# Dupilumab treatment in Type 2 CRSwNP: nasal cytology evaluation before and after six months' follow-up and correlation with clinical response to biological therapy.

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**Abstract**

**Objective.** The European Position Paper on Rhinosinusitis and Nasal Polyps 2020 (EPOS2020), the EUFOREA consensus on biologics for CRSwNP with or without asthma (2021) and the ARIA-ITALY multisciplinary consensus on nasal polyposis and biological treatments report guidelines for the use of biological treatment in chronic rhinosinusitis with nasal polyps (CRSwNP), all referring to the need of evidence of Type-2 inflammation to select patients for these therapies. During follow-ups of biological therapies, the same guidelines do not actually consider peripheral blood eosinophilic count nor local count of eosinophils in nasal mucosa as a marker of response to treatment. Nasal cytology is a useful methodology to evaluate inflammation at the level of nasal mucosa and it can assess the presence of eosinophilic flogosis in nasal mucosa. We performed the evaluation of nasal cytological aspects in patients with Type-2 CRSwNP eligible to biological treatment with Dupilumab to monitor during the first 6 months of therapy.

**Materials and methods.** We selected 20 patients who were candidate to start Dupilumab treatment for CRSwNP (according to EPOS2020 guidelines and Italian Agency of Medicines – AIFA – prescribing information). We performed nasal cytological sampling before the beginning of biological therapy (T0) and then during therapy at 2, 4 and 6 months (T1, T2, T3) after the first administration. We also performed on each patient nasal endoscopy for Nasal Polyp Score (NPS) evaluation, 22-item Sinonasal Outcome Test (SNOT-22) questionnare, and olfactometry with Sniffin' Sticks Identification test.

**Results.** Data showed a decreasing eosinophilic count in nasal cytology from T0 to T3 and this was associated with the improvement of other standardized parameters for the evaluation of biological therapy's response.

**Conclusion.** Nasal cytology is a good tool for monitoring nasal mucosa eosinophilic inflammation and it may be used routinely during Dupilumab therapy's follow up, as the peripheral eosinophilic count cannot be used for this purpouse for its known lack of correlation with treatment response.

## INTRODUCTION

Prescribing indication for biological therapy in CRSwNP are described in EPOS2020, EUFOREA2019 and, for Italy, in ARIA Italian Guidelines of 2021.(1–3)

In each paper, the first fundamental criteria for prescribing biological therapies is the presence of Type-2 inflammation in considered patients. Type-2 inflammation can be determined using one of the following criteria: tissue eosinophils ≥10/HPF (high power field) or blood eosinophils ≥ 250 cells/ µl or total IgE ≥ 100 kU/L.

Other considered criteria include the impairment of smell with remark of anosmia, the need for systemic corticosteroids or contraindication to systemic steroids, a significantly impaired quality of life with SNOT-22≥ 35 or ≥ 40 (depending on the document considered), the presence of asthma as a comorbidity.(2)

When our study started, only Dupilumab (Dupixent) had been approved for treatment of CRSwNP.

Dupilumab is a fully human monoclonal antibody that inhibits signaling due to interleukin-4 (IL-4) and interleukin-13 (IL-13), which are, together with interleukin-5 (IL-5), substantially the main mediators of type 2 inflammation.(4,5)

IL-4 and IL-13 are mediators with both common and diversified roles: IL-4 is the main factor in the polarization of T-lymphocytes CD4+ naive towards Th2 lymphocytes, it also conditions the switching of B cells towards the production of IgE and activates eosinophil chemotaxis; IL-13 also affects switching to IgE production and activates eosinophil chemotaxis but also determines the hyperplasia of the goblet cells and increases the contractility of the smooth muscle.(6) This direct mechanism of IL-13 on the production of nasal mucus causes changes in the rheological characteristics of the mucus, with alterations that determine some important pathological aspects of CRSwNP. IL-13 also alters ciliated cell differentiation, increases the proportion of secretory cells and decreases ciliary beat frequency in a time- and dose-dependent manner. (7)

In nasal mucosa and other target organs of Type-2 inflammation such as lungs, the effector cell is considered to the eosinophil, with a fundamental role of recruiting and chemotaxis driven by (IL-5).(8,9)

In the already mentioned guidelines, the monitoring of response to biological therapy with Dupilumab involves the use of nasal endoscopy through the use of the Nasal Polyps Score (NPS), a subjective questionnaire as the 22-items Sinonasal Outcome test (SNOT-22), the assessment of smell impairment using a validated test such as the Sniffin' Sticks Smell Test or the UPSIT test (University of Pennsylvania Smell Identification Test). The degree of response to biological therapy is also

assessed on the reduction of need of oral/systemic corticosteroids and on the improvement of comorbidities (e.g. asthma).

