

# Biases related to periostin levels in chronic rhinosinusitis with nasal polyposis: a systematic review\*

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## Abstract

**Background:** The role of periostin in the pathophysiology of chronic rhinosinusitis with nasal polyposis (CRSwNP) has been debated in the literature, with several authors proposing periostin as a potential biomarker or therapeutic target. However, the mechanisms regulating the systematic or local periostin production in both CRSwNP patients and controls remain elusive.

**Methodology:** Any factors reported to affect periostin expression in polyp tissue samples, nasal mucosa samples, serum and nasal secretions were considered as primary outcomes in this systematic review. Interactions or synergistic effects between bias factors were considered as secondary outcomes.

**Results:** Eosinophilic CRSwNP, large polyp size and radiological severity were found to be high-risk, positive bias factors for periostin levels in polyp tissue samples, while the role of atopy and asthma has been debated. Immunotherapy and eosinophilic endotype were identified as biases for serum periostin measurements, while steroids and non-steroidal anti-inflammatory drug exacerbated respiratory disease remain of unclear risk. Bronchial asthma, eosinophilic endotype and immunotherapy have been reported to bias periostin measurements in nasal secretions.

**Conclusions:** The relevant literature is extremely limited and little is actually known about the intrinsic or extrinsic factors affecting periostin measurements. The synthesis of the existing literature should be done with cautiousness.

**Key words:** periostin, bias, nasal polyp, serum, nasal secretions

## Introduction

Periostin is an extracellular matrix protein, which is considered as one of the main contributors to collagen fibrinogenesis in response to injury and inflammation<sup>(1)</sup>. It has been postulated to participate in the pathophysiology of several types of eosinophilic and chronic allergic inflammation, including chronic rhinosinusitis with nasal polyposis (CRSwNP)<sup>(2-7)</sup>. However, the mechanisms regulating the systematic or local periostin production in both CRSwNP patients and controls remain elusive.

The identification of the intrinsic or extrinsic factors affecting periostin measurements is crucial for comparisons between study groups. The present study aims at addressing the biases that may interfere with the results of studies on the role of periostin in the pathophysiology of CRSwNP. The literature was reviewed in order a) to identify the confounding factors related

to the levels of periostin in tissue samples, nasal secretions or serum, b) investigate if the methods used to measure periostin are comparable, c) recognize any association between different CRSwNP phenotypes and endotypes with periostin levels in nasal polyp (NP) tissue samples, nasal secretions or serum and d) discover any effect that may be exerted to periostin levels in tissue samples, nasal secretions or serum by concurrent or previous medical treatment.

## Materials and methods

### Eligibility criteria

This review adhered to the recommendations of the PRISMA (Preferred Reporting Items of Systematic Reviews and Meta-analysis) statement<sup>(8)</sup>. Both prospective and retrospective studies addressing any factors that could affect periostin measurements

in NP samples, nasal mucosa, nasal secretions or serum, thus acting as bias, were eligible for inclusion.

## Information sources and search

A review of the literature was conducted via the PubMed database of the US National Library of Medicine ([www.pubmed.org](http://www.pubmed.org)) and The CENTRAL of Cochrane Library. The search terms “periostin and sinusitis”, “periostin and sinus”, as well as “periostin and nasal and polyp” limited for the time period 1990-December 2022, and filtered for humans, attributed 58, 40 and 34 results respectively. Earlier studies were excluded, because of limitations in the available laboratory methods and the acquisition of the manuscripts' full texts. Three studies written in Chinese were excluded due to language barrier. No relevant/similar reviews were found in the Cochrane library.

## Study selection

The studies were initially screened through their abstracts. Studies referring to antrochoanal polyps, inverted papilloma, cystic fibrosis, primary ciliary dyskinesia, pediatric populations or cell cultures were excluded. Full text manuscripts were then screened for relevance. Literature review was expanded manually to the articles suggested by the search engine as similar, and the citation lists of included full text studies. Full text articles or parts of larger studies were excluded in case they referred to mixed CRS cohorts (CRSwNP and CRSsNP presented as a unique study group), because mRNA and protein levels of periostin have been reported to be significantly lower in chronic rhinosinusitis without nasal polyposis (CRSsNP) than in CRSwNP patients<sup>(3-5)</sup>. Studies on periostin levels in sputum were also excluded, as well as studies on specimens with enhanced mucosal injury due to post-operative collection. Studies lacking information on concurrent medical treatment, or studies on populations of  $\leq 10$  patients were excluded too. This minimum sample size was calculated according to the recommendations for determination of sample size in Health Sciences (WON 2018) using the equation

$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\epsilon^2}$$

for  $z$  equivalent to 95% Confidence Interval,  $\epsilon$  equal to 10% margin of error (for the calculation and comparison of periostin mean values in study subgroups) and population proportion ( $\hat{p}$ ) of 2.5% for CRSwNP<sup>(9)</sup>.

## Data collection process

Two investigators (MR and OA) independently decided on the eligibility of the studies and extracted data from full text manuscripts. Minor discrepancies were discussed and resolved.

