# Neutrophilic inflammation in chronic rhinosinusitis

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**Purpose of review**
Over the last years, extensive research has been done on neutrophils and their contribution in chronic rhinosinusitis (CRS), and made it clear that they are more than just a bystander in this disease. In this article, we will review all recent publications on this topic and look to what the future hold regarding therapeutics targeting the neutrophilic inflammation in CRS.

**Recent findings**
Evidence is growing that the presence of neutrophils are associated with a worse disease outcome in certain CRS patient groups. They are highly activated in type 2 inflammations and exhibit damaging properties through their proteases, contributing to the chronicity of the disease. Several recent studies identified useful biomarkers and targets for future therapeutics.

**Summary**
The findings we review in this manuscript are of utmost importance in unraveling the complexity of CRS and provide us with the necessary knowledge for future clinical practices.

**Keywords**
chronic rhinosinusitis, neutrophils, therapeutics, type 2 inflammation

## INTRODUCTION

Chronic rhinosinusitis (CRS) is an increasing health problem affecting up to 15% of the population in western countries. The complexity of underlying inflammatory patterns complicates the understanding of major pathways suitable for personalized medicine approaches and causes less or nonresponsiveness to current treatments in a substantial group of the patients. CRS patients are phenotypically classified as CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) [1,2]. Neutrophilic responses have long predominantly been associated with type 1–type 3 CRSsNP, while type 2 CRSwNP was considered a predominant eosinophilic disease [3–9]. However, recent endotype-focused studies have challenged this polarized image, showing a more versatile cytologic picture in both CRSsNP and CRSwNP patients [10▪▪,11]. In CRSsNP, about 50% of the patients present a type 2 response with eosinophils significantly increased and activated, while the neutrophilic inflammation is not different compared with the nontype 2 counterpart [11]. As the type 2 CRSsNP patients have worse clinical outcomes by means of recurrence, comorbid asthma and reduced smell/taste, neutrophils cannot be associated with a more severe clinical outcome in CRSsNP. Interestingly, more and more studies over the last decade report the existence of a mixed eosinophilic–neutrophilic inflammation, associated with high type 2 responses and a worse clinical outcome in about 26–36% of CRSwNP patients [4,10▪▪,12–17].

## NEUTROPHIL INFILTRATION AND SURVIVAL IN CHRONIC RHINOSINUSITIS

It is generally accepted that IL-17 is the driving force of neutrophilia in CRSwNP through stimulation of both neutrophilic infiltration and survival [18]. In addition, IL-17 causes the upregulation of CXCR-1 and CXCR-2, and the release of IL-6, IL-8 and G-CSF, further stimulating neutrophil infiltration [19,20]. IL-17 is, therefore, often used as a marker for tissue neutrophilia in CRS. iNKT17 (type 17 invariant natural killer cells) cells were significantly increased in Asian neutrophilic nasal polyps, and its differentiation from native iNKT cells was stimulated in

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## KEY POINTS

- Neutrophils are present and activated in severe type 2 CRSwNP, where they interact with eosinophilic inflammation, establishing a mixed inflammation.
- Neutrophils can contribute to the CRS pathophysiology via increased proteolytic activity of elastase and cathephin G, or via NETosis.
- The presence of a neutrophilic inflammation in CRS is often associated with a worse disease outcome, glucocorticosteroid resistance and recurrence after surgery.
- Current therapies targeting the neutrophilic activation have been tested in other airway diseases and look promising to use in CRS as well.

neutrophilic homogenates, suggesting a role for iNKT17 cells in the feedback mechanism of local neutrophilic inflammation in Asian CRSwNP [21]. However, the most severe Caucasian patient group shows a predominant type 2 inflammation with a vast neutrophilic inflammation but low IL-17 levels, not linking type 17 to neutrophilia and disease severity [4,10▪▪,22]. Interestingly, recent in-vitro studies point to a potential role for Charcot–Leyden crystals (CLCs) to the neutrophilic infiltration in severe type 2 CRSwNP as CLC-stimulated nasal polyps released significantly higher concentrations of neutrophil attractant proteins (TNFα, IL-6 and IL-8) and an increased neutrophilic migration was observed towards CLC-stimulated epithelial cells of CRSwNP patients [17].

