The Clinical Stage of Allergic Rhinitis is Correlated to Inflammation as Detected by Nasal Cytology

Matteo Gelardi[1], Cristoforo Incorvaia[*,2], Maria Luisa Fiorella[1], Paolo Petrone[1], Nicola Quaranta[1], Cosimo Russo[1], Paola Puccinelli[3], Ilaria Dell'Albani[3], Gian Galeazzo Riario-Sforza[2], Eleonora Cattaneo[3], Gianni Passalacqua[4], Franco Frati[3] and the Italian Academy of Nasal Cytology[§]

[1]*University Department of Otolaryngology, Bari, Italy*

[2]*Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy*

[3]*Medical and Scientific Department, Stallergenes, Milan, Italy*

[4]*Allergy and Respiratory Diseases, University Of Genoa, Genoa, Italy*

Abstract: Allergic rhinitis (AR) is the most common allergic disease. The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines classify AR according to its duration and severity and suggest recommended treatments, but there is evidence that these guidelines are insufficiently followed. Considering the validity of histopathological data, physicians are more likely to be persuaded by such information on AR. Thus, we attempted to define the severity of AR by nasal cytology on the basis of the ARIA classification. We examined 64 patients with AR caused by sensitization to grass pollen. We clinically defined AR according to the ARIA classification and performed nasal cytology by Rhino-probe sampling, staining and reading by optical microscopic observation. Clinically, 22 (34.4%), 21 (32.8%), 10 (15.6%), and 11 (17.2%) patients had mild intermittent, moderate-to-severe intermittent, mild persistent, and moderate-to-severe persistent AR, respectively. Nasal cytology detected neutrophils in 49 patients, eosinophils in 41 patients, mast cells in 21 patients, and lymphocytes or plasma cells in 28 patients. The patients with moderate-to-severe AR had significantly more mast cells and lymphocytes/ plasma cells than those with mild AR.

Our findings demonstrate that the ARIA classification of AR severity is associated with different cell counts in nasal cytology; especially, moderate-to-severe AR shows significantly increased counts of mast cells and lymphocyte or plasma cells. The ease of performing nasal cytology ensures is feasibility as an office AR diagnostic procedure for primary care physicians, able to indicate when anti-inflammatory treatments, such as intranasal corticosteroids and subcutaneous or sublingual allergen immunotherapy, are needed.

Keywords: Allergic rhinitis, clinical severity, nasal cytology, eosinophils, mast cells, lymphocytes, plasma cells.

INTRODUCTION

Allergic rhinitis (AR) is the most common hypersensitivity disease, with a still rising worldwide prevalence [1]. In the US it is estimated that up to 60 million people suffer from AR [2], and this represents a significant burden from both the societal and individual perspective. As far as the physicians are concerned, most patients with AR are managed by primary care physicians [3], who refer only a small fraction of patients to the allergists. The factor mainly influencing such decision is the clinical form and severity of AR. In 2001 the Allergic Rhinitis and its Impact on Asthma (ARIA) document introduced a new classification of AR, based on its duration and severity [4]. This classification includes a measurement of the frequency and duration of the symptoms. Intermittent AR (IAR) is

defined by symptoms occurring for <4 days/week or <4 consecutive weeks. Persistent AR (PER) is defined by symptoms occurring for >4 days/week and >4 consecutive weeks. Additionally, a severity scale of mild to moderate-severe (based on the AR impact on both daily activities and quality of life) was included. Each clinical form has a recommended treatment, such as antihistamines, intranasal or oral corticosteroids, and subcutaneous or sublingual allergen immunotherapy [4]. However, the reported data indicate that primary care physicians are reluctant to follow the established guidelines [5].

Considering the validity of cytological data, one may argue that physicians are more likely to be persuaded by such information on AR. Thus, we addressed this study to define the severity of AR by nasal cytology on the basis of the ARIA classification.

METHODS

We examined 64 patients (35 men and 29 women, mean age 35.3 years) with AR caused by sensitization to grass pollen, and 18 normal subjects. AR was clinically defined according to the ARIA classification and sensitization to grass pollen was demonstrated by a positive skin prick test

*Address correspondence to this author at the Allergy/Pulmonary Rehabilitation, ICP Hospital, Via Bignami 1 - 20126 Milan, Italy; Tel: + 39 02 57993289; Fax: +39 0257993315;
E-mail: cristoforo.incorvaia@gmail.com

[§]Rachele Boccardo, Corso Bocciolini, Giuseppe Caruso, Andrea Ciofalo, Danilo Dall'Olio, Massimo Landi, Enrico Maffezzoni, Donatella Poletti, Lorenzo Salerni, Marilena Tomaiuolo, Leonardo Troiano.

