Letter to the Editor

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Added value of indirect immunofluorescence intensity of automated antinuclear antibody testing in a secondary hospital setting

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To the Editor,

Indirect immunofluorescence (IIF) on human epithelial (HEp-2) cells still remains the gold standard method for antinuclear antibody (ANA) detection [1]. As IIF is time consuming, requires considerable expertise, and suffers from intra- and inter-laboratory variance, automated systems for ANA analysis are being introduced in laboratories [2, 3]. Quantitative data generated by automated image acquisition facilitates standardized reading of IIF.

ANAs can be found in a variety of diseases and their occurrence does not necessarily indicate the presence of disease at all. In addition to autoimmune rheumatic diseases, ANAs are found in other clinical settings such as organ specific auto-immune disease, infectious disease and lymphoproliferative disorders [4]. In a clinical setting where the pre-test probability of ANA associated rheumatic disease (AARD) is generally low, as in primary care, the added value of a positive ANA test is lower as compared to secondary and tertiary care situations where pre-test probabilities of AARD are often higher [5]. In a recent study, Schouwers et al. demonstrated the clinical added value of likelihood ratios (LR) based on fluorescence intensity (FI) test result intervals [6]. In their study, samples from a well-defined AARD group at diagnosis were analyzed. However, in a routine secondary setting like ours, up to 40% (personal data) of the samples received for ANA detection in AARD patients are follow-up samples. In addition, ANA IIF investigation is routinely requested for patients under treatment with biologicals. In this population, ANAs are often found and might be associated with the development of clinical overt AARD [7, 8]. It is obvious that exclusion of this specific patient population will have an influence on calculated LRs. Moreover, in their diseased control group, Schouwers et al. excluded patients with rheumatoid arthritis. It is well known that ANAs can be present in sera of patients with rheumatoid arthritis. These ANAs, however, are not well characterized and do not have any diagnostic value for rheumatoid arthritis. Finally, as ANA IIF is performed in the context of multiple diseases varying from AARD to auto-immune liver diseases (e.g. auto-immune hepatitis, primary biliary cirrhosis), it is of interest to know the value of ANA IIF for the diagnosis of AARD when these patients groups are included.

Recently, we introduced the NOVA View® (Inova Diagnostics, Inc., San Diego, CA, USA) digital IIF microscope in our lab. To assess whether the automated quantitative reading of FI is of clinical added value in our setting, we performed a diagnostic evaluation. ANAs were detected using NOVA Lite® HEp-2 ANA kit (Inova Diagnostics) in a well-established group of 102 patients with AARD at the time of diagnosis or follow-up [female/male: 95/7; mean age (range): 52.8 years (19–89); diagnosis: n=27, follow-up: n=75], 169 rheumatic disease control patients (RDCG) [i.e. patients with "other" rheumatic disease (e.g. rheumatic arthritis, spondyloarthritis, etc.)] [female/male: 114/55; mean age (range): 59.2 years (17–89)] and 224 diseased controls (DCG) [i.e. patients from other clinical disciplines, e.g. gastroenterology (including auto-immune

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hepatitis, primary biliary cirrhosis), nephrology, etc.; for whom an ANA analysis is requested, but with no rheumatic disease of any kind] [female/male: 129/*95*; mean age (range): 53.8 years (4–93)]. The group of AARD consisted of patients with systemic lupus erythematosus (n=31), cutaneous lupus erythematosus (n=4), Sjögren syndrome (n=32), systemic sclerosis (n=14), mixed connective tissue disease (n=4), polymyositis (n=1) and undifferentiated AARD (n=6). Diagnosis of specific AARD was based on international consensus classification criteria [9–13]. The study was approved by the local Ethics Committee. Sample dilution and slide processing was carried out on QUANTA-Lyser 2 (Inova diagnostics). Goat anti-human IgG specific fluorescein-labeled (FITC) conjugate containing 4′-6-diamidino-2-phenylindol (DAPI) was used. Slides were read using NOVA View® (Inova Diagnostics) digital IIF microscope (software version 2.0.3.2.). Statistical analysis was performed using MedCalc software (Medcalc Software BVBA, Ostend, Belgium; version 11.6.1).

