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Letter to the Editor

Feasibility of flow cytometry in the rhinologist's clinic

Attuabilità della citometria a flusso nella pratica rinologica

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Dear Editor,

The assessment of the inflammatory pattern in patients with rhinitis is conventionally performed by nasal cytology [1]. However, some studies have investigated nasal inflammation by flow cytometry, even though most aimed at evaluating issues far from conventional rhinology [2-8].

Flow cytometry allows to define a series of additional aspects in comparison with traditional nasal cytology, including cellular volume and density, the antigenic and genetic cellular pattern, and the functional state, such as activation. Moreover, flow cytometry is automated and well standardised, so it may be considered as a precise and accurate method to analyse the cellular pattern in nasal inflammation. On the contrary, it is usually considered expensive and laborious, as requires adequate machinery and well-trained staff.

The current experience was determined to evaluate the feasibility of performing flow cytometry in the rhinologist's clinic. For this purpose, we chose a real-world model such as a clinical setting: 41 consecutive patients (23 males, 18 females, mean age 38.7 years) were visited at a rhinology clinic in two consecutive days and enrolled. All had nasal complaints that need thorough otorhinolaryngological evaluation. Patients were visited, and nasal scraping, endoscopy and lavage were carried out.

Nasal scraping for traditional cytology was performed according to validated criteria [1]. Nasal lavage was performed by slowly instilling 10 mL sterile isotonic saline into each nostril using a 10 mL syringe, while the subject reclined the head and closed the soft palate. The solution was retained for approximately 10 s in the nasal cavities without swallowing. After that, it was expulsed by forward flexing the head, lightly exhaling and rinsing the lavage liquid into a sterile plastic beaker. Patients were strictly instructed to collect only secretions from the nose in the sterile beaker, whereas secretions deriving from the mouth had to be spit into the lavatory. Immediately after collection, NL-fluid was cytocentrifuged and the cell pellet was suspended in flow cytometry buffer (PBS, 0.09% sodium azide, 1% heat inactivated FBS) and stained with antibodies to CD3, CD4, CD14, CD15, CD294, CD203c, and HLA-DR, DP, DQ for 20 minutes at room temperature in the dark. Cells were washed with flow cytometry buffer, resuspended in 0.5% paraformaldehyde, and stored at 4°C in the dark. Samples were acquired within 24 hrs on a flow cytometer (Cytomics

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Table I. Frequencies of inflammatory cells recovered from nasal lavage and visualised by cytofluorimetry.

Cell type	No. of positive cells /mL [mean (SE)]			
	All patients (41)	Allergic patients (16)	Non-allergic patients (25)	P value
CD14+ cells (monocytes)	8.56 (6.80)	19.69 (17.37)	1.44 (0.45)	0.16
CD15+ cells (neutrophils)	25.73 (6.47)	27.94 (5.88)	24.32 (10.01)	0.025
CD294+/230c- cells (eosinophils)	6.46 (1.34)	9.31 (2.19)	4.64 (1.62)	0.03
CD294/203c+ cells (basophils)	0.39 (0.16)	0.81 (0.39)	0.12 (0.07)	0.042
CD3+ cells (T lymphocytes)	8.45 (1.68)	9.25 (3.33)	7.92 (1.81)	1.00
HLA DR+, DP+, DQ+ cells	12.29 (2.20)	14.19 (4.25)	11.08 (2.43)	0.99

FC 500, Beckman Coulter Diagnostics, Brea, CA, USA). Isotype-matched single colour controls were used to control for nonspecific staining and to set analysis gates. CD3 positive cells were defined as T lymphocytes, CD3-CD4 positive cells were T helper lymphocytes, CD14 positive cells were monocytes, CD15 positive cells were neutrophils, CD294 positive and CD230c negative cells were eosinophils, CD294/203c positive cells were basophils, and HLA-DR, DP, DQ positive cells were activated cells.

Table I shows the flow cytometric data. Neutrophils were the most common inflammatory cell recovered by cytofluorimetry. Patients were subdivided in two groups considering allergy: 16 were allergic and 25 non-allergic. Allergic patients had significantly more abundant cellular infiltrate, including neutrophils, eosinophils, and mast cells than non-allergic ones ($p = 0.025$, 0.03, and 0.042 respectively). Comparing outcomes from nasal cytology and cytofluorimetry, there was good agreement, especially for mast cells detectable only by cytofluorimetry as well as for activated cells (HLA-DR, DP, DQ+).

Therefore, the current real-world experience demonstrates that nasal cytofluorimetry may be considered to be a reliable test to assess inflammatory cells infiltrating the nasal mucosa in clinical practice. In addition, cytofluorimetry allows to define the activation state of cells and more precisely detect mast cells. On the other hand, cytofluorimetry needs adequate machinery, trained staff and is more expensive. For these reasons, nasal cytofluorimetry should be reserved to investigational studies at present.

On the other hand, the current experience has some limitations, including the limited number of patients, cross-sectional design and lack of symptom severity assessment. Thus, further studies should be conducted to respond to these unmet needs.

In conclusion, nasal cytofluorimetry may represent a reliable and precise tool for investigating cellular inflammation in patients with nasal disorders.

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