The influence of inhibitors of apoptosis proteins (IAPs) on chronic rhinosinusitis with nasal polyps*

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Abstract

Background: Inhibitors of apoptosis proteins (IAPs) modulate the inflammatory process, and may facilitate the formation of chronic rhinosinusitis with nasal polyps (CRSwNP). This study aimed to observe if IAPs were differently expressed between patients with CRSwNP and controls, and to correlate the expression of IAPs with some inflammatory markers, as with the response to nasal corticosteroids in patients with CRSwNP.

Methodology: We obtained nasal biopsies from patients with CRSwNP (n=27) and controls (n=16). qRT-PCR measured the expression of IAPs and caspases, while Luminex assay measured the concentration of cytokines. Unpaired parametric tests and Principal Component Analysis (PCA) were used for statistical analysis.

Results: We observed lower expression of IAP genes (XIAP, BIRC2/IAP1, and BIRC3/IAP2) in CRSwNP patients compared to controls, and we identified that patients with bad response to corticosteroids presented lower levels of BIRC2/IAP1, XIAP, BCL2, CASP9, and IL-17, and higher levels of CASP7 and TGF-β.

Conclusions: IAPs expression was downregulated in CRSwNP, and was associated with poorer response to nasal corticosteroids. The present findings suggest the importance of IAPs as a link between environment and the host inflammatory responses, and this pathway could be explored as a potential new target therapy for patients with CRSwNP.

Key words: inhibitor of apoptosis protein, nasal polyps, rhinosinusitis, inflammation, corticosteroid

Introduction

Chronic Rhinosinusitis (CRS) has a worldwide prevalence between 5-12%[1]. It is associated with a highly deleterious impact on quality of life[2] and increased socioeconomic burden, with an estimated total cost of over 30 billion dollars anually in the USA[3].

In addition, CRS is frequently associated with several diseases, especially asthma and Non Steroidal Anti-Inflammatory Drugs-Exacerbated Respiratory Disease (N-ERD). The relationship between CRS and asthma is undeniable: the incidence of asthma is five times higher among patients with CRS than in the general population[4], and the association of asthma is related to poorer outcomes in CRS, with higher chances of surgical recurrence[5]. Patients with N-ERD also present worse sinonasal symptoms, higher disease extension, and higher predisposition to recurrence[5,6].

Mechanisms involving mucociliary clearance, epithelial barrier, immune response, and tissue remodeling are orchestrated for nasal mucosa homeostasis. Changes in one of these stages can induce chronic inflammation[7]. Several endotypes of CRS have been described, with different stages of the characteristics of inflammation[2,7-9].

CRS has been classically divided into two leading phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal

polyps (CRSsNP)[2]. The predominant symptoms in patients with CRSwNP differ from those with CRSsNP: the first group is characterized by changes in smell and nasal obstruction, while the latter preferably complain of pain[10]. More importantly, patients with CRSwNP tend to present less controlled disease[10] and higher predisposition to surgical recurrence[5] than patients with CRSsNP. In 2020, EPOS[1] classified CRS in primary (without a definitive cause) or secondary to an specific disease. Primary CRS is further classified according to extension (localized or diffuse) and to endotype dominance (type-2 versus non-type-2). CRSwNP is considered a phenotype of diffuse primary type-2 CRS.

The predominant method to classify type-2 or non-type-2 CRS is the number of eosinophils/high power field (400x) at histology from nasal biopsies. There is a great variability of how CRS is endotyped in the literature, with the number of eosinophils ranging from 5 up to 70/HPF[11]. In 2020, EPOS recommended that patients with nasal polyps with more than 10 eosinophils/HPF should be considered to have eosinophilic CRS.

Innate and adaptative immune responses are already known as essential mediators for CRSwNP[2,7-9]. Epithelial cells produce innate cytokines such as TSLP (thymic stromal lymphopoietin), IL-25, and IL-33, which recruit type-2 innate lymphoid cells (ILC2s). ILC2s are essential promoters of type-2 cytokines, such as IL-5, IL-4, and IL-13[12,13], and recruit inflammatory cells to the tissue. The interaction between nasal mucosa and recruited inflammatory cells leads to persistent inflammation[14], and some signaling pathways, such as MAPK (Mitogen-Activated protein kinase) and NF-κB (nuclear factor-κB) have significant roles in this process[15]. Eosinophil infiltration and quantity at the sinonasal mucosa are central markers for CRS prognosis. Even so, more recent articles pointed out that not only Type-2, but several other cytokines are increased in CRSwNP[2,7-9,16,17], suggesting that CRSwNP may present a mixed inflammatory profile, or that different inflammatory endotypes can finally result in the phenotype - CRSwNP.

Besides the inflammatory process, CRSwNP may also present changes in other esssential cascade pathways. Küpper et al.[18] have shown that caspase 3, caspase 9 and p53 were all significantly decreased in CRSwNP samples in comparison to nasal mucosa from control group, suggesting the importance of the these factors in inducing the proliferation and perpetuation of epithelial and inflammatory cells during nasal polyp's formation.