It has been demonstrated that the neutrophil lifespan increased through inhibition of cell apoptosis at sites of inflammation [23,24]. We recently showed deceased apoptosis of neutrophils in CRSsNP environment compared with CRSwNP and controls [10▪▪]. In Asian patients, both numbers of neutrophils and rates of apoptosis correlated with G-CSF in nasal polyps and suppressed apoptosis could be reversed by anti-G-CSF treatment [25]. These observations were recently confirmed in Caucasian patients with low-type 2 CRSwNP [26▪]. It has been shown that co-stimulation of neutrophils with GM-CSF, TNF-α and IL-4 leads to the generation of long-living populations of neutrophils; and the concentration of all these mediators have been reported to be increased in CRS mucosa [27,28].

## NEUTROPHILIC ACTIVATION AND IMPACT ON CHRONIC RHINOSINUSITIS

Although mature neutrophils are dominant in the blood of CRSwNP patients, a significant shift towards activated neutrophils ($CD16^{high}$, $CD62L^{dim}$) is observed in the tissue of CRSwNP, suggesting that neutrophils get activated once they enter the CRSwNP microenvironment [10▪▪,29–31]. These activated neutrophils showed higher proteolytic activity via cathepsin G and elastase in severe type 2 CRSwNP [10▪▪]. These mediators enhance secretion and activation of IL-1 family cytokines as IL-1β, IL-33 and IL-36γ in an extremely efficient manner [32]. IL-1β and IL-33 are also key players in the induction of type 2 responses as they function as chemoattractant for Th2 cells and stimulate the production of type 2 cytokines in eosinophilic nasal polyps [33–35]. In CRS, IL-36γ promotes the secretion of IL-8 and IL-17 from tissue neutrophils, reinforcing a positive feedback loop and their own recruitment [12]. In addition, neutrophilic serine proteases have a direct negative effect on the nasal epithelial barrier integrity and elastase can initiate goblet cell metaplasia and increased mucus production [36–38]. Several recent studies on elastase-induced mucus production revealed involvement of the TRAF6/autophagy regulatory axis, TNFα-converting enzyme-epidermal growth factor receptor signaling (TACE-EGFR) pathway and miR-146a [39–41]. Substrates for neutrophilic proteases are elastin, collagen and fibronectin, which are major components of the extracellular matrix, and their degradation could be linked to tissue remodeling in CRS [42]. (Fig. 1)

The phagocytic function of neutrophils and superoxide anion production is significantly more impaired in cystic fibrosis (CF) patients with CRSwNP, compared with patients with CRSsNP [43]. Another mechanism to clear invaders is the formation of NETs (neutrophil extracellular traps). Those NETs can be formed via different processes (viable NET formation or NETosis), but are generally nicotinamide adenine dinucleotide phosphate (NADPH)-dependent and consists of neutrophil DNA associated with granule proteins [44,45]. The expression of NADPH-oxidase subunit p67phox was found to be expressed in eosinophils and neutrophils but not in macrophages in CRSwNP tissue [46]. However, studies about the presence of NETs in the tissue are contradicting, although NETs are observed in secretions of exacerbated eosinophilic CRSwNP patients [44,47,48]. It was recently found that – in addition to multiple microorganisms – CLCs evoke NETosis *in vitro* [17,44]. Therefore, it is likely that CLC deposition in tissue and secretions might contribute to NETosis in CRS patients. The pathway of NET formation and outcome is highly dependent on the individual micro-organism identity, pathogen size and additional stimuli [44,49,50]. Moreover, *S. aureus* – present in 67% of CRSwNP patients – has been found to degrade NETs to promote its own survival [51].