## Data items

Any factors reported to affect periostin expression in NP tissue samples, in samples of non-polypoidal mucosa (taken from the

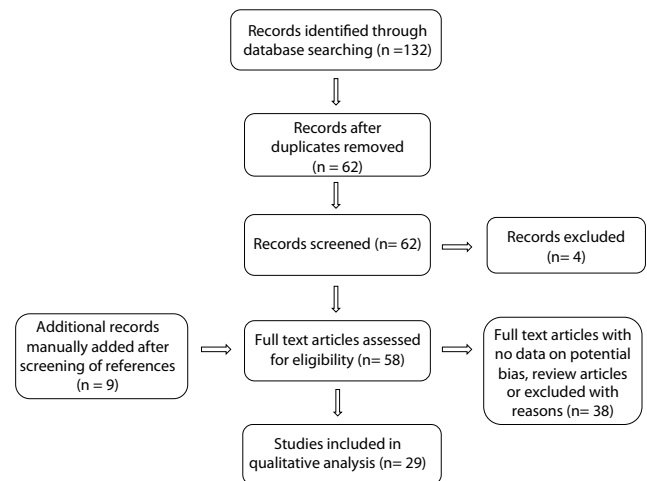


Figure 1. The study selection flowchart.

uncinate process, the inferior or middle turbinate), in serum, or in nasal secretions were considered as primary outcomes. The study aimed at identifying factors that may act as biases both within and across individual studies in the following domains: 1) Bias due to confounding factors, 2) bias arising from CRSwNP diagnosis and sampling, 3) bias in selection of the study participants, 4) bias due to therapeutic interventions, 5) bias arising from measurement of the outcome and 6) bias in selection of the reported result. Interactions or synergistic effects arising from the combination of the aforementioned factors in CRSwNP were considered as secondary outcomes.

## Additional analysis

Factors reported to affect periostin expression in tissue, nasal secretion or serum samples from patients with CRSsNP were extracted.

## Summary measures and synthesis of results

An evaluation regarding the predicted direction of bias toward the overestimation or underestimation of the measured periostin levels and the likely magnitude of bias was sought after synthesis of the results. The risk of bias concerns was graded into high, low and unclear risk. Within individual studies statistical significance was attributed to two-sided  $p < 0.05$ .

## Results

### Study selection

The total number of records identified through database searching ( $n=132$ ) was reduced into 29 studies following the procedure presented as a flowchart in Figure 1.

### Study characteristics

Data on possible bias regarding periostin measurements in tissue samples, nasal secretions and serum of patients with

CRSwNP could be extracted from 16, 4 and 11 studies respectively (Table 1). All tissue samples were taken intraoperatively during endoscopic sinus surgery (for patients) and septoplasty or other nasal operations (for controls). The vast majority of studies used reverse transcription polymerase chain reaction (RT-PCR) for mRNA and enzyme-linked immunosorbent assay (ELISA) for protein quantification.

## Results of individual studies

### Possible bias due to confounding factors

1. **Bronchial asthma:**
  - Effect on concentrations in polyp tissue samples: The effects of asthma and its severity on periostin mRNA and protein measurements have been debated as non-significant<sup>(2,10)</sup> or significantly upregulating<sup>(11)</sup>.
  - Effect on concentrations in nasal secretions: Baseline periostin expression in nasal secretions has been positively correlated with asthma<sup>(12)</sup>.
  - Effect on serum concentrations: The presence of asthma has not been identified as an independent predictor of periostin serum levels in patients with CRSwNP<sup>(13,14)</sup>. Vice versa, the role of CRSwNP as a confounding factor for serum periostin levels in asthmatic populations has been debated<sup>(15-17)</sup>.
2. **Atopic status:** (allergic rhinitis, atopic dermatitis and atopic keratoconjunctivitis):
  - Effect on concentrations in polyp tissue samples: Tissue periostin expression has been reported to be either irrelevant to atopic status (determined on the basis of skin prick tests)<sup>(3)</sup> or significantly higher in allergic rhinitis patients than controls<sup>(18)</sup>.
  - Effect on concentrations in serum: To the best of our knowledge no studies have investigated atopy as a confounder for serum periostin measurements in CRSwNP patients.
3. **Body mass index (BMI):** Although a moderate negative association between serum periostin levels and BMI has been reported in the general population as well as in asthmatic patients<sup>(14,19-21)</sup>, no studies have been conducted on CRSwNP populations.
4. **Smoking:** Multivariate regression analysis demonstrated no association between smoking and serum periostin levels in CRSwNP patients<sup>(13)</sup>.
5. **Demographic characteristics and surgical history:** The present literature reports no correlation between tissue periostin gene expression or serum periostin levels and patient age<sup>(2,13)</sup>, gender<sup>(2,13,14)</sup>, or number of prior surgeries<sup>(2,13,14)</sup>. In the study of James et al.<sup>(14)</sup> a multiple regression analysis supported that higher serum periostin levels were associated with older age (>60 years) in both non-asthmatic and asthmatic subgroups.