IAPs (inhibitors of apoptosis proteins) are a family of proteins known for blocking apoptosis. Among the mammalian IAPs, cellular IAP1 (cIAP1), cellular IAP2 (cIAP2), and X-linked IAP (XIAP) are the most intensiley studied[19,20]. Although the IAPs were first described for their role in controlling the apoptosis cascade, only XIAP can directly bind caspase-3 and caspase-9[19,20]. Recent studies have stressed the importance of IAPs in other cellular signaling processes[14,21,22], among them, inflammation[21-23].

IAPs have a baculoviral repeat domain (BIR) that binds to tumor necrosis factor (TNF) receptor-associated factors (TRAF), which will finally lead to an increase in both pro-inflammatory and pro-survival proteins[20].

IAPs regulate innate and adaptative immune response by canonic and non-canonic NF-κB signaling[21]. The inhibition of cIAP1 and cIAP2 lead to the proliferation and differentiation of B lymphocytes and dendritic cells and regulate pro-inflammatory cytokine production[21]. All these discoveries bring new perspectives for new treatment approaches for chronic inflammatory diseases by controlling or regulating IAPs.

Topical corticosteroid is the first option to treat CRSwNP[2]. There is high-quality evidence that it decreases sinonasal symptoms, reduces nasal polyps, and improves the quality of life. Essentially, corticosteroids reduce pro-inflammatory cytokines, inhibit the recruitment of inflammatory cells[1,2,24], and increase apoptosis of both eosinophils and neutrophils[25]. However, many patients persist with symptoms even after appropriate treatment[26]. Up to this moment, it is known that corticosteroid resistance in airway disease may occur due to altered activation of the glucocorticoid receptor (GR) or increased activity of NF-κB[13,27–30].

We postulated if IAPs could be associated with the presence of CRSwNP, and if they could influence the response to medical treatment. Thus, the present study aimed to compare the expression of IAPs between patients with CRSwNP and controls, to associate the expression of IAPs with the response to topical corticosteroid (mometasone furoate), and to correlate the expressions of IAPs and some inflammatory markers in patients with CRSwNP.

Materials and Methods

This study included patients aged 18 to 60 years, of both genders diagnosed with CRSwNP, according to EPOS 2012 criteria[31]. We excluded patients with unilateral symptoms, secondary CRSwNP (with associated primary ciliary dyskinesia, cystic fibrosis, or granulomatosis), or suspected tumors. We also excluded patients under any medical treatment for CRSwNP (including topical, inhaled or systemic corticosteroids, antihistamines and macrolides) within the month before the screening visit of the study. The control group comprised patients without sinonasal symptoms who underwent rhinoplasty for aesthetic reasons. We excluded patients with associated diseases, especially those characterized by eosinophilia, such as allergic rhinitis, asthma, and atopic dermatitis. Patients from both groups were invited to participate, and those who signed the Informed Consent were included in the study. The study was approved by local IRB, under the number CAAE 62763316.7.0000.5440.

Patients with CRSwNP

The patients in this group were invited to fill SNOT-22 and then underwent nasal endoscopy at the first visit:

Table 1. Demographic data of patients with CRSwNP.

Characteristics	Total Sample	Bad Responders	Good Responders
Sex			
Male / Female	10 / 17	2 / 10	8 / 7
SNOT - 22			
Before Treatment	49.2 ± 25.1	45.6 ± 23.6	52.1 ± 26.8
After Treatment	33.3 ± 27.7	45.8 ± 29.3	23.3 ± 22.7
Endoscopic Score			
Before Treatment	7.4 ± 2.9	7.9 ± 2.7	6.9 ± 3.2
After Treatment	5.7 ± 2.1	5.7 ± 3.3	5.8 ± 3.2
CT scan Score	14.6 ± 6.6	15.3 ± 6.6	14.1 ± 6.9
Previous Sinonasal Surgeries	5 (18%)	2 (16%)	2 (13%)
Associated Diseases			
Asthma	11 (40%)	6 (50%)	5 (33%)
N-ERD	4 (14%)	2 (16%)	2 (13%)
Smoking	5 (18%)	3 (25%)	2 (13%)
# Eosinophils in the tissue	52.3 ± 47.6	58.8 ± 38.2	46.7 ± 55.2

1. SNOT-22 (SinoNasal Outcome Test), validated in Portuguese[32]. We considered a previous study identifying 8 points as the minimal clinically important difference (MCID) for medical treatment[33], and included only patients with scores higher than 9.
2. Lund-Kennedy score[1] to grade nasal endoscopic findings.
3. The patients were also scheduled to a sinusal Computed Tomography (CT), and it was graded according to Lund-Mackay scan score[34].

At the first visit, two biopsies were obtained from the nasal polyp: a) one was kept for histopathology to confirm the diagnosis and to count the number of eosinophils in the tissue; and b) the other was placed in RNAlater and kept in -80°C for posterior RNA and protein analyses.

