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Integrating phenotypes and endotypes in chronic rhinosinusitis: a combined clinical and experimental approach.

Approccio sinergico clinico-sperimentale per l'integrazione di fenotipo ed endotipo nella rinosinusite cronica.

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Abstract [italiano]

La rinosinusite cronica rappresenta un argomento ampiamente dibattuto nel panorama scientifico rinologico internazionale. Pur essendo sotto i riflettori da quasi 40 anni, le innumerevoli teorie eziopatogenetiche che si sono avvicendate negli anni non sono altro che la dimostrazione che si tratti di un disordine complesso.

Siamo ormai giunti al momento in cui i principi e i criteri che hanno definito la rinosinusite cronica negli anni passati sono messi in discussione. La relativamente recente classificazione dicotomica di rinosinusite cronica con e senza polipi nasali si è dimostrata nel tempo troppo semplicistica per spiegare completamente le manifestazioni cliniche della malattia e i meccanismi patogenetici sottostanti.

Questa premessa impone diverse considerazioni.

Dal momento che lo stesso fenotipo possa essere espressione di meccanismi patogenetici sostanzialmente diversi e fenotipi diversi l'espressione dello stesso meccanismo, un approccio terapeutico “one-size-fit-all” si è rivelato insufficiente in una porzione non trascurabile di pazienti. Allo stesso modo, le sperimentazioni cliniche sugli interventi terapeutici soffrono intrinsecamente dello stesso bias di selezione. Ciò si traduce in studi con risultati eterogenei e discordanti e metanalisi spesso deboli, incapaci di generalizzarli.

Oltretutto, nel tentativo di dare un taglio classificativo ad un livello biomolecolare, un approccio diagnostico e prognostico limitato esclusivamente a parametri clinici, oggettivi e soggettivi, fallisce inevitabilmente. Tuttavia, ad oggi, non sono ancora disponibili altri marcatori più efficaci per monitorare l'andamento della malattia.

Trattandosi di una patologia apparentemente molto frequente, responsabile di un significativo disagio sulla qualità della vita ed un impatto economico considerevole, si rende quanto più necessaria un'appropriatezza diagnostica e terapeutica per un'adeguata allocazione delle risorse nell’ambito degli standard

della medicina di precisione. E questo è particolarmente vero per i pazienti refrattari che sono spesso sottoposti a trattamenti ripetuti con risultati incerti.
Inoltre, la definizione biomolecolare dei sottotipi di rinosinusite cronica è purtroppo ancora nelle sue fasi iniziali per poter garantire una solida traslazione nella pratica clinica e, sebbene gli sforzi siano intensi, gli endotipi proposti sono ampiamente sovrapposti e mancano di biomarcatori affidabili. Allo stesso modo, le terapie biologiche, introdotte per soddisfare la necessità di trattamenti più efficaci e mirati, anche se promettenti, arrivano in un momento in cui le fondamenta non sono ancora state gettate.
Tutti questi aspetti critici attuali riguardanti la rinosinusite cronica saranno discussi in dettaglio nella presente dissertazione.
Resta il fatto che c'è ancora molto lavoro da fare nel campo della rinosinusite. E certamente, lo sviluppo di sistemi per l'archiviazione e la condivisione uniforme delle esperienze di ciascun centro rinologico (in particolare per quanto riguarda i casi refrattari, l'aderenza terapeutica e gli effetti delle nuove terapie) potenzierebbe gli sforzi della comunità scientifica nella definizione di percorsi di cura integrati e mirati.

Studio #1

La rinosinusite cronica (CRS) rappresenta un argomento dibattuto nella letteratura rinologica internazionale a causa della sua alta prevalenza, dell'eterogeneità delle manifestazioni cliniche e della difficoltà a predire l'andamento della malattia. Recentemente l'attenzione della ricerca nella CRS si è spostata verso l'identificazione di sottotipi biologici che possano giustificare l'eziologia e la variabilità clinica. Tuttavia, queste analisi risultano ancora costose e limitate nell'impiego per scopi di ricerca, per cui non applicabili su larga scala e nella pratica clinica quotidiana. Per questo motivo ci siamo domandati se fosse possibile ottenere una stratificazione del paziente rinosinusitico solo sulla base di indagini di primo livello. L'eterogeneità intrinseca della malattia ci ha messo di fronte ad una vasta quantità di dati obbligandoci a trovare strategie di

archiviazione addizionali. Presentiamo quindi il frutto del nostro lavoro, il RhinoBank, principalmente per due motivi. Crediamo che sia uno strumento di facile impiego a disposizione di chiunque tratti questa patologia ed un sistema efficace da sfruttare nella ricerca clinica.

Studio #2

Studi in letteratura hanno evidenziato che il punteggio basale del Sinonasal Outcome Test 22 (SNOT-22) influenza l'outcome chirurgico nella rinosinusite cronica (CRS) ed hanno suggerito che un approccio SNOT-22-mediato potrebbe migliorare la comprensione delle aspettative dei pazienti dopo il trattamento. Il presente studio mirava a verificare questa ipotesi in una popolazione italiana di CRS. In 457 pazienti con CRS, trattati con chirurgia endoscopica endonasali dopo fallimento della terapia medica massimale, sono stati calcolati la percentuale di raggiungimento della differenza minima clinicamente rilevabile (MCID) e la percentuale di miglioramento relativo dopo l'intervento chirurgico. Inoltre, è stato studiato l'impatto di diversi fattori sul punteggio dello SNOT-22 preoperatorio e postoperatorio. Il miglioramento dei sintomi si è verificato nella maggior parte dei pazienti ed era direttamente proporzionale alla SNOT-22 basale. Il 79,7% dei pazienti ha raggiunto l'MCID e la percentuale di miglioramento relativo è stata del 50,1%. Le implicazioni psicologiche e sociali hanno influenzato significativamente i punteggi dello SNOT-22. Un'analisi di regressione multipla ha mostrato che la storia di precedenti interventi chirurgici, asma, score endoscopo preoperatorio e SNOT-22 basale hanno statisticamente predetto il punteggio dello SNOT-22 postoperatorio ($R2 = 0,229$). Sottoporre i pazienti con CRS a SNOT-22 prima dei trattamenti chirurgici potrebbe quindi aiutare ad informarli sui probabili esiti, sebbene sia fortemente influenzato dalla percezione individuale. Sono necessari ulteriori studi per identificare un set efficace di parametri soggettivi e oggettivi per la valutazione dei risultati.

Studio #3

La rinosinusite cronica (CRS) deriva da un ampio spettro di meccanismi infiammatori. La discriminazione tra profilo eosinofilo e non eosinofilo dell'infiammazione rappresenta, se non altro, un approccio di endotipizzazione di prima linea. Lo scopo dello studio era di verificare il grado di correlazione tra diversi metodi di quantificazione di eosinofilia tissutale. 33 pazienti con CRS sottoposti a chirurgia endoscopica endonasale e 30 controlli sottoposti a interventi chirurgici non-CRS sono stati arruolati. Ogni paziente è stato valutato per le comorbidità cliniche rilevanti. Prelievo di sangue venoso, biopsia nasale su processo uncinato (UP) e citologia nasale standard su turbinato inferiore (IT) sono stati eseguiti per valutare l'infiltrazione eosinofila. Lo scraping del meato medio (MM) è stato aggiunto essendo questa regione anatomica cardine delle manifestazioni della CRS. Le differenze nella conta degli eosinofili nel sangue ($p = 0,0001$), UP ($p < 0,0001$), IT ($p = 0,01$) e MM ($p = 0,0006$) sono risultate statisticamente significative tra casi CRS e controlli. Il test di Spearman ha mostrato una debole correlazione tra conta eosinofila nel UP e nel sangue [$r = 0,34$, $p = 0,006$], una debole correlazione tra conta eosinofila nel UP e IT [$r = 0,30$, $p = 0,017$] e una correlazione moderata tra conta eosinofila nel UP e MM [$r = 0,51$, $p < 0,0001$]. Nessuna differenza statisticamente significativa è stata osservata nell'eosinofilia tissutale (sangue, UP, IT, conteggio degli eosinofili MM) in relazione a diversi parametri clinici e sistemi di scoring. Tuttavia, l'analisi della curva ROC ha predetto la CRS eosinofila con una sensibilità globalmente bassa. È interessante notare che, una volta esclusi i pazienti allergici dall'analisi, la sensibilità è ulteriormente diminuita per il campionamento citologico sull'IT e leggermente aumentata per il prelievo citologico del MM. Lo studio rappresenta un'esplorazione preliminare del ruolo della citologia nasale nella CRS. Sembra che l'esecuzione della citologia nasale nel MM fornisca informazioni più accurate sul grado di eosinofilia tissutale. Sono necessari replicazioni in coorti più ampie per verificare questi risultati e definire soglie accurate.

Scientific work […] It must be done for itself, for the beauty of science,
and then there is always the chance that a scientific discovery
may become, like the radium, a benefit for humanity

From Marie Curie, *The Discovery of Radium,*
Address by Madame M. Curie at Vassar College, May 14th, 1921

1. Basic concepts on chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is defined as a persistent symptomatic inflammation of the nasal and paranasal sinus mucosa, resulting from the interaction of multiple host and environmental factors.

It is one of the most commonly reported diseases, being estimated as the second most prevalent chronic health condition, affecting 12.5% of the United States (US) population [Hamilos DL, 2011], and with an overall prevalence of 10.9% in Europe [Hastan D at al., 2011].

The burden of CRS to society is considerable and related to loss of productivity, office visits and medical expenses. Costs of medical and surgical care for CRS are estimated at about 8.6 billion dollars yearly in the US [Bhattacharyya N, 2011].

CRS has been shown to have a considerable negative impact on several aspect of quality of life (QoL) [Birch DS et al., 2001] and has a greater impact on social functioning than chronic heart failure, angina or back pain [Gliklich RE et al., 1995; Suh JD et al., 2010].

CRS is clinically characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), and facial pain/pressure or reduction/loss of smell. Duration of symptoms has to be longer than 12 weeks, without complete resolution and likely with periodical exacerbations [Meltzer EO et al., 2004]. Nasal endoscopy and sinuses computed tomography (CT) are necessary for objective confirmation of the diagnosis due to quite high false-positive and false-negative rates arising from subjective criteria alone [Bhattacharyya N et al., 2010; Tomassen P et al., 2011].

The widespread adoption of the term “rhinosinusitis” in preference to “sinusitis” indirectly supports the perspective that a variety of noxa brought in through the

airway, or perhaps from the nasopharynx, acts on the nasal mucosa first, with secondary effects, direct and indirect, on the sinus mucosa [Van Crombruggen K et al., 2010]. In a very small percentage of cases, such as dental and iatrogenic sinusitis, this pathway is reversed with processes in the sinus cavity leading to secondary inflammation of the nose. CRS may also, in rare cases, develop secondary to inflammatory conditions intrinsic to the mucosa in the presumed absence of exogenous stimuli (e.g. granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, sarcoidosis). Lastly, CRS may occur in association with distinct host genetic factors (e.g. cystic fibrosis, CF) or systemic immunodeficiency [Fokkens WJ et al., 2012].

In the overwhelming majority of CRS cases, however, the aetiology and pathogenesis remain unclear. Idiopathic CRS has been typically divided into two distinct phenotypes based on endoscopic findings, CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). CRSsNP is more tightly linked to mechanical obstruction of the ostio-meatal complex (OMC), while CRSwNP is generally attributed to a more diffuse mucosal T helper 2 (Th2)-mediated response [Leung RM et al., 2011], though these broad phenotypes do not provide full insight into the potential underlying pathophysiologic mechanisms of CRS. CRS is a complex inflammatory disease with several variants resulting mainly from dysfunctional host-environment interactions [Kern RC et al., 2008]. Different attempts to sub-classify CRS have been based mainly on clinical and histopathological features [Han JK, 2013]. However, last trends support a deeper concept, that is that CRS consists of multiple biological subtypes, or “endotypes”, which are defined by distinct cellular and molecular mechanisms that might be identified by corresponding biomarkers and might differ in therapeutic responses [Akdis CA et al., 2013]. The characterisation of the heterogeneity of the underlying inflammatory process should then define the treatment plan. In other words, a specific kind of medication should be used for a specific kind of sinus inflammation.

Complications of CRS are rare and are largely due to effects on the surrounding bone. Generally, these are far less documented in the literature than those associated with acute infection and inflammation. They include bone erosion and expansion due to mucocoeles or polyps, osteitis and metaplastic bone formation and occasionally optic neuropathy [Fokkens WJ et al., 2012]. In some cases, they may be considered as simply a manifestation of the natural history of the chronic condition. Indeed, osteitis, that is frequently associated with CRS [Lee JT et al., 2006; Videler WJ et al., 2011], should be regarded as part of the pathophysiological process of CRS rather than a real complication. It has been suggested that the irregular bony thickening, resulting from bone remodelling and neo-osteogenesis, is a sign of inflammation of the bone, which in turn might maintain a mucosal inflammation [Kennedy DW et al., 1998].

Medical therapy remains the cornerstone of CRS management and relies on combinations of antibiotics and oral or topical corticosteroids. The most recent medical treatment evidences and recommendations are reported in *Table 1-1* and *1-2* [Fokkens WJ et al., 2012]. While these combinations are often effective in relieving symptoms, at least temporarily, they are rarely curative. In individuals failing to respond to medical therapy, surgical management is required, in the form of endoscopic sinus surgery (ESS), to remove diseased tissue and clear obstructed sinus drainage passages. ESS restores sinus health with complete or moderate relief of symptoms in about 80% of patients with recurrent or medically unresponsive CRS [Senior BA et al., 1998]. Approximately 20% of operated patients fails ESS [Hopkins C et al., 2009], requiring multiple surgeries, and success in revision cases falls to 69.8% [King JM et al., 1994]. Middle turbinate lateralisation, adhesions and scar formation in the middle meatus, an incompletely resected uncinate process and retained ethmoid cells are frequent findings in patients undergoing revision surgery [Ramadan HH, 1999].

Those patients who complain persisting signs and symptoms of CRS, despite technically adequate endoscopic sinus surgery and well-leaned, specific, medical treatment, are considered to have a refractory or difficult-to-treat CRS [Desrosiers

M, 2004]. Many reports point out a role for more radical or extended surgeries in this group of patients, with the aim of reducing the high inflammatory load [Bassiouni A et al., 2012], combined with medical therapies, more determined by the individual experience of the single rhinologic centre/physician than by standardized clinical trial [Fokkens WJ, 2010] (*Table 1-3* and *1-4*). Novel treatment strategies are spreading but their efficacy is still unpredictable and needs to be proven on a large scale.

Table 1–1 Treatment evidence and recommendations for adults with CRSsNP

Therapy	Level	Grade	Relevance
steroid - topical	Ia	A	yes
nasal saline irrigation	Ia	A	yes
bacterial lysate (OM-85 BV)	Ib	A	unclear
oral antibiotic therapy short term < 4 weeks	II	B	during exacerbations
oral antibiotic therapy long term ≥ 12 weeks	Ib	C[%]	yes, especially if IgE is not elevated
steroid - oral	IV	C	unclear
mucolytics	III	C	no
proton pump inhibitors	III	D	no
decongestant oral / topical	no data on single use	D	no
allergen avoidance in allergic patients	IV	D	yes
oral antihistamine added in allergic patients	no data	D	no
herbal en probiotics	no data	D	no
immunotherapy	no data	D	no
probiotics	Ib(-)[§]	A(-)[°]	no
antimycotics - topical	Ib(-)[§]	A(-)[°]	no
antimycotics- systemic	no data	A(-)[°]	no
antibiotics - topical	Ib(-)[§]	A(-)[°]	no

[Adapted from Fokkens WJ et al., 2012]

Some of these studies also included patients with CRSwNP
Acute exacerbations of CRS should be treated like acute rhinosinusitis
[§] Ib(-): Ib study with a negative outcome
[°] A(-): grade A recommendation not to use
[%] Level of evidence for macrolides in all patients with CRSsNP is Ib and strength of recommendation C, because two double-blind placebo-controlled studies are contradictory; indication exist for better efficacy in CRSsNP patients with normal IgE (recommendation A). No randomized clinical trials exist for other antibiotics

Table 1–2 Treatment evidence and recommendations for adults with CRSwNP

Therapy	Level	Grade	Relevance
topical steroids	Ia	A	yes
oral steroids	Ia	A	yes
oral antibiotic therapy short term < 4 weeks	Ib and Ib(-)[§]	C[#]	yes, small effect
oral antibiotic therapy long term ≥ 12 weeks	III	C	yes, especially if IgE is not elevated, small effect
capsaicin	II	C	no
proton pump inhibitors	II	C	no
aspirin desensitisation	II	C	unclear
furosemide	III	D	no
immunosuppressants	IV	D	no
nasal saline irrigation	Ib, no data in single use	D	yes for symptomatic relief
topical antibiotics	no data	D	no
anti IL-5	no data	D	unclear
phytotherapy	no data	D	no
decongestant topical / oral	no data in single use	D	no
mucolytics	no data	D	no
oral antihistamine in allergic patients	no data	D	no
antimycotics - topical	Ia(-)[%]	A(-)[°]	no
antimycotics - systemic	Ib(-)[§]	A(-)[°]	no
anti leukotrienes	Ib(-)[§]	A(-)[°]	no
anti-IgE	Ib(-)[§]	A(-)[°]	no

[Adapted from Fokkens WJ et al., 2012]

Some of these studies also included patients with CRSsNP

[#] Short term antibiotics show one positive and one negative study, therefore recommendation C

[§] Ib(-): Ib study with a negative outcome

[%] Ia(-): Ia level of evidence that treatment is not effective

[°] A(-): grade A recommendation not to use

Table 1–3 Treatment evidence and post-operative recommendation for adults with CRSsNP

Therapy	Level	Grade	Relevance
steroid - topical	Ia	A	yes
nasal saline irrigation	Ia	A	yes
nasal saline irrigation with xylitol	Ib	A	yes
oral antibiotic therapy short term < 4 weeks	II	B	during exacerbations
nasal saline irrigation with sodium hypochlorite	IIb	B	yes
oral antibiotic therapy long term ≥ 12 weeks	Ib	C[%]	yes, especially if IgE is not elevated
nasal saline irrigation with baby shampoo	III	C	no
steroid - oral	IV	C	unclear
antibiotics - topical	Ib(-)[§]	A(-)[°]	no

[Adapted from Fokkens WJ et al., 2012]

Some of these studies also included patients with CRSwNP

[§] Ib(-): Ib study with a negative outcome

[°] A(-): grade A recommendation not to use

[%] Level of evidence for macrolides in all patients with CRSsNP is Ib and strength of recommendation C, because two double-blind placebo-controlled studies are contradictory; indication exist for better efficacy in CRSsNP patients with normal IgE (recommendation A). No randomized clinical trials exist for other antibiotics

Table 1–4 Treatment evidence and post-operative recommendation for adults with CRSwNP

Therapy	Level	Grade	Relevance
topical steroids	Ia	A	yes
oral steroids	Ia	A	yes
oral antibiotic therapy short term < 4 weeks	Ib	A	yes, small effect
anti IL-5	Ib	A	yes
oral antibiotic therapy long term ≥ 12 weeks	Ib	C[%]	yes, only when IgE is not increased
oral antihistamines in allergic patients	Ib	C	unclear
furosemide	III	D	no
nasal saline irrigation	no data	D	unclear
anti leukotrienes	Ib(-)[§]	A(-)[°]	no
anti-IgE	Ib(-)[§]	C[#]	unclear

[Adapted from Fokkens WJ et al., 2012]

Some of these studies also included patients with CRSsNP

[%] Level of evidence for macrolides in all patients with CRSsNP is Ib and strength of recommendation C, because two double-blind placebo-controlled studies are contradictory; indication exists for better efficacy in CRSsNP patients with normal IgE (recommendation A). No randomized clinical trials exist for other antibiotics

[§] Ib(-): Ib study with a negative outcome

[°] A(-): grade A recommendation not to use

[#] Because positive level III evidence

2. Unmet needs in chronic rhinosinusitis

Chronic rhinosinusitis represents a hot and debated topic in rhinology. Despite being in the limelight since almost 40 years, in trying to get to the bottom of the issue, the impression is that, at times, there is no rhyme or reason.

We have now reached the point where the principles and criteria that defined chronic rhinosinusitis in the past years are questioned. The quite recent dichotomic classification of CRS with and without nasal polyps has proved to be too simplistic to fully explain CRS manifestations and the underlying pathogenetic mechanisms. This premise opens several issues.

Being either the same phenotype expression of substantially different pathogenic mechanisms or different phenotypes the expression of the same mechanism, a one-size-fit-all therapeutic approach turned out to be insufficient in a non-negligible proportion of patients. Similarly, clinical trials on therapeutic interventions intrinsically suffer from the same selection bias for treatment. This results in studies with heterogeneous and discordant outcomes and often weak meta-analysis unable to generalize them.