### Possible bias arising from CRSwNP diagnosis and sampling

1. **Tissue samples:** Periostin mRNA and protein levels have been found to be significantly increased in polyp samples compared to non-polypoidal mucosa samples from the unciniate process, septum, inferior turbinate, middle turbinate, or lateral nasal wall of the same CRSwNP patients<sup>(2,5)</sup>. In fact, no significant regional variation in periostin gene or protein expression has been found between non-polypoidal mucosa of the septum, inferior turbinate, middle turbinate, ethmoid labyrinth or lateral nasal wall in either CRSwNP patients or control subjects. Even more interestingly, samples from non-polyp mucosal subsites of CRSwNP patients demonstrated equivalent periostin gene and protein expression compared to each similar subsite in controls<sup>(2,4)</sup>.
2. **Tissue processing after collection:** Most studies have quantified periostin mRNA and protein levels in tissue homogenates, which involves mechanical micro-disruption of fresh tissue and permeabilized cell membranes. Thus, possible within-study bias related to differences in the localization of periostin anywhere between epithelial, subepithelial, basement membrane and lamina propria levels among subgroups cannot be evaluated<sup>(22-24)</sup>. Moreover, while most studies have used tissue samples which were stored immediately after collection at  $-80^{\circ}\text{C}$  until time of RNA or protein extraction<sup>(10,25)</sup>, a combination with tissue homogenizer preparations, where samples were deparaffinized, rehydrated, centrifuged and used to acquire a supernatant for measurements has also been described<sup>(10)</sup>.
3. **Diagnosis of eosinophilic CRSwNP (E-CRSwNP):** The most common criterion for the classification of CRSwNP to eosinophilic is for eosinophils to comprise more than 10% of the inflammatory cell population<sup>(5,26,27)</sup>. However, other criteria have been applied too. In the study of Wei et al.<sup>(11)</sup> the cut-off value for the eosinophilic phenotype was set at 7 eosinophilic cells/HPF (400 $\times$ ). Shiono et al.<sup>(23)</sup> established E-CRSwNP diagnosis on 3 criteria 1) blood eosinophilia (>6% of total white blood cells), 2) presence of soft tissue density in the olfactory cleft on computed tomography (CT), and 3) presence of soft tissue density in the posterior ethmoid sinus. Finally, according to the JESREC classification, a score is determined as the sum of scores for four items (unilateral or bilateral disease, presence of nasal polyposis, blood eosinophilia>5%, and dominant shadow of ethmoid sinuses in CT scans). The diagnosis of E-CRS is made if the JESREC score is  $\geq 11$  points<sup>(28,29)</sup>. These differences in the definition of E-CRSwNP may represent within-study or across-studies biases of yet unknown risk and direction. No studies have so far compared periostin levels across different E/non-E-CRSwNP classifications.
4. **Daytime variations of serum periostin:** Serum periostin levels

Table 1. Estimate of factors that could act as potential bias affecting the measurements of periostin in CRSwNP patients due to confounding factors, bias arising from CRSwNP diagnosis and sampling, bias in selection of study participants, bias due to therapeutical interventions, bias arising from measurement of the outcome and bias in selection of the reported result. The arising risk of bias, the predicted direction of bias and an estimation of whether the bias refers to within-studies or across-studies comparisons are also presented.

