ICAP CLASSIFICATION PATTERNS UNDER MAGNIFYING GLASS OF MULTIPLEX ANTIBODIES DETECTIONS

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OBJECTIVES

In 2009, the American College of Rheumatology ANA Task Force position statement recommended the indirect immunofluorescence assay (IIF) using Hep-2 cells as the "gold standard" for primary antinuclear antibodies (ANA) detection. Each laboratory can decide whether a competent or expert level can be used to interpret and report the results. For an accurate diagnostic, in both cases, the autoantibodies confirmatory tests are necessary to complete the puzzle, together with clinical manifestations. Starting from ICAP classification patterns we assessed a new multi-parameter Microblot-Array (MBA) technique that could shed light for the clinician on the way of diagnosis.

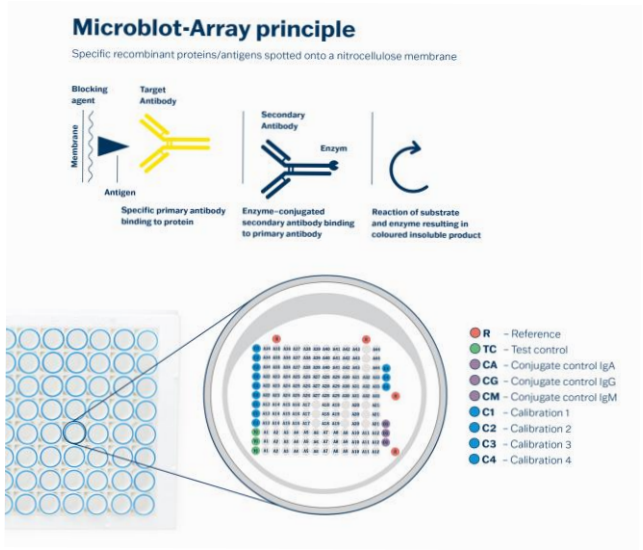

Figure 1 Microblot-Array Principle. MBA well configuration of antigens, controls and calibrators spots

METHODS

For a period of three months, 120 ANA Hep-2 positive samples with different patterns and titers were tested using the MBA technique. IIF slides (Nova Lite Hep-2 ANA kit, Werfen, Spain) were incubated with QUANTA-Lyser 2 (Inova Diagnostics, USA). Reading and interpretation of slides were done manually, with a Nikon E200 microscope equipped with Mshot MSX2-C camera. The MBA ANA plus kit contains 44 recombinant antigens which are spotted in triplicate on a nitrocellulose membrane and fixed at the bottom of each well, in an ELISA plate format (TestLine Clinical Diagnostics, Czech Republic). MBA plate was processed on an automated ELISA DS2 system (Dynex Technologies, USA). Data analysis was performed using a reader that measures the color change of the spot and the results are evaluated by interactive software. The MBA principle is easy and presents many benefits (Figure 1).

CONCLUSIONS

RESULTS

From 120 samples tested on both IIF ANA Hep-2 and MBA ANA Plus, 70 samples (59%) with titer >= 1/320 were positive on MBA, while 50 samples (41%) with 1/80 and 1/160 titer, had no positive antigen on MBA (Figure 2). The predominant pattern identified was AC-4, followed by AC-5, AC-2, AC-1 and AC-3. Of cytoplasmic patterns, the AC-21 was the only one presented in our study at this moment (Figure 3). All of these patterns, exception AC-2, revealed at least one antigen on MBA ANA plus (Figure 4). The pattern AC-2, associated with DFS-70 antibody, was negative on MBA at 1/80, 1/160 and 1/320 IIF ANA Hep-2 titer.

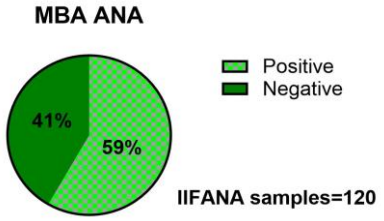

Figure 2 Positive and negative percentage of total IIF ANA Hep-2 samples on MBA ANA plus

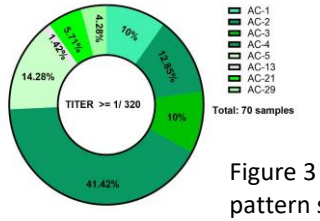

Figure 3 Percentage of pattern samples (ANA Hep-2 titre >= 1/320)

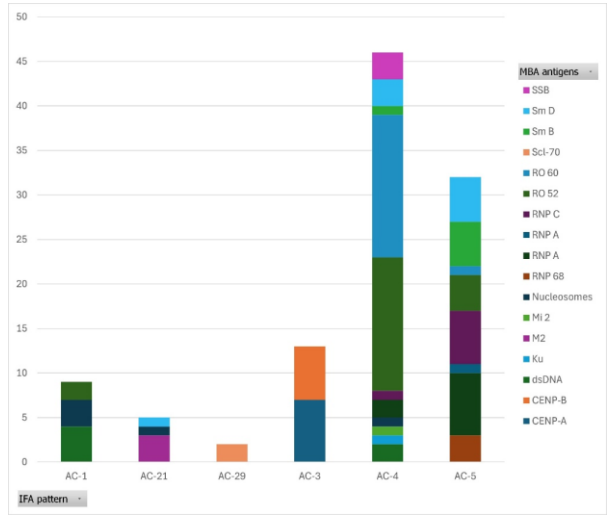

Figure 4 Most frequently ANA Hep-2 patterns associated with MBA antigens

- The negative results from MBA for week positive IIF titers (1/80 and 1/160) may indicate that ANA is found in healthy individuals or different pathology and this should be taken into account when interpreting IIF ANA Hep-2 results.
- Antigens associated with different ICAP patterns started from 1/320 were confirmed by this new generation immunoblot array.
- For AC-2 (DFS-70), further studies are needed.

Bibliography

Carolien Bonroy, Martine Vercammen, Edward K.L. Chan, Jan Damoiseaux et al., Detection of antinuclear antibodies: recommendations from EFLM, EASI and ICAP, Clin Chem Lab Med 2023; 61 (7): 1167-1198