After the initial evaluation, patients with CRSwNP received mometasone furoate spray flasks, and they were instructed to apply four sprays in each nostril daily for 60 days. At the second visit, the patients returned their empty flasks to check compliance to treatment, and SNOT-22 and Lund-Kennedy endoscopy scores were reassessed.

Finally, we obtained Delta-SNOT-22, i.e., the difference in SNOT-22 before and after treatment. Patients with Delta-SNOT-22 less or equal to 9 were considered bad responders to mometasone furoate as they did not fulfill MCID, and those with Delta-SNOT-22 higher than 9 were considered good responders.

Control group

For the control group, the patient confirmed the absence of sinonasal symptoms on the day of surgery and signed the informed consent. During surgery, nasal endoscopy confirmed the absence of sinonasal diseases, and a biopsy from the middle turbinate was collected. The sample was placed in RNAlater and stored at -80°C for later analyses.

Molecular analysis

All samples were labeled and placed in flasks RNAse-free, containing RNAlater, and stored at Multiuser Laboratory –Ribeirão Preto Medical School – University of São Paulo.Total RNA and proteins were extracted with AllPrep DNA/RNA/Protein Mini Kit (Qiagen), according to the manufacturer's protocol. DNA-free Removal Kit (Invitrogen) was used during the extraction process to avoid contamination with genomic DNA.

After the extraction, total RNA was quantified at NanoDrop2000c spectrophotometer (Thermo Scientific), and 300 ng of total RNA was used to synthesize cDNA with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

Real-time quantitative PCR (qPCR) was performed at ViiA7 Real-Time PCR System (Applied Biosystems) to assess the gene expression of IAPs (XIAP, BIRC2/IAP1, and BIRC3/IAP2) and of caspases (CASP3, CASP7, CASP9 and BCL2). Corresponding TaqMan probes (Thermo Scientific) were obtained, with ACTB and B2M probes added as endogenous genes. The TaqMan probes efficiency was measured at 90 to 110%.

For qPCR reactions, we added 5 μL of TaqMan Fast Advanced Master Mix (Applied Biosystems), 0.5 μL of hydrolysis probe TaqMan 20X (Applied Biosystems), and 4.5 μL of diluted cDNA (1:5). All the reactions followed the parameters: 1 cycle of 50°C for 2 minutes, 1 cycle of 95°C for 20 seconds, 40 cycles of 95°C for 1 second and 60°C for 20 seconds. All the reactions were per-

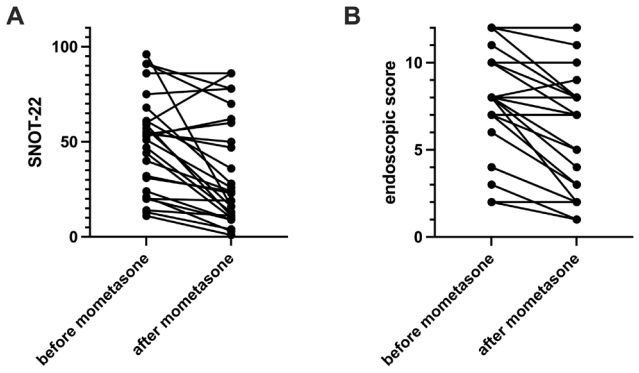

Figure 1. Clinical response to furoate mometasone in each patient with CRSwNP included in the study, analyzed by paired Student t-test. A) SNOT-22 (with a significant decrease from 49.2 25.1 to 33.3 27.7: CI95%: -24.17, -7.53; P-value<0.001); B) endoscopic score (with a significant decrease from 7.4 2.9 to 5.7 2.1; CI95%: -2.3, -0.9; P-value <0.0001).

formed in duplicate, and those with a standard deviation higher than 0.21 were repeated in a subsequent assay.

Thermo Fisher Cloud Software (Life Technologies Corporation) was used, and Relative Quantification (RQ, which is equivalent to ΔΔCt analysis) was measured for all the assays.

Total proteins were quantified from the samples by the Bradford method. The cytokines IFN-γ, IL-5, IL-33, IL-10, IL-17, and TGF-β were measured with Luminex assay, according to the manufacturer recommendations (Merck®). Luminex 200 equipment was used for this analysis, and the results obtained were measured at Luminex Xponent and Miliplex Analyst Software.

Histological analysis

Histological sections were stained with hematoxylin and eosin (HE) to obtain the number of eosinophils. Three representative areas were randomly chosen, and the number of eosinophils was counted in each of them at a 400x magnifying field in a Zeiss Primo Star microscope. The mean number of eosinophils was considered.

Statistical analysis

We compared the expression of IAPs and caspases between the groups CRSwNP and controls with parametric unpaired tests. Within the group of patients with CRSwNP, we also compared the expression of IAPs and caspases between the patients with good response to corticosteroids and those who did not respond well to treatment, using parametric unpaired tests. The P-value <0.05 was considered as statistically significant.

Principal Component Analysis (PCA) was used to correlate the data obtained from IAPs, caspases and cytokines, to evaluate the relationship between those biomarkers better. The Software JMP SAS version 16.0 (SAS Institute Inc., Cary, NC, USA) was used for this purpose.