Possible bias	Polyp tissue			Nasal secretions			Serum			
	Risk of bias	Direction of bias	Within/ across studies bias	Risk of bias	Direction of bias	Within/ across studies bias	Risk of bias	Direction of bias	Within/ across studies bias	
Confounding factors	Bronchial asthma	?	+	both <sup>(2,10,11)</sup>	high	+	both <sup>(12)</sup>	no	no	no <sup>(13,14)</sup>
	Atopy	?	+	both <sup>(3,18)</sup>			Lack of studies			Lack of studies
	BMI			Lack of studies			Lack of studies			Lack of studies
	Smoking			Lack of studies			Lack of studies	no	no	no <sup>(13)</sup>
	Age	no	no	no <sup>(2,13)</sup>			Lack of studies	no	no	no <sup>(2,13)</sup>
	Gender	no	no	no <sup>(2,13,14)</sup>			Lack of studies	no	no	no <sup>(2,13,14)</sup>
	Previous surgeries	no	no	no <sup>(2,13,14)</sup>			Lack of studies	no	no	no <sup>(2,13,14)</sup>
CRSwNP	NP samples	Non polypoidal samples of CRSwNP patients comparable to controls <sup>(2,4,5)</sup>			Non applicable			Non applicable		
	NP processing	Tissue homogenates disable histological localization <sup>(10,22-25)</sup>			Non applicable			Non applicable		
	Eosinophilic diagnosis	Four categorization methods have been used <sup>(5,11,23,26-29)</sup>			JESREC classification <sup>(28,29)</sup> and Shiono et al. <sup>(23)</sup> criteria			JESREC classification <sup>(28,29)</sup> and Shiono et al. <sup>(23)</sup> criteria		
Patient selection	Polyp size	high	+	both <sup>(10,11)</sup>			Lack of studies			Lack of studies
	Postoperative SNOT-22 score	high	+	both <sup>(10)</sup>			Lack of studies			Lack of studies
	Radiological severity	E:high non-E:?	+	both <sup>(5-7,10,31)</sup>			Lack of studies			Lack of studies
	AFRS			Lack of studies			Lack of studies			Lack of studies
	NERD	?	-	both <sup>(10,18,22)</sup>			Lack of studies	?	+	both <sup>(12,13)</sup>
	IL-4	?	+	both <sup>(3,5,11)</sup>			Lack of studies			Lack of studies
	IL-5	?	+	both <sup>(3,5,34)</sup>			Lack of studies			Lack of studies
	IL-13	?	+	both <sup>(3-5,11)</sup>			Lack of studies			Lack of studies
	Eosinophil values	high	+	both <sup>(5,7,10,11,27,31,35)</sup>			Lack of studies	high	+	both <sup>(24,27,29)</sup>
IgE	high	+	both <sup>(5)</sup>			Lack of studies	no	no	no <sup>(15)</sup>	
cysteinyl leukotrienes			Lack of studies			Lack of studies			Lack of studies	
Interventions	Steroids			Lack of studies	no	no	both <sup>(12)</sup>	?	-	both <sup>(12,13)</sup>
	Immunotherapy			Lack of studies	high	-	both <sup>(12)</sup>	high	-	both <sup>(12,36)</sup>
	Doxycycline			Lack of studies	no	no	both <sup>(12)</sup>			Lack of studies
	Antihistamines			Lack of studies			Lack of studies			Lack of studies
	ACEIs & ARBs			Lack of studies			Lack of studies			Lack of studies

?= unclear, no= no of relevant studies, BMI= body mass index, NP= nasal polyp, AFRS= allergic fungal rhinosinusitis, NERD= Non-steroidal anti-inflammatory drug exacerbated respiratory disease, IL= interleukin, ACEIs= angiotensin-converting enzyme inhibitors, ARBs= angiotensin receptor blockers; += positive direction of bias; -= negative direction of bias

have been found to progressively decrease during the day in both asthmatics and controls<sup>(30)</sup>. There are currently no studies assessing any similar variation in CRSwNP patients.

### Possible bias in selection of patients into the study

1. *Clinical characteristics:* Periostin measurements in NP tissue samples have been significantly and positively associated with polyp size<sup>(10,11)</sup> and postoperative SNOT-22 scores<sup>(10)</sup>.

Table 2. Correlation between periostin and IL-4, IL-5, IL-13.

Study	Comparison	Result	Method
<b>Relative expression of periostin/IL-4</b>			
Wang et al. 2015 <sup>(3)</sup>	mRNA in NP tissue homogenates	p=0.016	ELISA for protein expression, in situ hybridization, RT-PCR for RNA expression
	Protein in NP tissue homogenates	p=0.0078	
Kim et al. 2017 <sup>(5)</sup>	mRNA in E-NP tissue homogenates	r=0.35, p=0.049	qRT-PCR for mRNA expression, ELISA for protein expression
	mRNA in NE-NP tissue homogenates	NS	
	Protein in E-&NE-NP tissue homogenates	NS	
Wei et al. 2018 <sup>(11)</sup>	Protein in NP tissue homogenates	r=0.5552, p<0.001	qRT-PCR, Western blotting, ELISA
<b>Relative expression of periostin/IL-5</b>			
Wang et al. 2015 <sup>(3)</sup>	mRNA in NP tissue homogenates	NS	ELISA for protein expression, in situ hybridization, RT-PCR for RNA expression
	Protein in NP tissue homogenates	NS	
	Protein in NP tissue homogenates of IL-5high patients	p=0.013	
	Tissue periostin expression was significantly higher in IL-5high than in IL-5low patients with CRSwNP	p=0.017	
Gevaert et al. 2017 <sup>(34)</sup>	Protein in NP tissue homogenates	r=0.665, p<0.01	ELISA
Kim et al. 2017 <sup>(5)</sup>	Protein levels in E-NP tissue homogenates	r=0.488, p=0.039	qRT-PCR for mRNA expression, ELISA for protein expression
	Protein levels in NE-NP tissue homogenates	NS	
<b>Relative expression of periostin/IL-13</b>			
Seshardi et al. 2015 <sup>(4)</sup>	mRNA in NP tissue homogenates	p<0.05	Semi qRT-PCR
Wang et al. 2015 <sup>(3)</sup>	mRNA in NP tissue homogenates	p=0.023	ELISA for protein expression, in situ hybridization, RT-PCR for RNA expression
	Protein in NP tissue homogenates	p=0.0078	
Kim et al. 2017 <sup>(5)</sup>	mRNA in E-NP tissue homogenates	r=0.835, p<0.001	qRT-PCR for mRNA expression, ELISA for protein expression
	mRNA in NE-NP tissue homogenates	NS	
	Protein in E-NP & NE-NP tissue homogenates	NS	
Wei et al. 2018 <sup>(11)</sup>	Protein in NP tissue homogenates	r=0.7214, p<0.001	Aptamer-based proteomic array and whole transcriptomic analysis

NP= nasal polyp, E= eosinophilic, NE= non-eosinophilic, IL= interleukin, RT-PCR: reverse transcription polymerase chain reaction, qRT-PCR: quantitative real time PCR, ELISA: enzyme-linked immunosorbent assay.