Moreover, considering the attempt of giving a classification cut at a biomolecular level, a diagnostic and prognostic approach exclusively limited to subjective and objective clinical parameters is inevitably failing in many ways. However, to date no other more effective markers are available to monitor the trend of the disease.

The fact of dealing with an apparently very frequent pathology responsible for a strong discomfort on the QoL and a substantial economic impact requires a diagnostic and therapeutic appropriateness for an adequate allocation of resources within the standards of precision medicine. And this is especially true for uncontrolled patients who are often subjected to repeated and undetermined treatments.

Besides, the biomolecular definition of CRS subtypes is still in its early stages to guarantee a solid translation into clinical practice and, although the efforts are intense, the proposed endotypes are widely overlapping and lack reliable biomarkers. Equally, biological therapies, introduced to meet the need for more effective and targeted treatments, albeit promising, come at a time when the foundations have not been laid, yet.

All these current critical aspects concerning CRS will be discussed in detail in the chapters to follow.

It remains that there is still a lot of work to be done in the field of rhinosinusitis. And certainly, the development of systems for a uniform archiving and sharing of the experiences of each rhinological center (especially with regard to refractory cases, adherence to treatments and outcomes of novel therapies) would enhance the efforts of the scientific community in defining integrated and targeted care pathways.

2.1. Epidemiology and burden of chronic rhinosinusitis

Assessing the impact of CRS on society is a demanding endeavor. One must estimate the proportion of a given population affected by CRS and simultaneously evaluate the degree to which individual health and well-being are influenced. Although CRS is clinically characterized by sinonasal symptoms, the impact on each individual is likely more comprehensive, which involves additional health-related domains. Emerging trends in clinical research are beginning to elucidate not only the extent and the reach of the symptomatology of CRS but also which symptoms are most bothersome or most valued by the individual and the society [DeConde AS et al., 2016].

Epidemiology – CRS is considered a common disease with an estimated prevalence of 10-15% [Halawi AM et al., 2013]. The question remains whether this often-quoted prevalence is indeed an accurate approximation. Being able to estimate CRS prevalence in a reliable and reproducible way has its value because it would allow a more adequate allocation of resources and would provide a measure for the impact of time and regional variations on the disease. Understanding the real prevalence of CRS has become a challenge for several reasons. First, systematic diagnostic criteria for CRS have only been developed relatively recently [Fokkens W et al., 2007; Rosenfeld RM et al., 2007]. Thus, older epidemiological studies are barely specific and hard to compare. Notwithstanding that a correct diagnosis of CRS is based on the combination of both symptomatic criteria and objective features of tissue inflammation, most of the investigations aimed at defining the prevalence of CRS are carried out only through surveys with questionnaires. And that is because objective measures are not always applicable on a large scale. However, a purely symptoms-based diagnosis, while showing a wide sensitivity, risks having a high false-positive rate

(due to the overlap of CRS symptoms with other diseases equally common, such as allergic rhinitis) leading to an overestimation of the nosological picture.

National surveys are benchmark tools for estimating disease prevalence across a society as they use complex sampling methods and large sample sizes to increase generalizability. Results recorded in the US by the National Health Interview Survey (prevalence of 12.1%) [Blackell DL et al., 2014] and in Canada by the National Population Healthy Survey (prevalence of 4.5%) [Chen Y et al., 2003], obtained on the basis of a diagnosis of sinusitis provided by a health care in the previous year, show a wide discrepancy however; this may be related to several factors such as the lack of distinction between acute and chronic sinusitis, the different accessibility to health care and the variable diagnostic threshold of physicians. In Europe, the prevalence esteem through the Global Allergy and Asthma European Network (GAL2EN) in agreement with the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS), which includes the criterion that symptoms should last for over 12 weeks, reaches 10.9% (with a regional variation range from 6.9% to 27.1%) [Hastan D et al., 2011] (*Figure 2-1*). Again, diagnoses were not confirmed by the objective findings of sinus inflammation and therefore an overestimation cannot be excluded.

To increase the specificity of CRS diagnosis, guidelines recommend objective radiographic or endoscopic confirmation. In fact, it was shown that in a group of subjects with self-diagnosed CRS only in 2% CRS was actually confirmed [Eross E et al., 2007]. Another study based on International Classification of Diseases (Ninth Revision) codes found that only 1.96% of subjects received the diagnosis of CRS in a single year [Shashy RG et al., 2004]. Another report demonstrated that up to 30% of subjects complaining of subjective symptoms had no radiological evidence of CRS disease [Bhattacharyya N, 2006]. All these results suggest that relying only on patient reporting might lead to an exaggerated prevalence estimate but conclusions remain speculative. Indeed, all these studies report on geographically isolated patient populations, 1-3 times smaller than survey-based investigations and with potential observation and selection bias. The

follow-up study to the GAL2EN survey complicates the discussion even further [Lange B et al., 2013]. An otorhinolaryngology evaluation was performed on respondents of the original study and 30% of the CRS diagnoses were overturned by an otolaryngologist. Curiously, 15% of patients who initially did not meet the criteria of the questionnaire were diagnosed with CRS by the otolaryngologist, resulting in a net increase in the number of overall CRS diagnoses, which could reflect the potential for minimization of symptoms or objective disease (e.g. polyps) without symptoms.

In the light of these observations, it is likely that the true prevalence estimate of CRS will probably remain imperfect, with a range of findings across studies, and that should be considered with a grain of salt around 10-12%.

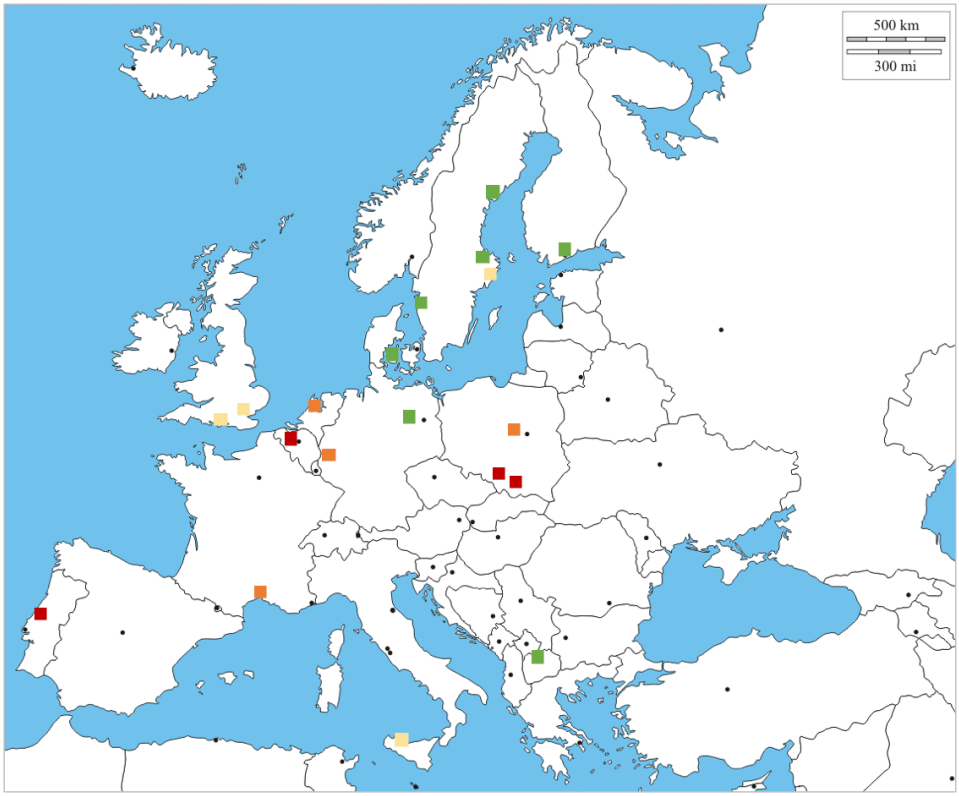

Figure 2-1 Prevalence of self-reported CRS by EPOS criteria throughout Europe
Mean prevalence was estimated at 10.9% (green <9%; yellow 9-12%; orange 12-15%; red >15%). [Adapted from Hastan D et al., 2011]

Burden to individual – The clinical burden of CRS on an individual can be debilitating. Physicians have always focused on cardinal symptoms, such as nasal congestion, purulent discharge, hyposmia and pain/facial pressure to establish a correct diagnosis and to monitor treatment outcomes. However, CRS produces a much broader range of troubles, that extend beyond the sinonasal symptoms, compromising the well-being status. Recent evidence supports that extra-rhinologic symptoms critically impact CRS patients' QoL. QoL has become an important end point in clinical studies because it quantifies the impact of the disease on daily functioning, indeed [Meltzer EO et al., 2004].

Sinus-specific QoL tools are composite questionnaires that aim to capture all the debilitating aspects of CRS. Some of these instruments are stratified into domains, others are summed into one cumulative score. The rationale behind domains is to measure more than a single aspect of health.

Major emerged complaints from researches on CRS impact on QoL were sleep impairment, cognitive dysfunctions and an overall decline in performances.

Poor sleep at night is a recurring issue in CRS patients and typically the subjective severity of CRS is closely related to sleep quality. Examination of the Sinonasal Outcome Test 22 (SNOT-22) showed that patients, in whom CRS impacted on the quality of sleep and on the psychological sphere, more frequently elect surgical intervention over medical therapy [DeConde AS et al., 2014]. Similarly, the close correlation between sleep quality and CRS burden emerges by applying other non-CRS-specific questionnaires (e.g. Pittsburg Sleep Quality Index) to the point that CRS impact on sleep is comparable to that of obstructive sleep apnea syndrome (OSAS) and higher than that of narcolepsy [Alt JA et al., 2013]. Sinus surgery has been proven to guarantee significant improvement in both sleep quality and fatigue/body pain, other often complained symptoms by CRS patients [Alt JA et al., 2014; Chester AC et al., 2008].

Less attention has been given so far to cognitive disorders such as poor concentration and altered memory. A recent case-control study, performed using the Cognitive Failure Questionnaire, has shown that cognitive functions of CRS

patients are frankly worse than those of the control population. However, studies on the subject are still scarce and the mechanisms that correlate the inflammatory sinusal status with cognitive function still require further investigations [Soler ZM et al., 2015].

Burden to society – The relative high prevalence of CRS, coupled with significant symptoms and QoL impact, leads to the assumption that CRS represents also a burden to society. Understanding the relative impact on society determined by the CRS requires a comparison with other types of illnesses through a common meter. Health utility value (HUV) is a metric that quantify the way in which an individual assesses his health status with a range from 0 (death) to 1 (perfect health). Determining the HUV of an intervention allows also for calculation of quality-adjusted-life-years (QALY), which represent both the number of years of life that would be added and the quality of those years. QALY are now considered the standard metric for cost-effectiveness research [DeConde AS et al., 2016].

A study by Soler [Soler ZM et al., 2011], which included CRS patients unresponsive to medical therapy electing surgical intervention, highlighted first that baseline HUV was extremely low (0.65) when compared to other medical conditions, such as Parkinson's disease, coronary heart disease, congestive heart failure and chronic obstructive pulmonary disease. Secondly, an improvement by 0.087 in HUV occurred after primary surgery and 0.062 after revision surgery. Considering that 0.03 is the minimum increase that allows to appreciate a benefit on the clinical level, ESS has proved to be an intervention with a strong impact in terms of QALY, even higher than other well-studied interventions, including continuous positive airway pressure for OSAS.

The direct costs of CRS to US society are estimated to be high, at $8.6 billion per year [Bhattacharyya N et al., 2011]. The indirect costs are represented by the loss of productivity, which is measured through absenteeism (missed work), presenteeism (reduced work performance) and lost leisure time due to CRS [Rudmik L et al., 2014]. A preliminary cost-effectiveness analysis showed that

ESS guarantees an economic advantage in terms of direct/indirect costs and a QALY gain of 20.50 (compared to 17.13 of medical therapy) in patients with refractory CRS. These analyses found that with 74% certainty surgery ultimately represents the cost-effective decision over continued medical therapy and that ESS becomes the cost-effective intervention within the third year after surgery [Rudmik L et al., 2015].

2.2. Outcomes assessment in chronic rhinosinusitis

Outcomes evaluation is of utmost relevance in any field of medicine for several aspects, including helping patients in understanding the benefits of a treatment, making physicians responsible for their care, empowering decision-making processes of both patients and caregivers, allowing appropriate allocation of resources and, consequently, improving the health system [Rudmik L et al., 2017]. Moreover, the assessment of treatment results is topical for CRS, as mentioned before, because of the high prevalence, the economic burden on the health system and the important repercussions on QoL of affected patients.

The first line treatment of CRS is primarily based on medical therapy. High-level evidence supports the treatment of CRSwNP with intranasal steroids, oral steroids and saline irrigation, while the common medical regimen recommended for CRSsNP includes intranasal steroids, saline irrigations and antibiotics (e.g. macrolides). ESS is the surgical treatment of choice and is normally reserved for patients who do not respond satisfactorily to medical therapy (approximately 38-51% of patients) [Fokkens WJ et al., 2012].

In recent years, much effort of researchers has been focused on studying parameters that might act as effective evaluation tools to quantify CRS treatment outcomes and, by consequence, able to provide a prognostic value. Therefore, a number of objective and subjective aspects of CRS has been reviewed [Ting F et al., 2018].

Among objective traits, endoscopic and radiologic scoring systems are the most frequently applied, such as the Lund-Kennedy score [Lund VJ et al., 1995] and the Lund-Mackay score [Lund VJ et al., 1993]. Other measurements include those coming from smell and nasal respiratory functional test [Kohli P et al., 2017; Whitcroft KL et al, 2017]. Contrarily, subjective features are represented by the complained symptoms and their impact on QoL. To quantify this aspect, different

symptoms/QoL-based questionnaire were introduced, among which the most common, specific for CRS, are the Rhinosinusitis Task Force (RSTF), the Rhinosinusitis Disability Index (RSDI), the Chronic Sinusitis Survey (CSS) and the SNOT-22 [Sedaghat AR et al., 2018] (*Table 2-1*). Moreover, these questionnaires have been widely used as tools for assessing CRS treatment outcomes. Indeed, it appears from literature that basal preoperative SNOT-22 score is one of the major factors affecting the outcome [Hopkins C et al., 2015] and several studies have shown its prognostic role in terms of improving symptomatology and risk of disease recurrence [Rudmik L et al., 2016].

Despite these questionnaires might surely help in monitoring CRS evolution, due to the chronic nature of the disease and the discrepancy between objectively-tested signs (both endoscopic and radiologic) and reported symptoms [Hopkins C et al., 2015], to date, no universally accepted method of outcomes evaluation exists. Furthermore, SNOT-22 and in general all QoL-questionnaire are based exclusively on subjective parameters, therefore endowed with a high inter-individual variability and influenced by aspects not strictly related to the pathology, such as the patient's psycho-social habitus.

In this context, Hopkins et al. evaluated a list of potential parameters that, once merged, could be used to appropriately evaluate the outcome after CRS treatments. A set of 15 items, over 4 domains, including SNOT-22 repeated over time with some additional questions and the Lund-Kennedy score were defined at the end as adequate to predict the outcome of the disease [Hopkins C et al., 2018] (*Table 2-2*). Other recent studies highlighted a close correlation between symptoms and burden of inflammation (expressed as tissue eosinophilia), suggesting that a high burden of inflammation correlates with a worse symptomatology [Lal D et al., 2018].

More studies are certainly needed to find a simple, practical and effective evaluation tool for CRS, implementing the knowledge of pathophysiological mechanisms underlying the different expressions of this disease. Eventually, this will lead to identify new histopathological-biomolecular markers able to classify

CRS patients into homogeneous subgroups, to enforce endotype-driven therapies and possibly provide an objective parameter of response to treatment.

Table 2–1 Sinonasal Outcome Test 22 (SNOT-22)

	No problem	Very mild problem	Mild or slight problem	Moderate problem	Severe problem	Problem as bad as it can be	5 most important items
1) Need to blow nose	0	1	2	3	4	5	□
2) Nasal blockage	0	1	2	3	4	5	□
3) Sneezing	0	1	2	3	4	5	□
4) Runny nose	0	1	2	3	4	5	□
5) Cough	0	1	2	3	4	5	□
6) Post-nasal discharge	0	1	2	3	4	5	□
7) Thick nasal discharge	0	1	2	3	4	5	□
8) Ear fullness	0	1	2	3	4	5	□
9) Dizziness	0	1	2	3	4	5	□
10) Ear pain	0	1	2	3	4	5	□
11) Facial pain/pressure	0	1	2	3	4	5	□
12) Decreased sense of smell/taste	0	1	2	3	4	5	□
13) Difficulty falling asleep	0	1	2	3	4	5	□
14) Wake up at night	0	1	2	3	4	5	□
15) Lack of a good night's sleep	0	1	2	3	4	5	□
16) Wake up tired	0	1	2	3	4	5	□
17) Fatigue	0	1	2	3	4	5	□
18) Reduced productivity	0	1	2	3	4	5	□
19) Reduced concentration	0	1	2	3	4	5	□
20) Frustrated/Restless/Irritable	0	1	2	3	4	5	□
21) Sad	0	1	2	3	4	5	□
22) Embarrassed	0	1	2	3	4	5	□

[Adapted from Hopkins C et al., 2009]

Table 2–2 Final item list (achieving consensus as essential) for inclusion in Core Outcome Set for CRS and proposed measurement tools.

Domain	Item	Proposed measurement tool
Patient reported symptoms and QoL	Overall symptom severity	SNOT-22 repeated over time
	Frequency of symptoms	
	Duration of symptoms	Additional question required to address frequency of symptoms
	Duration of treatment effect	
	Sense of smell	
	Runny nose / Nasal discharge (anterior or posterior)	
	Nasal obstruction / Blockage / Congestion	
	Disease specific quality of life	
Control of disease	Overall control of disease	Need for systemic medication (steroid or antibiotic)
	Need for surgery	Progression to surgery
	Endoscopic appearance (including presence/quality of pus, presence and size of polyps, edema, crusting, inflammation)	Lund-Kennedy score
Impact on daily activity	Ability to perform normal activities	SNOT-22 (or specific measures of productivity)
Acceptability of treatment and side effects	Compliance with treatment	Measurement of compliance and side effects
	Acceptability of treatment	
	Side effects of treatment (including medical and surgical)	

[Adapted from Hopkins C et al., 2018]

2.3. "Uncontrolled" chronic rhinosinusitis

The definition of uncontrolled CRS patient is certainly complicated. Bousquet at al. introduced the term "severe chronic upper airway disease" (SCUAD) to define those patients with allergic, non-allergic and occupation rhinitis and CRS, whose symptoms are inadequately controlled despite treatment following internationally validated guidelines [Bousquet J et al., 2009]. Precisely, EPOS defines difficult-to-treat CRS as patients who do not reach an acceptable level of control despite adequate surgery, intranasal corticosteroid treatment and up to 2 short courses of antibiotics or systemic corticosteroids in the last year [Fokkens WJ et al., 2012]. Thus, the combination of ongoing symptoms and objective endoscopic finding of mucosal edema, as well as the use of cumulative systemic medications for over 3 months in a year would define an uncontrolled patient (*Table 2-3*). Literature has been reporting for quite some time now that approximately 20% of operated patients respond unsatisfactorily to ESS with concomitant medical therapy and eventually require a revision surgery [Fokkens WJ et al., 2012]. Furthermore, this percentage seems to be even higher according to more recent publications, reaching an average of 40% of CRS patients appearing uncontrolled at 3-5 years after ESS [van der Veer J et al., 2017].

While it is logical to attribute the causes of these failures to a marked inflammatory state, on the other, it is unreasonable to think that all uncontrolled patients are suffering from a severe form of respiratory disease. For this reason, the assessment of uncontrolled patients should consider a set of associated risk factors for refractory disease, that will be discussed in detail below (wrong diagnosis, ineffective therapy, inappropriate therapy, dysfunctional sinus, severe disease) [Sivasubramaniam R et al., 2017]. Investigating these factors obviously requires a much more comprehensive and meaningful work-up that, besides a

thorough history and endoscopic/radiologic examination, includes blood tests and tissue biopsy (*Table 2-4*).

Wrong Diagnosis – This happens when physician accepts that the CRS is a primary condition of the airway without taking into account the potential for broader issues with immunity or associated conditions. This is often a common cause for the uncontrolled CRS patient. Definitely worth noting, among autoimmune diseases, eosinophilic granulomatosis with polyangiitis (EGPA) and hypereosinophilic syndrome should be always assessed in uncontrolled patients with increased eosinophilic count and signs/symptoms of organ involvement [Butt NM et al., 2017], as it will alter the management paradigm. Other rarer disorders that require exclusion in refractory CRS include immunodeficiency (mainly isolated IgA deficiency) [Chiarella SE et al., 2017], ciliary dysfunction (cystic fibrosis, primary ciliary dyskinesia) [Hamilos DL, 2016; Demarco RC et al., 2013], gastro-esophageal reflux disease [Leason SR et al., 2017], severe inhalant allergy (central compartment atopic disease) [DelGaudio JM et al., 2017] and IgG4-related rhinosinusitis [Hanaoka M et al., 2017].