Results

Initially, 61 patients were recruited: 41 with CRSwNP and 20 controls. Ten patients from the CRSwNP group were excluded because they lost follow-up, and eight patients were excluded due to poor quality of samples (four from CRSwNP and four from control groups). The final numbers of patients analyzed were 27 with CRSwNP (mean age 46±12 years; 17 female) and 16 controls (mean age 29.8±9 years; 14 female). The groups were significantly different regarding age (unpaired Student t-test, CI 95%: -25.7;-118, P-value<0.001). Asthma was present in 11 (40%) of patients, and N-ERD in 4 (14%); five patients had undergone at least one previous surgery. Twenty of 27 CRSwNP patients (74%) presented eosinophilic polyps, according to EPOS 2020 classification (> 10 eosinophils/ high power field)[1]. The mean number of eosinophils in the tissue at high powered field (400x) was 52.3±47.6.

Before treatment with furoate mometasone, the mean SNOT-22 was 49.2±25.1, the mean endoscopic score was 7.4±2.9, and the mean CT scan score was 14.6±6.6. Overall, CRSwNP patients presented a diffuse sinonasal disease and a significant negative impact on quality of life (Table 1).

After treatment with mometasone, there was a significant improvement in quality of life (SNOT-22 decreased from 49.2±25.1 to 33.3±27.7, CI95%: -24.2;-7.5, P-value<0.001) and in the endoscopic score (from 7.4±2.9 to 5.7±2,1, CI95%: -2.3;-0.9, P-value <0.0001).

As pointed out in Figure 1, some patients responded well to mometasone, while others presented identical clinical and endoscopic scores as before treatment. After this analysis, we divided the CRSwNP patients into two subgroups, the first composed of patients who had Delta SNOT-22 higher than 9 (Good Responders: N= 15 patients), and the latter composed of patients with Delta SNOT-22 equal or below 9 (Bad Responders: N=12 patients). The subgroups presented the same proportion of associated diseases, including asthma and N-ERD.

Molecular results

PCR analysis revealed that patients with CRSwNP had significantly lower expression of BIRC2 (0.7±0.3 CRSwNP vs. 0.9±0.2 controls; CI95%: 0.05,0.4; P-value<0.01), BIRC3 (0.6±0.5 CRSwNP vs. 0.9±0.5 controls; CI95%: 0.04,0.6; P-value<.05) and XIAP (0.5±0.2 CRSwNP vs.0.8±0.2 controls; CI95%: 0.2,0.4; P-value<0.0001) in comparison to controls (Figure 2).

On the other hand, the caspases gene expression was not significantly different between CRSwNP patients and controls (BCL2: 1.0±1.9 for CRSwNP vs. 1.6±1.7 for controls; CI95%: -0.6,1.8); (CASP3: 0.8±0.3 for CRSwNP vs. 0.8±0.3 for controls; CI95%: -0.2,0.1); (CASP7: 1.2±0.4 for CRSwNP vs. 0.9±0.3 for controls; CI95%: -0.5,-0.02); and (CASP9: 1.1±0.6 for CRSwNP vs. 1.0±0.4 for controls; CI95%: -0.4,0.3).

Regarding pro-inflammatory cytokines, CRSwNP presented

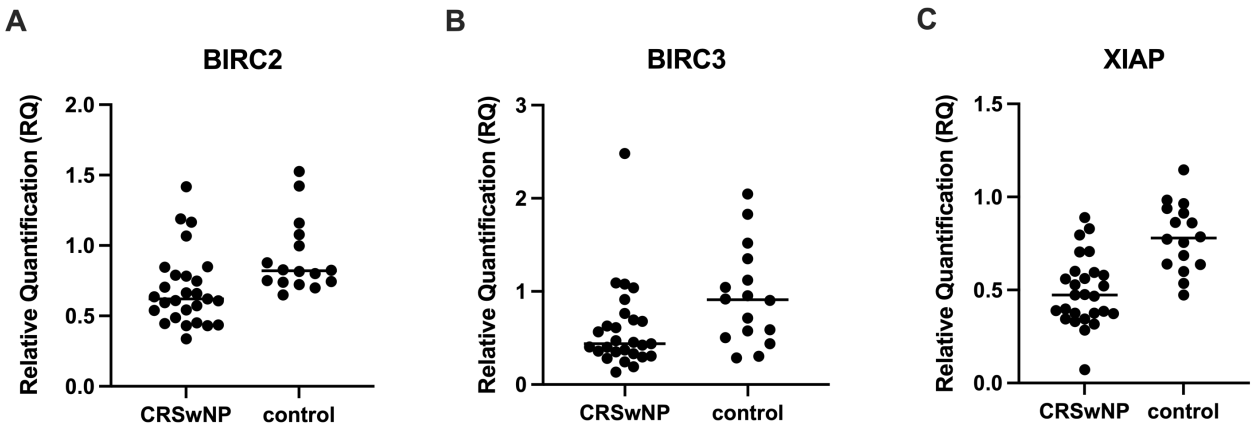

Figure 2. Comparison of IAPs expression between CRSwNP patients and controls, analyzed by unpaired Student t-test. A) BIRC2, B) BIRC3, and C) XIAP.

