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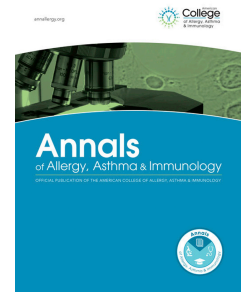
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Letter to the Editor

Standardization of the nasal cytology in the work-up of allergic rhinitis.

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Allergic rhinitis (AR) is a common disorder characterized by typical nasal symptoms and IgE-mediated inflammation (1). Nasal type 2 inflammation is initiated by the causal allergen exposure (2). Mediators and cytokines are deeply involved in the cascade of inflammatory phenomena. A typical pattern of inflammatory cells infiltrates the nasal mucosa, including eosinophils, neutrophils, and mast cells. However, the paradigmatic characteristic of allergic inflammation is its reversibility as it depends on the allergen exposure. Actually, pathophysiology of allergic inflammation may be investigated by two main lines of research: the natural allergen exposure (typical model is pollen allergy) and the experimental allergen exposure (typical model is allergen challenge). Consistently, a clear-cut association between allergen exposure and nasal inflammation has been documented exhaustively using these criteria in the past (3-6).

In clinical practice, the AR diagnosis is based on the consistency between clinical history and the assessment of allergen-specific IgE (7). Nasal allergic inflammation may be documented by nasal cytology as previously proposed (8), even though the main criticism is the lack of standardized criteria. In this regard, a series of statistical criteria should be required to standardize a test (9).

On the basis of this background, we aimed to standardize the nasal cytology testing in a group of 52 symptomatic and untreated allergic patients (26 males, mean age 32 years) evaluated in wall pellitory pollen season and in 52 healthy subjects (23 males, mean age 27 years).

Nasal cytology included: sampling, processing and microscope reading. Sampling required the collection of cells from the surface of nasal mucosa by nasal scraping performed with a plastic device (Nasal Scraping[®], EP Medica, Italy). Samples were collected from the middle portion of the inferior turbinate under anterior rhinoscopy in both nostrils. The sample obtained was immediately smeared on a glass slide and air-dried. The sample was stained using the common May-Grünwald-Giemsa for 30 minutes. The stained sample was read at light microscopy at high magnification (1000x). ~~The number of inflammatory cells was considered as a total number of any cell type per microscopic field (as mean of 10 fields).~~ Inflammatory cells i.e. eosinophils, neutrophils, and mast cells were counted and reported as the mean number in 10 "high power field" (HPF). Slides were

27 examined by two investigators blinded to the identity of each sample. The disagreement between
28 observers was good as <10%. The reproducibility was previously assessed in other groups of
29 patients (3-6).

30 All data, but age, were reported as medians with inter-quartile range; age as mean with standard
31 deviation (SD). Any statistically significant difference in the mean values or in the median values of
32 each continuous variable between patients and controls was evaluated with the Unpaired t test or
33 Mann-U Whitney test, respectively. The best cut-off-point of each inflammatory cell count able to
34 discriminate between patients and controls was estimated on the basis of the receiver operator
35 characteristic (ROC) curve analysis. Statistical significance was set at $p < 0.05$, and the analyses
36 were performed using GraphPad Prism software.

37 The perception of nasal symptoms was evaluated by a visual analogue scale (VAS): AR patients
38 had higher VAS score than controls: 7.5 (7.0-8.0) and 0 (0-1.0) respectively ($p < 0.0001$).

39 The number of each inflammatory cells (i.e. eosinophils, neutrophils, and mast cells) was
40 significantly higher ($p < 0.0001$, each comparison) in AR patients than in controls (Figure 1A).

41 Nasal eosinophils were identified in all AR patients and in only 3 out of 52 controls (only one
42 eosinophil in each control). The median values of eosinophil count were higher ~~between~~ in AR
43 patients ~~and~~ than in controls being 15 (11-21) and 0 (0-0) eosinophils/~~10~~-HPF, respectively
44 ($p < 0.0001$), (Figure 1A). On the basis of this result, we evaluated the best cut-off of nasal
45 eosinophil count able to discriminate between AR patients and controls. On the basis of the ROC
46 curve analysis, we identified 2 nasal eosinophils as the optimal cut-off value; the performance was
47 excellent: the AUC corresponded to a discrimination of 1.00 [95% Confidence interval (95%CI):
48 1.00 – 1.00] (Figure 1B). Also, sensitivity, specificity, positive likelihood ratio (PPV) and negative
49 likelihood ratio (NPV) were excellent. Indeed, the probability of a subject having AR when the
50 number of nasal eosinophils was at least 2 (PPV) and the probability of a subject not having AR
51 when the number of nasal eosinophils was lower than 2 (NPV) were 100%; in addition, the negative
52 LR, i.e. the ratio between $1 - \text{sensitivity}$ divided by specificity, was 0 confirming that the lower the

53 value, the more informative the test. Of note that, at least 2 nasal eosinophils were found in all AR
54 patients and in none of the controls.

