

Approach to Laboratory Diagnostics of ANCA Associated Vasculitis (AAV) in Italian Clinical Laboratories

Radice A¹, Trezzi B², on behalf of Forum Interdisciplinare per la Ricerca sulle Malattie Autoimmuni

¹Microbiology Inst., ²Clin. Immunol & Renal Unit, S. Carlo B. Hospital, Milan; Italy.



BACKGROUND: ANCA detection is fundamental to the diagnosis of AAV. The request for autoantibody-testing has increased in the last years and highly performing assays/technologies, as well as testing-strategies, have been developed.

AIM: the survey is part of a project endorsed by the “European Autoimmunity Standardisation Initiative” (E.A.S.I., www.easi-network.com), with the financial support of Phadia-ThermoFisher Italia, aimed to report and compare the daily practice in European countries.

METHODS:

- ❖ 59 multi-choice questionnaires were sent to 300 laboratories through the Country, introduced by an e-mail with the link to a web-site;
- ❖ out of the 145 returned polls (48.3%), 112 were validated and statistically evaluated by using the online software Survey Monkey (www.surveymonkey.com).

Preliminary results: the percentage of responders is probably underestimated due to the consolidation processes of the autoimmunity testing implemented in recent years. This makes it quite difficult to get updated lists of the Centres actually performing these investigations. Most of the laboratories involved in the survey belong to public, certified structures of large size (58% >1.000.000 tests/yr), while the percentage focused on the autoimmunity and ANCA-testing is detailed in Fig.1 and Tab.1.

Autoimmunity is only rarely independent or part of the Clinical Immunology (9%), often it is a subspecialty of the Clinical Pathology (53%), Biochemistry (24%) or Microbiology (7%). Medical doctors (46%) as well as biologists (54%) are in charge for the ANCA diagnostics.



Tab. 1: ANCA test/week

ANCA request/week	Response Percent
< 5	13.8%
5 - 15	39.4%
15 - 30	22.0%
30 - 50	15.6%
> 50	9.2%

ANCA-testing: ANCA are detected by IFT on Et-OH-fixed granulocytes/leukocytes in ≈ 75% of the centers, generally followed by the MPO and PR3-specific immunoassays. Specificities other than MPO and PR3-ANCA are rarely looked for (5%), mainly for research purposes.

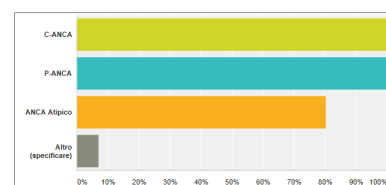


Fig. 2: reported ANCA-IFT patterns

Fig. 1: autoimmunity tests/year

IFT-ANCA is performed using commercial kits, at a screening dilution of 1:10 (38%) or 1:20 (60%), followed by titration of the +ve samples in 36%. The slides are evaluated by two independent observers in half of the cases. Although cellular substrates were mainly neutrophils, in a number of labs mosaics consisting of granulocytes and HEp-2 cells are used. Titer/intensity and staining pattern are reported in 75% & 95%, respectively. **IFT on HCHO-fixed cells** is performed never, ever or in selected cases in 24%, 35%, 31%, respectively.

MPO/PR3 specific test are always performed with commercial kits, usually by using the suggested cut-off (85%) and results are mostly quantitatively reported (80%). The different MPO/PR3 immunoassays are listed in Fig. 3. Only a very few specialists answered the question about the definition of “significant” ANCA test increase.

The relatively high proportion of IFT on formalin-fixed cells is mostly due to the employment of commercially available substrates with Et-OH & HCHO-fixed cells in the same well.

The definition of **atypical pattern** was especially controversial, identifying all those granulocyte-specific pictures other than the “classic” C-ANCA/P-ANCA (29%), mixed patterns (9%), as well as P-ANCA without confirmation on HCHO-fixed cells or MPO/PR3 immunoassays (27%). 70% of the responders uses a strategy **to avoid interfering factors**, such as ANA testing on HEp-2/2000 cells, HCHO-IFT, or other.

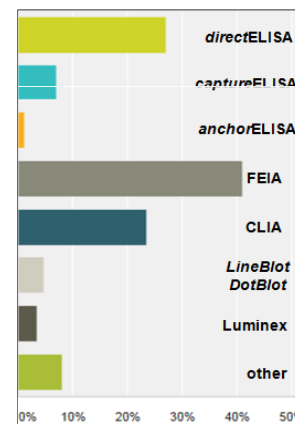


Fig. 3

Test performed on ANCA request (new pts)	Response Percent
IFT + PR3 + MPO	37.3%
IFT, confirmation by PR3 or MPO	6.0%
IFT, confirmation by PR3 and MPO	28.9%
MPO + PR3 only	16.9%
IFT only	1.2%
PR3 + MPO, confirmation by IFT	
other	9.6%

Tab. 2

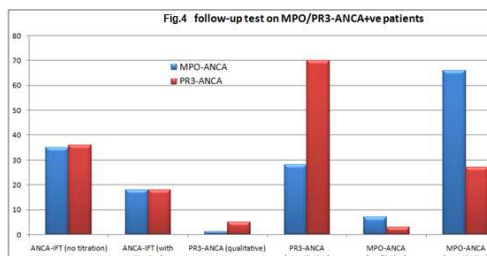


Fig.4 follow-up test on MPO/PR3-ANCA+ve patients

ANCA testing strategy: the algorithms applied for new pts or during the f-up are listed in Tab. 2 & Fig. 4.

Samples are reported as ANCA positive on different basis (Fig. 5).

There was no agreement on how to report P-ANCA/PR3+ve and C-ANCA/MPO+ve samples.

Rules defining the shortest time interval between serial ANCA tests are generally not applied. Clinical information were considered extremely useful, but rarely available. Urgent ANCA test is rarely coded, however results are supplied within 24 hours all days (7%), Monday to Friday (26%) or in critical clinical settings (severe renal and/or lung involvement, 11%). Unexpectedly, an alternative algorithm, consisting on screening by highly sensitive MPO & PR3-ANCA followed by IFT for confirmation of the +ve, is rarely applied. When appropriate, 90% of the laboratory specialists add comments to help clinical interpretation. Automation is widely used in Italian labs; according to recently collected data, 90% of the IFT & 99% of the immunometric assays are performed by slide-processors and/or fully automated systems.

Conclusions: most of the Italian labs perform ANCA test according to the International recommendations, and laboratory specialists show a high awareness of the importance of ANCA testing and reporting in the diagnosis of AAV.

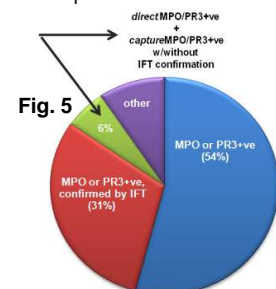


Fig. 5