Table 2. Comparison of pro-inflammatory cytokine levels between patients with CRSwNP and controls; analysis with unpaired Student t-test.

Cytokine	CRSwNP (mean±SD)	Control (mean±SD)	CI95%	P-value
IFN-γ	2.6±0.5	2.3±0.3	-0.6,-0.03	<0.05
IL-10	0.3±0.06	0.3±0.06	-0.06,0.01	NS
IL-17A	1.8±0.3	2.0±0.2	-0.001,0.4	NS
IL-33	1.3±1.1	2.4±2.7	-0.1,2.4	NS
IL-5	0.6±0.06	0.5±0.05	-0.1,-0.01	<0.05
TGF-β	115.4±157.1	8.5±3.9	-186.77,-27.1	<0.01

significantly higher protein levels for IFN-γ, IL-5, and TGF-β compared with controls, while there was no significant difference in other cytokines (Table 2).

We then evaluated the expression of IAPs and caspases in CRSwNP patients according to clinical outcome. We observed that patients with inadequate response to nasal corticosteroids had significantly lower levels of BIRC2 compared to those who responded better to this treatment (0.5±0.1 in the Bad Responders group vs. 0.8±0.3 in the Good Responders, CI 95% -0.4,-0.02, P-value< 0.05). The other genes did not differ significantly.

We also evaluated the effect of IAPs on other molecular parameters. For this purpose, we included all the analyzed genes and the proteins from CRSwNP patients and controls in a dendrogram (Figure 3).

PCA analysis revealed that two principal components explain 43.4% of the variance: component 1 explain 28.9% and component 2 explain 14.5% (Figure 4). Component 1 correlated positively to the variables IL-17A, XIAP, BIRC2, CASP9, and BCL2 and negatively to CASP7 e TGF-β. Component 2 was associated positively with the variables CASP3, IFN-γ, IL-5, and IL-10 and negatively with BIRC3 and IL-33. We observed that six patients with CRSwNP presented a molecular pattern similar to those presented by controls (named Cluster 1), and 19 other patients with CRSwNP showed a different molecular pattern (called Cluster 2).

Next, we compared the clinical parameters in these two Clusters of patients with CRSwNP. These two clusters presented similarity in all parameters except one, Delta SNOT-22. Delta SNOT-22 was significantly higher in Cluster 1 than in Cluster 2 (37.0±26.5 for Cluster 1 vs. 7.4±13.5 for Cluster 2, P-value<0.05, Effect size 0.503, Table 3), showing that patients in Cluster 1 presented a better response to nasal corticosteroids than patients in Cluster 2.

In addition to clinical response to medical treatment, the groups also significantly differed in some molecular markers. Patients from Cluster 2 (Bad Responders to topical corticosteroid) had significantly lower levels of XIAP (0.7±0.2 for Cluster 1 vs. 0.4±0.1 for Cluster 2, P-value<0.05), BIRC2 (1.0±0.3 for Cluster 1 vs. 0.6±0.1 for Cluster 2, P-value<0.0005), BCL2 (3.2±3.3 for Cluster 1 vs. 0.3±0.2 for Cluster 2, P-value<0.05), CASP9 (2.0±0.8 for Cluster 1 vs. 0.8±0.2 for Cluster 2, P-value<0.001), IL-17A (2.2±2.2 for Cluster 1 vs. 1.0±0.1 for Cluster 2, P-value<0.005), and IL-33 (0.6±0.1 for Cluster 1 vs. 0.6±0.1 for Cluster 2, P-value<0,05, Table 4). On the other hand, these patients presented significantly higher expression of CASP7 (0.9±0.3 for Cluster 1 vs. 1.3±0.4 for Cluster 2, P-value<0.05) and significantly increased concentration of TGF-β (18.2±24.6 for Cluster 1 vs. 146.1±169.1 for Cluster 2, P-value<0.01, Table 4).

To confirm the PCA analysis, we correlated each IAP to each cytokine with the Spearman test. We observed a significant

