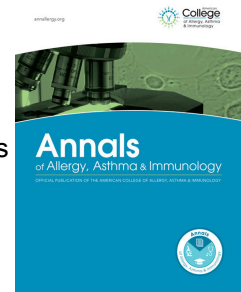


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VEGF/VEGFRs nasal expression is reduced by Mepolizumab in chronic rhinosinusitis with nasal polyposis

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Abbreviations:

AAR: active anterior rhinomanometry

a.u.: arbitrary units

CRSwNP: Chronic rhinosinusitis with nasal polyposis

GINA: Global Initiative for Asthma

IL-5: Interleukin 5

IL-5R α : Interleukin 5 receptor α

MCID: minimal clinically important difference

PoC: Proof of Concept

SNOT-22: sino-nasal outcome test-22

Th2: T helper type 2

VEGF: vascular endothelial growth factor

VEGFR1: vascular endothelial growth factor receptor 1

VEGFR2: vascular endothelial growth factor receptor 2

Word count: 960

Figure: Figure 1. Expression of VEGF, VEGFR1, and VEGFR2.

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a phenotype of chronic rhinosinusitis characterized by the presence of nasal polyps. To date, medical therapy and surgery can manage the disease in most of the cases.¹ However, despite improvements in surgical techniques, CRSwNP is characterized by relapses in severe cases, thus leading to the study of biological drugs such as Mepolizumab, antagonizing IL-5. Experimental and clinical evidence demonstrates that CRSwNP is driven by a type 2 immune response and displays eosinophil infiltration, mucosal inflammation, and hyperplasia of the epithelium.² Besides, it has been described that vascular endothelial growth factor (VEGF) is strongly upregulated in the mucosa of CRSwNP patients, promoting edema and epithelial cell growth in nasal polyps when compared to normal mucosa.³ Notably, other reports demonstrated that IL-5 stimulates VEGF production, which may influence immune cells' activity in inflammatory conditions.⁴

In this Proof of Concept (PoC), we evaluated the expression of VEGF and its receptors (VEGFR1, VEGFR2) in five patients (three females and two males, mean age 57.8±8.4) affected by bilateral, relapsing after Endoscopic Sinus Surgery, CRSwNP treated for three months with Mepolizumab. Clinical evaluation of the symptomatology associated with nasal polyposis was also carried out to highlight signs of improvement following Mepolizumab administration.

Patients were affected by severe eosinophilic asthma, meeting the requirements to receive Mepolizumab; nobody reported nonsteroidal anti-inflammatory drug intolerance. Mepolizumab treatment was performed by subcutaneous injection of 100 mg every four weeks for three times.

CRSwNP was diagnosed by nasal endoscopy with a 0° rigid endoscope, and the severity of symptoms was evaluated by the sino-nasal outcome test-22 (SNOT-22). Nasal resistance was studied by active anterior rhinomanometry (AAR) performed by the subject after 30 minutes of sitting in the waiting room. Cytological samplings were obtained by scraping (Nasal Scraping® - EP Medica, Italy) the superficial layers of the middle turbinate mucosa.

Intranasal corticosteroids were taken by patients during the study and discontinued 14 days before AAR and nasal scraping. Oral corticosteroids were not used by the patients during the study. Part of the scraping material was frozen at -80°C , processed to obtain a total lysate, then employed to assess the expression of VEGF, VEGFR1, and VEGFR2 by Western blot analysis. The values of VEGF and its receptors were compared with six age-matched healthy subjects (three females and three males, mean age 57.5 ± 6.18), not affected by nasosinus al pathologies.

All indices were measured before starting Mepolizumab therapy and 16 weeks after the first administration (immediately before the fourth application).

Statistical analysis was performed using student's t-test for independent and paired data. $P < 0.05$ were considered statistically significant. The values are reported as means $\pm$ standard deviation.

Results show that VEGF levels in untreated CRSwNP patients were significantly higher compared to controls ($P < 0.001$). VEGFR1 expression was strongly induced in CRSwNP vs. healthy subjects ($P < 0.01$). Similarly, VEGFR2 was also upregulated in CRSwNP with respect to controls ($P < 0.001$). After Mepolizumab treatment, VEGF, VEGFR1, and VEGFR2 expressions significantly decreased, in comparison with baseline values ($P < 0.05$ for all). When compared to healthy controls, VEGF expression after Mepolizumab treatment was still significantly higher ($P < 0.05$), whereas no differences were detectable in VEGF receptors (VEGFR1: $P = 0.34$; VEGFR2: $P = 0.63$) (Table 1).