- No significant correlations between NP tissue periostin levels and the difference between pre-post-operative SNOT-22 scores, or the difference between pre-post-operative endoscopic scores have been documented<sup>(10)</sup>.
- Radiological disease severity:** A significant positive correlation between Lund-Mackay CT subtractive or added scores and tissue periostin protein and m-RNA expression has been advocated for patients with E-CRSwNP<sup>(5,6,31)</sup>, while it has been debated in mixed eosinophilic and non-eosinophilic populations<sup>(6,7,10,31)</sup>.
  - Special CRSwNP phenotypes:**
    - Allergic fungal rhinosinusitis (AFRS): To the best of our knowledge, there are no studies comparing AFRS with CRSwNP patients. Periostin levels have been reported to be significantly higher in NP samples of AFRS patients compared to sinus tissue samples of CRSwNP patients and healthy controls. They have also been positively correla-

ted with Lund-Mackay and CT bone erosion scores<sup>(32)</sup>.

- Non-steroidal anti-inflammatory drug exacerbated respiratory disease (NERD):
  - Effect on concentrations in polyp tissue samples: Stancovic et al.<sup>(22)</sup> reported that, when assessed with qRT-PCR, periostin mRNA expression in NP samples was significantly higher in the CRSwNP than the NERD group. On the contrary, the same authors reported that comparisons based on microarray data, yielded no significant difference. Immunohistochemical as well as sandwich ELISA based analyses of NP samples also failed to attribute significant differences in periostin protein production between CRSwNP and NERD patients<sup>(10,18)</sup>.
  - Effect on concentrations in serum: While Maxfield et al.<sup>(13)</sup> report no association, De Schryver et al.<sup>(12)</sup> found that baseline periostin serum levels were positively correla-

ted with NERD.

4. *Special CRSwNP endotypes as possible bias*: Four distinct but overlapping classification schemes have emerged to define endotypes within the CRSwNP phenotype: 1) type-2 cytokine-based, 2) eosinophil-based, 3) immunoglobulin (Ig)E-based, 4) cysteinyl based approach<sup>(33)</sup>.

• **Type-2 cytokine-based approach**:

Throughout the known CRSwNP inflammatory endotypes, different type-2 cytokines may resume the orchestrating role and presumably interplay with periostin in different ways.

- Interleukin-4 (IL-4): A significant positive correlation between tissue periostin and IL-4 expression, especially in E-CRSwNP has been reported (Table 2)<sup>(3,11)</sup>.
- IL-5: Available data seem to support a significant positive correlation between tissue periostin and IL-5 expression<sup>(3,34)</sup>, as well as an important association between high tissue periostin expression and the most severe subtype of CRSwNP characterized with high mucosal IL-5 expression, high frequency of asthma comorbidity and recurrent disease<sup>(3)</sup> (Table 2). Tissue periostin expression has been reported to be significantly higher in IL-5<sup>high</sup> than in IL-5<sup>low</sup> CRSwNP patients<sup>(3)</sup>. However, it should be noted that local IL-5-related upregulation of periostin is not reflected in the serum<sup>(3)</sup>.
- IL-13: Periostin mRNA and protein levels have been found to correlate positively and significantly with those of IL-13 in NP tissues<sup>(4,11)</sup> (Table 2).
- IL-25 and IL-33: The possible interactions of these type-2 cytokines with periostin have not been investigated yet in CRSwNP patients.

• **Eosinophil-based approach**

*Tissue concentrations*: Periostin tissue mRNA and protein levels have been found to be significantly higher in eosinophilic than non-eosinophilic NP tissues<sup>(5,11,27,31)</sup>, even between subsets with similar goblet cell hyperplasia and collagen deposition<sup>35</sup>. A significant positive correlation between tissue eosinophil values and periostin protein/mRNA levels has been observed in CRSwNP patients by several research groups<sup>(7,10,35)</sup>. Periostin has been found to be mainly localized in periostin-positive mast cells, which have been hypothesized to play a key role in the production of periostin and are significantly increased in eosinophilic compared with non-eosinophilic polyp samples<sup>(5)</sup>. Xu et al.<sup>(27)</sup> reported that the expression levels of periostin measured by Western blotting were comparable between non-eosinophilic polypoidal and non-polypoidal samples.