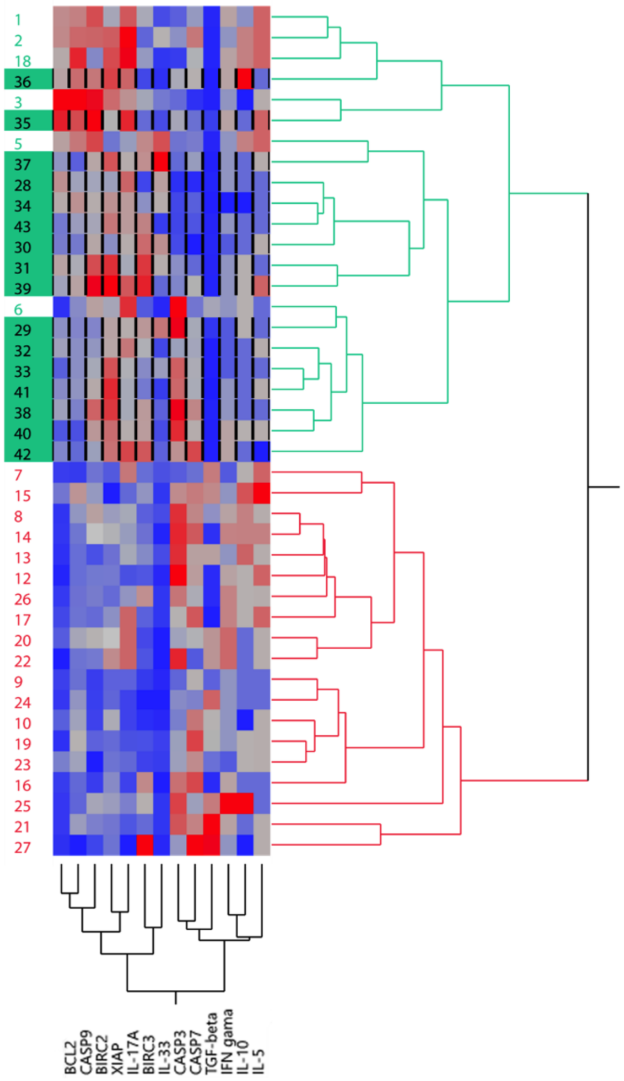

Figure 3. Cluster dendrogram and respective heatmap representing all the participants including CRSwNP and controls) at vertical and variables at horizontal. Green: cluster 1 (highlighted participants are controls, and those without highlights are patients with CRSwNP). Red: cluster 2, composed only of CRSwNP patients.

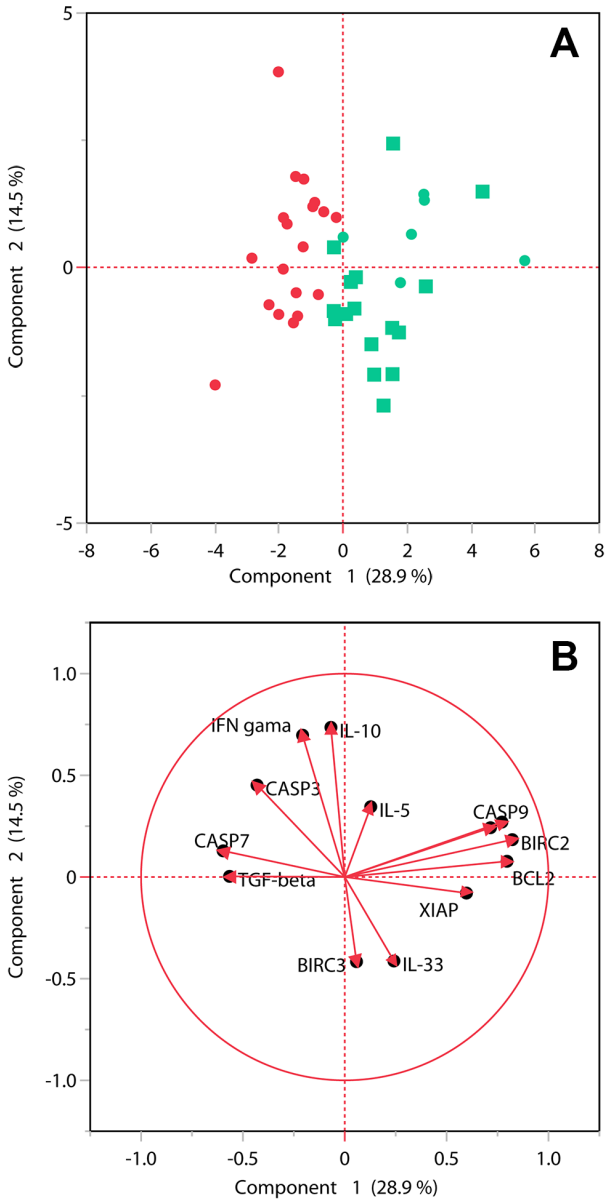

Figure 4. A) Distribution of CRSwNP patients (circles) and controls (squares) in principal components 1 and 2. Participants in this study were divided into two clusters: Green – cluster 1 (including both patients with CRSwNP and controls); Red – cluster 2 (composed only of patients with CRSwNP). B) Principal Component Analysis (PCA). A bi-dimensional graphic illustrates the projection of the variables (represented as arrows) in components 1 and 2, showing their association. The variables XIAP, BIRC2, CASP 9, BCL2, and IL-17A relate to component 1, and the variables CASP3, IFN-γ, IL-5, and IL-10 relate to component 2.

positive correlation between IL-17A and BIRC2 and XIAP, a significant positive correlation between IL-33 and all three IAPs, and a significant negative correlation between TGF-β with BIRC2 and XIAP (Table 5).