ANAs were determined in 102 samples from AARD patients and 393 control samples (RDCG and DCG). Using a screening dilution of 1/80 and the FI cut-off proposed by the manufacturer [i.e. negative result: FI<49 Light Intensity Units (LIU)], 91.2% of the AARD patients were ANA positive compared to 46.8% of the controls. In the patients, the FI ranged from 16 to 5349 LIUs (median: 652), whereas in the controls, the FI ranged from 1 to 3278 LIUs (median 39). Figure 1 summarizes the distribution of the FI at the 1:80 screening dilution across the different patients groups. Kruskal-Wallis analysis with post-hoc pair-wise comparison showed significant differences in FI between the different groups (p<0.01), as well as between the AARD group and the patient control group taken RDCG and DCG together.

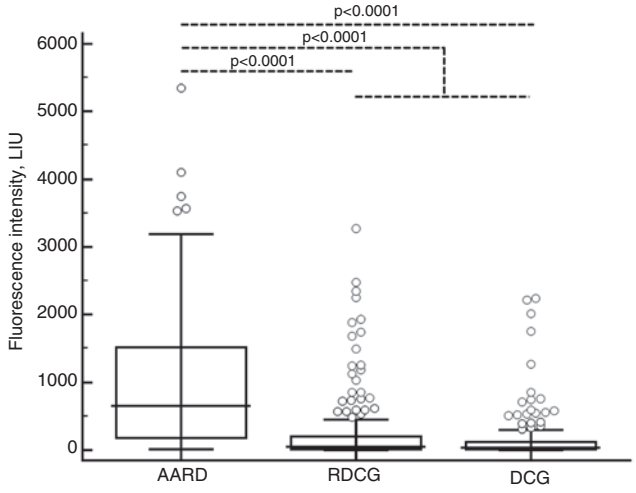

Figure 1: Distribution of the fluorescence intensities for the different patient groups.
Box and Whisker plots are presented. AARD, ANA associated rheumatic disease; RDCG, rheumatologic disease control group; DCG, disease control group.

LRs (i.e. the likelihood (%) for patients with AARD divided by the likelihood (%) for controls) were calculated for different ANA IIF FI test result intervals. RDCG and DCG patients were combined for this analysis. The results are presented in Figure 2. The probability of AARD increased with FI and LRs of 0.17, 0.61, 1.0, 2.9, and 8.3 for FIs of <49, 49–150, 151–300, 301–1000 and >1000 were obtained, respectively. 40.2% of the patients with AARD had a FI>1000. In total, 53.2% of the controls had a FI<49 (LR 0.17) and another 22.4% between 49 and 150 (LR 0.61).

By omitting positive ANA IIF results in patients with auto-immune liver diseases (n=7) or treated with biologicals (n=22), LRs for the different result intervals increased up to 0.15, 0.57, 1.0, 4.8 and 16, respectively. In the control group, 56.3% of the controls had a FI<49 (LR 0.15) and another 23.7% between 49 and 150 (LR 0.57).

Our study shows that reporting FIs is of clinical value in a routine secondary hospital setting. The added value of the FI lies in the standardized quantification of FI that can be used to generate information regarding likelihood for disease.

By including patients with "other" rheumatologic diseases in the diseased control group, and known follow-up patients under immunosuppressive medication, in the AARD group lower LRs were obtained compared to the study by Schouwers et al. [6]. Our LRs seem to be pattern dependent as a high proportion (51.0%) of the positive DCG patients show a speckled pattern with a FI between 301 and 1000. On the other hand, patterns that are less prevalent in the DCG and RDCG, such as the homogenous (35.3%) and centromere (4.9%) patterns, tend to show higher LR. As antibodies that react with a limited part of the cell, such as centromeres, have lower FIs than samples with antibodies that react with larger parts of the cell, higher LR are already obtained with lower FI intervals. For the NOVA View®, it is important to remark that the FI is only a measure for nuclear and not for cytoplasmatic IIF staining. This results in an underestimation of the LR for AARDs that are associated with cytoplasmatic patterns (e.g. Jo-1 and polymyositis).