using allergen extracts from Stallergenes (Milan, Italy). To be included, patients must be untreated, so to avoid influence of drugs on rhinocytogram. In all subjects nasal cytology was performed by anterior rhinoscopy, using a nasal speculum and good lighting. The collection technique consisted of scraping from the middle portion of the inferior turbinate, using a Rhino-probe (Arlington Scientific, Springville, Ut, USA). Then, the obtained material was placed on a glass slide, fixed by air drying and stained by the May-Grunwald Giemsa method, that allows to detect all the cellular components of the nasal mucosa. The slide was observed by a Nikon E600 light microscopy (Nikon Canada, Toronto, Canada) equipped with a digital camera Nikon Coolpix 3:34" (Nikon Canada) for the acquisition of microscopic images. For the rhinocytogram analysis, 50 microscopic fields were read at a magnification of 1,000x to assess the presence of cells. Cell counts were carried out by a semi-quantitative grading as proposed by Meltzer and Jalowayski [6], but using the percent of each cell type in place of the original 1+, 2+, 3+ and 4+ grading.

The association between the severity of rhinitis and the kind of cells detected in the nasal mucosa was analyzed by the Fisher exact test, a p value <0.05 being considered significant.

RESULTS

Table **1** shows the distribution of AR severity according to ARIA classification: 67.2% of patients had IAR (34.4% mild and 32.8% moderate-severe) and 32.8% had PER (15.6% mild and 17.7% moderate-severe). Twenty-three (35.9%) of patients had AR from at least 5 years. The most common symptoms were sneezing, runny nose and nasal blockage. The cells most commonly detected by nasal

cytology were neutrophils (76.6% of patients) and eosinophils (64.1% of patients). The most frequent comorbidities were sinusitis (40% of patients) and asthma (26% of patients).

Table **2** shows the cells detected by nasal cytology according to ARIA classification of AR: neutrophils were present in 49 patients, eosinophils in 41 patients, mast cells in 21 patients, and lymphocytes or plasma cells in 28 patients. The patients with moderate-severe AR had significantly more mast cells (p=0.014) and lymphocytes or plasma cells (p= 0.026) than those with mild AR. A negligible number of such cells was found in slides from normal subjects. Fig. (**1**) shows the typical nasal cytology results in patients with mild and moderate-to-severe AR as well as in normal subjects.

DISCUSSION

AR is one of the most common diseases both in children and adults [1, 2], and thus represent a major challenge in primary care. The ARIA guidelines were introduced in 2001 [4], and recently updated [7], to provide evidence-based recommendations for the diagnosis and management of AR. However, it has been reported that the application of ARIA guidelines is far from optimal. For example, a survey in Belgium showed that general practitioners overtreated 49% of patients with mild and/or intermittent AR, while they undertreated 30% of those with moderate/severe persistent AR [8]. In a recent paper the ARIA board stated that the implementation strategies need to be improved, and possible interventions to achieve this goal were suggested, such as encouraging physicians to understand how and why the recommendations were made, and informing also patients

Table 1. Distribution of Clinical Forms of AR According to Disease Duration

ARIA level	< 2 Years	2-5 Years	5-10 Years	> 10 Years	Total
Intermittent					
Mild	66.7% (6/9)	50% (8/16)	21.7% (5/23)	18.8% (3/16)	34.4% (22/64)
Moderate-severe	33.3% (3/9)	31.3% (5/16)	30.4% (7/23)	37.5% (6/16)	32.8% (21/64)
Persistent					
Mild	0.0% (0/9)	12.5% (2/16)	26.1% (6/23)	12.5% (2/16)	15.6% (10/64)
Moderate-severe	0.0% (0/9)	6.3% (1/16)	21.7% (5/23)	31.3% (5/16)	17.2% (11/64)
Total	12.5% (8/64)	25% (16/64)	37.5% (24/64)	25% (16/64)	100% (64/64)

Table 2. Cells Detected by Nasal Cytology According to ARIA Classification of Rhinitis