Ineffective therapy – The first-line treatment for CRS considers the use of intranasal corticosteroids associated with nasal saline irrigations and systemic drugs, such as antibiotics. The effectiveness of topical therapy depends on the penetration into the paranasal sinuses and the actual distribution within the sinuses depends in turn on several factors, including anatomy and surgical status, type of delivery device, head position, respiratory cycle and carrier vehicles [Thomas WW 3rd et al., 2013]. Several studies have shown that unoperated patients have inconsistent and very limited sinus distribution regardless of the position of the head, the volume or the type of device used. ESS increases the possibility of penetration into the sinuses [Harvey RJ et al., 2008]; however, more extensive interventions (for example the modified endoscopic Lothrop procedure) are needed to improve irrigation of the frontal sinus [Barham HP et al., 2016].

The type of device used to irrigate and administer topical steroids is just as important because evidence shows that “high volume” irrigations provide better drug distribution. Although the term “high volume” is not precisely defined, > 100 ml devices are shown to have better delivery into the sinuses [Pynnonen MA et al., 2007].

Inappropriate therapy – This predisposes to the use of inappropriate medications for the type of CRS. One of the most common is the use of long-term macrolides in eosinophilic CRS and the use of steroids in steroid-non-responsive CRS (non-eosinophilic CRS). The evidence for the use of systemic corticosteroids is plenty for CRSwNP and only as an option in CRSsNP [Fokkens WJ et al., 2012]. The difficulty arises because eosinophilic CRS can occur in the absence of nasal polyps and still respond very well to the steroid; and vice versa, some forms of non-eosinophilic CRS can occur as CRSwNP and therefore not suitable to respond to steroids [Snidvongs K et al., 2012]. There is also paucity of evidence for the use of topical antibacterials, oral and topical antifungals in the routine CRS. Moreover, the inappropriate use of antibiotics might favor resistant microorganisms and thus account for further treatment failures.

Dysfunctional sinus – Sinuses needs to have an effective mucociliary clearance to allow adequate drainage. Dysfunctional sinuses thus lead to treatment failure and recurrent inflammation. It is well documented that chronic inflammation drives mucosal remodeling which displays at histopathology as thickening of the basement membrane, fibrosis and squamous metaplasia and affects up to 80% of refractory CRS [Snidvongs K et al., 2012]. If some of these mucosal changes might be arrested or reduced by medical treatments, some others are irreversible (such as the loss of ciliary function) leading to ongoing sinus symptoms despite the original inflammation being resolved. Formation of “sumps” in the maxillary and occasionally in sphenoid sinus is another feature observed in uncontrolled patients. Localized edema and purulent discharge are seen through nasal

endoscopy in the floor of the maxillary sinus despite a wide antrostomy or in the sphenoid sinus below the level of the ostial opening. This feature is secondary to dysfunctional sinus mucosa and arduous debridement of sinus floor by high-volume irrigations due to the gravity dependent location (indeed, this feature is not seen in ethmoid or frontal sinuses) [Sivasubramaniam R et al., 2017].

Severe disease – In rare cases, patients do not have any other confounding systemic disorders and have been adequately treated by medical and surgical therapy and still remain uncontrolled; they simply have a severe form of CRS. Indicators of such patients include high tissue and blood eosinophilia, increased IgE (> 1000 IU/L) and difficult-to-control asthma. If an asthmatic patient, with eosinophilic disease, is not well controlled on maximal inhaled corticosteroid, then his upper airway is unlively to be controlled with intranasal steroids, no matter how effectively they are delivered. Similarly, if asthma is requiring large doses of systemic steroids to control, that is a marker that CRS will also be difficult to control [Sivasubramaniam R et al., 2017]. Aspirin-exacerbated respiratory disease (AERD) is an example; it represents a distinct clinical entity with the triad of asthma, aspirin intolerance and CRSwNP and accounts for 9.7% of all CRSwNP patients. The clinical manifestations of CRSwNP and asthma in AERD typically present during the third or fourth decade of life, which contrasts with the early childhood onset of CRSwNP and asthma in aspirin-tolerant patients. Additionally, AERD tends to occur more commonly in patients who do not demonstrate an atopic history and are female. The majority of these cases will exhibit severe asthma and extensive CRS, showing greater resistance to corticosteroids and requiring extensive sinus surgery to control symptoms [Kennedy JL et al., 2016]. Osteitis is another feature of chronic and severe CRS, likely to be the result of neo-osteogenesis and bone remodeling processes [Snidvongs K et al., 2014].

Table 2–3 Assessment of clinical control of CRS

Features	Controlled (all present)	Partly controlled (at least one present)	Uncontrolled (three or more present)
Nasal blockage	Not present or not bothersome	Present on most days of the week	Present on most days of the week
Rhinorrhea/Post-nasal drip	Little and mucous	Mucopurulent on most days of the week	Mucopurulent on most days of the week
Facial pain/headache	Not present or not bothersome	Present	Present
Smell	Normal or only slightly impaired	Impaired	Impaired
Sleep disturbance/fatigue	Not impaired	Impaired	Impaired
Nasal endoscopy (if available)	Healthy or almost healthy mucosa	Diseased mucosa (nasal polyps, mucopurulent secretions, inflamed mucosa)	Diseased mucosa (nasal polyps, mucopurulent secretions, inflamed mucosa)
Systemic medication needed to control disease	Not needed	Need of a course of antibiotics or systemic corticosteroid in the last three months	Need of long-term antibiotics or systemic corticosteroids in the last month

[Adapted from Fokkens WJ et al., 2012]

Table 2–4 Proposed investigations for CRS and the rationale behind them

	Test	Rationale
Primary investigations prior to initial treatment	Full blood count with differentials	Eosinophilia
	ESR and CRP	Broader autoimmune screen
	ImmunoCAP testing (formerly RAST)	Atopic status and fungal antigen assessment for AFRS/ABPA
	Total IgE	Degree of atopic disease
	Mucosal biopsy	Degree of tissue eosinophilia Eosinophil activation: Charcot-Layden/eosinophil aggregates Remodeling changes: squamous metaplasia, fibrosis and BM thickening Fungal hyphae: AFS/ABPA
Secondary investigations for uncontrolled patients	Serum eosinophil	Eosinophil > 0.3 as abnormal Eosinophil to eosinophilic Marker of severe disease Potential for secondary hypereosinophilic syndromes EGPA
	ESR and CRP	Broader autoimmune disorder
	ImmunoCAP analysis	Define atopic status
	Dust, fungal, animal, grasse/tree mix	Define perennial, seasonal, environmental sensitization Fungal antigen for AFRS/ABPA
	Total IgE, IgG, IgM, IgA	Humoral deficiency (CVID and specific deficiency) Degree of atopic disease
	cANCA, pANCA (with MPO and PR3), ENA	Autoimmune disorders (particularly EGPA and GPA)
	ACE	Sarcoidosis

[Adapted from Sivasubramaniam R et al., 2017]

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RAST, radioallergosorbent test; Ig, immunoglobulin; ANCA, anti-neutrophil cytoplasmic antibody; ENA, extracted nuclear antibody; ACE, angiotensin-converting enzyme; AFRS, allergic fungal rhinosinusitis; ABPA, allergic bronchopulmonary aspergillosis; BM, basal membrane; EGPA, eosinophilic granulomatosis with polyangiitis; CVID, Common Variable Immunodeficiency; GPA, granulomatosis with polyangiitis

2.4. Rethinking chronic rhinosinusitis phenotypes

As mentioned, CRS is a broad clinical syndrome that is defined by mucosal inflammation of the nose and the paranasal sinuses. This inflammatory condition is commonly divided into two phenotypes based on the presence or absence of nasal polyps (CRSwNP and CRSsNP) and current treatment regimens are based on this phenotypic classification [Fokkens WJ et al., 2012]. However about 38 to 51% of CRS patients still fail to respond to those recommended therapies [Lal D et al., 2009; Baguley C et al., 2014] and their limitations highlight the clinical variability that characterize CRS as a whole, even within the CRSwNP and CRSsNP phenothypes. Increasing evidence suggests that the heterogeneity in CRS manifestations may be explained by a variety of disparate molecular and cellular pathways that result in the mucosal inflammation of CRS [Akdis CA et al., 2013] (*Figure 2-2* and *2-3*). The improved understandings of different pathophysiologic mechanisms in CRS have allowed for the identification of disease variants as endotypes. While by convention, CRS phenotyping differentiates disease variants with observable clinical features, endotyping relies on immunohistologic biomarkers involved in disease pathophysiology to create defined subtypes. Compared to phenotyping, endotyping provides a more comprehensive approach to classify CRS variants because it emphasizes the upstream pathophysiologic factors that determine and influence the clinical manifestations of disease. The current interest in CRS endotypes draws from prior research effort in asthma, a similarly heterogeneous inflammatory disorder involving epithelium of the lower airways. Over the last decades, the development of asthma endotypes based on the biologic mechanisms of inflammation have not only enhanced descriptive diagnostic schemes but have also streamlined the application of targeted biologic treatments for patients with disease refractory to conventional therapies. Biologic therapy, primarily through monoclonal

antibodies, provides highly effective treatment alternatives for severe and resistant asthma due to the targeting of specific biomarkers and thus the underlying causes of inflammation in certain endotypes. The similarities in inflammatory mechanisms between asthma and CRS have thus raised the possibility of using biologic therapy for certain CRS endotypes.

Four distinct, but overlapping, classification schemes have been proposed for identifying endotypes within the CRSwNP phenotype (Type 2 cytokine-based, eosinophil-based, B cell/IgE-based, cysteinyl leukotriene-based), which will be further discussed in detail. Conversely, very little is known about non-type 2 endotypes. Cytokines, such as IL-17, interferons, TNF-α and IL-22, are also expressed in patients with CRSsNP/CRSwNP and can be associated with specific bacterial stimuli but have not shown clear associations with comorbidities or clinical outcomes. Studies demonstrating a beneficial effect of their antagonism are currently lacking and studies in asthmatic patients with anti-TNF and anti-IL-17 have not shown benefits either [Bachert C et al., 2018].

Type 2 cytokine-based approach – CRS with and without nasal polyps in Western countries have historically been differentiated by distinct inflammatory cytokine profiles. A type 1 inflammation characterized by the presence of neutrophils, elevated IFN-γ and Th1 cells is generally associated with CRSsNP (*Figure 2-2*). A type 2 inflammation, characterized by a high presence of eosinophils, mast cells, basophils, Th2 and comorbid associations with asthma and atopy is evident in approximately 80-85% of western CRSwNP patients (*Figure 2-3*). Within the type 2 inflammatory milieu, multiple cytokines, including IL-5, IL-4, IL-13, have been shown to drive the immunologic pathways central to CRSwNP pathophysiology [Bachert C et al., 2015]. Released by type 2 innate lymphoid cells (ILC2), Th2 cells and mast cells, IL-5 is a common cytokine that coordinates the local influx, maturation and survival of eosinophils.

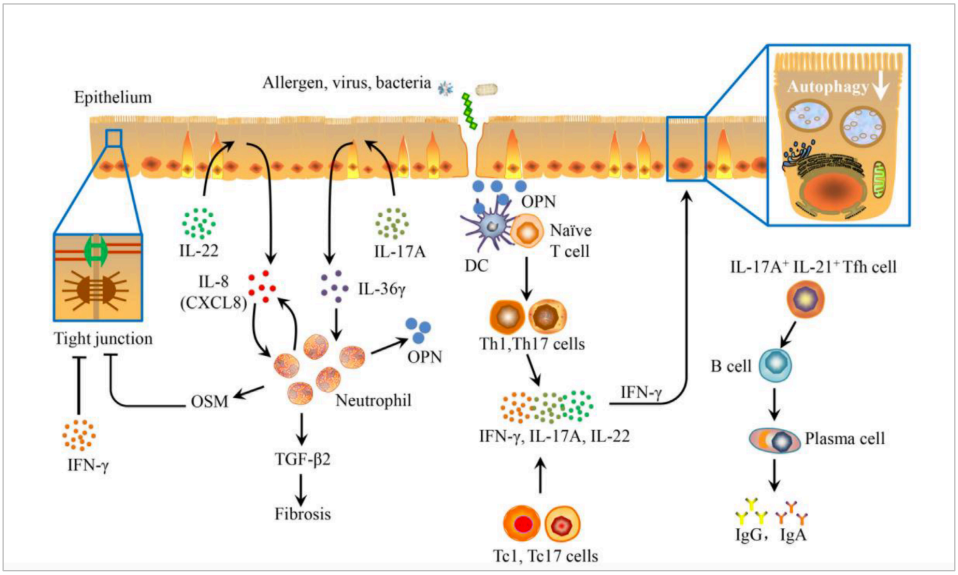

Figure 2-2 Type 1 and 3 immune responses and chronic rhinosinusitis

An overview of the type 1 and 3 immune responses in human chronic rhinosinusitis based on available evidence. In response to environmental stimuli, epithelial cells secrete osteopontin (OPN); OPN-stimulated dendritic cells (DCs) induce type 1 T helper (Th1) and type 17 T helper (Th17) cell differentiation. Th1 and Th17 cells as well as type 1 cytotoxic T (Tc1) and type 17 cytotoxic T (Tc17) cells orchestrate non-eosinophilic inflammation through production of interferon γ (IFN-γ), interleukin (IL)-17A and IL-22. IL-17A upregulates the expression of IL-36γ in epithelial cells, and IL-36γ can act on neutrophils and further exaggerates neutrophilic inflammation by inducing IL-8/CXCL8 production from neutrophils. IL-22 induces epithelial cells to produce IL-8/CXCL8, then IL-8/CXCL8 act on neutrophils. Neutrophils can also produce oncostatin M (OSM), OPN and transform growth factor β2 (TGF-β2). TGF-β2 may be involved in fibrosis. IFN-γ and OSM may disturb epithelial barrier function by diminishing the expression of epithelial cell tight junction proteins. IFN-γ can induce activated but insufficient autophagy, leading to apoptosis of nasal epithelial cells. IL-17A+IL-21+ Tfh cells initiate B cell differentiation into plasma cells that produce IgG and IgA.

[Reprinted with permission from Cao PP et al., 2019]

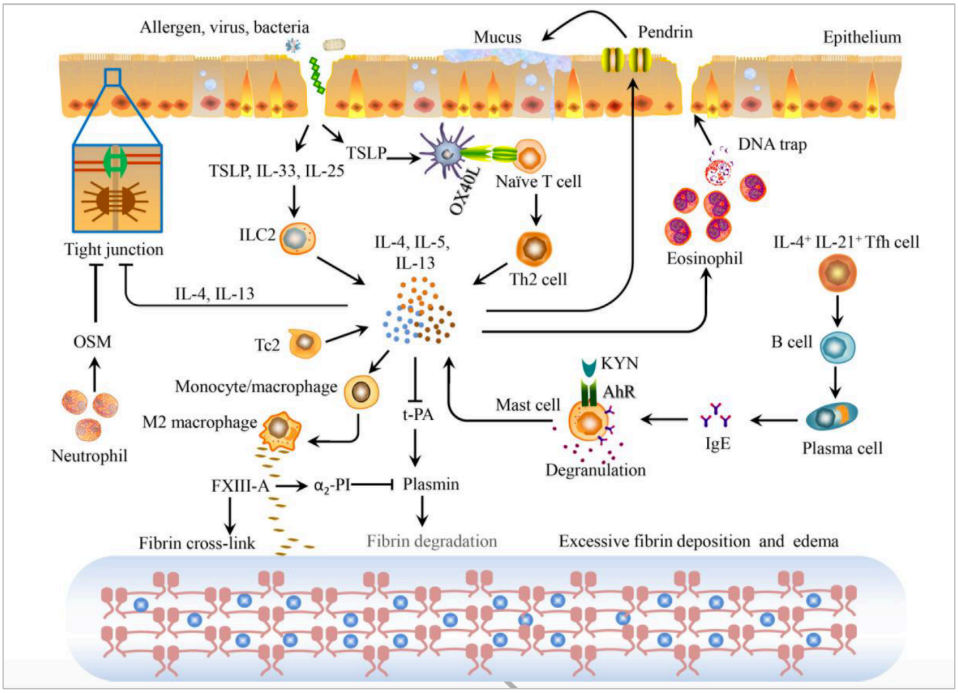

Figure 2-3 Type 2 immune responses and chronic rhinosinusitis

An overview of the type 2 immune responses in human chronic rhinosinusitis based on available evidence. After stimulation with innate immune activating stimuli, cytokines or injurious environmental agents such as proteases, epithelial cells produce thymic stromal lymphopoietin (TSLP), and perhaps in some cases interleukin (IL)-33 or IL-25, which activate type 2 innate lymphoid cells (ILC2s). Epithelial cell-derived TSLP up-regulates OX40L expression on dendritic cells (DCs), then DCs initiate the differentiation of naïve T cells into type 2 T helper (Th2) cells. Th2 cells, ILC2 and type 2 cytotoxic T (Tc2) cells orchestrate eosinophilic inflammation through production of type 2 cytokines. IL-4+IL-21+ T follicular helper (Tfh) cells initiate the differentiation of B cells into plasma cells, then mast cells are activated by IgE which is produced locally by plasma cells; the kynurenine (KYN)/aryl hydrocarbon (AhR) axis may be involved in this process. In addition, mast cells can also produce type 2 cytokines. Th2 inflammation can induce monocytes/macrophage differentiation into M2 macrophages. M2 macrophages produce coagulation factor XIII-A (FXIII-A) that induces excessive fibrin deposition by cross-linking of fibrin and via the antifibrinolytic pathways through binding α2-plasmin inhibitor (α2-PI, also known as a2 antiplasmin) to fibrin. Meanwhile, tissue plasminogen activator (t-PA) levels are reduced in Th2 inflammation, causing impaired plasmin generation, which, in turn, decreases fibrinolysis. These events collectively result in retention of water and formation of edema in polyps. Th2 cytokine-mediated pendrin expression may increase mucus production. The cytokines IL-4 and IL-13 can diminish the expression of epithelial cell tight junction proteins. Neutrophil-derived oncostatin M (OSM) and eosinophil-derived DNA traps may also contribute to epithelium disruption.

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The pivotal role of IL-5 was demonstrated by Tomassen et al., who correlated different phenotypic manifestations of CRS to various patterns of immune cytokines. In this cluster analysis, patients with high levels of IL-5 had the highest prevalence of nasal polyps and asthma. Contrarily, patients with low IL-5 primarily consisted of CRSsNP patients. Patients with moderate levels of IL-5 exhibited variable expressions of nasal polyps and asthma comorbidity. This study suggests that for diagnostic purposes, elevated IL-5 in CRS universally indicates the presence of nasal polyps, whereas nasal polyps do not necessarily indicate an elevation of IL-5 [Tomassen P et al., 2016] (*Figure 2-4*). Similarly, IL-4 and IL-13 are leading cytokines of the type 2 inflammation and share many overlapping functions, such as driving Th2 cells differentiation. As such, IL-4 and IL-13 both actively promote adaptive Th2 response through activation of B cells and the local production of immunoglobulins, especially IgE [Bachert C et al., 2015]. In particular, these patients may exhibit increased local IgE without evidence of elevated systemic IgE levels. Lastly, epithelial cells have been recognized as an active component of the immune response. Beside serving as a physical barrier at the interface between environment and mucosa, epithelial cells respond to environmental triggers by releasing cytokines capable of coordinating a type 2 inflammation. These epithelial cell-derived cytokines, which include IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), assist in activating the adaptive Th2 cascade, as well as stimulating basophils, mast cells, eosinophils and ILC2 [Poposki JA et al., 2017]. Increased populations of ILC2s have been observed in CRSwNP versus CRSsNP and due to their cytokine-producing propensity, ILC2s are thought to be integral in the type 2 immune response.

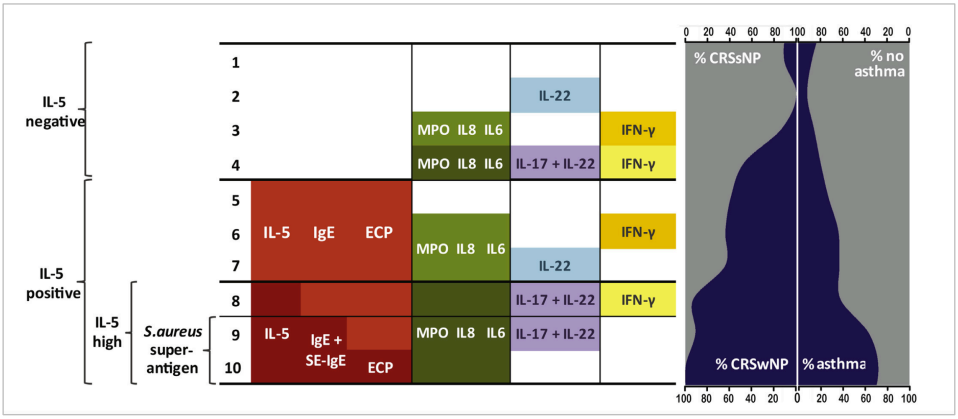

Figure 2-4 Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers

Simplified graphic depiction of clusters and their characteristic cytokine profile, as well as the distribution of CRSsNP versus CRSwNP and asthma. For cytokines, white indicates no increased concentration, light colors indicate moderately increased concentrations, and dark colors indicate strongly increased concentrations. Horizontal lines indicate groups of clusters, as determined by IL-5, SE-IgE, and CRSwNP and asthma characteristics.