In summary, we observed that Bad Responders to corticosteroids presented lower levels of BIRC2, XIAP, BCL2, CASP9, IL-17A, and IL-33, and higher levels of CASP7 and TGF-β.

Discussion

The present study aimed to evaluate the influence of IAPs in CRSwNP pathophysiology and to assess the effect of IAPs on the response to nasal corticosteroids.

Initially, we compared IAPs expression in nasal polyps from patients with CRSwNP and nasal mucosa from patients without sinonasal symptoms. We observed a significantly decreased expression in all IAPs in nasal polyps compared to controls. IAPs were initially described in the literature to reduce cell death by inhibiting apoptosis. However, recent studies show that

Table 3. Comparison of clinical parameters in each cluster of patients with CRSwNP; analysis with Mann-Whitney.

	Cluster 1 (n = 6)				Cluster 2 (n = 19)				P-value	Effect size (r)
	Mean	SD	Med	IQR	Mean	SD	Med	IQR		
Nasal endoscopy (baseline)	5.3	3.3	5.0	6.5	7.8	2.8	8.0	3.0	0.11	0.318
Delta Endoscopy Score*	1.5	1.4	1.5	3.0	1.7	1.8	1.0	3.0	0.97	0.007
Lund-Mackay score	12.0	6.2	15.5	12.3	14.7	6.8	16.0	11.0	0.29	0.211
SNOT-22 (baseline)	46.7	32.0	50.5	55.8	48.9	24.7	53.0	37.0	0.82	0.045
Delta SNOT-22**	37.0	26.5	37.5	46.3	7.4	13.5	8.0	16.0	0.0119	0.503
Number of eosinophils	45.4	43.0	50.0	83.5	57.7	50.4	50.0	83.0	0.67	0.078

SD = standard deviation; Med = median; IQR = interquartile range. Effect size (r) = 0.1 to <0.3, small; 0.3 to < 0.5, medium; >= 0.5, large; MCID: minimal clinically important difference. *Delta Endoscopy = Endoscopy score before – Endoscopy score after treatment; **Delta SNOT-22 = SNOT-22 before – SNOT-22 after treatment.

Table 4. Comparison of IAP genes, genes related to apoptosis (both measured with qPCR), and cytokines' concentrations (measured with Luminex) between Clusters 1 and 2; analysis with Mann-Whitney.

	Cluster 1 (n = 6)				Cluster 2 (n = 19)				P-value	Effect size (r)
	Mean	SD	Med	IQR	Mean	SD	Med	IQR		
IAP genes										
XIAP	0.7	0.2	0.7	0.3	0.4	0.1	0.4	0.2	0.0143	0.5
BIRC2	1.0	0.3	1.1	0.4	0.6	0.1	0.6	0.2	0.0002	0.6
BIRC3	0.6	0.2	0.6	0.4	0.6	0.5	0.4	0.3	0.16	0.3
Genes related to caspases										
BCL2	3.2	3.3	2.4	3.3	0.3	0.2	0.3	0.2	0.0100	0.5
CASP3	0.7	0.3	0.6	0.4	0.9	0.2	0.9	0.4	0.07	0.4
CASP7	0.9	0.3	0.9	0.5	1.3	0.4	1.3	0.5	0.0428	0.4
CASP9	2.0	0.8	1.8	1.4	0.8	0.2	0.7	0.3	0.0007	0.6
Cytokines										
IFN-γ	2.4	0.1	2.3	0.3	2.7	0.6	2.6	0.6	0.40	0.2
IL-5	0.3	0.05	0.3	0.1	0.3	0.1	0.3	0.1	0.19	0.3
IL-10	2.2	0.3	2.2	0.5	1.7	0.3	1.6	0.4	0.63	0.1
IL-17A	2.2	2.2	1.3	2.0	1.0	0.1	1.0	0.1	0.0034	0.6
IL-33	0.6	0.06	0.6	0.1	0.6	0.06	0.6	0.04	0.0240	0.5
TGF-β	18.2	24.6	8.2	16.7	146.1	169.1	78.8	197.5	0.0056	0.6

SD = standard deviation; Med = median; IQR = interquartile range. Effect size (r) = 0.1 to <0.3, small; 0.3 to < 0.5, medium; >= 0.5, large.

IAPs are crucial inflammatory modulators, especially in innate immune responses[20]. The increase in IAP expression has been observed in several tumors[20-22], and SMAC mimetic compounds are being tested as potential drugs for treating cancer[21,35,36]. In contrast, the decrease in IAPs is related to poor response to bacterial infection, and chronic inflammation. Labbé et al.[36] tested the effect of SMAC mimetics on IAPs expression using a model for peritonitis. They observed that, while acute stimulation with SMAC mimetics significantly decreased inflammation, prolonged stimulus with this drug led to increased inflammasome activation, thus amplifying inflammation. Other studies show that animals with IAP deficiencies develop an exacerbated chronic inflammatory process by an inadequate innate immune response, blocking the NOD (nucleotide-binding oligomerization domain-containing protein) protein signaling pathway[20,37]. Thus, an adequate balance in IAP expression is essential for

Table 5. Correlation between expression of each IAP and protein concentration of each cytokine evaluated, measured by Spearman test.