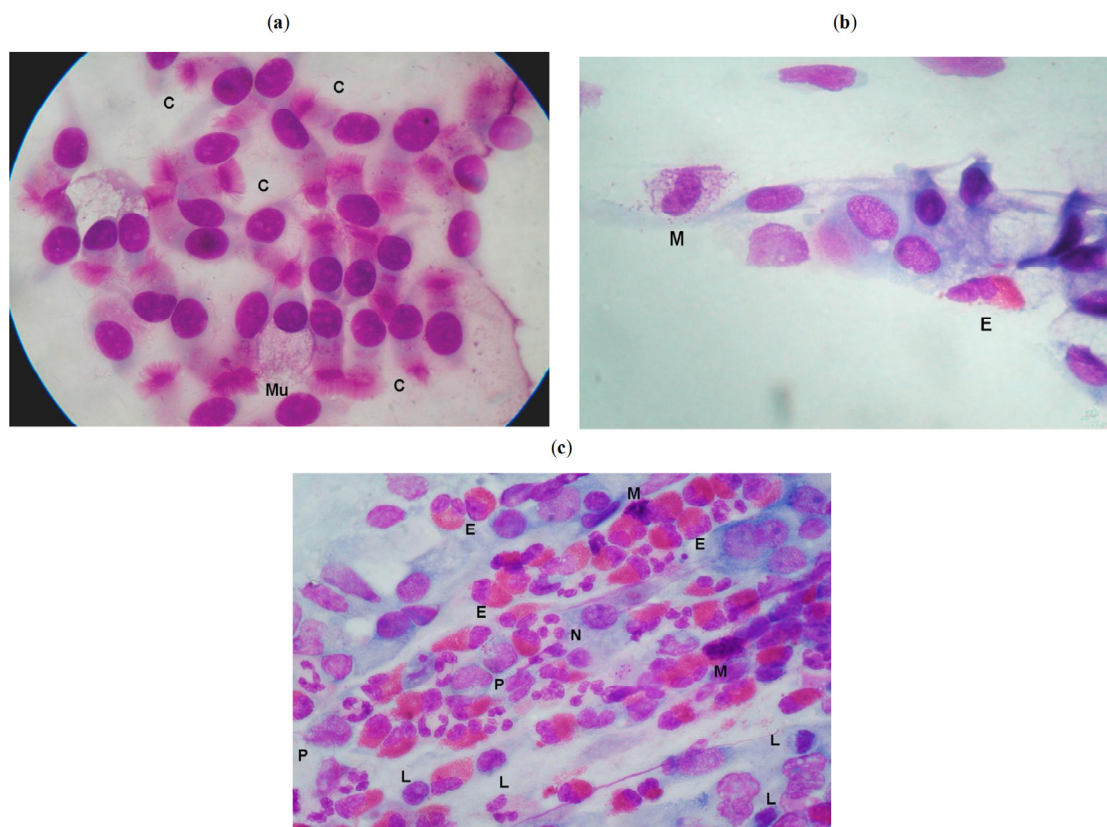| Cells	Intermittent AR		Persistent AR		Total
	Mild	**Moderate-Severe**	**Mild**	**Moderate-Severe**	
Neutrophils	52.4% (11/21)	81.8% (18/22)	100.0% (9/9)	91.7% (11/12)	76.6% (49/64)
Eosinophils	47.6% (10/21)	63.6% (14/22)	77.8% (7/9)	83.3% (10/12)	64.1% (41/64)
Mast cells	4.8% (1/21)	40.9% (9/22)	22.2% (2/9)	75.0% (9/12)	32.8% (21/64)
Limphocytes/Plasm cells	4.8% (1/21)	63.6% (14/22)	44.4% (4/9)	75.0% (9/12)	43.8% (28/64)