In clinical monitoring of patients who are addressed to therapy with Dupilumab, therefore, to date there are no parameters that allow the monitoring of the trend of local eosinophils' level in nasal mucosa, as it also not monitored even at a systemic level. This is due to the fact that transient increases in blood eosinophilia are generally known and peripheral eosinophils count does not meet any correlation with clinical response to biological therapy. (10–12)

To date, local and systemic eosinophilia associated with type 2 inflammation do not represent follow-up parameters, despite its finding being fundamental both from a theoretical and clinical point of view, for eligibility of patients to Dupilumab therapy.

Nasal cytology represents a useful, inexpensive and easy-to-apply diagnostic method to better detail the phenotypic characteristics of mucosal flogosis. In fact, it allows to detect and quantify the cell population within the nasal mucosa at a given time. As it is replicable at any time without any discomfort or adverse effect for patients, it can be performed at different times before and during medical treatments to assess changings in nasal mucosa flogosis associated with the considered therapy.

The technique involves sampling, processing and microscope reading. Sampling requires the collection of cells from the surface of nasal mucosa that is usually done by a sterile disposable curette. Samples should be collected from the middle portion of the inferior turbinate where the ratio ciliate/mucinous cells is usually expected to be of 4:1. This totally painless procedure is performed under anterior rhinoscopy, with an appropriate light source. The sample staining is executed using the common May-Grünwald-Giemsa (MGG). The stained sample is read at optical microscopy with a 1000x objective and with oil detecting the presence of inflammatory elements (eosinophils, mast cells, neutrophils and lymphocytes) in nasal mucosa.(13)

Nasal cytology has also been already used to characterized CRSwNP and its degree of severity before the introduction of biological therapies for CRS with a significant predictive value on risk of relapses and a role in the non-surgical management of the disease.(14)

With these considerations, we conducted the present study to evaluate changings in nasal cytology comparing samples collected before Dupilumab therapy was started and then samples collected at two, four and six months after the beginning of monoclonal therapy.

## MATERIALS AND METHODS

We evaluated 20 patients affected by severe CRSwNP who met the necessary criteria for indication to Dupilumab monoclonal antibody therapy and which had never received any biological therapy for CRS or asthma or atopic dermatitis. Patients were evaluated before starting the treatment (T0) with the following assessments:

- Blood sampling for elevated peripheral blood eosinophilic count or elevated total IgE values.
- Nasal endoscopy, for NPS measurement.
- Assessment of the severity of symptoms using SNOT-22;
- Olfactometry using Sniffin' Sticks (Identification test).

Furthermore, medical history was collected for comorbidity of asthma and for the use of systemic and oral corticosteroids in the last year.

We have added the nasal cytological evaluation to all these tests routinely performed in patients with CRSwNP.

Nasal cytology was performed by nasal scraping with the use of a Rhinoprobe from the body of inferior turbinate. We used the May-Grumwald-Giemsa stain. The samples were read by semi-quantitative reading using an optical microscope. (15,16)

The patients were then re-evaluated with nasal cytology and with other listed above tests at 2 months, 4 months and 6 months from the start of the therapy itself (T1, T2, T3).

## RESULTS

Patients enrolled were all affected by severe Type-2 CRSwNP (Tab.1). Eight patients were male, and 12 patients were female. Mean age was of 53.8 ± 13.5. Each patients had undergone at least one surgical procedure for nasal polyposis with a mean of 1,65 procedures.

Twelve patients had asthma has comorbidity and three patients and ASA-intolerance. All except one patient had need of oral or systemic corticosteroid during the last 12 months.