[Reprinted with permission from Tomassen P et al., 2016]

Eosinophil-based approach – Another way of endotyping CRS is through the identification of the predominant immune cells in the inflamed sinonasal mucosa. This type of classification basically sees the juxtaposition of eosinophilic predominant and neutrophilic predominant CRS subtypes. Western patients with nasal polyps generally exhibit an eosinophilic mucosal infiltrate, while nasal polyps from Asian patients are associated with a predominant neutrophilic infiltrate [Ba L et al., 2011].

Several potential molecular pathways may modulate eosinophil-mediated CRSwNP. Eosinophils express more than 30 cytokines and chemokines, which are rapidly released following cellular activation and lead to a unique inflammatory signature. Eosinophilic mucin is another aspect frequently associated with nasal polyps in allergic fungal rhinosinusitis (AFRS), AERD and not otherwise-categorized eosinophilic chronic rhinosinusitis (ECRS). These phenotypes, however, seem different in patient's demographics, comorbidity with

asthma and clinical responses to therapy. Indeed, a study demonstrated that ECRS was defined by significantly elevated levels of IL-5 and IL-13, whilst AERD demonstrated a greater increase of IL-4 [Steinke JW et al., 2013]. As a result, while an eosinophilic predilection is evident in several phenotypically distinct CRSwNP forms, additional molecular endotyping is necessary to precisely characterize these variants.

The role of fungi in ECRS remains controversial. The presence of fungi within sinonasal mucus is largely dependent on the techniques used for isolation, treatment, and culture of the collected specimens [Montone KT, 2016]. It has been otherwise demonstrated that in fungi sensitized CRSwNP patients, fungi induce IL-4 production [Porter PC et al., 2014]. Lastly, other markers such as periostin, a proinflammatory mediator, have been found to be significantly elevated in AFRS patients [Laury AM et al., 2014].

B cell/IgE-based approach – Elevated IgE levels are observable in all forms of CRSwNP, except AERD, and may therefore serve as a broader approach to endotype CRSwNP. In particular, local IgE may be a stronger driver of disease pathophysiology than systemic IgE. Indeed, it seems from literature that systemic IgE levels do not correlate with polyp tissue eosinophilia [Kim JW et al., 2007]. Conversely, local nasal IgE production was demonstrated to drive nasal polyps' development and to be correlated with a greater prevalence of comorbid asthma. Of interest, the production of Staphylococcus aureus enterotoxin-specific IgE has been found to be associated with some of the highest local concentrations of IgE and asthma prevalence [Tomassen P et al., 2016].

Cysteinyl leukotriene-based approach – AERD represents a specific category within the CRSwNP phenotype due to its intrinsic association with two other comorbidities, asthma and intolerance to aspirin or other non-steroidal anti-inflammatory drugs, all inhibitors of the cyclooxygenase (COX)-1 enzyme. From a pathophysiological point of view, AERD has been related to enzymatic defects

in eicosanoids metabolism, including a functional reduction of COX enzymes and an upregulation of the 5-lipoxygenase and leukotriene C4 (LTC_4) synthase pathways. This metabolic imbalance results in a reduced production of anti-inflammatory prostaglandin E2 and a marked increase of pro-inflammatory leukotrienes (produced from the 5-lipoxygenase and LTC_4 synthase cascades) [Laidlaw TM et al., 2016]. High levels of leukotrienes activate downstream important effector cells, including eosinophils and mast cells, which in turn stimulate the inflammatory response within the respiratory mucosa. Other cytokines implicated in AERD inflammatory milieu are IL-4, IL-33 and IFN-γ, demonstrating that overlapping mechanisms exist between AERD and CRSwNP in aspirin-tolerant patients.

2.5. Experimental models for chronic rhinosinusitis

The greatest challenge in developing novel and efficient therapies for CRS is represented by the high variability in the etiology, microbiology, genetics and immune response between individuals and the scarce feasibility of clinical experimentation due to practical and ethical issues.

In order to get a better understanding of the pathological mechanisms underlying this complex disease, experimental preclinical models would be extremely useful [Kara CO, 2004]. A short review of available models is reported below. However, at the state of the art, there is no ideal preclinical model that can be universally adopted to resemble the features of human CRS in an accurate and reproducible way. It is therefore important to choose, among the wide range of available models, the one that best suits the specific aim of each experimental study (*Table 2-5*).

The use of omics technologies represents another effective way for better exploring and defining CRS endotypes.

Suffix "ome" derives from "chromosome" and today includes: *genomics* (a branch of genetics that studies the sequencing and analysis of an organism's genome), *transcriptomics* (the study of complete set of RNA transcripts that are produced by the genome), *proteomics* (the systematic identification and quantification of the complete complement of proteins – the proteome – of a biological system like cell, tissue, organ, biological fluid, or organism), *metabolomics* (the scientific study and analysis of the metabolites produced by a cell, a tissue, or an organism) and *epigenomics* (the study of all the epigenetic changes in a cell).

More in general, the omics approach and the application of systems biology methods provide unbiased tools allowing for better understanding of pathophysiology and for developing "precision medicine" approaches (*Figure 2-*

2). In contrast with the more general but still used one-size-fits-all approach, precision medicine considers a specifically targeted therapy that includes specific biological profiles together with patient's exposure and lifestyle. The omics technologies are contributing to the identification of new biomarkers that compose these biological profiles and consequently to the development of targeted biological therapies [Galeone C et al., 2018].

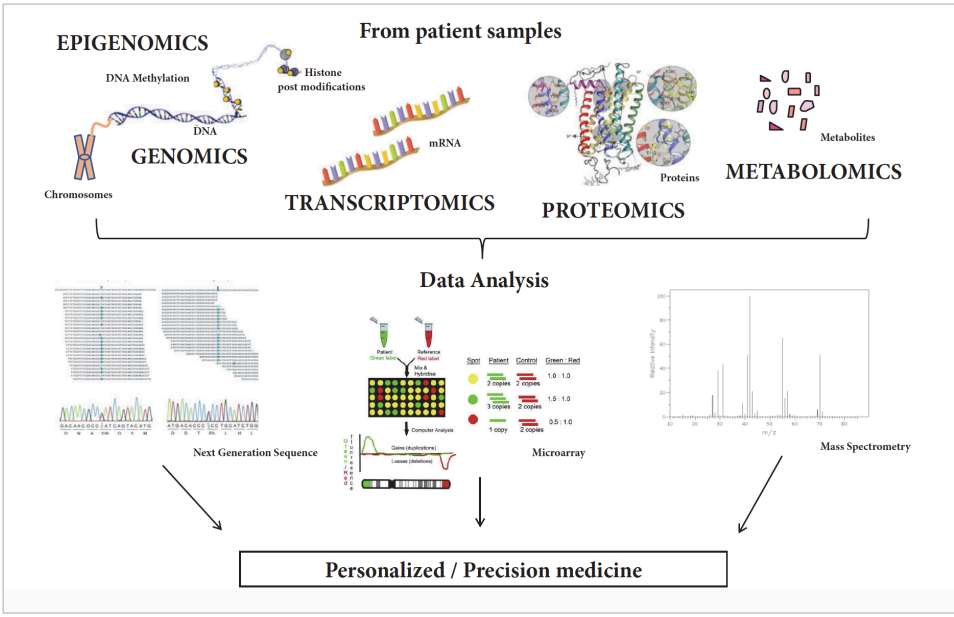

Figure 2-5 From omics technology to personalized medicine
From DNA microarray to Next Generation Sequencing (NGS), system biology provides for management and data analysis. This path is moving forward to the development of "precision medicine" approaches.
[Reprinted with permission from Galeone C et al., 2018]

In vivo models – Animal models have been essential to deepen knowledge on CRS pathophysiology. They enable to study the dynamics of the immune response and the inflammatory process in a complex systemic environment [Al-Sayed AA et al., 2017]

Over the years, different models have been proposed. There are considerable variations in sinonasal anatomy and in the immunologic responses among different species that must be taken into account when choosing the most appropriate animal model for each experimental requirement.

The most extensively used experimental model has been rabbit. There is a strong similarity between rabbit and human both in the immune response and in the sinonasal morphology: the paranasal sinuses are well-pneumatized and have a favorable size to perform surgical procedures. The prototypical animal model of sinusitis was created by Hiding in 1941 in rabbit by performing surgical antrostomy through or adjacent to the natural ostium of the maxillary sinus. Later on, sinusitis has been induced in rabbits by sinusal ostium mechanical obstruction and by injection with pathogenic bacteria (Staphylococcus Aureus or Bacteroides Fragilis) following allergic sensitization (e.g. with ovoalbumine and other food allergens) [Johansson P et al., 1988]. The so-obtained models have been used to study the microbiology of sinusitis, the inflammatory alterations found both locally and systemically in sinusitis and to compare the effects of drugs administered via systemic or topical route. Sheep models, showing a sinonasal disease pattern similar to humans, have also been described. One of greatest pitfall of these models is that the so-induced sinusitis tends to have an acute course, being inadequate for the study of chronic inflammatory alterations and the development of nasal polyps.

In the last decades, as the research was proceeding at a molecular and genetical level, rodent (mouse and rat) models have been adopted. They are less expensive and easier to handle, experimental murine-specific reagents are commercially available, and an important advantage is the ability to genetically manipulate to obtain transgenic or knockout mice.

Despite considerable differences between mice and humans in sinonasal anatomy (e.g. the maxillary sinus is not completely enclosed in the maxillary bone, being often referred to as “maxillary recess”), and physiology (the muco-ciliary drainage follows different pathways), the respiratory epithelium is similar, so

murine models are particularly indicated to study epithelial remodeling, inflammatory cell infiltration and collagen deposition. In 1998 Bomer developed the first murine model of acute sinusitis [Bomer K et al., 1998]; in 2001 Jacob was able to induce chronic rhinosinusitis [Jacob A et al., 2001] and in 2006 Lindsay developed a mouse model of chronic eosinophilic rhinosinusitis using Aspergillus fumigatus extract with intraperitoneal injection and subsequent nasal challenges [Lindsay R et al., 2006]. These models have provided invaluable information on the immune cascade and mediators involved and on the genetics of CRS.

However, an important limitation of in vivo models is the difficulty in translating these findings into human pathology. Despite promising results at a preclinical level, therapeutic strategies could be not so effective in humans, due to intrinsic differences between the two experimental systems. To be more accurate in translation it should be taken into consideration the exact mechanisms of inflammation and immune responses occurring in humans. These mechanisms need to be clarified at individual cell level.

In vitro models – The culturing of cells directly deriving from human sinonasal epithelium permits to investigate the CRS processes at a cellular level and to avoid the translational gap that subsists with animal models. The main disadvantage is the loss of the complex systemic immune response that can be found in vivo.

Several culturing methods have been described over the years. Organ and tissue explant cultures consist, respectively, in the culturing of all cells within an organ or of a tissue sample explanted from an organ. In this model the normal tissue architecture and the complex cell-to-cell interactions are preserved, but it is difficult to distinguish the direct role of nasal epithelial cells from that of the surrounding cells, such as fibroblasts. Cell cultures are established by enzymatical dissociation of cells from the human epithelium and their subsequent plating on a Petri dish coated in a culture medium (F12 serum-free medium supplemented by specific growth factor) [Wu R et al., 1985]. Under these conditions, cells tend to

grow in a flat configuration, forming monolayers. Monolayer cell cultures have been used to discover the increased production of pro-inflammatory cytokines in response to bacterial lipopolysaccharide and to test the cellular behavior in response to topical antibiotics and glucocorticoids. The main limitation of this traditional model is that the nasal epithelial cells, when growing in monolayers, tend to lose their differentiation and undergo a squamous transformation within 2-3 weeks, losing their ciliary activity and mucin production. This causes an important limitation in long term studies, and therefore in the translational application of the findings.

To overcome this problem multiple solutions have been proposed. The use of cell lines has historically been taken into consideration. Cell lines are immortalized cells that are allowed to grow and divide freely in a culture medium. The only commercially available nasal epithelial cell line is RPMI 2650. It was established from an anaplastic squamous cell carcinoma of the nasal septum in a 52-year-old male, showing similarities to the nasal epithelial cells in karyotype, mucous production and surface cytokeratines [Moorhead PS et al., 1965]. However, due to differences in the growth pattern and in inflammatory responses, and to the mixed mesenchymal and epithelial phenotype, this cell line has been considered not appropriate for CRS studies [Ball SC et al., 2015].

An innovative option is represented by the culturing of cells in three-dimensional models. This growth pattern can be obtained by many different techniques, classified into scaffold-based, which utilize a biological or synthetical 3D geometric network as a matrix for cell attachment and migration, and non-scaffold-based techniques, which utilize physical forces to aggregate cells in spherules. Three-dimensional cultures more closely resemble the features of the complex in vivo environment, and they enable cells to maintain their differentiation longer. In alternative, cells can be grown in specific air-liquid interface (ALI) culture systems, which keep the basal surface in contact with the culture medium and the apical surface with air [Schmidt D et al., 1996]. These

innovative culture's methods recapitulate more accurately the features of human CRS and open the way to interesting future applications.

Table 2–5 Comparison between CRS in vivo and in vitro models

In vivo models	
Abilities	Limitations
▪ Provides a good overview of the inflammatory process ▪ Better illustration of the dynamic immune response to microorganisms ▪ Good for studying the genetic causes for sinusitis	▪ Cannot predict individual cell response during inflammation or response to therapy. Therefore, doesn't translate human response accurately ▪ Chronic inflammation is difficult to produce ▪ Has a great ethical burden
In vitro models	
Abilities	Limitations
▪ Can measure individual cell response. Therefore, more accurately translates to human responses ▪ Chronic inflammation can be achieved reliably ▪ Achieves the principles of refinement, reduction and replacement	▪ Depends on the technique of culturing ▪ Can't account for the dynamic immune response that occurs during inflammation

[Adapted from Al-Sayed AA et al., 2017]

2.6. Biological treatments in chronic rhinosinusitis

The number of severe refractory CRS patients has enhanced, during time, the search for novel treatment strategies. Moreover, the inadequate knowledge of CRS pathogenetic mechanisms has long involved the improper application of both pharmacological (antibiotics, massive use of steroids) and surgical treatments (incomplete surgeries), contributing to the rate of uncontrolled cases.

With the introduction of disease-modifying therapies for severe asthma (i.e. biological antagonists), and given the frequent association between asthma and CRSwNP, it was a logical step to verify the effect of these new treatments on nasal polyps and the impact of patients QoL. This transition then paved the way for further studies to verify whether these treatments could be considered a potential therapeutic option for CRS.

These biological agents should work, in theory, as specific antagonists of an inflammatory pathway, overcoming the side effects of long-term steroidal therapy [Sweeney J et al., 2016].

Currently 3 major groups of biologics have been studied in terms of efficacy and safety in adult patients targeting different aspects of airways inflammatory cascade and will be further discussed. However, different from severe asthma, no registration for any of these drugs has been achieved for the indication on nasal polyps. Phase 3 studies with mepolizumab, omalizumab, dupilumab and benralizumab are currently being performed or in preparation [Bachert C et al., 2017].

Despite the encouraging results reported in literature about monoclonal therapies in CRS, the lack of large randomized clinical trials, the short treatment periods and the small sample size are crucial factors that need to be considered in drawing conclusions. Moreover, there are no reported data about the cost-effectiveness of these therapies in CRS, making it mandatory that further studies evaluate this

important aspect, too [Tsetsos N et al., 2018]. These assumptions lead to an important reflection in daily clinical practice: the indication of a biological therapy in CRS is dependent on the coexistence of asthma and its severity, in order to justify costs and clinical benefits.

Targeting IgE pathway – Omalizumab, a recombinant humanized monoclonal antibody, selectively binds to free circulating IgE, interfering with the activation of effector cells such as mast cells, basophils and dendritic cells, by decreasing IgE receptors' expression. It is now approved in US and Europe as a treatment options for severe allergic asthma. It has been investigated in multiple randomized control trials for CRSwNP with comorbid asthma in allergic and nonallergic patients. The most recent study showed that omalizumab significantly decreased total nasal polyp score and sinus opacification on CT scan, and improved nasal symptoms in both allergic and non-allergic subjects, independent of serum IgE levels [Gevaert P et al., 2013]. Conversely, an earlier study that included CRSwNP and CRSsNP was not able to demonstrate significant improvement in outcomes with omalizumab versus placebo [Pinto JM et al., 2010]. The conflicting results may support the role of local IgE in CRSwNP and suggest a variability within CRS subtypes.
Cost and toxicity of omalizumab remain the major obstacles to widespread adoption in CRS. Althought side-effects of anti-IgE therapy are rare (nasopharyngitis, headache), since doubts about the potential effect of induction of tumours, cardiovascular disease and anaphylaxis have not been dispelled yet, to date it seems more reasonable and cautious to adopt mainly for severe asthma, which has an uncommon but not insignificant mortality [Lam K et al., 2016].

Targeting IL-5 pathway – Reslizumab and Mepolizumab are both anti-IL-5 monoclonal antibodies. They interrupt the terminal differentiation of bone marrow progenitors into mature eosinophils. Literature reports the efficacy of Reslizumab in treating both poorly controlled eosinophilic asthma [Castro M et

al., 2011] and CRSwNP patients [Gevaert P et al., 2006]. Similarly, Mepolizumab showed a reduction in nasal polyp and sinus opacification CT scores in a CRSwNP cohort [Gevaert P et al., 2011]. Despite evidence of nasal IL-5 levels reduction after Reslizumab treatment, the effects of Mepolizumab seem to be independent of nasal IL-5 levels, thus restricting the use of this parameter as predictor biomarker [Lam K et al., 2016]. Mepolizumab showed good tolerability with nasopharyngitis as the only reported adverse event.
Conversely, Benralizumab serves as a humanized afucosylated monoclonal antibody that targets IL-5 receptors located on eosinophils, basophils and their progenitors. A randomized placebo-controlled phase I trial in eosinophilic asthma demonstrated a reduction in eosinophil count in the airway mucosa, sputum, bone marrow and peripheral blood [Laviolette M et al., 2013].

Targeting IL-4/IL-13 pathway – Dupilumab is a humanized monoclonal antibody directed against the alpha subunit of IL-4 receptor, common to both IL-4 and IL-13 receptors. Targeting simultaneously both IL-4 and IL-13, it causes a more comprehensive inhibition of the type 2 inflammatory pathway. This antibody has shown its benefits in patients affected by allergic asthma and atopic dermatitis. [Laviolette M et al., 2013; Beck LA et al., 2014]. Bachert et al. reported encouraging results in patients affected by CRSwNP refractory to intranasal corticosteroids; the addition of Dupilumab to mometasone furoate reduced endoscopic nasal polyp burden after 16 weeks. [Bachert C et al., 2016]. Dupilumab showed good tolerability without serious adverse events related to the drug. Injection site reactions, nasopharyngitis and headache were the most frequent reported events.

Other possible strategies to target inflammation in CRS are currently under investigations.
Increasing research on the regulation of the type 2 inflammatory response in CRSwNP has additionally focused on the role of the mucosal epithelium, which

not only provides a mechanical barrier to the external environment, but also actively stimulates the host innate and acquired immune responses through cytokine production. These epithelial cell-derived cytokines, including TSLP, IL-33 and IL-25, have shown the capacity to activate ILC2s, a subset of innate immune cells which release significant amounts of type 2 cytokines, including IL-5 and IL-13, in the absence of specific immune activation [Lee M et al., 2017]. TSLP, IL-33 and IL-25 also influence acquired immune responses by fostering Th2 lymphocyte differentiation with an amplified type 2 cytokine response. Overall, epithelial cell-derived cytokines promote important upstream mechanisms that drive the type 2 inflammation observed in CRSwNP. Targeting these key biomolecules involved in these immunologic pathways may ultimately offer more effective pharmacologic methods to alter the inflammatory responses in CRSwNP. AMG 157, a human monoclonal antibody that binds human TSLP and prevents its receptor interaction, showed efficacy in decreasing allergen-induced bronchoconstriction and eosinophil levels in sputum and blood of asthmatic patients [Gauvreau GM et al., 2014]. In addition, the effects of blocking the IL-33 pathway are undergoing investigation in a phase I clinical trial utilizing AMG 282, a monoclonal antibody that inhibits binding of IL-33 to the ST2 receptor, for possible therapeutic use in atopic asthma and CRSwNP [Liu X et al., 2009].