*Serum concentrations*: On the basis of univariate regression analysis and binary logistic models, Xu et al (2017)<sup>(27)</sup> supported that serum periostin levels were significantly

increased in the E-CRSwNP subgroup of their population (compared to the non-eosinophilic), with the diagnosis of E-CRSwNP being based on eosinophils comprising >10% of the inflammatory cell population. Among authors who applied the JESREC criteria, Ninomiya et al.<sup>(24)</sup> reported a significant association between serum periostin levels and JESREC-defined severity of E-CRSwNP, while Nakayama et al.<sup>(29)</sup> found no significant differences between eosinophilic and non-eosinophilic CRSwNP with regard to either periostin mRNA or protein concentrations.

• **Immunoglobulin (Ig)E-based approach**.

Elevated levels of IgE are seen in all forms of CRSwNP, except NERD. A strong positive relationship between periostin protein levels and total IgE levels in eosinophilic NP tissue homogenates has been reported<sup>(5)</sup>, while no correlation was found between periostin and IgE concentrations in the serum<sup>(15)</sup>. Serum IgE levels in the patients with the diffuse-type periostin expression (where periostin was expressed throughout the lamina propria starting just below the basement membrane of the NP) were significantly higher than the superficial type (where periostin was detected only in the subepithelial layers between the basement membrane and the nasal gland)<sup>(23)</sup>.

• **Cysteinyl-based approach**

There are no studies investigating any possible correlation between periostin levels and cysteinyl leukotrienes.

**Possible bias due to therapeutical interventions**

To the best of our knowledge there are no reports on the effects of relevant local or systemic treatments on tissue samples. The limited available data refer to serum and nasal secretions.

1. *Steroids*: The effect of oral steroids on periostin concentrations in serum has been debated as either non-significant<sup>(13)</sup> or negative 4 weeks after the initiation of treatment<sup>(12)</sup>, while the effect on nasal secretions has been found non-significant in the one relevant study published in the literature<sup>(12)</sup>. Interestingly, after cessation of oral steroid treatment, periostin expression in both serum and nasal secretions mounted above baseline<sup>(12)</sup>. One study reported that steroid nasal spray usage has also no effect on serum periostin<sup>(13)</sup>.
2. *Immunotherapy*: Serum periostin was significantly reduced by omalizumab after 8 weeks of treatment<sup>(12)</sup> as well as by dupilumab, 2-4 weeks after initiation of treatment, with the maximum reduction being observed at week 52<sup>(36)</sup>. Nasal periostin levels were significantly reduced by mepolizumab 8 weeks after treatment initiation<sup>(12)</sup>.
3. *Doxycycline*: Periostin reduction in nasal secretions after 4 weeks of treatment with doxycycline did not reach statistical significance<sup>(12)</sup>.
4. *Antihistamines*: To the best of our knowledge there are no

published data investigating this question.

5. *Systematic treatments interfering with the renin-angiotensin pathway*: There is no study measuring the effects of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) on periostin concentrations in CRSwNP patients. However, it is of note that, in a retrospective study conducted by Brook et al.<sup>(37)</sup>, patients with CRSwNP and concurrent asthma on treatment with ACEIs or ARBs demonstrated a significant prolongation of the mean time to revision sinus surgery compared to other asthmatics. For the cohort of patients without asthma, however, the use of ACEIs or ARBs had no effect on the mean time to revision sinus surgery.

#### **Possible bias arising from measurement of the outcome**

1. *Standardization issues*: As far as periostin is concerned, there is a lack of standardization of samples' processing and assay workflow, which prohibits direct comparisons of periostin levels resulting from different platforms (either electrochemiluminescence or ELISA)<sup>(25)</sup>.
2. *Periostin isoforms measured*: Periostin can appear in multiple differentially spliced isoforms and according to studies on asthmatic patients, the proportions of periostin isoforms may vary between different tissues and disease states<sup>(25)</sup>. With the available ELISA platforms presenting differences in monoclonal antibodies affinities for periostin isoforms, different isoform mixtures may attribute substantially different numerical results<sup>(16)</sup>.

#### **Possible bias arising from selection of the reported result**

1. *Publication bias*: Publications supporting periostin as a possible biomarker or therapeutic target are usually favoured.
2. *Statistical bias*:
  - omitted variable bias: With the current lack of literature specifying the factors affecting periostin measurements, there is also a lack of evidence-based list of variables that should be included in a multivariate analysis.
  - susceptibility bias: The causative role of periostin in polyp formation has been challenged<sup>(3,5,38)</sup>. Further studies are needed in order to clarify the role of periostin in the pathogenesis of nasal polyposis.

#### **Synthesis of results**

The results of our study are summarized in Table 1. The quantitative synthesis of our results was prohibited by the lack of standardization of the periostin laboratory samples processing, by the differences in monoclonal antibodies affinities for periostin isoforms between different ELISA methods and by the very limited number of studies conducted for each potential bias factor.

