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# Contents

JIACI Journal of Investigational Allergology and Clinical Immunology

# PREMIO PROFESOR ALBERTO OEHLING

La SEAIC, en agradecimiento a la labor desarrollada por el Profesor Alberto Oehling, uno de los pioneros de la Alergología en España y fundador de la revista *Journal of Investigational Allergology and Clinical Immunology*, ha decidido convocar bianualmente los premios "Profesor Alberto Oehling".

## BASES DE LA CONVOCATORIA

1. Este premio tiene por objetivo incentivar la publicación de artículos originales de calidad en el Journal of *Investigational Allergology and Clinical Immunology*, órgano oficial de la SEAIC.
2. Se concederá un primer premio de 5.000 euros y un accésit de 2.000 euros.
3. Optarán a los premios todos los artículos originales publicados en el JIACI en el periodo de tiempo comprendido desde el 1 de octubre del año del último congreso de la SEAIC hasta el 30 de septiembre del año del siguiente congreso, en los que al menos un firmante sea Socio de la Sociedad Española de Alergología e Inmunología Clínica, salvo deseo expreso de los autores de no optar al mismo.
4. No podrán optar a estos premios los artículos publicados en forma de casos clínicos o comunicaciones cortas (Practitioner's Corner), editoriales, cartas o revisiones.
5. El jurado que realizará la selección de los dos trabajos premiados estará presidido por el Presidente de la SEAIC y constituido, además, por los Editores Jefe y los Editores Asociados del JIACI. Su decisión será inapelable.
6. El premio podrá quedar desierto si así lo considera el jurado.
7. La entrega de los premios se realizará en un acto que se celebrará durante el Congreso de la SEAIC. Los autores designarán a la persona del equipo que recogerá el premio y que deberá ser un miembro numerario de la SEAIC.

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## Case Reports



## Letters to the Editor

# Effect of Different Therapeutic Strategies on Olfactory Outcomes in Patients With Chronic Rhinosinusitis With Nasal Polyps: A Systematic Review

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## Abstract

*Introduction:* Olfactory impairment is one of the cardinal symptoms of chronic rhinosinusitis with nasal polyps (CRSwNP). However, the effect of currently available therapeutic options on the recovery of the sense of smell is not well defined. The aim of this systematic review was to compile evidence on the impact of medical, surgical, and biological treatment on olfactory outcomes in patients with CRSwNP.
*Methods:* This review was conducted by 2 reviewers according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. The quality of evidence of all the studies included in the qualitative synthesis was evaluated using the Critical Appraisal Skills Programme (CASP).
*Results:* Forty-four studies were included in the qualitative synthesis. These assessed sinonasal surgery (n=23), biologics (n=15), and conventional medical treatment (n=6). The methodological quality was moderate-to-high in most. Overall, significant improvements in the sense of smell were detected with all the interventions analyzed and measured using an objective tool, a subjective tool, or both. However, most studies used different outcome measures, thus hindering comparisons between interventions, and data on clinically relevant changes were missing.
*Conclusion:* Oral corticosteroids, biologics, and sinonasal surgery improve the olfactory impairment associated with CRSwNP. However, the heterogeneous nature of existing studies does not allow accurate comparisons.