Future research interest may furthermore continue to expand targeting effector cells involved with type 2 inflammatory responses. To this point, the sialic acid immunoglobulin-like lectin (Siglec) group of cell-surface proteins might present such a target. Among them, Siglec-8 is uniquely expressed by human eosinophils, mast cells, and basophils. Engaging this structure with antibodies has the therapeutic potential to neutralize all three cell types and thus address a wide array of type 2 inflammatory disorders. In particular, targeting of Siglec-8 has been found to result in apoptosis in human eosinophils and inhibition of mediator release from human mast cells without affecting their survival [Schleimer RP et

al., 2016]. At present, anti-Siglec-8 treatments are entering phase II of clinical trials for use in CRSwNP patients.

Due to the relatively localized nature of most CRS disease and topical accessibility, locally administered therapies would be preferable to decrease systemic risks. One possible target is GATA-3, which is the transcription factor controlling the production of IL-4, IL-5, and IL-13 in Th2 cells. Because GATA-3 is overexpressed in patients with asthma, nasal polyps and atopic eczema, inhibition of GATA-3 has the potential to greatly reduce the Th2 burden. A GATA-3 DNAzyme applied through inhalation or spray have shown mucosal lymphocyte uptake and decreased GATA-3 RNA, which have led to decreased IL-5 production [Bachert C et al., 2015].

Lastly, platelets have increasingly emerged as promising biologic targets for patients with AERD. Increasing evidence suggests that activated platelets influence the inflammatory state of AERD by amplifying the generation of cysteinyl leukotriene, forming aggregates with circulating levels of inflammatory leukocytes and enhancing leukocyte recruitment to local tissue sites. Several clinical trials are ongoing to assess the effects of platelet-targeted therapies on various clinical endpoints of AERD. Such investigational therapies presently include prasugrel and ifetroban, which selectively inhibit the P2Y12 receptors and T prostanoid receptors, respectively, and thereby block the downstream platelet-associated mechanisms of inflammation in AERD [Laidlaw TM et al., 2015].

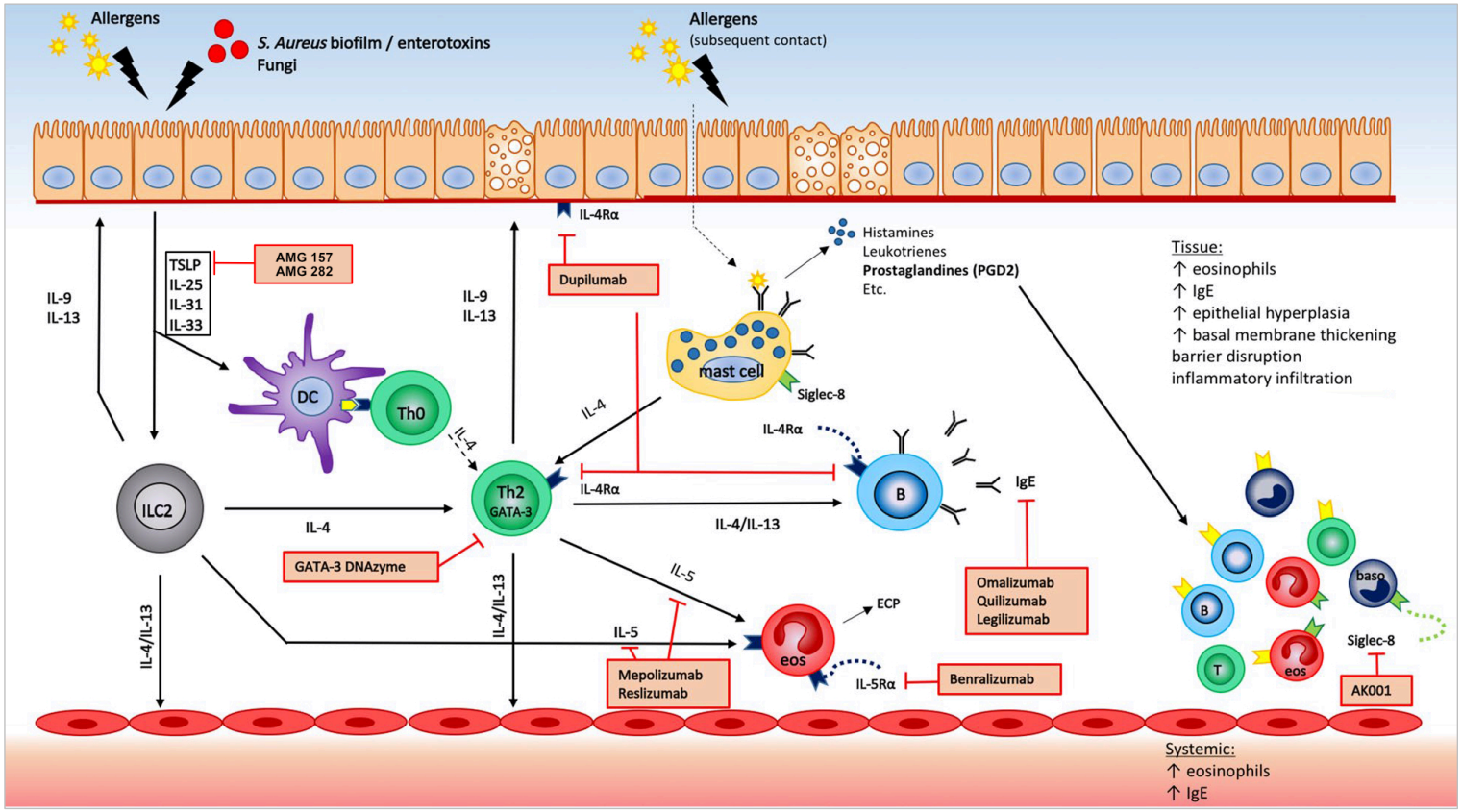

Figure 2-6 Type 2 inflammation and biologicals

B, B cell; Th, T helper cell, ILC2, type 2 innate lymphoid cell; DC, dendritic cell, eos, eosinophil; bas, basophil; ECP, eosinophilic cationic protein. [Adapted with permission from De Greve G et al., 2017]

3. Experimental studies

The following section includes three original manuscript, each built around a different clinical-instrumental approach to chronic rhinosinusitis.

Study #1 (Implementing strategies for data collection in chronic rhinosinusitis) is an information-technology project for the realization of an ad hoc CRS database (RhinoBank) to allow accurate electronic archiving of clinical data. RhinoBank represents the first step of a much wider plan of CRS awareness through a network at a national level. The main objective is to create a detailed monitoring of the disease, in terms of treatment optimization and adherence. The study has been published in 2018 in ACTA Otorhinolaryngologica Italica (PMID: 29984798, DOI 10.14639/0392-100X-1993). The database has now been transferred to the Italian Academy of Rhinology (IAR) to be distributed among selected Italian rhinological centers for a preliminary validity test.

Study #2 (Prediction of endoscopic sinus surgery outcomes in chronic rhinosinusitis) arises from the clinical need to measure the results of CRS combined medical and surgical treatment in our tertiary care center. Data from 2017 speak of over 600 annual endoscopic sinus surgery procedures. Applying failure rates described in literature, this would translate into over a hundred patients with poor clinical control of the disease, which requires frequent hospital admissions, revision surgeries and individual/social burden. To date, outcomes analysis in CRS is mainly based on a mere subjective evaluation. Paradoxically, we may deal with patients with discrete endoscopic findings but poor control of symptoms, as well as patients with suboptimal surgical outcomes that report an acceptable level of control. This work (currently being reviewed in an international journal) has allowed us to compare our data with reference case

studies, to quantify the effectiveness of our treatments and also to make us aware of the current outcomes' evaluation methods. Probably part of their limitations is a consequence of the complexity of CRS classification, which has led for long to group under the same clinical phenotype different biomolecular aspects that would deserve, instead, to be taken into consideration when staging the disease.

Study #3 (Exploring the role of nasal cytology in chronic rhinosinusitis) aimed at verifying the effectiveness of nasal cytology as a surrogate method for quantification of tissue eosinophilia. The premise is that, even with considerable efforts by the scientific community, reliable molecular markers defining different CRS endotypes have not been identified yet. If nothing else, the eosinophilic inflammation profile is the first discriminative feature that can actually guide current therapeutic choices. Despite controversies, tissue eosinophilia is defined on the basis of histopathological findings on nasal biopsy, which may not be either easily accessible or the first step of the diagnostic workup. That is why other alternative methods for tissue eosinophilia quantification have been investigated. This work (currently being reviewed in an international journal) allowed us to add our experience to available data in literature on the role of nasal cytology in chronic rhinosinusitis.

3.1. Implementing strategies for data collection in chronic rhinosinusitis

Abstract

Chronic rhinosinusitis (CRS) is a debated topic in the international rhinologic literature because of its high prevalence, heterogeneity of clinical manifestations and unpredictability of disease course. Recently, the focus in CRS research has moved to identify biological subtypes that might explain its aetiology and clinical variability. However, these analyses are still expensive and limited to scientific purposes, so that they cannot be used on a large scale in daily practice. For this reason, we wondered if it was possible to define a risk stratification for CRS patients based only on first level investigations. The heterogeneity of the disease has given us a large amount of data compelling to find an additional storage system. Herein, we present the results of our work, the RhinoBank, as we believe that it is an easy-to-use tool for those professionals dealing with CRS and an effective system to exploit in clinical research.

Keywords

Chronic rhinosinusitis, Phenotypes, Endotypes, Database, Clinical trials

Chronic rhinosinusitis (CRS) is a frequent disease. The true prevalence is challenging to be accurately estimated because it depends on the epidemiological methodology employed. However, according to studies based on large-scale questionnaires, it ranges from around 10 to 12% in Europe and the US. Moreover, CRS represents a burden both to individuals and society [Bachert C et al., 2015]. In recent years, with the demand to justify therapeutic failures, the scientific community has begun to critically review the diagnostic criteria for CRS and realised that they were not sufficient to explain the heterogeneity of the disease. There is, in fact, a broad spectrum of rhinosinusitis manifestations, ranging from

simple paranasal sinus dysventilation to frank nasal polyposis, which is not adequately taken into account by the phenotypic classification based on guidelines. The classic dichotomy between CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) is too simplistic to explain a disorder that is actually considered as a complex multifactorial disease grounded on the interplay between gene-susceptibility and the exposome (microbiota, immunity, epigenetics, nutrition) [Tomassen P et al., 2016; Divekar R et al., 2017; Adnane C et al., 2017].

In the attempt to overcome this limit, we gradually shifted to a different perspective for which the clinical phenotypes in reality is nothing but the emerging part of a massive iceberg.

All these considerations were inherited from the pulmonology field. Studies on asthma endotyping have been mentioned since 2008, when the literature began to put a new focus on pathogenetic mechanisms, recognising the complexity and variability of chronic inflammatory disorders of the airways [Anderson GP, 2008]. All these efforts have been made to correlate the clinical phenotype to the course of the dis- ease and its response to therapies [Agache I et al., 2012]. In 2013, the concept of endotyping in CRS first appeared [Akdis CA et al., 2013]. This consensus is the expression of the consciousness that CRS heterogeneity is supported by multiple biological subtypes (endotypes), each of which is defined by a distinct pathophysiological mechanism, determined equally by a well-defined genetic-environmental interaction. Each endotype should be in a theoretical line identified by a biomarker, to be intended both as diagnostic marker and as prognostic and therapeutic indicator. To find a highly predictive biomarker, a long series of key requirements for reproducibility, accessibility and stability must be met. In truth, we are still facing with the lack of an ideal biomarker that identifies CRS endotypes, allows a precise estimation of the severity of inflammation and predicts possible therapeutic responses. Therefore, it is likely that only a combination of biomarkers will be adequate to characterise each specific CRS subtype [Bachert C et al., 2016]. It is intrinsic to the concept of a

multifactorial disease, as CRS, the existence not only of multiple predisposing factors (risk factors), but also of other concomitant pathological conditions (pre and comorbidities) that contribute in shaping the phenotype. Differentiation of pre- and comorbid and risk factors is not easy, because of the variability in disease definitions, the lack of longitudinal studies that establish temporal relationship between exposure and disease onset and the difficulty of assessing the dose-effect size on disease severity.

Furthermore, the opportunity to attest the effectiveness of "standard" therapies is limited by the wide variability of treatment types, patient selection and outcome assessments.

A non-negligible number of prior studies, which reported high proportions of patients improving following medical and/or surgical treatments, were, however, retrospective analyses, which deduced subjective parameters or collected results though unverified surveys [Rimmer J et al., 2014]. There was no standard for categorising preoperative status, extent of disease or surgical outcomes, and many of these studies were unable to interpret the clinical relevance of a specific treatment or further delineate subgroups of patients who did or did not experience improvement. In addition, single institution studies have been criticised for the potential lack of generalisability to patients' population, an issue at least partially addressed by incorporating a multi-institutional study design [Smith TL et al., 2010; Fadda GL et al., 2016]. In the last years, the introduction of validated disease-specific quality of life (QoL) and general health-related QoL outcomes instruments allowed building a standard assessment of CRS patients. Notwithstanding, in our opinion, the exclusive evaluation of outcomes based on symptomatic and objective scores (endoscopic, radiological) may be limitative. A previous prospective-designed publication showed that other clinical factors (such as asthma, ASA intolerance, prior sinus surgery etc.) were found to be important predictors of outcomes. Our idea is that patients affected by CRS should be framed as a whole, going beyond a sole rhinological point of view in a multidisciplinary perspective [Gelardi M et al., 2017]. Consequently, a very

frequent disease associated with multiple variables generates a large amount of data that should be collected. It clearly emerges that there is a need to establish a systematic approach for data collection and evaluation of outcomes.

Our tertiary care institution is working toward this direction and has created a CRS online database, called RhinoBank. Its advantages are many. First of all, it allows storing data in a single solution with the possibility of easily retrieving previously stored data. In addition, it provides the physician all the information at a glance, allowing location of missing data in a very simple way. Lastly, it enables data sharing with other work centres.

The aim of this letter is to present the efforts of our work in search of active collaboration. We are aware that the database can be further upgraded thanks to suggestions or implementations from other experts in the field. The database now contains only basic clinical information that can be routinely obtained in any hospital. It is not envisaged to store third level parameters such as genetic or biomolecular markers. This will be the next step, dictated by the possibility to perform a more detailed analysis in our institute. The proposal is to spread this data collection system to other national centres to obtain large and uniform cohorts of patients. The goal is to overcome that lack of constant parameters, that is a critical element inside systematic reviews that hinders the possibility to draw conclusions on clinical practice [Rimmer J et al., 2014].

The database is at disposal for consultation at:

https://www.rhinobank.eu/demo/admLoginWin.asp

(Account access: Username, Admin; Password, demo000)

3.2. Prediction of endoscopic sinus surgery outcomes in chronic rhinosinusitis Peaks and troughs of using the Sinonasal Outcome Test 22 (SNOT-22)

Abstract

Previous studies highlighted that baseline Sinonasal Outcome Test 22 (SNOT-22) score affects surgical outcomes in chronic rhinosinusitis (CRS) and suggested that a SNOT-22-based approach might ameliorate patients' understanding of expectations after treatment. Our study aimed at verifying this hypothesis in an Italian CRS population. In 457 CRS patients, treated with endoscopic sinus surgery after failure of maximal medical therapy, the percentage of achieving a minimal clinical important difference (MCID) and the percentage of relative improvement after surgery were calculated. Moreover, the impact of several factors on preoperative and postoperative SNOT-22 score was investigated. Symptoms improvement occurred in the majority of patients and was directly proportional to baseline SNOT-22. 79.7% of patients achieved the MCID and the percentage of relative improvement was 50.1%. Psychological and social-functioning implications significantly affected SNOT-22 scores. A multiple regression showed that history of previous surgery, asthma, preoperative endoscopic and SNOT-22 scores statistically predicted the postoperative SNOT-22 score (R2 = .298). Submitting CRS patients to SNOT-22 prior to surgical treatments might help to inform about their probable outcomes, although it is strongly influenced by individual perception. Further studies are needed to identify an effective set of subjective and objective parameters for outcomes evaluation.

Keywords

Sinonasal Outcome Test-22 (SNOT-22), Chronic rhinosinusitis, Endoscopic sinus surgery, Outcome prediction, Quality of life

Introduction

Since the advent of nasal endoscopy, the evaluation of treatment outcomes in patients affected by chronic rhinosinusitis (CRS) has been a matter of debate. The pioneers of endoscopic sinus surgery (ESS) demonstrated surgical success rates around 90-95% [Smith TL et al., 2005]. These results are far from the actual rates reported in literature. This is easily explained in two ways. As ESS was introduced recently in the 1980s and it was not so widespread practice, there is a lack of long-term follow-up studies able to describe the real surgical effect [Rice DH, 1989]. Moreover, the evaluation method of outcomes was based on qualitative scales, often estimating changes only on one or few items of the CRS symptoms criteria, lacking a global assessment of improvement [Chester AC et al., 2007; Terris MH et al., 1994]. Finally, and this is partly also a current issue, the cohorts of patients were inhomogeneous, including in the analysis cases of acute rhinosinusitis, massive nasal polyposis or recurrent sinusitis after external procedures [Stammberger H et al., 1990]. The 1990s witnessed the clinical application of the biopsychosocial model [Engel GL, 1977]. This theory supported that, in order to understand and respond adequately to patients suffering, clinicians should attend simultaneously to the biological, psychological and social dimensions of illness. Practically, it was a way of considering the patient's subjective experience as an essential contributor to accurate diagnosis, health outcomes and humane care [Borrell-Carrió F et al., 2004]. In accordance with this philosophy, a "quality of life revolution" was observed in different areas of medicine [Smith TL, 2017] and several quality of life (QoL) questionnaires have been developed to quantify the individual and societal burden of chronic diseases. This paradigm shift occurred also for CRS. Since then, rhinologists have used several specific symptoms-based scores to evaluate treatment outcomes in CRS patients, such as the Sinonasal Outcome Test 22 (SNOT-22) [Soler ZM et al., 2010]. Applying these tools, it emerged that around 20-30% of CRS patients do not experience significant improvement after surgery, although the impact of ESS on QoL is generally reported as positive [Smith TL et al., 2010]. Moreover, other studies have

quantified the 5-years risk of revision surgery to be 10-20%, while the presence of certain comorbidities, such as asthma and aspirin sensitivity, along with other factors like high baseline CT stage or incomplete sinus dissection have been associated with elevated revision rates between 25-40%. However, despite the presence of known risk factors for revision surgery, evidence for several of these clinical characteristics have failed to reliably predict ESS outcomes [Smith TL et al., 2010]. Contrarily, it seems from previous regression studies that baseline SNOT-22 is one of the most important factors affecting the outcome [Hopkins C et al., 2015] and several studies suggested its prognostic role in terms of achievement of improvement and risk of revision surgery [Rudmik L et al., 2016]. In light of these observations, the presented study aimed at verifying in an Italian CRS population whether SNOT-22 could assist physicians in predicting surgical outcomes, improving shared decision-making process and ameliorating patients' understanding of their QoL expectations after treatment. The primary outcomes included the measurement of the percentage of patients receiving a minimal clinical important difference (MCID) and the percentage of relative improvement (RI) after surgical treatment.

Materials and methods

This prospective study was conducted according to the declaration of Helsinki and was previously approved by the Institutional Review Board of the hospital.
Clinical data were obtained from a population of 457 patients affected by CRS operated in the same tertiary care center in the period 2015-2018.
Enrolled patients were adult subjects affected by bilateral CRS undergoing ESS as primary procedure after failure of maximal medical therapy [Fokkens WJ et al., 2012]. Exclusion criteria were previous trauma, congenital facial malformations, systemic autoimmune diseases, ciliary dyskinesia, head and neck malignancies or history of previous radiotherapy, any other nasal surgery performed concomitantly.

All surgical procedures were performed by the same 4 surgeons with more than 10 years of experience in ESS.

Postoperative medical therapy consisted in nasal irrigation with saline solution and intranasal corticosteroid [Fokkens WJ et al., 2012], delivered with a high-volume squeeze bottle device [Snidvongs K et al., 2012]. A perioperative short term of oral corticosteroid was also administered. An oral antibiotic therapy was suggested only in selected cases. Patients were followed at 15 days, 1, 3, 6 and 12 months after surgery. Each patient was evaluated before surgery and during follow-up visits using a set of objective and subjective (self-assessed) measurements. Data obtained in the preoperative assessment and during the last follow-up visit (12 months) were collected for analysis.

Concerning the objective evaluation, the Lund-Kennedy (LK) [Lund VJ et al., 1995] and the Lund-Mackay (LM) [Lund VJ et al., 1993] scales were used. The evaluation between preoperative and postoperative LM scores was not possible, because CT scan is not routinely performed after surgery unless required for particular clinical conditions.