SNOT-22 was found higher than 50 in all patients (mean value 64.3) and every patient suffered of anosmia or iposmia with a mean value of Sniffin’ Sticks Identification test of 7.8.

| | M/F | Age (yo) | Previous surgery (n) | Asthma | AERD | Need of OCS during last year |
|---|---|---|---|---|---|---|
| ***1*** | M | 69 | 1 | yes | no | yes |
| ***2*** | F | 53 | 1 | yes | no | yes |
| ***3*** | M | 49 | 3 | no | no | yes |
| ***4*** | F | 54 | 1 | yes | no | yes |
| ***5*** | M | 60 | 1 | no | no | yes |
| ***6*** | F | 43 | 1 | no | no | yes |
| ***7*** | F | 46 | 2 | no | no | yes |
| ***8*** | F | 26 | 1 | yes | no | yes |
| ***9*** | M | 40 | 1 | yes | yes | yes |
| ***10*** | F | 71 | 1 | yes | no | yes |
| ***11*** | M | 68 | 1 | no | no | yes |
| ***12*** | M | 40 | 3 | yes | no | yes |
| ***13*** | F | 47 | 3 | yes | no | yes |
| ***14*** | F | 51 | 1 | yes | no | yes |
| ***15*** | M | 74 | 1 | no | no | yes |
| ***16*** | F | 48 | 1 | yes | no | yes |
| ***17*** | F | 74 | 5 | no | no | yes |
| ***18*** | F | 70 | 1 | yes | no | yes |
| ***19*** | M | 40 | 2 | yes | yes | yes |
| ***20*** | F | 52 | 2 | no | yes | no |

**Tab.1** A brief report of the main characteristics of patients included in the study.

Data from nasal cytology shows at T0 a reduction of the ratio between ciliate/mucinous cells with an increasing of the last type. At T0, nasal mucosa presented a high number of eosinophils in cytological samples (mean value: 15 cells/HPF). (Tab.2)

We compared these results with nasal cytological examination at T3, when ciliated cells appeared to be normally represented in nasal scraping samples, with a decreasing of mucinous cells. Eosinophils were significally reduced at T3 with a mean value of 0.75 cells/HPF, though they did not completely disappear from nasal mucosa. Performing a statical analysis using Mann-Whitney U test we found $p<0.0001$ comparing results from cytological evaluation at T0 and at T3.

| T0 | | | | T3 | | | |
|---|---|---|---|---|---|---|---|
| | **Ciliated cells** | **Goblet cells** | **Eosinophils** | | **Ciliated cells** | **Goblet cells** | **Eosinophils** |
| | ++++ | +++ | ++ | | ++++ | + | / |
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**Tab.2** Results from nasal cytology samples read with a semiquantitive reading.

We report the trend of different parameters (SNOT-22, NPS, Identification Test) and of nasal cytology and it seems that the latter has a correlation with the formers in terms of indicators of therapy response. (Fig.1)