**Key words:** CRSwNP. Olfaction. Impairment. Biologics. Surgery. Corticosteroids.

## Resumen

*Introducción:* El deterioro del olfato es uno de los síntomas cardinales de la rinosinusitis crónica con pólipos nasales (RSCcPN), pero el efecto de las opciones terapéuticas actualmente disponibles sobre la recuperación del sentido del olfato no está bien definido. El objetivo de esta revisión sistemática es recopilar datos sobre el impacto de los tratamientos médicos, quirúrgicos y biológicos en los resultados sobre el olfato de los pacientes con RSCcPN.
*Métodos:* La revisión se llevó a cabo de acuerdo con las directrices *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA), y el proceso fue realizado por dos revisores. La calidad de la evidencia de todos los estudios incluidos para la síntesis cualitativa se evaluó mediante el Critical Appraisal Skills Programme (CASP).
*Resultados:* Se incluyeron cuarenta y cuatro estudios para la síntesis cualitativa (que evaluaban la cirugía sinonasal [n=23], los productos biológicos [n=15] o el tratamiento médico convencional [n=6]), la mayoría de ellos con una calidad metodológica de moderada a alta. En general, se detectaron mejoras significativas en el sentido del olfato con todas las intervenciones analizadas medidas mediante una herramienta objetiva o subjetiva (o ambas). Sin embargo, la mayoría de los estudios utilizaron diferentes pruebas de medición de resultados, lo que dificultó las comparaciones entre intervenciones, y se ofrecían datos sobre el cambio clínicamente relevante.
*Conclusion:* Los corticosteroides orales, los fármacos biológicos y la cirugía sinonasal mejoran la alteración olfativa asociada a la RSCcPN, pero la elevada variabilidad entre los estudios existentes no permite realizar comparaciones precisas.

**Palabras clave:** RSCcPN. Olfato. Deterioro. Fármacos biológicos. Cirugía. Corticosteroides.

## Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a complex disorder characterized by chronic inflammation of the sinonasal mucosa and presence of nasal polyps [1], which confer a significant long-term symptom burden [2]. It affects about 4% of the population globally [3] and, in most patients, is associated with type 2 inflammation, a pathway involved in other airway diseases. For this reason, CRSwNP often co-occurs with asthma and/or nonsteroidal anti-inflammatory drug–exacerbated respiratory disease (N-ERD) [4]. Among the range of clinical symptoms usually present in CRSwNP, olfactory impairment is a common complaint that can be troublesome and substantially impact on patients' quality of life [5].

The conventional approach to improving olfactory outcomes consists of medical treatment with intranasal corticosteroids (INCS), nasal washing, antibiotics, and/or oral corticosteroids (OCS) [6]. In refractory cases, endoscopic surgical resection of nasal polyps is recommended, and biological agents were recently approved as an alternative treatment for these cases [7]. However, despite the increasing number of studies assessing olfactory outcomes in patients with CRSwNP [8], the effect of currently available therapeutic options on olfactory recovery is not well defined.

The aim of this systematic review, then, was to analyze the literature in order to compile and summarize current evidence on the effect of medical, surgical, and biological treatment of olfactory dysfunction associated with CRSwNP.

## Methods

This review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [9] and the recommendations of the Cochrane handbook for systematic reviews [10]. The search protocol was entered in the International Prospective Register of Systematic Reviews (PROSPERO) of the National Institute for Health Research under number CRD42022336668.

### *Search Strategy*

The research question was defined using the PICO structure. The population comprised patients with CRSwNP, and the interventions considered included biologic therapies, medical therapies, and surgery. Outcome was the change in the sense of smell at different timepoints after surgery or after the beginning of the treatment measured with 1 or more of the following psychophysical and/or subjective tests: Sniffin' Sticks test, Connecticut Chemosensory Clinical Research Center (CCCRC) test, Brief Smell Identification Test (BSIT), University of Pennsylvania Smell Identification Test (UPSIT), Barcelona Smell Test-24 (BAST-24), visual analog scale (VAS), Likert scale, and smell item of the 22-item Sinonasal Outcome Test (SNOT-22). The comparator was the change in outcome after the intervention, or another intervention, or placebo.

A search strategy that also included Medical Subject Heading (MeSH) terms was developed (Table S1). Searches for publications in English and/or Spanish were performed using the PubMed, Web of Science, and SCOPUS databases on April 1, 2022, with a publication timeframe that ran from January 2014 to March 2022.