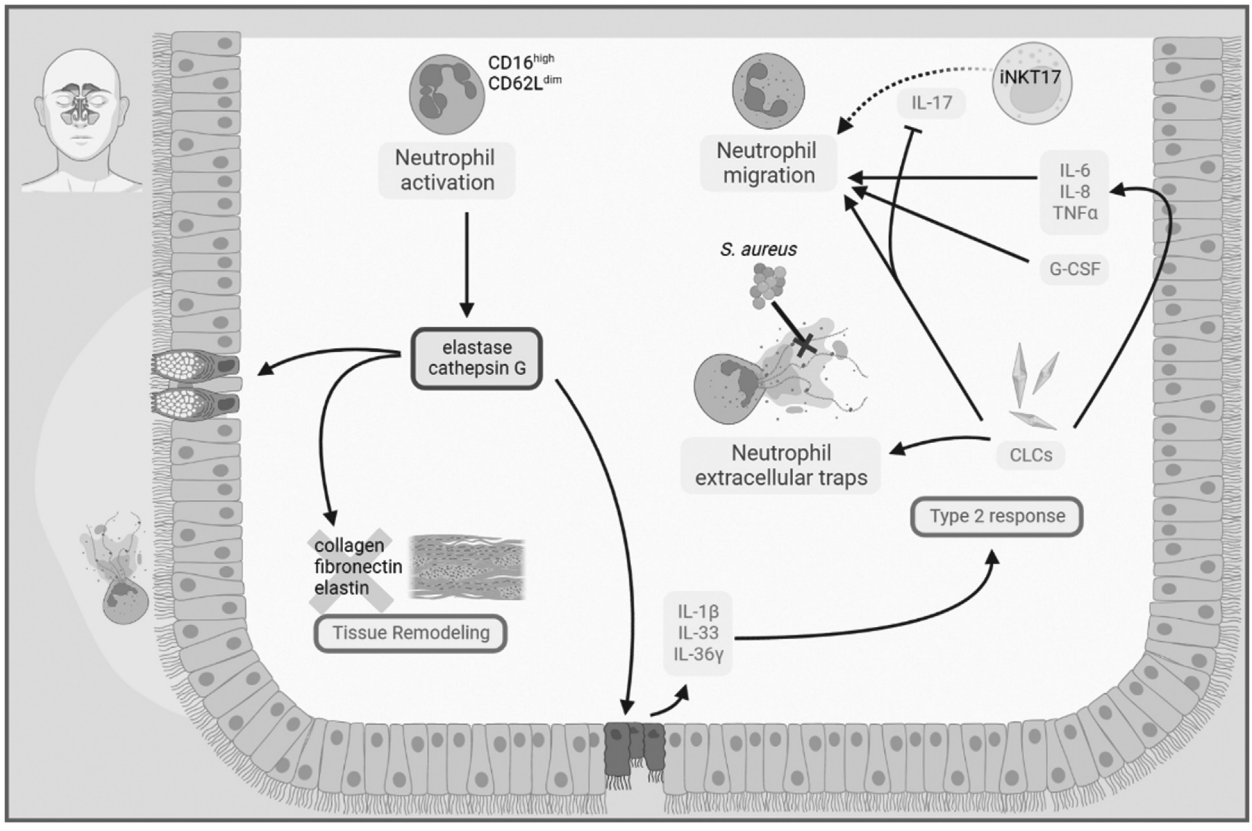


**FIGURE 1.** Contribution of neutrophilic inflammation to the pathophysiology of chronic rhinosinusitis. CLCs possibly overrule IL-17 in the recruitment of neutrophils in type 2 CRS, while G-CSF may regulate neutrophilic migration in nontype 2 CRS. Increased neutrophilic activation and proteolytic activity of elastase and cathepsin G cause increased mucus production, tissue remodeling via degradation of collagen, fibronectin and elastin; and release of type 2 promoting cytokines through epithelial cell damage. The type 2 immune response subsequently activates eosinophils and establishes a positive feedback loop between eosinophils and neutrophils in severe type 2 CRSwNP. CLCs, Charcot–Leyden crystals; CRS, chronic rhinosinusitis; G-CSF, granulocyte colony-stimulating factors; iNKT17, type 17 invariant natural killer cells.

