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

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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 subspeciality 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.

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 Tab. 2 & Fig. 4.

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.