55 Similar results were found for nasal neutrophils: a statistically significant difference in the median
56 values of nasal neutrophil count was found between AR patients and controls being 12.0 (9.0-18.5)
57 and 1 (0-2) neutrophils in 10-HPF, respectively (Figure 1A) ($p < 0.0001$). The best cut-off of nasal
58 neutrophils number able to discriminate between AR patients and controls was 5; again, the
59 performance was excellent: the AUC corresponded to a discrimination of 0.991 (95%CI: 0.949-
60 0.998), (Figure 1B). Also, sensitivity, specificity, positive LR and negative LR were very good: the
61 probability of a subject having RA when the number of nasal neutrophils was at least 5 (PPV) and
62 the probability of a subject not having AR when the number of nasal neutrophils was lower than 5
63 (NPV) were 96%; in addition, the positive LR was 25, indicating that given a positive test result,
64 the probability of having the disease is high, whereas the negative LR was 0.04, indicating that
65 given a negative test result, the probability of having the disease is low.

66 Mast cells were only found in nasal specimens from patients with AR and none of the controls had
67 this cell type (Figure 1A). The best cut-off of nasal mast cells number able to discriminate between
68 AR patients and controls was 1; the performance was very good: the AUC corresponded to a
69 discrimination of 0.875 (95%CI: 0.796-0.932), (Figure 1B). Also, sensitivity, specificity, positive
70 LR and negative LR were very good.

71 Taking into account the fact that the nasal cell counts are predictive of AR, we evaluated the
72 performance of a “combo-test”: we considered a positive “combo-test” when nasal eosinophils ≥ 2
73 and neutrophils ≥ 5 and mast cells ≥ 1 whereas a negative test in the remaining cases. The
74 performance of the test was as follows: sensitivity 0.71, specificity 1.00, PPV 1.00, NPV 0.78,
75 negative LR 0.29. Comparing the performance of the “combo-test” and that of the nasal eosinophil
76 counts, we found that this latter seems to be better suggesting that nasal eosinophil counts could be
77 sufficient to discriminate between AR patients and controls.

78 This case practice did not incorporate patients with non-allergic rhinitis as the cytology profile may
79 be different as previously reported (10).