Cytokine	BIRC2		BIRC3		XIAP	
	Spearman ρ	Prob>|ρ|	Spearman ρ	Prob>|ρ|	Spearman ρ	Prob>|ρ|
IFN-γ	0.042	0.7916	-0.10	0.5218	0.01	0.9484
IL-10	0.11	0.4877	-0.04	0.7883	-0.13	0.4195
IL-17A	**0.55**	**0.0002***	0.056	0.7297	**0.54**	**0.0002***
IL-33	**0.53**	**0.0004***	**0.44**	**0.0040***	**0.33**	**0.0352***
IL-5	0.12	0.4386	-0.04	0.7952	-0.18	0.2469
TGF-β	**-0.44**	**0.0037***	-0.28	0.0793	**-0.67**	**<.0001***

mucosal homeostasis.

As the present results suggested that chronic inflammation in CRSwNP may be associated with decreased expression of IAPs, we next tested if these proteins could influence medical response to nasal corticosteroid. Nasal corticosteroids were chosen because they are considered the gold-standard treatment for CRSwNP[1], with an excellent cost-benefit ratio and a low rate of side effects[38]. Yet, mechanisms related to poor response to nasal steroids in CRSwNP remain incompletely understood.

To answer this question, we treated patients with mometasone furoate, and measured Delta SNOT-22. We could confirm the positive effect of nasal corticosteroids on CRSwNP, as the mean SNOT-22 in this cohort decreased from 49.2±25.1 to 33.3±27.7. Yet, 15 (55.5%) patients responded very well to mometasone furoate (with Delta SNOT-22 valued above MCID), while 12 (44.5%) had Delta SNOT-22 values lower than MCID, or even worsted their symptoms after the treatment. No clinical parameter (N-ERD, asthma, endoscopic or tomographic extension of the disease, or number of eosinophils in tissue) were related to the medical response. In the literature, many patients persist with symptoms even after optimized treatment[26].

Next, we characterized the inflammatory pattern of CRSwNP in our Brazilian cohort. We detected that the mean number of eosinophils in the tissue was 52.3, and 20 out of the 27 participants had eosinophilic nasal polyps compatible with the Caucasian inflammatory profile[1,2,16,39]. Yet, we observed a significant increase in T1 (IFN-γ), T2 (IL-5), and remodeling (TGF-β) biomarkers in nasal polyps compared to control nasal mucosa. This mixed inflammatory profile, observed in our study, has also been described in other cohorts[17,40,41].

Together, these cytokines promote migration and proliferation in epithelial cells, and increase cellular permeability and eosinophil recruitment. In addition, TGF-β induces the epithelium to change to a more mesenchymal phenotype in a process known as EMT (epithelial-mesenchymal transition)[42,43].

Finally, we correlated IAPs expression to medical response to corticosteroid using PCA analysis. We detected that patients with worst responses to corticosteroids were those with lower expression of BIRC2 and XIAP when compared to those with a better outcome. Thus, the decreased expression in IAP was related to CRSwNP and poor response to corticosteroid treatment. PCA analysis also revealed that lower BIRC2 and XIAP were strongly associated with lower expression of BCL2, CASP9, and IL-17A and higher expression of TGF-β. Together, these results indicate that lower IAP expression can be related to increased inflammation, especially in remodeling, and decreased apoptosis. This pattern could be easily associated with CRSwNP and a poor response to topical corticosteroids.

Nadella et al.[35] showed that SMAC mimetics decreased IAPs expression and enhanced the M2 phenotype in cell cultures of murine macrophages, which could be related to bacterial persistence inside the macrophages. Our findings, together with the studies previously described [20,35–37], suggest that chronic decrease in IAPs could dysregulate macrophage activity and innate immune response, leading to a chronic inflammatory process with T2 phenotype and facilitating bacterial persistence in the tissue.

The main limitation of this study was the number of patients, especially those who fulfilled all the steps of this project. About one quarter of the patients lost follow up, most of them because COVID-19 pandemics started when we were still recruiting the patients.

Conclusion

Decreasing IAP might be an essential process in CRSwNP pathophysiology, changing immune homeostasis between environment and the host inflammatory responses. This process can induce the formation of nasal polyps and affect their response to corticosteroids. Further studies will contribute to analyzing the real impact of IAPs in CRSwNP physiopathology, and if this pathway could be explored as a potential new target therapy for patients with CRSwNP.

Authorship contribution

The authors IMP and FCPV idelized the project. IMP also collec-

ted all the clinical measures and samples from the patients. MZF, AABM, LECMS, DMG, FMF, RBM were responsible for laboratory assays and statistical analysis. IMP, ET, EA, RBM, DMG, WTA-L and FCPV analyzed all the collected data, discussed the results and wrote the final article.

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Conflict of interest

The authors state they don't have any conflict of interest directly related to this article.

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