The role of NETs on the CRS disorder is still poorly understood. However, it is clear that NETs display a dual role in homeostasis by protecting the host from infectious diseases via killing bacteria, while they are also likely causing pathologic alternations [44]. It was recently shown that dsDNA released during NETosis may directly contribute to the pathogenesis, by inducing a type-2 immune response [52]. In secretions of eosinophilic CRSwNP patients, NETs – in addition to EETs (eosinophil extracellular traps) and CLCs – were found to increase the mucus viscosity, leading to plug formation, hampering mucociliary clearance and eventually airway damage [53]. Elevated production of NETs was also found to be associated with disease severity in cystic fibrosis and chronic obstructive pulmonary disease (COPD) patients; and NETs could have pro-inflammatory effects on macrophages or stimulate tissue remodeling of the extracellular matrix via degradation of elastin, collagen and fibronectin by neutrophilic proteases [42,44,50,54]. Interestingly, it has been shown that NETs aggregate (aggNETs) under high neutrophil densities, and that cytokines and chemokines trapped in these aggNETs are degraded via serine proteases. These findings suggest that aggNETs promote the resolution of neutrophilic inflammation and could prevent exacerbation of chronic inflammations [55]. However, if this is truly the case in CRS remains to be investigated.

## NEUTROPHILIC HETEROGENEITY IN CHRONIC RHINOSINUSITIS

Increasing evidence over the past decade has demonstrated an unexpected phenotypic heterogeneity and functional versatility within the neutrophil population. Multiple neutrophil subsets based on their pro-inflammatory function (N1 vs. N2), increased survival, maturation state and potential to phagocytize and to form NETs were recently described in inflammatory diseases [27,29,38,56–59]. Understanding the heterogeneity of neutrophils in CRS

could help in understanding their contribution across endotypes. So far, subsetting of neutrophils in CRS lead to the identification of IL-9-producing neutrophils and an activated subset of CD16$^{high}$ CD62L$^{dim}$ neutrophils [10▪▪,29,60]. Also in CRSwNP, neutrophils were found to be a major source of oncostatin M. In addition, its role in neutrophil polarization, oncostatin M is also able to impair the epithelial barrier in CRSwNP patients, implying an additional role for neutrophils in impairing barrier function [61]. A majority of those cells also expressed arginase 1, suggestive of a N2 phenotype. IL-33 treatment of neutrophils resulted in a polarization of the neutrophil and also to the elective production of type 2 cytokines, including IL-4, IL-5, IL-9 and IL-13 [62]. In asthma, a subset of CXCR4$^{high}$ neutrophils – prone to go into NETosis, and IL-5Rα-expressing neutrophils have been described [63,64]. However, no evidence for IL-5Rα expression has been found in the tissue of CRS so far.

## NEUTROPHILIC CONTRIBUTION TO CHRONIC RHINOSINUSITIS SEVERITY

Multiple studies reported that the presence of neutrophils in subepithelial regions of nasal polyps is associated with refractory CRS [65–69,70▪]. Markers of severe or moderate neutrophilic inflammation were associated with elevated levels of IL-8 and high proportions of difficult to treat CRS [71]. Recent studies proposed the delta neutrophil index (DNI) as a useful early predictor for determining the need for surgical intervention in patients with CRS [72]. Increased concentrations of neutrophil-derived MMP-9 had a negative impact on the patient's quality of life and increased the time of healing and regeneration of tissues after endoscopic sinus surgery [73]. Moreover, the concentrations of neutrophilic elastase correlated significantly with CRS-MRI scores [74]. In CRSsNP, there was a significant relationship between neutrophilia and improvement in sleep latency and sleep efficacy after surgery, although this relationship might be circumstantial [75]. In Asia, difficult-to-treat CRS had higher glutathione disulfide levels, which correlated positively with IL-8 in the tissue [76]. However, other studies showed that only increased eosinophilic infiltration and levels of IL-5, and not IL-8 or neutrophilic infiltration correlated with long-term recurrent CRSwNP [77]. Recurrence in CRSwNP patients correlate with the ratio ECP/myeloid peroxidase (MPO) in nasal polyp tissue and peripheral eosinophil/neutrophil ratio, more likely to be attributed to excessive levels of ECP as decisive factor in this balance [78,79]. Other studies find no association between SNOT-22 scores with neither mucosal eosinophilia nor neutrophilia [80].