#### **Additional analysis**

There is only one study addressing possible periostin-related bias in CRSsNP. Maxfield et al.<sup>(13)</sup> reported that asthma comorbidity did not significantly affect serum periostin levels in CRSsNP patients. Studies on factors that may affect periostin measurements in CRSsNP, including eosinophilic/non-eosinophilic subtypes, are currently lacking.

#### **Discussion**

According to the results of this study, tissue eosinophilia, large polyp size and radiological severity affect periostin levels in polyp tissue samples as bias factors of high-risk and positive direction. Immunotherapy and blood eosinophilia were identified as high-risk biases for serum periostin measurements. Regarding most of the potential bias, however, the existing literature is either controversial or lacking and further research is needed (Table 1).

Protein and mRNA periostin levels have been consistently found to be significantly higher in eosinophilic than non-eosinophilic polyp tissues<sup>(5,11,27,31)</sup>. Thus, the distribution of eosinophilic and non-eosinophilic endotypes should be considered as a high-risk bias factor when comparing periostin levels among CRSwNP populations. Regarding the underlying mechanism, Wang et al.<sup>(3)</sup> reported that periostin is unlikely to contribute significantly to the recruitment of eosinophils into NPs based on their findings that tissue periostin expression was not correlated with eotaxin or tissue eosinophil cationic protein expression or tissue or blood eosinophil numbers. However, a role for periostin in eosinophil localization was supported in the same study, based on experiments on periostin adherence to eosinophils after they had been stimulated with IL-5<sup>(3,39)</sup>. A positive correlation between periostin levels and polyp size, as well as radiological CRSwNP severity has been repeatedly reported for the eosinophilic endotype<sup>(5,10,11,31,40)</sup>. This correlation has been, however, debated for mixed eosinophilic and non-eosinophilic populations<sup>(6,7,10,31)</sup> (probably due to heterogeneous representation of eosinophilic and non-eosinophilic endotypes) and has not been sufficiently studied for non-E-CRSwNP.

As far as other CRSwNP endotypes are concerned, the role of AFRS as a possible source of bias needs further investigation, while the role of NERD is debated. Regarding the latter, the discrepancies noted in the limited relevant literature could be attributed first to the substantial heterogeneity in the inflammatory signatures between its 3 recently identified inflammatory subendotypes<sup>(41)</sup> (associated with elevations in IL-5, IL-13 and interferon- $\gamma$ ) and second, the laboratory methods used<sup>(22)</sup>. A positive correlation has been demonstrated between tissue periostin and IL-4, -5 and -13 expression<sup>(3-5,11,34)</sup>. However, a positive correlation between two molecules does not by itself prove any causative association and further studies are needed before deciding on the pathophysiological mechanism that

produces the positive result. Ex-vivo studies have supported the role of periostin production stimulator only for IL-4 and IL-13<sup>(3-5,11)</sup>, while IL-5 is more likely a periostin production aftermath<sup>(5,11)</sup>. A bidirectional causative and consequential role for IL-4 and IL-13 has also been demonstrated by Wei et al.<sup>(11)</sup>. According to their study, IL-4 and IL-13 first stimulate nasal epithelial cells to secrete periostin. In turn, nasal epithelial-cell-derived periostin stimulates dendritic cells to increase expression of IL-4/IL-13 mRNA levels and promotes Th2 inflammation in CRSwNP<sup>(11)</sup>. IL-5, on the other hand, has only been identified as a periostin sequelae. Kim et al.<sup>(5)</sup> supported that the production of periostin by mast cells, through an intermediate step of epithelial-derived TSLP, also activates mast cells to produce IL-5 and induce Th2 inflammation via dendritic cells, while Wang et al.<sup>(3)</sup> reported that IL-5 did not increase periostin expression in polyp tissue. Regardless of the mechanisms underlying this correlation the distribution of IL-4, -5 and -13 endotypes should be taken into consideration when comparing periostin levels among CRSwNP subpopulations. Further studies are needed to investigate the possible interactions of other type-2 cytokines with periostin in CRSwNP.

The effects of bronchial asthma on the concentrations of periostin in NP samples, nasal secretions and serum of patients with CRSwNP, seem to be debated. This may be attributed to the fact that the existing literature has not assessed the question regarding the heterogeneous asthma phenotypes, or endotypes. During stable asthma periods, serum periostin levels were reported to be significantly higher in eosinophilic patients with severe, adult-onset asthma<sup>(15,17)</sup>, and poorer lung function<sup>(14)</sup>. Similarly to non-asthmatics, asthmatic patients with E-CRSwNP have been reported to exhibit significantly higher serum periostin levels than non-E-CRSwNP or controls<sup>(16,27)</sup>. Further studies, which will take into consideration these significant differences between different asthma and CRSwNP endotypes are needed to determine the power of asthma as a possible confounding factor for periostin levels in patients with CRSwNP.