For subjective evaluation, the Italian version of the Sino-Nasal Outcome Test-22 (I-SNOT-22) [Mozzanica F eta al., 2017] was used. It is the most frequently employed in clinical practice because it is simple, intuitive and takes a few minutes to complete [Morley AD et al., 2006]. It represents a questionnaire structurally composed of 22 CRS-related items scored from 0 to 5 (total score range 0-110, higher scores represent worse symptoms), which evaluates the severity of complaints that patients have been experiencing over the past weeks due to CRS [Hopkins C et al., 2009]. SNOT-22 items can be divided into 2 categories: questions about physical symptoms (items 1-12) which cover rhinologic as well as ear and facial symptoms, and questions about health and QOL (items 13-22) which cover sleep function and psychological issues [Abdalla S et al., 2012].

Similar to Rudmik [Rudmik L et al., 2015], the cohort of patients was divided into 10 groups according to baseline SNOT-22 score. These groups were based on 10-

points increments of the SNOT-22 score (patients who scored less than 10 were excluded since they had no chance to receive an MCID). The chance of receiving a MCID improvement, that in SNOT-22 is defined as a reduction of 9 points after ESS [Chowdhury NI et al., 2017], was estimated. The percentage of RI for each preoperative SNOT-22 group was then calculated with the formula [(mean postoperative SNOT-22 score – mean preoperative SNOT-22 score)/mean preoperative SNOT-22 score] x 100 [Rudmik L et al., 2015].

Statistical analysis

The results were given as arithmetic mean ± standard deviation. The Kolmogorov Smirnoff test was used to test the normality of distribution. Parametric tests were used to evaluate the differences among the groups. In particular, ANOVA test with Tukey post-hoc test and Chi-square test were used when appropriate to compare the groups. A multiple regression analysis was run to predict the SNOT-22 postoperative score from age, sex, smoking habit, asthma, allergy, aspirin intolerance, LK score, LM score, history of previous surgery for CRS and preoperative SNOT-22 score. A significance level of 0.05 for all testing was used. Statistical analyses were performed using the SPSS 25.0 package.

Results

A total of 457 CRS patients were consecutively enrolled. Among them, 34 patients were lost at follow-up. The remaining 423 patients attended the scheduled follow-up visits for 12 months and were considered eligible for analysis. The mean age of the cohort was 47.4 ± 13.5 years (range 18-86 years). 112 patients were asthmatic (26.5%), 156 patients were allergic to common inhalants (36.9%), while 31 patients complained aspirin intolerance (7.3%). 225 patients were affected by CRSwNP (53.2%), while the remaining 198 (46.8%) were affected by CRSsNP.

The mean preoperative SNOT-22 score was 48.9 ± 20.8 (range 13-106), while the mean preoperative SNOT 1-12 score was 30.8 ± 10.3 (range 9-56). The

preoperative SNOT 1-12 score accounted for the total SNOT-22 for 67.4% (percentage of the SNOT-22 related to rhinologic symptoms). The mean preoperative LK score was 5.6 ± 2.8 (range 0-12), while the mean preoperative LM score was 11.5 ± 6.6 (range 0-24). The mean postoperative SNOT-22 score was 22.9 ± 17.9 (range 1-75), while the mean postoperative SNOT 1-12 score was 14.3 ± 9.5 (range 1-41). The postoperative SNOT 1-12 score accounted for the total SNOT-22 for 70.7%. The mean postoperative LK score was 1.7 ± 2.1 (range 0-10). These differences were found significant at Student t test ($p = 0.001$ for SNOT-22 score; $p = 0.001$ for SNOT 1-12 score; $p = 0.001$ for the percentage of SNOT-22 related to rhinologic symptoms, and $p = 0.001$ for LK score).

Based on baseline SNOT-22 score, 10 different groups of patients were defined. The sample sizes for each preoperative SNOT-22 group appeared to follow a normal distribution ($p = 0.132$ at Kolmogorov-Smirnoff test), with the largest groups composed by patients with baseline SNOT-22 scores between 20-69 (*Figure 3-1*).

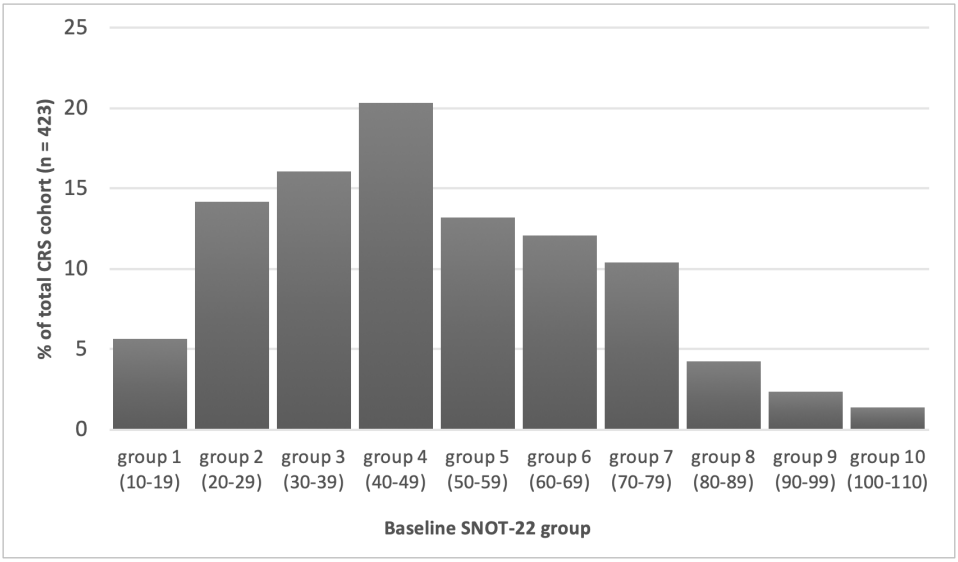

Figure 3-1 Distribution of the study population according to the baseline SNOT-22 score

Clinical characteristics, as well as preoperative subjective and objective scores are depicted in *Table 3-1*.

Postoperative SNOT-22 score was found significantly improved in each of the 10 groups at paired Student t test ($p = 0.001$ for all the comparisons). 79.7% of the total cohort achieved a MCID improvement after ESS. Among patients who achieved a MCID, the percentage of RI was 62.7%. When considering the total cohort (including also those who did not achieved a MCID) the percentage of RI was 50.1%. The MCID and the percentage of RI obtained from each of the 10 groups, as well as pre- and postoperative SNOT-22 scores are reported in *Table 3-2*. A clear distinction of behavior is observed between patients with baseline SNOT-22 score greater or less than 30. In particular, the mean percentage of achieving a MCID in groups 3-10 is 91.6% with an average 56.8% of RI. Contrarily, the mean percentage of achieving a MCID in groups 1-2 is 44.2% with an average of 38.9% of RI.

Significant differences in the number of patients achieving the MCID were demonstrated at Chi-square test ($p = 0.001$). In detail, patients in groups 1-2 achieved a MCID with a significant less frequency than those in the other groups. Furthermore, also the percentage of RI among the 10 groups was significantly different at ANOVA test ($p = 0.002$) and patients in group 2 scored significantly lower than those in group 6, 8 and 10 ($p = 0.013$, $p = 0.022$ and $p = 0.039$ respectively at Tukey post-hoc test). Interestingly, the percentage of the SNOT-22 score related to nasal symptoms was significantly different among the 10 groups both in the pre and post-treatment conditions ($p = 0.001$ and $p = 0.001$ respectively at ANOVA test). In particular, at baseline, the SNOT 1-12 score accounted for the 88.5% of the SNOT-22 total score in group 1, while it accounted for the 51.9% in group 10. These differences were found significant at Tukey post-hoc test. In the post-treatment assessment, the SNOT 1-12 score ranged from 88.0% of the SNOT-22 total score in group 1 to 54.5% in group 9. These differences were found significant at Tukey post-hoc test.

Each of the 10 groups was further divided into two subgroups according to the presence of polyps. The results of SNOT-22 scores obtained before and after the surgery, as well as the probability of achieving a MCID and the percentage of RI are reported in *Table 3-3* and *3-4*. No differences between CRSwNP and CRSsNP patients in the postoperative SNOT-22 score ($p = 0.177$), the percentage of the SNOT-22 score related to rhinologic symptoms in the pre- ($p = 0.366$) and post-treatment ($p = 0.300$) conditions, and the percentage of the RI ($p = 0.162$) were demonstrated at Student t. Moreover, no difference in the probability of achieving a MCID was demonstrated at Chi-square test ($p = 0.215$). On the contrary, a significant difference in the baseline SNOT-22 score was found at Student t test ($p = 0.010$). Precisely, patients affected by CRSsNP scored significantly better than those affected by CRSwNP.

A multiple regression was run to predict the postoperative SNOT-22 score from gender, age, smoke, asthma, LK, LM, previous surgery, allergy, aspirin intolerance, preoperative SNOT-22 score. Some of these variables statistically predicted the postoperative SNOT-22 score, $F(9, 423) = 6.423$, $p = 0.001$, $R2 = .298$. An history of previous surgery for CRS was found to be the most important predictor ($B = 6.277$, $p = 0.009$). Other factors predicting ESS outcomes included the presence of asthma ($B = 5.286$, $p = 0.045$), preoperative LK score ($B = 0.937$, $p = 0.040$) and preoperative SNOT-22 score ($B = 0.326$, $p = 0.001$).

Table 3–1 Pre-treatment clinical features of the study population classified in 10 groups based on baseline SNOT-22 score.

	Group 1 (10-19)	Group 2 (20-29)	Group 3 (30-39)	Group 4 (40-49)	Group 5 (50-59)	Group 6 (60-69)	Group 7 (70-79)	Group 8 (80-89)	Group 9 (90-99)	Group10 (100-110)
n (%)	24 (5.7)	60 (14.2)	68 (16.1)	86 (20.3)	56 (13.2)	51 (12.1)	44 (10.4)	18 (4.3)	10 (2.4)	6 (1.4)
Age, mean ± SD (min-max)	48.3 ± 16.6 (18-73)	49.1 ± 15.4 (18-71)	47.7 ± 16.5 (18-80)	50.4 ± 12.3 (21-86)	47.1 ± 12.7 (22-77)	46.2 ± 11.1 (27-67)	43.6 ± 10.9 (24-65)	42.1 ± 11.1 (24-61)	42.2 ± 6.6 (34-53)	47.3 ± 9.1 (41-59)
Sex (M:F)	16:8	52:26	44:24	54:32	28:28	33:18	22:22	8:10	2:8	2:4
Asthma, n (%)	10 (41.7%)	14 (23.3%)	18 (26.5%)	18 (20.9%)	18 (32.1%)	8 (15.7%)	14 (31.8%)	4 (22.2%)	8 (80%)	0 (0%)
Allergy, n (%)	8 (33.3%)	25 (41.7%)	22 (32.6%)	18 (20.9%)	26 (46.4%)	23 (45.1%)	20 (45.5%)	10 (55.6%)	4 (40%)	0 (0%)
ASA intolerance, n (%)	2 (8.3%)	4 (6.7%)	2 (2.9%)	8 (9.3%)	6 (10.7%)	5 (9.8%)	0 (0%)	4 (22.2%)	0 (0%)	0 (0%)
CRSwNP, n (%)	14 (58.3%)	33 (55%)	36 (52.9%)	36 (41.9%)	30 (53.6%)	26 (50.9%)	26 (59.1%)	12 (66.7%)	6 (60%)	6 (100%)
LK score, mean	4.9	4.9	6	6.1	5.8	5.4	5.3	5.4	5.6	5.7
LM score, mean	11.3	10.6	11.5	13.6	11.6	11.3	7.8	12.4	14.6	10.3

M, male; F, female; ASA, acetylsalicylic acid; CRSwNP, chronic rhinosinusitis with nasal polyps; LK, Lund-Kennedy; LM, Lund-Mackay

Table 3–2 Probability of patients with CRS achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22 score	% SNOT 1-12 over preop. SNOT-22	Postop. SNOT-22 score	% SNOT 1-12 over postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 24	16 ± 2.2	88.5%	8.7 ± 3.8	88.0%	33.3% (n = 8)	- 44%
Group 2 (20-29) n = 60	24.8 ± 2.9	82.1%	16.3 ± 12.2	75.6%	55% (n = 33)	- 33.8%
Group 3 (30-39) n = 68	34 ± 2.7	76.4%	17.5 ± 13.6	79.9%	82.4% (n = 56)	- 49%
Group 4 (40-49) n = 86	44.5 ± 3.1	67.2%	22.9 ± 16.8	66.8%	86.1% (n = 76)	- 48.9%
Group 5 (50-59) n = 56	54.2 ± 2.8	58.9%	25.9 ± 15.8	63.8%	85.7% (n = 48)	- 52%
Group 6 (60-69) n = 51	65.4 ± 3.2	57.5%	23.1 ± 19.2	69.9%	92.1% (n = 47)	- 64.6%
Group 7 (70-79) n = 44	73.9 ± 2.8	55.8%	35.6 ±21.9	62.4%	86.3% (n = 38)	- 51.6%
Group 7 (80-89) n = 18	82 ± 2	53.7%	32.8 ± 21.4	68.6%	100% (n = 18)	- 60.2%
Group 9 (90-99) n = 10	94.6 ± 3.6	53.5%	43.8 ± 21.6	54.5%	100% (n = 10)	- 53.8%
Group 10 (100-110) n = 6	104 ± 2.4	51.9%	26.7 ± 14.5	69.4%	100% (n = 6)	- 74.3%
Total n = 423	48.9 ± 20.8	67.4%	22.9 ± 17.9	70.7%	79.7% (n = 338)	- 50.1%

SNOT, Sinonasal Outcome Test; MCID, minimal clinical important difference; RI, relative improvement

Table 3–3 Probability of patients with CRSwNP achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22	Postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 14	16.9 ± 2.1	7.7 ± 4.5	42.9% (n = 6)	- 54.7%
Group 2 (20-29) n = 33	25.6 ± 2.7	16.9 ± 15.1	54.6% (n = 18)	-33.7%
Group 3 (30-39) n = 36	33.1 ± 2.5	17.1 ± 9.1	77.8% (n = 28)	- 48.1%
Group 4 (40-49) n = 36	44.5 ± 3.3	23.8 ± 18.1	88.9% (n = 32)	- 46.8%
Group 5 (50-59) n = 30	54.3 ± 2.7	27.4 ± 18.2	80% (n = 24)	- 48.9%
Group 6 (60-69) n = 26	64.3 ± 3.4	21.4 ± 19.2	92.3% (n = 24)	- 66.6%
Group 7 (70-79) n = 26	74 ± 2.9	36.6 ± 26.1	76.9% (n = 20)	- 50.1%
Group 7 (80-89) n = 12	81.5 ± 2.1	23.2 ± 16.2	100% (n = 12)	- 71.6%
Group 9 (90-99) n = 6	92 ± 3.8	39.7 ± 18.1	100% (n = 6)	- 56.8%
Group 10 (100-110) n = 6	104 ± 2.2	26.7 ± 14.5	100% (n = 6)	- 74.3%
Total n = 225	48.9 ± 20.7	22.9 ± 18.4	78.2% (n = 176)	- 50.9%

Table 3–4 Probability of patients with CRSsNP achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22	Postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 10	14.8 ± 2.1	10.0 ± 1.9	25.0% (n = 2)	- 30.6%
Group 2 (20-29) n = 27	23.9 ± 2.7	15.4 ± 7.6	55.6% (n = 15)	- 33.8%
Group 3 (30-39) n = 32	35.1 ± 2.9	18.0 ± 17.5	87.5% (n = 28)	- 50%
Group 4 (40-49) n = 50	44.4 ± 3.1	22.2 ± 15.9	84.0% (n = 42)	- 50.3%
Group 5 (50-59) n = 26	54.2 ± 2.2	24.1 ± 12.7	92.3% (n = 24)	- 55.6%
Group 6 (60-69) n = 25	66.4 ± 2.5	24.9 ± 19.5	92.0% (n = 23)	- 62.5%
Group 7 (70-79) n = 18	73.8 ± 3.5	34.0 ± 14.9	100% (n = 18)	- 53.8%
Group 7 (80-89) n = 6	83 ± 3.1	52.0 ± 17.6	100% (n = 6)	- 37.4%
Group 9 (90-99) n = 4	98.5 ± 0.6	50.0 ± 27.7	100% (n = 4)	- 49.4%
Group 10 (100-110) n = 0	/	/	/	/
Total n = 198	47.6 ± 19.3	23.1 ± 17.3	82.8% (n = 162)	- 49.2%

SNOT, Sinonasal Outcome Test; MCID, minimal clinical important difference; RI, relative improvement

Discussion

Chronic rhinosinusitis affects a large portion of the world population determining a significant impairment of QoL [Fokkens WJ et al., 2012]. Current studies report that about half of CRS patients remain symptomatic despite first-line pharmacological therapy [Lal D et al., 2009; Young LC et al., 2012].

Consequently, patients and physicians have to make a decision whether to either continue with medical therapy alone or undergo ESS followed by ongoing pharmacological therapy. On the one hand, Steele et al. showed that 57% of patients electing continued medical therapy failed to improve 1 MCID with a mean relative score improvement of 16%. Moreover, 1 in 5 patients experienced deterioration by >1 MCID [Steele TO et al., 2016]. On the other, although surgical benefits are much more remarkable [Smith TL et al., 2005; Soler ZM et al., 2010; Smith TL et al., 2010; Soler ZM et al., 2018], the decision to face the surgical iter cannot disregard the evaluation of the related risks and costs. To date a tool able to identify patients who might benefit from surgery and the expected degree of improvement is still lacking. This is a natural consequence for not having available a standardized staging system that drives treatment choices.

Many reports investigated a number of factors which might influence the outcomes of CRS surgery. These account for patient-related factors (baseline SNOT-22, radiological extent of disease, presence of polyps, asthma or other comorbidities, gender, previous surgery) and operative factors (experience of surgeon, timing of surgery, postoperative management) [Le PT et al., 2018]. It seems from previous regression studies, and partly confirmed by our work, that baseline SNOT-22 is one of major factors affecting the outcome [Hopkins C et al., 2015]. In this sense, the advantage of submitting CRS patients to SNOT-22 prior to any surgical treatment could, in theory, help physicians to inform them about their probable outcomes after ESS. For simplicity, explaining to a patient that he is likely to receive a 50% reduction in his symptoms load will aid informed consent and optimize preference-based decisions.

The fact is that, luckily, the majority of patients experiences an improvement in symptoms after ESS, intended as a reduction of the SNOT-22 score after treatment ($p = 0.001$) [Smith TL et al., 2005; Soler ZM et al., 2010; Smith TL et al., 2010; Soler ZM et al., 2018]. We have shown that an improvement of symptoms occurs in all groups and that is directly proportional to the baseline SNOT-22 value. In other words, patients who have a worse preoperative symptomatology obtain the greatest range of score reduction after treatment. However, this statistical significance might not imply a clinical significance. Indeed, the MCID has been proposed to combat this conceptual vice by defining a threshold value by which a statistically significant result may also offer a clinically meaningful result. The MCID is the lowest degree of change that a patient will notice and for SNOT-22 score has previously been defined as 8.9 points in a 3-month postoperative score [Hopkins C et al., 2009]. However, what represents a clinically important change may vary from one individual to another and may not necessarily reflect the patients' expectation for improvement after treatment. As an example, a patient reaching a MCID of 9 points in the postoperative SNOT-22 may not be satisfied with this outcome due to a persistent measurable burden of disease, despite achieving a noticeable improvement. To overcome the MCID intrinsic limitation, the clinically significant change should be also outlined by a parameter expressing the true magnitude of the postoperative improvement, that is the percentage of RI. Hence, integrating these measurements together might optimize patient understanding and counselling. Rudmik et al. demonstrated that 80% of patients with a SNOT-22 score >30 improved by an average 48% following ESS [Rudmik L et al., 2015]. Similarly, in our series, patients with SNOT-22 score >30 showed a 91.6% chance of achieving the MCID with a mean 56.8% of RI. Also, a larger UK cohort showed a 66% chance of achieving a MCID with baseline SNOT-22 score >30 [Hopkins C et al., 2015]. On the other hand, patients with SNOT-22 <30 have less than half probability of achieving the MCID and a reduced degree of RI. That was evident in all the above-mentioned studies and confirmed in our series (44.2% mean MCID achievement, 38.9% mean RI). Therefore, although

the baseline SNOT-22 score and the chance of achieving the MCID is not intended to be used as an absolute threshold for eligibility for surgery, these global results suggest that a patient with low preoperative score might be less likely to benefit from surgery and caution should be paid when operating on patients with a score of <10. It is also true that only the categories of patients with lower baseline SNOT-22 values are likely to achieve a normal or near-normal status. Indeed, prior studies submitting SNOT-22 to patients with no sinus disease resulted in an average score of around 10 [Mozzanica F et al., 2017; Hopkins C et al., 2009; de Dorlodot C et al., 2015; de los Santos G et al., 2015; Kosugi EM et al., 2011]; conversely, patients with higher baseline SNOT-22 values, despite a good RI, are still left with a significant burden of disease and remain more symptomatic than healthy controls. To be honest, SNOT-22 groups on either extreme of the scoring scale contained small sample size in all the compared studies, which makes it difficult to provide accurate statistical results and introduce larger degrees of uncertainty around the means of these groups. Therefore, larger collaborative CRS databases should be developed to better define these categories of patients [Hopkins C et al., 2015; Castelnuovo P et al., 2018] and understand their behavior. Although our results are in line with the current literature, they should be interpreted with caution. First, though few in numbers, CRS patients with baseline SNOT-22 score <10 were excluded from the analysis because of their near-normal status. Moreover, since all surgical procedures have been performed in the Day Surgery division, patients with severe comorbid asthma are not included in the study population. This choice obviously affected the overall mean values of percentage of MCID achievement and RI. Second, all surgeries were performed by specialist rhinologists, minimizing the unfavorable outcomes due to surgical inexperience. Third, CRS is a dynamic disease characterized by fluctuating trend from quiescence to outbreaks. A one-off administration of a self-assessed questionnaire might not be enough reliable to assess the overall burden of the disease.