### *Study Selection and Data Extraction*

Two reviewers screened the title, abstract, and full text of all articles (one reviewer screened the records and the other checked the decisions) and applied eligibility standards based on the inclusion/exclusion criteria for selecting the studies. The final articles comprised systematic reviews with meta-analyses, clinical trials (both randomized and nonrandomized), post hoc studies of randomized trials, and observational studies focusing specifically on the effects of the medical, surgical, or biological treatment of CRSwNP on smell impairment measured using one of the previously mentioned tests. The exclusion criteria were systematic reviews without meta-analyses, case reports or case series, narrative reviews, studies on chronic rhinosinusitis without nasal polyps (CRSsNP) or mixed CRS (CRSwNP and CRSsNP), studies with patients presenting comorbidities not associated with T2 inflamation, studies with a sample size smaller than 25 patients (we estimated the sample size for between-group mean comparisons with an $\alpha$ level of 0.05 and a power of 80%, assuming a mean difference in the UPSIT score of 5 points and an SD of 4), publications where explicit olfactory outcomes could not be retrieved, and subanalyses of a study already included with repeated outcome data. The data extracted were recorded using a standardized Microsoft Excel® template by a single reviewer and validated by a second reviewer and included information about the study design and methodology, percentage of participants with asthma, N-ERD and previous surgery at baseline, follow-up time, outcomes before and after the intervention, data on olfactory status and clinically relevant changes when available, and conclusions.

### *Methodological Quality Assessment*

The quality of evidence of all studies included was evaluated to determine risk of bias using the Critical Appraisal Skills Programme (CASP) (https://casp-uk.net/casp-tools-checklists/). Two independent reviewers assessed both methodology and results using the appropriate checklist depending on the type of study. In the absence of a numeric score, the articles were classified as low-, medium-, or high-quality evidence according to the type of study and the number of questions in the corresponding checklist that were answered affirmatively or negatively.

## Results

A total of 1659 records were identified through the database searches. After eliminating duplicates and screening the title, abstract, and full text, we selected 44 publications for inclusion. The Figure shows the PRISMA diagram detailing the workflow of the screening process. Articles finally selected for the qualitative synthesis included clinical trials, subgroup and post hoc analyses, systematic reviews with meta-analyses, and observational studies. The methodological quality of the references reviewed is shown in Table S2a and Table S2b.