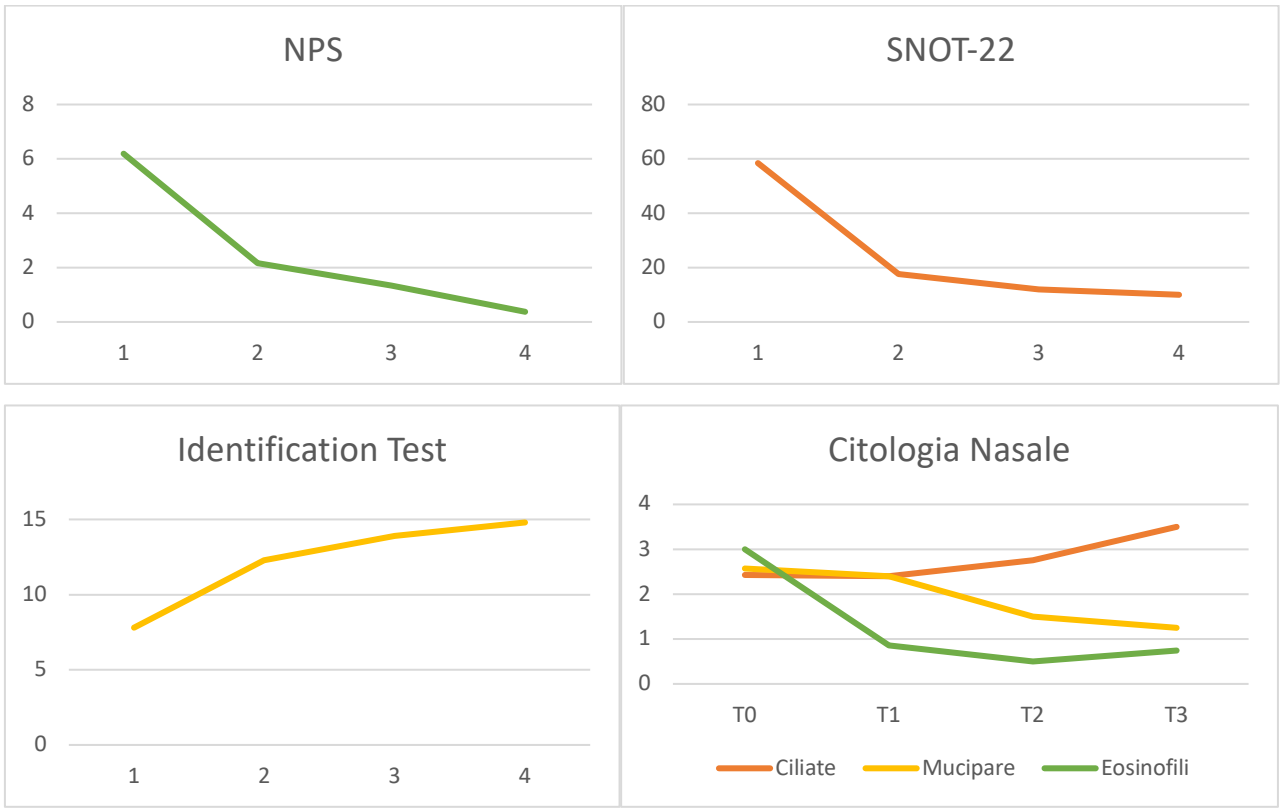


**Fig. 1** Trend of standardized parameters of evaluation of response to biological therapy with Dupilumab from T0 to T3 and trend of main cytological aspects considered (ciliated cells, mucinous cells, eosinophils).

## DISCUSSION

The use of biological therapy with Dupilumab in CRSwNP, which is directed against IL-4 and IL-13 receptor, has paved the way for the treatment of severe and relapsing forms of this disease, which otherwise cannot be fully controlled with endoscopic sinus surgery, even when correctly performed in order to restore ventilation of paranasal sinuses and allow an improved delivery of drugs for topical use.(2) Traditional medical therapies such as corticosteroids, administered topically, orally o with systemic administration, also resulted in a partial control of severe forms of CRSwNP.

Dupilumab is a targeted therapy that must be addressed to those patients who have the correct indication, which mean a higher probability to properly respond. Current international guidelines, such as EPOS2020 and EUFOREA, put a particular attention, in addition to clinical criteria, on the presence of type 2 inflammation, which represents a specific type of inflammatory response involving specific humoral and cellular mediators: one of the main players is the eosinophil cell. The increasing of peripheral blood eosinophilic count or the presence of eosinophils on nasal biopsy represents a fundamental prescribing information (as an alternative to the detection of elevated total IgE).

The lack of a monitoring system of this important type-2 inflammation “actor” that correlates with the trend of the other parameters used in the follow-up constituted the rationale of our study to investigate the inflammatory status of the nasal mucosa and its improvement induced by Dupilumab therapy.

At baseline, these patients appear to have marked eosinophilic cellularity in nasal mucosa, an aspect that substantially correlates with those indicated by the guidelines and in particular with the increased presence of eosinophils in nasal biopsies.

The finding obtained at the 6-months follow-up is that of a substantial correlation between the decreasing of the eosinophilic count in nasal cytology samples and the improvement of traditional parameters used for clinical evaluation such as NPS, SNOT-22, olfactometry.

Nasal cytology therefore represents a method that is easy to perform and repeatable which could allow the documentation of the improvement over time of the nasal eosinophilic inflammation during therapy with Dupilumab and which could therefore allow the monitoring of a parameter otherwise ignored in the follow-up but of extreme importance from the point of view of the control of type 2 inflammation in these patients. Moreover, nasal cytology had already been studied and classified in the so-called clinical-cytological grading to choose the correct medical therapy for each degree of severity.(14)

On the other hand, the routinary use of this diagnostic method would require a better characterization in cytological terms of type 2 patients especially before starting of biological therapy.(17)

**Conclusions.**

Nasal cytology is an underused tool in the follow-up of patients with severe Type-2 CRSwNP treated with biological therapies. Our data are intended to be a preliminar study and a further examination in the long term (e.g. twelve months of Dupilumab therapy) may be needed. If data will be confirmed by a larger sample, nasal cytology may be considered an indicator as responsiveness to biological therapy and a way to monitor local eosinophilic count in nasal mucosa, which is not actually being recorded in routinary clinical practice, but it may represent a valuable data.

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