The possible role of allergic rhinitis or atopic dermatitis as a bias when measuring nasal or systematic periostin levels in CRSwNP cohorts has not been investigated yet. Serum periostin levels have been reported to be comparable between allergic rhinitis patients and controls<sup>(13,14)</sup>, but tissue periostin expression has been debated as comparable or significantly higher in allergic rhinitis patients than controls<sup>(3,18)</sup>. In cohorts with bronchial asthma and allergic rhinitis, the combination of the two comorbidities did not have any effect on serum periostin levels, even when subgroups of different allergic rhinitis severity grades or various phenotypes of severe asthma were assessed<sup>(13,14,16,17)</sup>. On the other hand, there is limited evidence to support that awareness regarding atopic dermatitis and conjunctivitis is important. Serum periostin was significantly higher in patients with atopic dermatitis than patients with psoriasis vulgaris and

healthy controls, and positively correlated with disease severity and initiation of treatment<sup>(42)</sup>. Serum periostin was also significantly higher in patients with atopic keratoconjunctivitis than controls<sup>(43)</sup>.

Discontinuation of local as well as systemic glucocorticosteroids for at least 4 weeks before surgery has almost unanimously been adopted as inclusion prerequisite<sup>(3,5,6,10,26,27,31,34,40)</sup>, while some researchers excluded only patients with history of systemic use<sup>(2,7,16,18,22,29)</sup>. However, there are no studies exploring/quantifying the role of steroids as bias when measuring periostin levels in NP tissue samples. The limited literature refers to periostin concentrations in serum or nasal secretions and remains inconclusive. Until further studies clarify the role of steroids as possible bias factor for periostin measurements, it is recommended for studies to state the administration, duration, time after discontinuation and clinical response to steroids<sup>(12)</sup>. Although the relevant literature is limited, omalizumab, dupilumab, and mepolizumab have been shown to represent a source of bias with negative effect on periostin concentrations in serum and nasal secretions<sup>(12,36)</sup>. The role of ACEIs and ARBs as possible periostin-related bias factors also needs further investigation<sup>(37)</sup>. Brook et al.<sup>(37)</sup> explained the possibility of such an association through several overlapping pathways, all implicated in the pathogenesis of CRS, among them the downregulation of periostin expression through inhibition of the renin-angiotensin pathway<sup>(3,44-49)</sup>. These antihypertensive medications may possibly represent underestimated bias factors regarding CRSwNP research.

When attempting a synthesis of the existing literature, one should take into consideration that, CRSwNP patients and controls demonstrate comparable tissue periostin gene and protein expression in non-polypoidal mucosa samples, even samples from non-polypoidal sites of the ethmoid labyrinth<sup>(2,4)</sup>. This observation limits the role of periostin in the pathophysiology of mature NP and clearly indicates that comparisons between polypoidal samples from CRSwNP patients and non-polypoidal samples from healthy controls should be interpreted with caution, avoiding generalizations of the results in polypoidal samples to all sites and stages of the inflammatory process in CRSwNP. Quantitative synthesis and direct comparisons between numerical data across studies is prohibited for several reasons. First, the different criteria used for the classification of CRSwNP to eosinophilic, which seem to present substantial differences<sup>(23,28,29)</sup>. Comparative sensitivity studies that may verify the hypothetical bias and assess its risk grade and direction are needed. Second, there is lack of standardization between the different manufacturers' kits used for periostin measurements<sup>(16,25)</sup>. Although serum periostin levels quantified in asthmatic populations through different ELISA kits are strongly and significantly correlated<sup>(50)</sup>, some ELISA kits have been consistently attributing higher levels than others. For example serum periostin levels measured by the kit from Shino-test, Kanagawa, Japan have been reported to



be higher than those detected by the Elecsys® Roche Diagnostics<sup>(25)</sup>. To the best of our knowledge there are no such comparative studies in NP samples.

One of the main clinical implications of periostin-related research is its potential applications as a biomarker. Serum periostin has been identified as a possible biomarker of rapid decline in the pulmonary function of asthmatic patients<sup>(15,51)</sup>, a biomarker for type-2 airway inflammation in severe asthma<sup>(52)</sup>, and a biomarker for the presence of nasal polyposis in patients with asthma who had chronic rhinosinusitis<sup>(16)</sup>. The possible role of periostin as a biomarker of CRSwNP disease severity or as a prognostic factor for the response to surgical or medical treatment has been suggested by case-control studies. However, such a role has been questioned by the few relevant studies that have conducted multivariate analyses<sup>(27,53)</sup> and combinations of periostin with other molecules or findings have been proposed in order to achieve useful sensitivity and specificity values<sup>(26,31)</sup>.

## Conclusion

The thorough investigation of the sources of bias affecting periostin measurements is of outmost importance to understand the role of periostin in NP pathophysiology and evaluate its potential as a biomarker or therapeutical target. According to the results of the present review further studies on this field are needed.

## Authorship contribution

OAA: Eligibility of studies and data extraction, data analysis, review of the manuscript; WFA, IAE: Studies' collection, data analysis and review of the manuscript; MGR: Eligibility of studies and data extraction, data analysis, preparation and final review of the manuscript.

## Conflict of interest

No conflict of interest exists, for any of the authors.

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