From the clinical point of view, we registered an improvement of rhinosinusitis after Mepolizumab treatment. The mean values of the SNOT-22 score decreased even though the difference did not reach statistical significance; however, the score dropped beyond the minimal clinically important difference (MCID = 8.9 points)⁵ in all patients. The reduction of nasal flow resistance in AAR was significant in both nostrils ($P < 0.05$).

Our report points out that VEGF, VEGFR1, and VEGFR2 expression is markedly increased in patients with CRSwNP and that the administration of Mepolizumab, justified by severe asthma, decreased such expression and concurrently improved CRSwNP symptoms. Evidence from the literature suggests that VEGF may play a role in chronic airway inflammation acting as a pro-inflammatory cytokine, as it enhances Th2-mediated sensitization and inflammation in the mouse lung.⁶ Coherently, the amount of this growth factor, and its receptors, increases in the respiratory mucosa of patients with asthma, proportionally to the severity of the disease.³ Morphological analysis also reported that immunopositivity for VEGF, VEGFR1, and VEGFR2 is upregulated in nasal polyps.³ Our Western blot findings confirm this observation since VEGF levels were significantly higher in specimens from CRSwNP patients when compared to healthy mucosa. It has been shown that IL-5 stimulates eosinophils to release VEGF which, in turn, induces blood eosinophils to migrate into the mucosa and release the eosinophil cationic protein. These events could contribute to an enhancement of the inflammatory response mediated by IL-5.⁴ Mepolizumab neutralizes IL-5, thereby preventing IL-5 from binding to the α chain of the IL-5 receptor (IL-5R α) on the cell surface of eosinophils and reducing blood eosinophil levels. The significant reduction of VEGF and its receptors in the nasal polyps following brief treatment with Mepolizumab is in line with this observation. However, it cannot be excluded that alterations of VEGF pathway in CRSwNP physiopathology may involve other cell types different from eosinophils; it has been reported that VEGF receptors are expressed by epithelial cells, resident fibroblasts and other inflammatory cells in the mucosa of nasal polyps.⁷ Notably, among cells of the immune system, basophils respond to IL-5 by expressing its cognate receptor,⁸ and VEGF is stored in secretory granules of infiltrating basophils in the nasal polyps.⁹ It can be speculated that IL-5/IL-5R α axis may affect VEGF signalling in nasal polyps by acting on diverse cell contexts. Regarding clinical findings, SNOT-22 shows non-statistically significant reduction; whereas, AAR shows an improvement of flow resistance likely related to the reduction of mucosal

78 swelling by Mepolizumab. The efficacy of anti-IL-5 therapy on CRSwNP is being evaluated
79 with phase III clinical trials, and various biomarkers are proposed to identify patients
80 responders to monoclonal antibody therapy.¹⁰ The main limit of this PoC is the small sample
81 size, studies on larger patient populations will be required in order to better clarify molecular
82 mechanisms, and whether the VEGF pathway may be evaluated as an additional component
83 involved in CRSwNP.

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	Control	CRSwNP (Baseline)	CRSwNP (after 24 weeks)
VEGF (a.u.)	0.99±0.28	3.96±0.97*	2.51±0.27* [#]
VEGFR1 (a.u.)	0.98±0.26	3.36±1.33*	1.50±0.77
VEGFR2 (a.u.)	1.01±0.70	5.07±1.34*	1.59±1.31
SNOT-22		55.66±21.12	35.67±21.01
Resistance AAR left nostril (Pa/cm ³ /sec)		0.8±0.13	0.36±0.04 [#]
Resistance AAR right nostril (Pa/cm ³ /sec)		0.61±0.14	0.30±0.09 [#]

Table 1. Changes in VEGF, VEGFR1, VEGFR2, SNOT-22, and AAR

Comparison among controls, CRSwNP patients at baseline and after 24 weeks of Mepolizumab treatment. Values are reported as means ± standard deviation. A.u.: arbitrary units from densitometric analysis of immunoblots. * statistical significance vs Control; # statistical significance vs CRSwNP at Baseline.