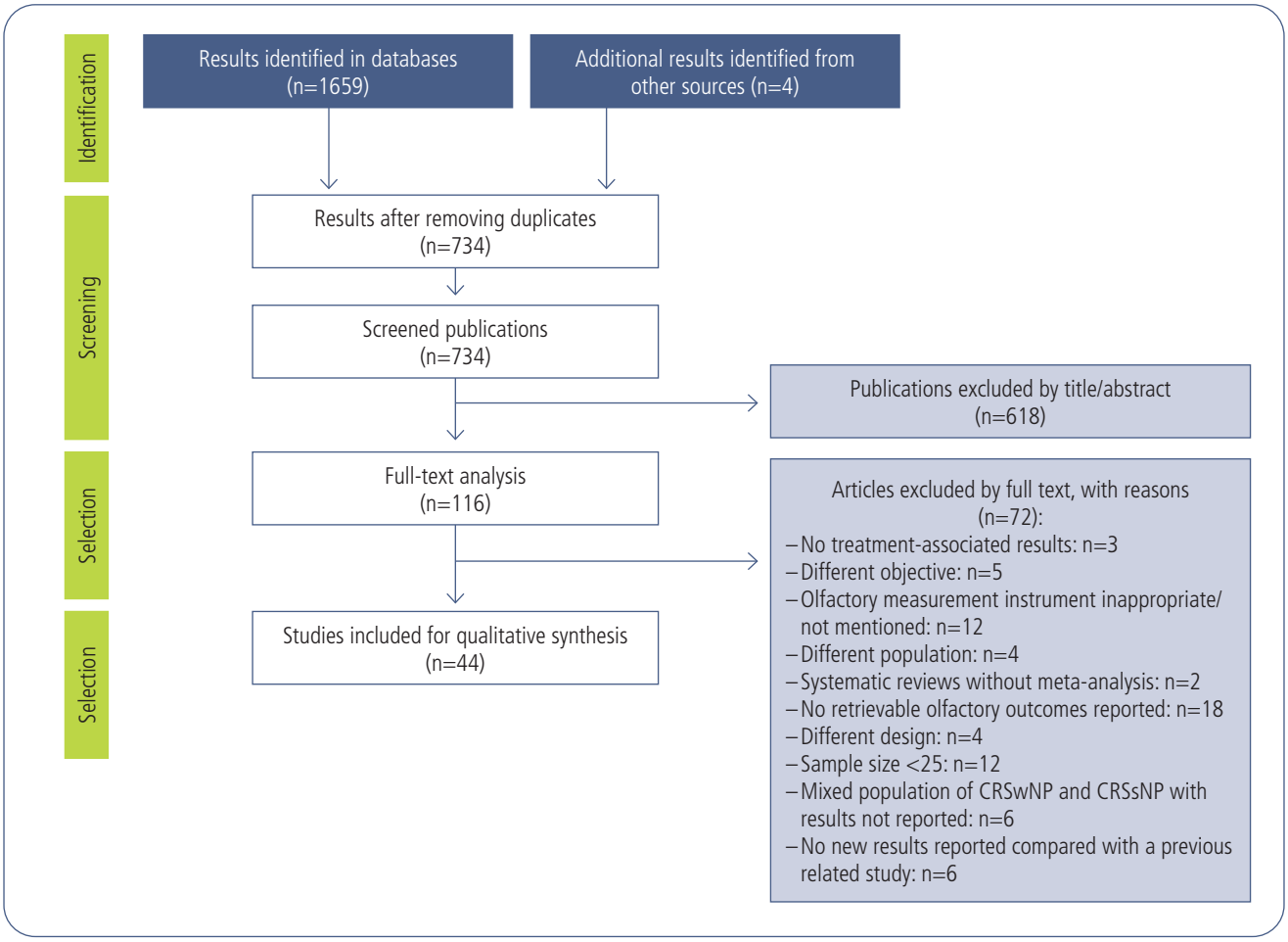


**Figure.** PRISMA diagram showing the selection flow of identified references. PRISMA indicates Preferred Reporting Items for Systematic reviews and Meta-Analyses; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

### *Effect of Biological Treatment on Olfaction*

Fifteen articles addressed biological treatment with dupilumab, mepolizumab, omalizumab, benralizumab, or reslizumab. Of these, 10 were randomized controlled trials (RCTs) and 5 were systematic reviews with meta-analyses. The population sample in most studies comprised patients with noncontrolled CRSwNP and an inadequate response to INCS and/or previous endoscopic surgery.

The UPSIT was used in almost all articles that addressed the efficacy of either dupilumab or omalizumab (n=13) and in the meta-analyses that included several biologics. In general terms, significant improvements in the UPSIT scores were detected after treatment with biologics (ranging from 16 to 52 weeks), and all the studies showed a statistically significant mean change from baseline (Table). In studies where the comparator was placebo, the least square mean difference (95%CI) in the UPSIT scores between the 2 arms at 24 weeks of follow-up ranged from 3.81 (1.38-6.24) (*P*=.0024) for omalizumab (POLYP 1 trial) [11] to 10.56 (8.79-12.34) (*P*=.0001) for dupilumab (LIBERTY NP SINUS 24 trial) [12]. According to 3 network meta-analyses and indirect comparisons, the mean difference in UPSIT scores (95%CI) for dupilumab versus omalizumab was 6.70 (4.67-8.73) [13], 7.21 (5.20-9.23) [14], and 6.70 (4.59-8.80) [15], all favoring dupilumab. The latter was also superior to mepolizumab, with a mean (95%CI) difference of 4.83 (2.43-7.22), and to benralizumab, with a mean (95%CI) difference of 8.01 (5.73-10.29). Besides the UPSIT test, the Sniffin' Sticks test was the only nonsubjective smell test used, in only 1 study, revealing a mean difference of 0.7 (–0.5 to 1.9) between mepolizumab and placebo at 24 weeks of treatment (*P*=.233) [16].