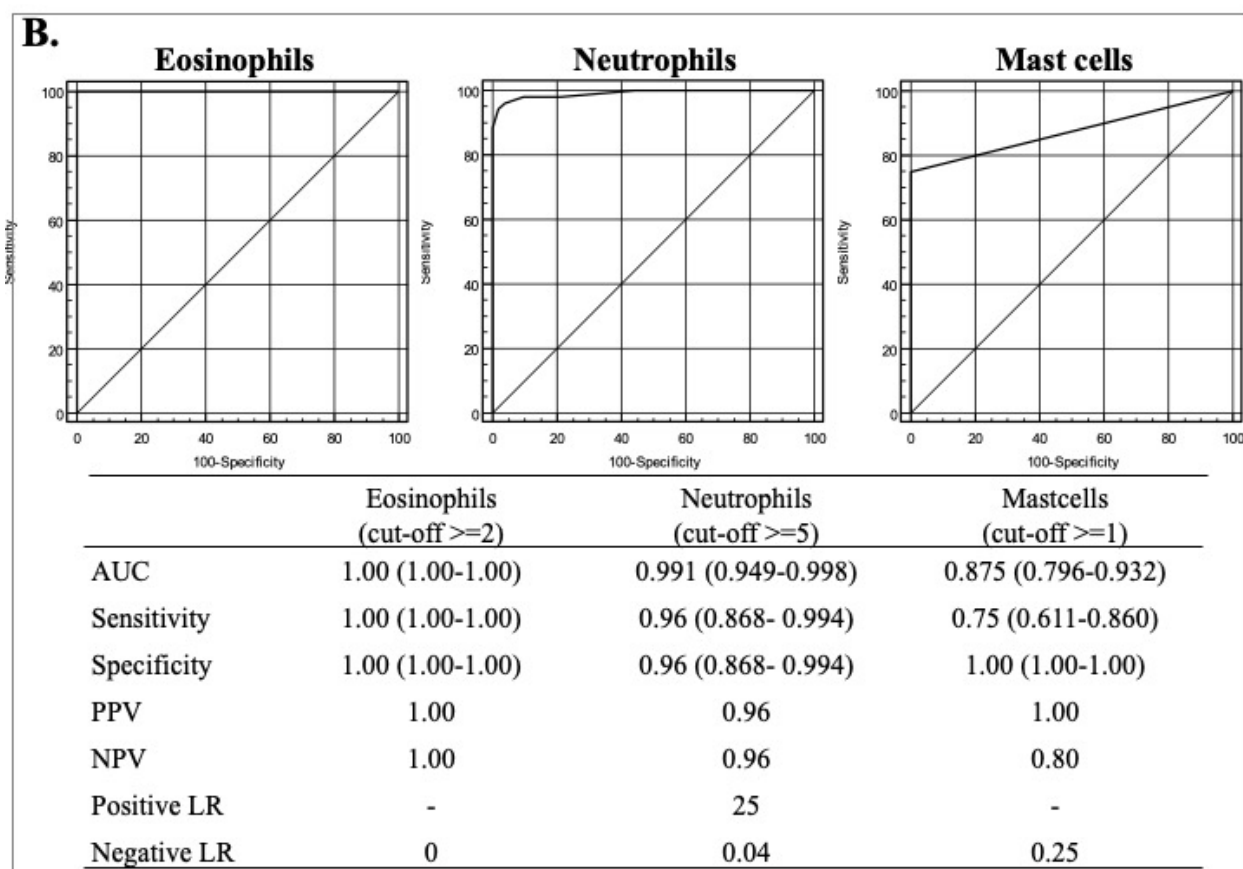
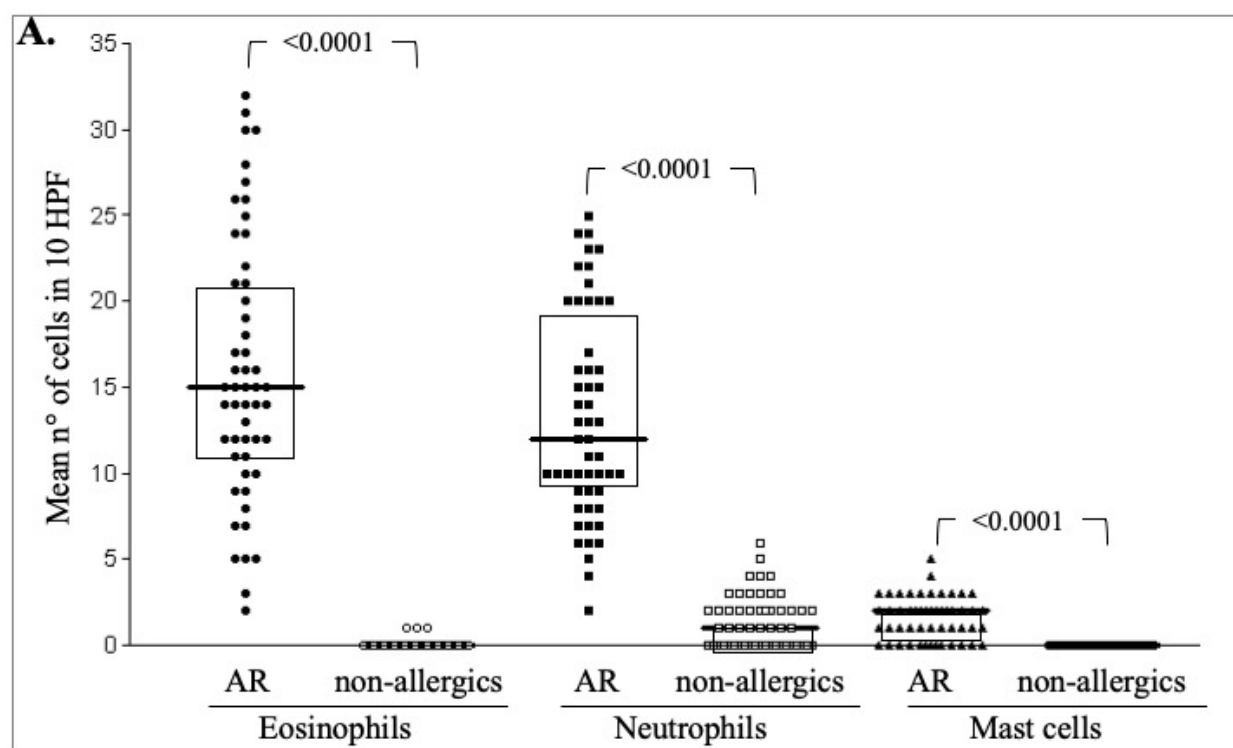
80 However, it has to be noted that the work-up of AR usually includes history and skin prick test. The
81 current study demonstrates ~~therefore~~ that nasal cytology is a standardized technique that may be
82 additionally performed in the work-up of AR patients. In addition, this test is necessary to diagnose
83 non-allergic rhinitis.

84 In conclusion, the present study showed that a series of requisites has been satisfied and these
85 proofs could be retained convincing for the use of nasal cytology in clinical practice.

86

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Figures Legend

Figure 1A – Number of eosinophils, neutrophils and mast cells in 10 high power fields (HPF).

Boxes represent upper and lower quartiles whereas the horizontal bar represents the median value

Figure 1B - Receiver operator characteristic (ROC) curve analysis to estimate the best cut off-points for eosinophils, neutrophils and mast cells number able to discriminate between patients with or without RA.