## BIOMARKERS FOR NEUTROPHILIC CHRONIC RHINOSINUSITIS

As it is becoming clear that neutrophils might have a true impact on CRS inflammation, recent studies tried to identify neutrophil biomarkers that could ease potential future therapeutics. A recent study evaluated trace elements and mineral status in association with mucociliary status and found that increased Se in the hair was associated with the number of neutrophils in nasal mucosa biopsies [81▪]. Increased levels of calprotectin in nasal secretions were associated with increased neutrophil presence in CRSwNP. Interestingly both concentrations of calprotectin and numbers of neutrophils were higher in patients who previously underwent at least three times functional endoscopic sinus surgery (FESS) [82]. Hypoxia-inducible factor 1α is also associated with neutrophilic inflammation in CRSwNP [83]. Another Asian study, however, showed that the clinical characteristics, blood cellular and biological markers could not effectively distinguish between eosinophilic or neutrophilic responses in CRSwNP [84]. Artificial intelligence for cellular endotyping of nasal polyps via whole-slide imaging was proposed as a promising technique to possibly evaluate patients' cellular endotype [85].

## TREATMENT STRATEGIES FOR NEUTROPHILS IN CHRONIC RHINOSINUSITIS

Glucocorticosteroids do target type 2 inflammatory responses better than nontype 2 responses; however, glucocorticosteroid (GCS) resistance has been observed even in patients with type 2 CRSwNP [15,86]. Despite improvements of patients' symptoms upon treatment with GCS, neutrophil-negative polyps had significantly greater reductions in bilateral polyp scores, nasal congestion scores and total symptom scores, compared with neutrophil-positive patients [15]. In addition, the use of topical steroids did not affect the neutrophil activation state in CRSwNP, reflected by the unaltered expression of CD16, CD62L, CD11b or ICAM-1, and did not influence NET formation [29,47,87,88]. GCS were even reported to prevent apoptosis of neutrophils and to promote neutrophilic inflammation [89,90].

The appearance of neutrophils might thus affect the treatment outcome of CRS patients and might be troublesome in specific CRS endotypes. More thorough studies are necessary to identify immunopathological patterns associated with neutrophilic inflammation and to unravel the driving factors of neutrophilia in CRS. These studies could provide a

crucial notion of potential interesting targets to interfere with the chronic neutrophilic inflammation in CRS. On the basis of current knowledge, a different therapeutical approach will be required to target neutrophils in CRSwNP compared with CRSsNP; and it will be more relevant to focus on CRSwNP. Because of its relationship with severe eosinophilic type 2 immune response is this patient group, it might be interesting to not focus on targeting the neutrophilic inflammation alone but rather consider targeting both the eosinophilic and neutrophilic inflammation as a whole [91]. Considering the neutrophilic inflammation on its own, it might be more relevant to focus on inhibiting neutrophil activation rather than reducing numbers of tissue neutrophils itself via inhibiting production, apoptosis or chemotaxis, as we did not observe decreased apoptosis or increased migration of neutrophils in nasal polyps. Neutrophil-specific therapeutics might be more relevant to use as add-on therapies in addition to the current biologicals against the eosinophilic type 2 inflammation in nasal polyposis. (Table 1).

**Table 1.** Summary of therapeutics against neutrophilic inflammation in chronic rhinosinusitis

| Therapeutics | Effects on neutrophilic inflammation | Positive/ promising in clinic |
|---|---|---|
| Glucocorticosteroids | No effect on activation and NETosis | No |
| | Prevent apoptosis | |
| Macrolides | Reduce IL-8 and ICAM expression | Yes |
| | Induce apoptosis | |
| Doxycycline | Impairs migration | Yes |
| | Induces apoptosis | |
| | Reduces MPO levels | |
| 17,18-EpETE | Inhibits production of IL-6, IL-8 | N/A |
| PF-1355 | Inhibitor of MPO | No |
| AZD9668 | Neutrophil elastase inhibitor | Yes |
| Nebulized heparin | Inhibits elastase, cathepsin G | Yes |
| | Blocks P-selectin and L-selectin | |
| Antigal-10 | Dissolution of CLCs | Yes |

CLCs, Charcot–Leyden crystals; NET, neutrophil extracellular traps.