In light of the above, two reflections arise. If we assume that a patient with a low baseline SNOT-22 score has a low probability of reaching the MCID and that a patient with a high baseline SNOT-22 score has a high probability of reaching the MCID, but not enough RI to become asymptomatic, either we are far from having an ideal treatment for CRS or SNOT-22 (in general QoL-based questionnaires) may not be a sufficiently effective tool to evaluate treatment outcomes. While, on the one hand, basic research efforts are aimed at discovering innovative targeted therapies [Bachert C et al., 2018], on the other, clinical practice efforts are focused on defining new comprehensive methods of outcomes evaluation. In particular, the attempt is to incorporate subjective and objective parameters since symptoms-based items are influenced by the psychological habitus and show a wide inter-individual variability. Indeed, our data show that in groups with low baseline SNOT-22 almost all of the SNOT-22 score is given by rhinologic symptoms, while in groups with high baseline SNOT-22 score rhinologic symptoms account only for about 50% of the global SNOT-22 value, suggesting that psychological and social-functioning aspects significantly affect the SNOT-22 score. Furthermore, Hopkins et al. demonstrated that when the sleep-psychological domain items dominate the total SNOT-22 score, ESS outcomes may be suboptimal. In fact, CRS patients that showed a moderately-severe total SNOT-22 score with high burden from sleep-psychosocial items may have less durable benefit after treatment, showing a statistically and clinically improvement at 3 months after ESS, followed by a worsening of symptoms at 6 months. For this reason, these patients may be counseled to expect less benefit than those in whom nasal subdomain scores predominate [Lal D et al., 2018]. In this context, Hopkins et al. obtained a long list of potential parameters revising the current literature. After an intricate statistical analysis, the 54 initial items were distilled down to a final core set of 15 items, over 4 domains, including the SNOT-22 repeated over time with some additional questions and the Lund-Kennedy score [Hopkins C et al., 2018]. This core outcome set (COS) represents the first "prototype" of evaluation tool for CRS able to integrate subjective and objective parameters, but

work is still necessary to make it actual for the clinical practice. In this regard, a recent study highlighted a close correlation between symptoms and burden of inflammation. A cohort of CRSsNP patients undergoing ESS was clustered in 4 preoperative SNOT-22-based groups. These groups were significantly different with respect to primary versus revision ESS status, number of previous sinonasal surgeries, asthma prevalence and total SNOT-22 scores. More interestingly, the cluster of subjects with the highest total preoperative SNOT-22 score had the highest tissue eosinophilia compared to the other symptomatic groups and a more frequent diagnosis of asthma, suggesting that a high burden of inflammation correlates with a worse symptomatology [Lal D et al., 2018].

Based on these preliminary observations, the integration of SNOT-22 scores and tissue histopathology could represent an innovative method to predict treatment outcome in CRS patients.

Further studies are needed to define a simple and effective evaluation tool for CRS outcomes, implementing the knowledge of pathophysiological mechanisms underlying the different expressions of this disease. Eventually, this will lead to identify new histopathological-biomolecular pathways able to classify the CRS patients into homogeneous subgroups, to establish endotype-driven treatments and possibly provide objective predictors of response to therapy.

3.3. Exploring the role of nasal cytology in chronic rhinosinusitis

Abstract

Chronic rhinosinusitis (CRS) results from a broad spectrum of inflammatory mechanisms. The discrimination between eosinophilic and non-eosinophilic profile of inflammation represents at least the first-line approach of endotyping. The aim of the study was to verify the degree of correlation between different methods of tissue eosinophilia quantification. 33 CRS patients undergoing endoscopic sinus surgery and 30 controls undergoing non-CRS surgeries were enrolled. Each patient was evaluated for relevant clinical comorbidities. Blood venous sampling, nasal biopsy on uncinate process (UP) and standard nasal cytology on inferior turbinate (IT) were performed to assess the eosinophilic infiltration. Middle meatus (MM) scraping was added being this anatomical region pivotal of CRS manifestations. Differences in eosinophil count in blood ($p = 0.0001$), UP ($p < 0.0001$), IT ($p = 0.01$) and MM ($p=0.0006$) were statistically significant between CRS cases and controls. Spearman's test showed a weak correlation between UP and blood eosinophil count [$r = 0.34$, $p = 0.006$], a weak correlation between UP and IT eosinophil count [$r = 0.30$, $p = 0.017$] and a moderate correlation between UP and MM eosinophil count [$r = 0.51$, $p < 0.0001$]. No significant statistical differences were observed in tissue eosinophilia (blood, UP, IT, MM eosinophil count) across different clinical parameters and staging scores. However, ROC curve analysis predicted ERCS with an overall low sensitivity. Interestingly, once excluded allergic patients from the analysis, sensitivity further decreased for cytological sampling on IT and slightly increased for cytological MM sampling. The study represents a preliminary exploration of the role of nasal cytology in CRS. It seems that performing nasal cytology in MM gives more accurate information on the degree of tissue eosinophilia. Replication

in other wide and unbiased cohorts is necessary to verify these results and to define accurate thresholds.

Keywords

Chronic rhinosinusitis, Eosinophilic chronic rhinosinusitis, Nasal cytology, Eosinophil, Endotype

Introduction

Chronic rhinosinusitis (CRS) is an umbrella term for different disease entities, each of which represents the downstream consequence of a specific immune-mediated inflammatory mechanism. That is why a blanket approach to treat CRS has been proven to be unsuccessful in some of the cases [van der Veen J et al., 2017]. The phenotypic dichotomy of CRS with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP) is progressively giving way to a deeper and complex biomolecular classification of CRS subtypes (or endotypes) [Dennis SK et al., 2016].

If on the one hand the therapeutic strategies are evolving towards a more focused immunomodulatory effect (i.e. monoclonal antibodies), on the other, despite research efforts are very intense, predictive molecular markers – that allow to define which CRS endotype would benefit of that specific treatment – are not available, yet.

To date, biological agents tested or already in use for moderate/severe inflammatory disorders of the airways and the skin are mainly based on targeting constituents of the T helper 2 (Th2) pathway (anti-IL-5, anti-IL-4, anti-IL-13, anti-IgE). Conversely, fewer treatments are available for the non-Th2 and non-eosinophilic cascades [Avdeeva K et al., 2018]. The evidence that the endotyping process should reach a higher precision level is attested by the number of non-responders to biological therapies [MacDonald KM et al., 2019]. Indeed, in absence of specific biomarkers, the eligibility of a patient for these kinds of treatment is at least established by defining the eosinophilic or non-eosinophilic

profile of inflammation, primarily evaluating blood eosinophils and serum IgE. However, clear cut-off values are not defined, other than the thresholds imposed by clinical trials [Pavord ID et al., 2012].

All these premises are even more vague when applied to CRS. A basic attempt of CRS endotyping is represented by the identification of the predominant immune cells in the inflamed sinonasal mucosa. A major distinction is between eosinophilic mediated CRS (ECRS) and non-eosinophilic mediated CRS (non-ECRS) [Dennis SK et al., 2016]. In turns, ECRS might be considered a further subtype of the much wider type 2 cytokine-based CRS endotype. The clinical interest in ECRS arises from the fact that it generally shows a poor response to medical and surgical therapies, making it a refractory form of CRS [Shah SA et al., 2016]. The therapeutic impact would be significant, as cases with intense eosinophilia would justify a more massive steroid treatment (oral or aggressive local) and, theoretically, also selected biological antagonists of the type 2 inflammation [Tajudeen BA et al., 2019].

Although no unanimous histopathological criteria exist for discriminating between ECRS and non-ECRS, it is current practice to define western ECRS when tissue eosinophil count is > 5 cells/HPF [Soler ZM et al., 2009]. Moreover, a tissue eosinophil count > 10 cells/HPF was demonstrated to correlate with poorer outcomes and overall prognosis [Soler ZM et al., 2010].

Obviously, the diagnosis requires to obtain tissue for histopathological analysis. As sinus mucosa needs to be collected, and not just nasal polyp samples, biopsies may not be straightforward or performed under local anesthesia. That is why different, less invasive, surrogates have been tested to prove their reliability to predict tissue eosinophilia. It is worth mentioning the JERSEC score which defines ECRS in presence of differently matched clinical criteria (endoscopic, radiologic and peripheral blood eosinophil count) [Tokunaga T et al., 2015]. Much less widespread among rhinologists is the assessment of the degree of eosinophilia through nasal cytology. This technique has been reported as an efficient method to differentiate among various forms of non-allergic rhinitis

[Heffler E et al., 2018]. However, it is still debated if it might be of interest also for defining CRS inflammatory profiles. The reason of hesitations is ascribable to the fact that this procedure, although easy, cheap and non-invasive is underused and, so far, no consensus or official recommendations have been defined. A recent study introduced nasal cytological assessment among the criteria of a clinical-cytological grading system predictive of nasal polyps' relapse [Gelardi M et al., 2017]. One could object to this method that sampling performed along the inferior turbinate might not precisely mirror the mucosal inflammatory status of CRS; indeed, this region, in addition to a different embryological origin, shows a morpho-histological structure that is not identical to that of the middle meatus [White LC et al., 2016; Pezato R et al., 2016]. Nevertheless, only a few controversial reports looked at the cellular inflammatory pattern of the different endonasal subsites in CRS.

In light of these premises, we wished to verify in a sample population (including CRS patients and controls) the existence of a correlation among the degree of tissue, blood and cytological eosinophilia. Moreover, the standard cytological data was integrated with the analysis of a cytological sample obtained from the middle meatus region. Lastly, by sorting the study population into cases (patients with CRS) and controls, we have investigated the existence of significant differences in the degree of eosinophilia and the association with the most typical clinical features related to CRS.

Materials and methods

This prospective study was conducted according to the declaration of Helsinki and was previously approved by the Institutional Review Board of the hospital.

Study population

Clinical data were obtained from patients affected by CRS who underwent endoscopic sinus surgery (ESS) at the same tertiary care center in the period between January 2018 and July 2018.

CRS was diagnosed according to the latest European guidelines [Fokkens WJ et al., 2012]. Each CRS case was assessed by SNOT-22 questionnaire for symptoms collection, Lund Kennedy (LK) score to objectively describe the endonasal condition, Lund Mackay (LM) score to define the degree of opacification of the sinuses and skin prick test to investigate allergic sensitization to common inhalants. Data concerning asthma, aspirin sensitivity and smoking habits were self-reported by patients. Exclusion criteria were genetic syndromes, congenital or acquired immunodeficiency, malignancy or history of the head and neck cancers, systemic autoimmune diseases and drug abuse.

Similarly, clinical data were obtained from patients scheduled for other non-CRS surgeries (septoplasty and dacryocystorhinostomy) in the same time-lapse and served as control group. Each control was assessed by SNOT-22 questionnaire, LK and LM scores to exclude CRS. Control patients affected by asthma and aspirin sensitivity were excluded a priori. Lastly, skin prick tests were performed to investigate allergic sensitization to common inhalants.

All cases and controls were considered eligible to enrolment only after a washout period of 15 days from oral and topical steroids and 1 month from oral antibiotics. All collected data have been entered in a specific CRS database as previously reported [Castelnuovo P et al., 2018].

Sampling steps

At the beginning of the surgical procedure under general anesthesia, prior to nasal decongestion, the following sampling steps were taken.

A peripheral blood venous sampling was performed from antebrachial vein in a test tube with vacutainer system, containing ethylenediaminetetraacetic acid (EDTA), for further blood and leukocyte formula count. White blood cells (WBC) were expressed both as absolute count (cell x 10^9/L) and percentage of the total WBC count.

A nasal cytological sampling was performed under endoscopic view according to the nasal scraping technique along the inferior turbinate (IT) and the middle

meatus – lateral nasal wall (MM) mucosa. The procedure consisted in gently swiping on the mucosal surface a disposable plastic nasal curette (Rhinoprobe®) equipped with a small distal collection chamber, so as to collect cells from the superficial epithelial layer. Once collected, samples were uniformly swiped on a slide, on the central area, avoiding too much pressure to prevent cellular damage or lysis. Slides were finally left to air-dry and then placed in slide holders for further analysis. Samples were then processed entirely under hood and colored with May Grunwald-Giemsa as described by Gelardi et al. [Gelardi M et al., 2016]. Slides were observed through an optical microscope (Nikon Eclipse 600®) at different magnifications (100x, 200x and 400x). Observed cells included intact respiratory epithelial cells, flaking cells and immune cells (eosinophils, neutrophils, mast-cells, macrophage and plasma cells) and were counted in 10 consecutive fields at 400x. Eosinophils were expressed both as mean of eosinophil cells per high-power field (HPF) 400x and percentage of eosinophils on total immune cells. This latter parameter was intended to incorporate also the effect of the neutrophilic degree of infiltration of the specimen.

A nasal histological sampling through mucosal biopsy was performed on the uncinate process (UP) at the same side of the cytological sampling. Care was taken not to remove only polypoid tissue. Samples were stored in formaldehyde tubes. All samples with dimensions of more than 0.4 mm were fixed in 10% buffered formalin, dehydrated by alcohol passages with increasing concentrations up to absolute ethanol, clarified in BioClear® and then included in paraffin. Histological sections were set up from each block with a thickness of 3 μm and subsequently stained with Hematoxylin-Eosin. A conventional morphological evaluation was carried out on all the samples according to the 2017 WHO classification criteria. Additional histopathologic features were taken into consideration as reported by Snidvongs et al. [Snidvongs K et al., 2012]. Moreover, immune cells count was performed in 5 HPF using a 400x objective corresponding to an area of 1 mm^2. Tissue eosinophil count was graded in three

classes as follows: <5 cells/HPF, 5-10 cells/HPF, >10 cells/HPF [Snidvongs K et al., 2012].

Statistical analysis

An ad hoc electronic database was created to collect all study variables. Qualitative data were summarized with absolute and relative frequencies. Mean and standard deviation (SD) or median and interquartile range (IQR) were used for quantitative variables with a parametric and non-parametric distribution, respectively. Chi-squared or Fisher exact test were used to detect any statistical differences for qualitative variables. Student's t and Mann-Whitney tests were used for quantitative variables following their parametric or non-parametric distribution. Spearman's correlation was used to assess the relationship between the different measurements of eosinophils. P-value less than 0.05 was considered statistically significant. *Stata 15 statistical software* was used for each statistical computation.

Results

The study group included 33 CRS patients and 30 controls. Demographic data are shown in detail in *Table 3-5*.

The CRS group comprised 21 cases of CRSwNP (63.6%) and 12 cases of CRSsNP (36.4%). Allergic sensitization was diagnosed in 13 CRS cases (39.4%), asthma in 14 CRS cases (42.4%) and aspirin intolerance in 3 CRS cases (9.1%). Sixteen CRS patients (48.5%) already underwent previous surgeries elsewhere. Median baseline SNOT-22 score was 30. Mean baseline LK and LM score were 6.1 and 13.5, respectively.

In subjects included as control group, rhinosinusitis, asthma and aspirin intolerance were excluded prior to enrollment. Only 5 controls (16.7%) showed allergic sensitization to inhalants at skin prick test.

The median blood eosinophil count was $0.3x10^9$/L in CRS group (min 0.03, max 1.14) and $0.2x10^9$/L in control group (min 0.01, max 0.36) [p = 0.0001]. The

median percentage of blood eosinophils was 3.9% in CRS group (min 0.4, max 13.3) and 2% in control group (min 0.2, max 6) [$p = 0.0008$]. Differences in blood eosinophil count were statistically significant between CRS cases and controls.

At the histopathologic analysis, we observed an increased overall degree of inflammation in UP CRS samples when compared to UP control samples [$p = 0.003$]. Eosinophil count in UP samples was demonstrated to be statistically different between cases and controls [$p < 0.0001$]. In detail, among CRS group, eosinophil count was <5 cells/HPF in 18 cases (54.5%), 5-10 cells/HPF in 3 cases (9.1%) and >10 cells/HPF in 12 cases (36.4%); among control group, eosinophil count was <5 cells/HPF in 29 cases (96.7%), 5-10 cells/HPF in 1 case (3.3%) and no controls showed an infiltration >10 cells/HPF.

Similarly, the cytological analysis exhibited a higher overall inflammatory infiltration in CRS cases than in controls; that was confirmed both at IT scraping [$p = 0.01$] and at MM scraping [$p = 0.0006$]. Median IT eosinophil count was 0.5 cells/HPF in CRS group and 0 cells/HPF in control group [$p = 0.0002$]. Median IT eosinophil percentage on total immune cells was 4.2% in CRS group and 0% in control group [$p = 0.002$]. Median MM eosinophil count was 0.3 cells/HPF in CRS group and 0 cells/HPF in control group [$p = 0.006$]. Median MM eosinophil percentage on total immune cells was 1.9% in CRS group and 0% in control group [$p = 0.01$]. On the whole, these data showed a statistically significant difference in terms of eosinophilic infiltrate between CRS cases and controls (*Table 3-6*).

We further investigated the degree of correlation at Spearman's test among the eosinophilic count in different tissues. The analysis showed a weak correlation, though statistically significant, between UP eosinophil count and blood eosinophil count [$r = 0.34$, $p = 0.006$]. Moreover, a weak correlation was evident between UP eosinophil count and IT eosinophil count [$r = 0.30$, $p = 0.017$] and a moderate correlation between UP eosinophil count and MM eosinophil count [$r = 0.51$, $p < 0.0001$]. Unexpectedly, the subgroup analysis displayed that in control group only the correlation between UP eosinophil count and IT cytology was maintained, whereas the opposite occurred in CRS group (loss of correlation between UP

eosinophil count and IT cytology and confirmed correlation between UP eosinophil count and MM cytology) (*Table 3-7*).

No significant statistical differences were observed in terms of tissue eosinophilia (blood, UP, IT, MM eosinophil count) across different clinical parameters, including sex, age, presence of nasal polyps, previous surgery, allergy, asthma and smoking habit. Similarly, no significant statistical differences were evident comparing UP, IT, MM eosinophil count and clinical staging scores (SNOT-22, LK score, LM score). Conversely, higher values of blood eosinophilia were associated with an increase in endoscopic and radiological scores [LK score, p = 0.03; LM score, p = 0.01].

The CRS group was then classified in ECRS and non-ECRS on the basis of the histopathological threshold (ECRS, eosinophil count ≥5 cells/HPF; non-ECRS, eosinophil count <5cells/HPF). The analysis of different clinical and biological parameters showed only a significant statistical difference between the two groups for the MM eosinophil count [MM eosinophils/HPF 400x, p 0.003; MM eosinophil percentage on total immune cells, p = 0.005] (*Table 3-8*). The absence of a statistical difference for asthma, aspirin intolerance and polyp phenotype might be justified by the small size of the analyzed sample.

ROC curve analysis on IT eosinophil count predicted ERCS with a sensitivity of 51.5% and specificity of 90% [positive predictive value (PPV) 85%; negative predictive value (NPV) 62.8%; area under the curve (AUC) 0.76, range 0.65-0.87], on IT eosinophil percentage on total immune cells with a sensitivity of 48.5% and specificity of 80% [PPV 72.7%; NPV 58.5%; AUC 0.72, range 0.59-0.84], on MM eosinophil count with a sensitivity of 42.4% and specificity of 90% [PPV 82.4%; NPV 58.7%; AUC 0.69, range 0.57-0.81], on MM eosinophil percentage on total immune cells with a sensitivity of 42.4% and specificity of 87.7% [PPV 77.8%; NPV 57.8%; AUC 0.67, range 0.55-0.80]. Once excluded allergic patients from CRS population, ROC curve analysis on IT eosinophil count predicted ERCS with a sensitivity of 11.1% and specificity of 90.9% [PPV 50%; NPV 55.6%; AUC 0.53, range 0.27-0.80], on IT eosinophil percentage on total

immune cells with a sensitivity of 11.1% and specificity of 81.8% [PPV 33.3%; NPV 52.9%; AUC 0.51, range 0.24-0.77], on MM eosinophil count with a sensitivity of 33.3% and specificity of 90.9% [PPV 75%; NPV 62.5%; AUC 0.81, range 0.61-1], on MM eosinophil percentage on total immune cells with a sensitivity of 55.6% and specificity of 81.8% [PPV 71.4%; NPV 69.2%; AUC 0.85, range 0.67-1].