Nevertheless, calculation of LRs for different FI test result intervals aids in the interpretation of automated ANA analysis and allows value-added reporting in a routine secondary hospital setting. Clinical laboratories should consider to report FI as well as the test result specific LR, as LR are a more efficient and applicable way to

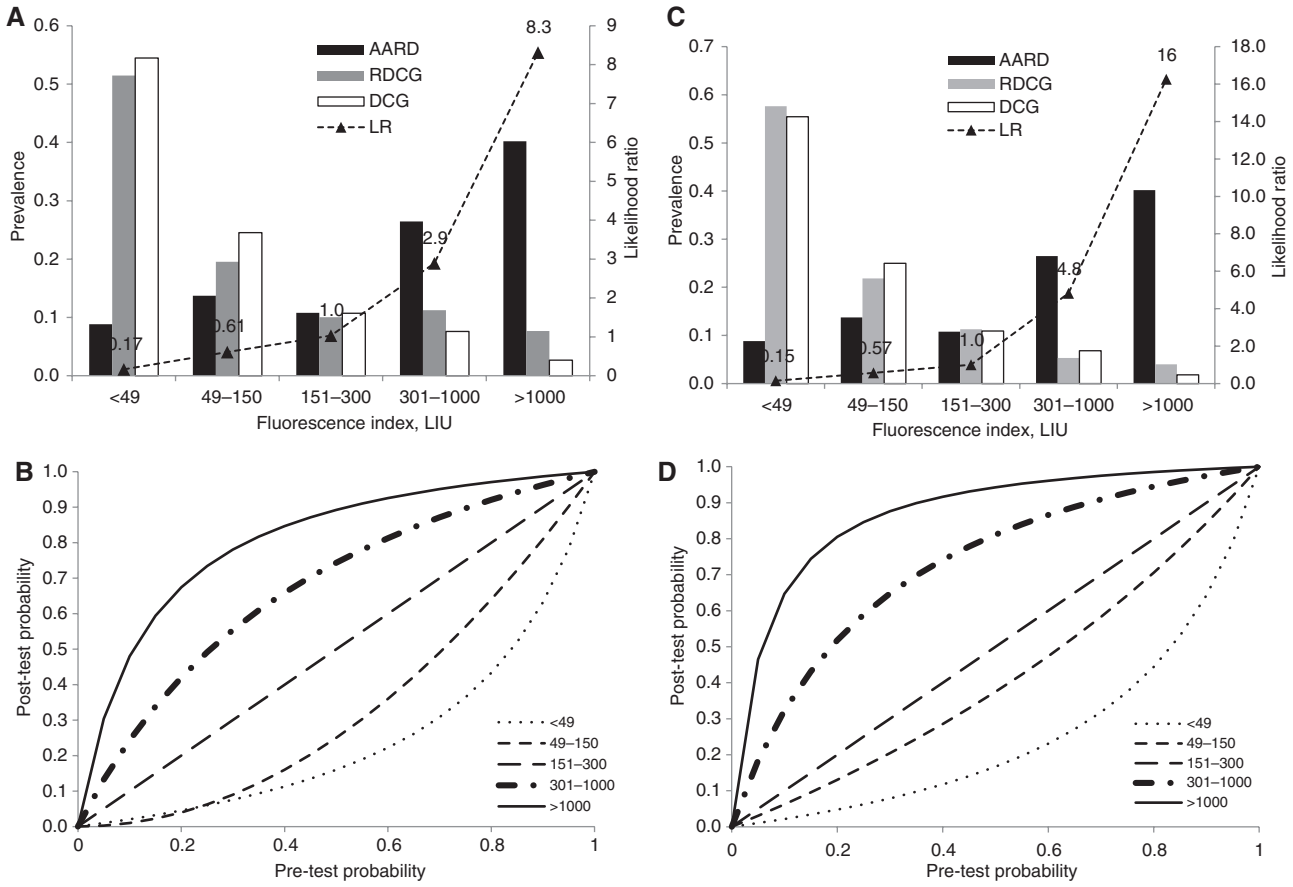

Figure 2: Likelihoods and LRs of fluorescence index test results for ANA associated rheumatic disease (AARD) given the disease status. (A and C) Prevalence (likelihoods) (left y-axis) of fluorescence index test result intervals (<49, 49–150, 151–300, 301–1000, >1000) given the disease status [AARD (black bars), RDCG (gray bars), other (open bars)] with (A) and without (C) inclusion of patients with auto-immune liver disease or treatment with biologicals. The triangles represent the LRs (second y-axis). (B and D) Post-test probability for AARD as a function of pre-test probability and of fluorescence index test result intervals (<49, 49–150, 151–300, 301–1000, >1000) with (B) and without (D) inclusion of patients with auto-immune liver disease or treatment with biologicals.

communicate diagnostic accuracy information to clinicians than sensitivity and specificity [14]. The probability that patients with a high ANA titer have AARD increases significantly if auto-immune liver disease and treatment with biologicals are excluded as a cause of a positive ANA IIF result. FI values <150 should not be considered as ANA positive (they rather exclude AARD).

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