There are conflicting data concerning the efficacy of macrolides in CRS. Macrolides are capable of reducing the expression of IL-8 and ICAM, and decreasing the bacterial load and biofilm formation and could, as such, diminish initial neutrophil recruitment in response to bacterial infection [92]. In addition, they induce neutrophil apoptosis, and long-term treatment with clarithromycin was shown to decrease IL-8 levels in Chinese CRSsNP patients, implying to interfere with recruitment [93]. Another antibiotic, doxycycline can impair neutrophil migration, induce apoptosis and modulate the oxidative burst of neutrophils [94,95]. In a randomized double-blind, placebo-controlled, multicenter trial on CRSwNP patients, doxycycline showed a moderate effect on nasal polyp score and symptoms for 12 weeks and significantly reduced levels of MPO, ECP and MMP-9 in nasal secretions [96]. Another study found that doxycycline had a beneficial role especially in patients without asthma, NERD or high levels of serum IgE before treatment [97]. Further considerations for dosing, duration of treatment and important side effects of macrolides and doxycycline have recently been reviewed by Lees *et al.* [98].

As we found that the neutrophilic inflammation in severe type 2 CRSwNP was independent of IL-17, therapeutics blocking the IL-17 pathway would most likely not be relevant in this patient group. Even in asthma, where IL-17 is known to play a role, clinical studies on IL-17-neutralizing antibodies did not prove to be effective or were terminated early. Also clinical trials with anti-TNFα in asthmatics were not convincing [99]. However, as we found the regulation of neutrophils in CRS to be different than in asthma, it might be interesting to perform clinical trials to antagonize IL-6, IL-8 or G-CSF on CRS patients, if these mediators are showed to be important. 17,18-Epoxyeicosatetraenoic (17,18-EpETE) inhibits TNFα-induced production of IL-6, IL-8 and mucin, and is, therefore, proposed as potential therapeutic approach for mucus hypersecretion and neutrophilic inflammation in nasal mucosa [100]. Treatment with intranasal recombinant IFNγ reduced neutrophilic activation in CRSwNP [101]. Of course, because of heterogeneity among CRS patients, it is likely that mediator-specific therapies will only be valuable for a subgroup of patients. Targeting neutrophilic activation locally instead of systemically could also reduce the risk of neutropenia.

Blocking the damaging capacity of neutrophils in CRS can be achieved by targeting its granule enzymes MPO or the serine proteases elastase and cathepsin G. PF-1355, a selective inhibitor of MPO was efficient in attenuating tissue injury in alveolitis [102,103]. A 4-week phase II trial with a neutrophil elastase inhibitor (AZD9668) showed clinical improvements in patients with bronchiectasis

[104]. Heparin inhibits neutrophilic elastase, cathepsin-G, blocks P-selectin and L-selectin; nebulized heparin treatment improved the outcome for patients with COPD and asthma [105]. Both MPO and elastase are also crucial mediators in NET formation, so these therapies may interfere with neutrophilic inflammation on several levels. NADPH oxidase, PAD 4 and Gasdermin D are also identified as crucial mediators in NET-formation, but their therapeutic utility has not been tested so far [103].

A study in mice could prevent CLC-evoked neutrophil infiltration by gal10-antibody treatment, causing the dissolution of CLCs [106]. Although this is an interesting approach, appropriate studies are needed to test this hypothesis in CRSwNP patients. In addition, it is unclear if and how neutrophilia contributes to clinical disease, and how it is affected by treatment targeting specific type 2 mediators, like anti-IL5(Rα) and anti-IL4/IL13R.

## CONCLUSION

Evidence is increasing that neutrophils are more than a bystander in CRS inflammation. We here summarized their possible involvement in the chronicity of the disease and reviewed their association with disease severity. As publications on neutrophils in CRS are increasing in both Asian and Western countries, we also discussed the therapeutic options to target neutrophilic inflammation in CRS.

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### Conflicts of interest

*C.B. is has received research funding and/or is a consultant for Sanofi, Regeneron, Genzyme, Novartis, and GSK. T.D. declares that he has no relevant conflicts of interest.*

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