Fig. (1). Typical rhinocytograms of normal nasal mucosa (**a**), mild AR (**b**), and moderate-to-severe AR (**c**). E = eosinophils, M = mast cells, L= lymphocytes/plasma cells, N = neutrophils.
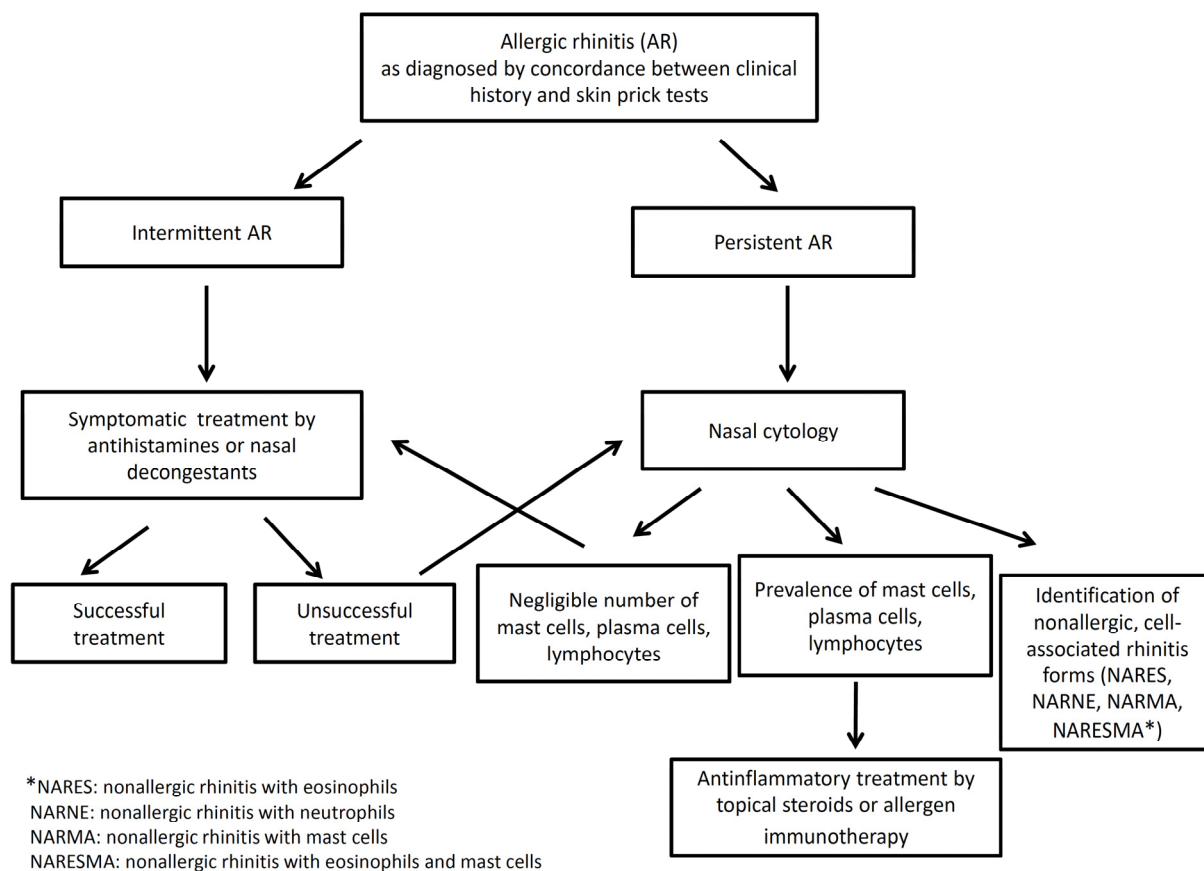

Fig. (2). Decision tree to prescribe the adequate treatment for allergic rhinitis based on data from nasal cytology.

about these guidelines to raise their awareness of optimal care and increase control of AR [9]. Considering AR in its pathophysiological aspects, it is long known that the exposure to the specific allergen by the model of a nasal challenge elicits infiltration of the nasal mucosa by inflammatory cells such as eosinophils and basophils [10]. It is also known that subjects with AR have a minimal persistent inflammation even in periods when they have no clinical symptoms [11]. A recent study demonstrated by nasal biopsies that patients with intermittent or persistent AR have different inflammatory profiles, with significantly increased mast cells and eosinophils in the nasal mucosa of the latter [12]. Of course, nasal biopsies are hardly feasible as a routine method to assess the inflammatory cells in the nose, while nasal cytology is simple to perform technique and provides clear data about the inflammatory cells involved in nasal allergy [13]. However, despite such characteristics, the use of nasal cytology as a diagnostic technique is rare. We feel that nasal cytology could help the physicians, including general practitioners and allergists, in assessing the clinical stage of AR in single patients. Therefore, we evaluated by nasal cytology a group of AR patients and investigated the possible relationship between the kind of cells detected by nasal cytology and the ARIA classification of AR.   Our findings demonstrate that the ARIA classification of AR severity is associated with different cell counts in nasal cytology; especially, moderate-to-severe AR shows significantly increased counts of mast cells and lymphocyte or plasma cells. The ease of performing nasal cytology ensures is feasibility as an office AR diagnostic procedure for allergists but also for primary care physicians. Treating the underlying inflammation in AR requires agents that have anti-inflammatory effects and proven clinical efficacy [11]. Thus, the data provided by nasal cytology should suggest the appropriate anti-inflammatory treatment, that include as options a topical treatment with corticosteroids [14, 15], or allergen specific immunotherapy in its forms of sublingual or subcutaneous administration [16-18], as suggested in the ARIA guidelines [7]. Fig. (**2**) shows a decision tree based on a certain diagnosis of AR and on data from nasal cytology. To make consistent such suggestion, this kind of findings needs to be confirmed in other forms of AR, and especially in mite-induced rhinitis. In fact, the continuous nasal inflammation occurring in mite allergy could provide more insight and could validate the clinical utility of nasal cytology particularly when treatment is concerned. Ultimately, whether the choice of the most adequate treatment according to cytological findings may improve the adherence to ARIA guidelines warrants to be evaluated in specific studies.

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