The cut-off level of ≥1.9% of MM eosinophil percentage on total immune cells produced the best sensitivity and specificity values (88.9% and 81.8%, respectively) with a positive likelihood ratio (LR+) of 4.9 and a negative likelihood ratio (LR-) of 0.1, with 85% of corrected classified cases (*Table 3-9*).

Table 3–5 Demographic data of control and CRS groups

		Control group n=30	CRS group n=33	*p* value
Males, n (%)		17 (56.7)	8 (24.2)	0.009
Mean (SD) age, years		52.1 (16.8)	52.7 (15.5)	0.88
CRS with nasal polyps, n (%)		-	21 (63.6)	-
Previous surgery for CRS, n (%)		-	16 (48.5)	-
Allergy, n (%)		5 (16.7)	13 (39.4)	0.05
Asthma, n (%)		0 (0.0)	14 (42.4)	<0.0001
Aspirin intolerance n (%)		0 (0.0)	3 (9.1)	0.24
Smoker, n (%)	No smoker	29 (96.7)	24 (72.7)	0.03
	Smoker	1 (3.3)	4 (12.1)	
	Former	0 (0.0)	5 (15.2)	
Median (IQR) SNOT-22 score		-	30 (25-42)	-
Mean (SD) LK score		-	6.1 (2.8)	-
Mean (SD) LM score		-	13.5 (5.7)	-

SD standard deviation, CRS chronic rhinosinusitis, IQR inter-quartile range, LK Lund-Kennedy, LM Lund-Mackay, SNOT-22 Sino-nasal outcome test 22

Table 3–6 Blood, histological and cytological features of control and CRS groups.

		Control group	CRS group	*p* value
Peripheral blood eosinophilia				
Median (IQR) blood eosinophil count, 10^9/L		0.2 (0.1-0.2)	0.3 (0.2-0.4)	**0.0001**
Median (IQR) blood eosinophils, %		2.0 (1.1-3.2)	3.9 (2.4-5.8)	**0.0008**
Uncinate process (UP) histological features				
Overall degree of inflammation, n (%)	Absent	14 (46.7)	5 (15.2)	**0.003**
	Mild	16 (53.3)	22 (66.7)	
	Moderate	0 (0.0)	6 (18.2)	
Inflammatory predominance, n (%)	Lymphoplasmacytic	16 (53.3)	27 (81.8)	**0.01**
	Absent	14 (46.7)	5 (15.2)	
	Eosinophilic	0 (0.0)	1 (3.0)	
Neutrophilic infiltrate, n (%)		4 (13.3)	4 (12.1)	1.0
Eosinophil count, n (%)	<5/HPF	29 (96.7)	18 (54.5)	**<0.0001**
	5-10/HPF	1 (3.3)	3 (9.1)	

	>10/HPF	0 (0.0)	12 (36.4)	
Inferior turbinate (IT) cytological features				
Median (IQR) eosinophils/HPF 400x		0 (0.0-0.2)	0.5 (0.0-1.3)	**0.0002**
Median (IQR) eosinophil percentage on total immune cells		0 (0.0-0.6)	4.2 (0.0-12.5)	**0.002**
Eosinophil grading, n (%)	<5%,	24 (80.0)	20 (60.6)	**0.05**
	5-19%	3 (10.0)	9 (27.3)	
	20-50%	3 (10.0)	1 (3.0)	
	>50%	0 (0.0)	3 (9.1)	
Median (IQR) mastcell count		0 (0-1)	3 (1-8)	**<0.001**
Median (IQR) neutrophil count		8 (2-43)	46 (8-300)	0.06
Median (IQR) macrophage count		2 (1-3)	3 (1-4)	0.25
Median (IQR) plasmacell count		0 (0-0)	0 (0-0)	0.17
Median (IQR) total immune cells		12 (4-50)	80 (20-409)	**0.01**
Middle meatus (MM) cytological features				
Median (IQR) eosinophils/HPF 400x		0 (0.0-0.2)	0.3 (0.0-3.5)	**0.006**
Median (IQR) eosinophil percentage on total immune cells		0 (0-4)	1.9 (0-30)	**0.01**
Eosinophil grading, n (%)	<5%,	24 (80.0)	18 (54.6)	**0.04**
	5-19%	4 (13.3)	4 (12.1)	
	20-50%	0 (0.0)	6 (18.2)	
	>50%	2 (6.7)	5 (15.2)	
Median (IQR) mastcell count		1 (0-1)	3 (1-12)	**<0.0001**
Median (IQR) neutrophil count		3 (2-13)	19 (4-200)	**0.04**
Median (IQR) macrophage count		1 (1-2)	3 (2-6)	**0.001**
Median (IQR) plasmacell count		0 (0-0)	0 (0-0)	0.33
Median (IQR) total immune cells		7 (4-18)	95 (13-253)	**0.0006**

Statistical difference is expressed as p value; significant results ($p<0.05$) are highlighted in bold. IQR interquartile range, UP uncinate process, IT inferior turbinate, HPF high power field, MM middle meatus

Table 3–7 Spearman's rank-order correlation between histological samples, blood tests and cytology.

	Uncinate process (UP) eosinophil count	
Spearman's rho	**rho**	***p* value**
Total population (n=63)		
blood eosinophil count, 10^9/L	0.34**	**0.006**
blood eosinophils, %	0.26*	**0.038**
IT eosinophils/HPF 400x	0.30*	**0.017**
IT eosinophil percentage on total immune cells	0.20	0.111
MM eosinophils/HPF 400x	0.51**	**< 0.0001**
MM eosinophil percentage on total immune cells	0.48**	**< 0.0001**
Control group (n=30)		
blood eosinophil count, 10^9/L	-0.17	0.36
blood eosinophils, %	-0.07	0.69
IT eosinophils/HPF 400x	0.40**	**0.03**
IT eosinophil percentage on total immune cells	0.26	0.16
MM eosinophils/HPF 400x	0.19	0.32
MM eosinophil percentage on total immune cells	0.11	0.56
CRS group (n=33)		
blood eosinophil count, 10^9/L	0.20	0.26
blood eosinophils, %	0.11	0.56
IT eosinophils/HPF 400x	0.04	0.83
IT eosinophil percentage on total immune cells	-0.06	0.74
MM eosinophils/HPF 400x	0.53**	**0.002**
MM eosinophil percentage on total immune cells	0.51**	**0.002**

Statistical difference is expressed as p value; significant results ($p<0.05$) are highlighted in bold.
IT inferior turbinate, HPF high power field, MM middle meatus

Table 3–8 Blood, histological, cytological and clinical differences between non-ECRS and ECRS group.

CRS group	Non-ECRS n=18	ECRS n=15	*p* value
Median (IQR) blood eosinophil count, 10^9/L	0.3 (0.2-0.4)	0.4 (0.2-0.6)	0.19
Median (IQR) blood eosinophils, %	3.7 (2.3-5.4)	3.9 (2.4-8.0)	0.42
Median (IQR) IT eosinophils/HPF 400x	0.4 (0.1-0.8)	0.7 (0-1.8)	0.66
Median (IQR) IT eosinophil percentage on total immune cells	4.6 (3-9.1)	3.4 (0-15)	0.94
Median (IQR) MM eosinophils/HPF 400x	0.05 (0-0.2)	3.3 (0.4-10.3)	**0.003**
Median (IQR) MM eosinophil percentage on total immune cells	0.2 (0-7.7)	23.3 (1.9-68.8)	**0.005**
Asthma, n (%)	8 (44.4)	6 (40.0)	0.80
Allergy, n (%)	7 (38.9)	6 (40.0)	0.95
Aspirin intolerance, n (%)	2 (11.1)	1 (6.7)	1.0
CRSwNP, n (%)	12 (66.7)	9 (60.0)	0.69

Statistical difference is expressed as p value; significant results ($p<0.005$) are highlighted in bold. IQR interquartile range, IT inferior turbinate, HPF high power field, MM middle meatus

Table 3–9 Sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of cytology performed on the inferior turbinate and middle meatus in the non-allergic CRS population.

non-allergic CRS n= 20	Best cut-off	Sensitivity, %	Specificity, %	LR+	LR-	Correctly Classified, %
IT eosinophil count in 10 consecutive HPF 400x	≥7	66.7	54.6	1.5	0.6	60.0
IT eosinophil percentage on total immune cells	≥1.3	66.7	45.5	1.2	0.7	55.0
MM eosinophil count in 10 consecutive HPF 400x	≥5	88.9	72.7	3.3	0.2	80.0
MM eosinophil percentage on total immune cells	≥1.9	88.9	81.8	4.9	0.1	85.0

IT inferior turbinate, HPF high power field, MM middle meatus, LR+ positive likelihood ratio, LR- negative likelihood ratio

Discussion

The term ECRS was first introduced in Japan in 2001 to identify a subgroup of patients with CRS and eosinophilic infiltration of nasal polyps, likely to occur consequently to eosinophils' dysregulation [Ishitoya J et al., 2010]. ECRS etiology encompasses a wide variety of stimuli (fungal antigens, allergens, bacteria, bacteria-derived superantigens) and possibly overlapping pathogenic mechanisms. Superantigen-induced inflammation, allergic and non-allergic fungal rhinosinusitis and aspirin-exacerbated respiratory disease are known processes in ECRS [Sok JC et al., 2006]. There is evidence that ECRS is associated with a greater symptom severity (and worse olfactory disfunction), extensive sinus disease and comorbidities (asthma), intermittent acute exacerbation of secondary bacterial infections [Shah SA et al., 2016]. Moreover, patients with ECRS seem to have a poorer response to medical and surgical treatments with high polyp recurrence rate and severely impaired quality of life [Lou H et al., 2018]. Therefore, it would be useful to be able to identify ECRS patients early, preferably within the outpatient setting, in order to guide overall long-term management and prognosis.

In daily practice, the diagnostic criteria for ECRS are based on clinical features. Traditional traits of ECRS include asthma (late-onset), nasal polyps, aspirin intolerance, high serum eosinophilia and IgE. Although the presence of polyps predicts high tissue eosinophilia, a remarkable number of CRSsNP show the same degree of eosinophilic inflammation (19%). [Snidvongs K et al., 2012] For this reason, the most reliable way to diagnose ECRS remains the histopathological assessment. However, relying on biopsy as the main diagnostic tool of ECRS opens several issues. (1) Unless adequately aware, pathologists' reports often conclude simply with "chronic inflammation" giving too much limited information [Snidvongs K et al., 2012]. (2) Obviously, the diagnosis requires to obtain enough tissue for histopathological analysis. As sinus mucosa needs to be collected, and not just nasal polyp samples, biopsies may not be straightforward or performed under local anesthesia. Moreover, biopsy – due to its intrinsic

invasiveness – is not an early step in CRS diagnostic workup. (3) To date, the definition of eosinophilia in CRS has not reached consensus among research [Lou H et al., 2018]. This controversy concerns both the method and the interpretation of the results. Actually, it is accepted practice to define western ECRS when tissue eosinophil count is >5 cells/HPF. Moreover, a tissue eosinophil count >10 cells/HPF was demonstrated to correlate with poorer outcomes and overall prognosis [Soler ZM et al., 2010].

To overcome the aforementioned disadvantages of biopsy, other types of biological samples have been considered as possible indirect assessments of tissue eosinophilia.

A number of studies demonstrated that there is an association between peripheral eosinophilia and tissue eosinophilia in paranasal sinuses. Similarly, our data confirmed this statistically significant correlation (p = 0.006), though weak (r = 0.341). The cut-point at >0.3 x 10^9/L or 4.4% of WBC is the one applied for provision of biological agents in asthma, though still within the normal range and with a reported negative predictive value of 67%. Other thresholds have been proposed to gain a better diagnostic reliability. However, their broad variability prevents from drawing firm conclusions. [Ho J et al., 2018; Sakuma Y et al., 2011]. The limits for which blood eosinophil count fails to be a reliable marker resides in the fact that increased circulating eosinophils may depend on other coexisting pathologies (parasitic infections, allergy, autoimmune disorders, adverse drug events, etc.) and that local eosinophilic activation often occurs without an increase in blood eosinophils. [Yao Y et al., 2017]. It is reasonable that on-site biomarkers might provide a more specific overview on airways cellular inflammatory pattern. In some studies, indeed, asthma subtypes are defined on induced sputum, a non-invasive well standardized procedure of bronchial cytological assessment, able to sort asthma into eosinophilic, neutrophilic, mixed-granulocytic or pauci-granulocytic subtypes [Simpson JL et al., 2006]. Similarly, the degree of nasal eosinophilia, together with other inflammatory cells, can be measured by cytological analysis. Numerous techniques have been described to

obtain nasal specimen for cytological assessment. Among them nasal scraping, performed along the medial aspect of the inferior turbinate, demonstrated advantages in terms of (1) less prevalence of blood derived artifacts than nasal brushing, (2) less time consuming and costly than sinus packs and micro-suction tubes, (3) lower percentage of apoptotic degenerated cells inside the collected sample than nasal lavage, (4) performing easiness comparable to nasal swab [Heffler E et al., 2018]. Although the technique has been validated as a semi-quantitative analysis for the diagnosis of cellular rhinitis and correlations have been demonstrated between nasal and bronchial inflammatory cytological patterns [McDougall CM et al., 2008], its role in CRS has not been clarified, yet. One controversial issue is linked to the sampling site. Some studies debate its usefulness when performed along the inferior turbinate. For example, De Corso et al. reported that inferior turbinate eosinophilic inflammation represents an early marker for severe CRSwNP [De Corso E et al., 2017]. Similarly, Gelardi et. al showed that the association of eosinophilic-mast cell inferior turbinate infiltration and the presence of asthma and aspirin sensitivity is correlated with an increased risk of polyp's relapse [Gelardi M et al., 2009]. Our analysis confirmed the existence of a statistically significant degree of correlation, albeit weak, between tissue eosinophilia and IT cytological eosinophilic count ($r = 0.30$ $p = 0.017$). To make matters complicated, She et al. demonstrated a lack of significant correlation between the total and individual inflammatory cell counts in inferior turbinate versus paranasal sinus mucosa, questioning the real diagnostic value of nasal cytology for CRS [She W et al., 2018]. If it is true that the term CRS has been coined precisely to express that every sinus inflammation [Meltzer EO et al., 2004] translates contextually into an inflammation of the nasal mucosa (and therefore also of the inferior turbinate) and that transcriptomics studies [Platt MP et al., 2011] showed a substantially overlapping gene expression profile of various nasal subsites, it is also true that clinical practice teaches that the phenotypic manifestations of CRS usually spare the inferior turbinates' mucosa. [White LC et al., 2016]. This aspect is the inspiring concept underlying the recent "reboot

approach" [Bachert C et al., 2018]. Again, She et al. demonstrated that about 66% of patients with CRS show a marked inflammation in the inferior turbinate but the inflammation is much more intense in maxillary sinus mucosa [She W et al., 2018]. Furthermore, the inflammatory response in the ethmoid sinus seems even more severe than in maxillary sinus or inferior turbinate in other series of chronic sinusitis patients [Kamil A et al., 1998]. These findings, together, suggest that paranasal sinuses, especially ethmoid, possibly play a pivotal role in CRS. It follows that sampling a typical site of CRS manifestation might be more representative of CRS-related inflammatory profile; moreover, let add that the cellular inflammatory pattern of the inferior turbinate can be clearly influenced by the coexistence of allergic and non-allergic rhinitis. More interesting data, observed by Armengot et al., is that a statistically significant correlation exists between ethmoid tissue eosinophilia and MM cytological eosinophilia, indeed [Armengot M et al., 2010]; the same moderate correlation emerged from our data ($r = 0.51$, $p < 0.0001$). However, the estimated accuracy of nasal cytology seems limited because overall sensibility values are low. Interestingly, once excluded allergic patients from the analysis, sensitivity further decreases for cytological sampling on IT and slightly increases for cytological MM sampling. This fact suggests that the allergy comorbidity can act as confounding factor and should be taken into account when interpreting nasal cytology findings. These results moreover lead to further reflections. Apparently, nasal cytology might not be the ideal screening test for ECRS due to low sensitivity values. However, when applied to patients clinically suspect for ECRS, this test might confirm the diagnosis and drive treatment selection. Lastly, it is reasonable to think that also the degree of neutrophilic infiltration produces an effect in terms of CRS classification. Thus, a more comprehensive grouping should account also for mixed-granulocytic and pauci-granulocytic CRS cases, apart from the classical ECRS and non-ECRS subtypes.

Of course, the study is somewhat limited. It represents a preliminary exploration of the role of nasal cytology in CRS in a relatively small population. The technical

choice has fallen upon nasal scraping because it was the only available in our center. Anyway, it remains that literature concerning this topic is limited and extremely variable in terms of sampling site and processing techniques, which makes it difficult to carry out comparisons and draw solid conclusions.

In summary, assuming that at present a re-classification of CRS is extremely urgent, together with the identification of reliable biomarkers, nasal cytology conceptually represents an interesting tool. In the same way as bronchial cytology for asthma, nasal cytology would allow a cellular profiling of CRS which, albeit embryonic, is a step forward the endotyping process and the thoughtful application of innovative biological therapies. Additionally, it shows several practical advantages, such as performing easiness, good tolerability and compliance, and limited costs. It is reasonable to think that performing nasal cytology in the middle meatus might give more accurate information on the degree of tissue eosinophilia in CRS. Next steps would be to verify these results across other wide and unbiased cohorts (eventually comparing different sampling methods) and to define thresholds values with the highest predictive index. However, at present, its semi-quantitative nature, the lack of standard cut-offs and the discrepancy of reported results limit its systematic use in CRS workup, while remaining undisputed its role in chronic rhinitis.

4. Future directions

A new paradigm to advance medical care is precision medicine. Precision medicine refers to the "ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology or prognosis of those diseases they may develop, or in their response to a specific treatment". Precision medicine supports a personalized, predictive, preventive and participatory approach to health, encouraging a convergence of omics, systems medicine, innovative health information technology and consumer-driven health care.

CRS endotype-driven treatment represents this need to adapt to the standards of precision medicine. However, despite considerable progresses in research, many questions remain open, mainly related to when, who and where to endotype. The lack of sensitive and specific biomarkers to identify different CRS endotypes is considered a major challenge to implement endotyping into clinical practice.

For the initial implementation, unanimity is achieved among experts on endotyping and biologicals being reserved for CRS patients with uncontrolled disease. However, some experts argue that biological treatment might alter disease progression, giving this treatment a potential role in the "prevention" of CRS patients to evolve towards a more severe phenotype. From this perspective, full endotyping of all CRS patients might become cost-effective in the future.

To establish the implementation of endotyping CRS patients in daily practice, scientific evidence should be developed to convince the health care system and associated organizations of the cost-effectiveness of this method. Therefore, solid data about the pharmaco-economics of CRS (without biologicals) are needed, including the cost of the "complications" of both medical treatment (for example side effects of oral steroids) and endoscopic sinus surgery.

Finally, if endotyping and biological treatment evolve to the next level of being implemented in general clinical practice, adaptation of the European guidelines with integration of the new biologicals in the treatment algorithms will be needed, taking into account regional differences reflecting the variation in organization and funding of health care systems across Europe.

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List of abbreviations

AERD, aspirin exacerbated respiratory disease
AFRS, allergic fungal rhinosinusitis
ALI, air-liquid interface
COX, cyclooxygenase
CRS, chronic rhinosinusitis
CRSsNP, chronic rhinosinusitis without nasal polyps
CRSwNP, chronic rhinosinusitis with nasal polyps
CT, computed tomography
ECRS, eosinophilic chronic rhinosinusitis
EDTA, ethylenediaminetetraacetic acid
EGPA, eosinophilic granulomatosis with polyangiitis
EPOS, European Position Paper on Rhinosinusitis and Nasal Polyps
ESS, endoscopic sinus surgery
GAL2EN, Global Allergy and Asthma European Network
HPF, high power field
IFN, interferon
Ig, immunoglobulin
IL, interleukin
ILC2, type 2 innate lymphoid cell
IQR, interquartile range
IT, inferior turbinate
LK, Lund-Kennedy
LM, Lund-Mackay
LT, leukotriene
MCID, minimal clinical important difference
MM, middle meatus
OMC, ostio-meatal complex
QoL, quality of life
RI, relative improvement
SD, standard deviation

Siglec, sialic acid immunoglobulin-like lectin

SNOT-22, Sinonasal Outcome Test 22

Th, T helper

TNF, tumor necrosis factor

TSLP, thymic stromal lymphopoietin

UP, uncinate process

US, United States

WBC, white blood cell

WHO, World Health Organization

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