 55% were proven false positives using ELISA and/ or formalin fixation techniques meaning the interference of ANA positivity in ANCA detection using IFA.[1]
PR3 and MPO, the antigens typically associated with ANCA, are primarily located within the cytoplasmic granules of neutrophils. Fixation of neutrophils with formalin preserves these granules and their contents within the cytoplasm, resulting in both anti-PR3 and anti-MPO antibodies displaying a C-ANCA pattern. In contrast fixation with ethanol leads to migration of MPO towards nuclear membrane by electrostatic interaction resulting in a perinuclear pattern (P-ANCA). Given that ANAs recognize nuclear antigens, some ANAs may produce a P-ANCA pattern similar to that seen with anti-MPO antibodies. Since the homogeneous pattern can be indicative of anti-ds DNA and histones antibodies, this could explain the positive correlation between the mentioned pattern and false positivity for ANCA that we found in our study .[2]
Another interesting finding in our study, is the association between higher ANA titers and the positivity of p-ANCA. This correlation have been mentioned in previous studies.[3]

CONCLUSION

this study points to the possible interference of ANA positivity in the interpretation of ANCA pattern. Indeed, Homogeneous ANA pattern with higher titer were shown to have more risk of producing a positive p-ANCA pattern.
We recommend to be cautious when reporting a positive ANCA result by IFA especially for patients with positive ANA. Additional techniques such as ANCA mosaic and ANCA typing may be useful to rule out the interference of ANA.
Moreover, the clinical context is crucial for the interpretation.

REFRENCES

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