In 10 of the 15 publications, 1 or more subjective outcome measures of olfaction were used. These outcomes were usually in line with those derived from the objective tests (Table). However, none of the studies included provided complete data on the clinically relevant change based on the olfactory condition, evaluated as the percentage of patients who were normosmic, hyposmic, and anosmic before and after treatment. Only a pooled analysis of the LIBERTY NP SINUS-24 and SINUS-52 phase 3 trials reported that 77.6% of 724 patients were anosmic at baseline, compared with 28% after treatment with dupilumab [17]. Detailed data for all outcomes and studies included are shown in the Table.

## Effect of Surgical Treatment on Olfaction

In 23 of the references included, the study intervention was sinus surgery, and most were prospective observational studies (n=17). The remaining articles were systematic reviews with meta-analyses (n=2), randomized or nonrandomized clinical trials (n=3), and a retrospective study (n=1). An objective olfactory test was used in 12 studies, whereas a subjective test was used in 11 studies. Six studies combined the use of an objective and subjective tool, and 8 included data on the percentage of patients with each clinical olfactory status before and after the intervention. Globally, all studies concluded that sinus surgery significantly improved the CRSwNP patient's perceived and measured sense of smell. The follow-up time ranged from 6 weeks to 12 years, and the population analyzed comprised mostly patients with CRSwNP refractory to medical treatment (Table S3). One study showed very long-term postoperative improvement in olfaction according to the BAST-24 test: at baseline, the median percentage (IQR) of smell detection, smell memory, and smell identification were 0 (0-5), 0 (0-5), and 0 (0-0), respectively, while at a 12-year follow-up, they were 65 (0-100) (*P*=.001), 15 (0-46.2) (*P*=.031), and 30 (0-55) (*P*=.001).

Two studies that reported separate CRSwNP and CRSsNP data observed a more pronounced response to surgery in patients with CRSwNP [18,19]. From the analyses including data on clinical olfactory status, 1 study reported a change in the proportion of anosmic patients, falling from 36.6% before surgery to 17.1% at 6 months after surgery. Almost half (46.5%) were hyposmic both before surgery and 6 months after surgery, while the proportion of normosmic patients rose from 17.1% before surgery to 36.6% at 6 months after surgery [20]. According to Bardaranfar et al [21], combined surgical and medical treatment had a better effect (CCCRC mean [SD] score: 1.10 [0.344] pretreatment vs CCCRC 7.0 [0.0] posttreatment) than surgery alone (CCCRC 1.33 [0.32] pretreatment vs CCCRC 6.37 [0.24] posttreatment), and these results correlated with clinical olfactory status (Table). In a different study, CRSwNP patients were significantly more likely to report complete restoration of smell or taste following sinus surgery than with medical management (23.8% vs 4.0%; *P*=.026) [22]. One trial compared the difference in olfactory outcomes between extensive endoscopic sinus surgery (EESS) and functional endoscopic sinus surgery (FESS). The mean (SD) difference in the VAS score 1 year after surgery was 6.00 (3.67) in the EESS group (n=23) and 3.30 (3.44) in the FESS group (n=24) (*P*=.015).

## Effect of Medical Treatment on Olfaction

Of the 44 references included in the qualitative synthesis, only 6 reported a medical intervention other than surgery or biologics, and all 6 were RCTs. Thus, 1 of these trials assessed the administration of oral prednisone for 2 weeks (30 mg daily for 4 days followed by a 2-day reduction of 5 mg) plus intranasal budesonide spray twice daily (400 µg) for 12 weeks. The control group did not receive the 2-week treatment with oral prednisone [23]. The combination of OCS and INCS improved smell and nasal congestion while decreasing nasal inflammation compared with the control group (*P*=.05). These results were in line with those of another study, in which recovery of olfaction was better when initial medical treatment consisted of a short course of oral dexamethasone and intranasal budesonide compared with INCS alone (*P*=.001) (Table) [24].

Kern et al [25] performed a sham-controlled trial with a sample of 300 refractory CRSwNP patients. Patients who received absorbable mometasone-eluting furoate 200 µg nasal spray combined with a mometasone nasal implant (1350 µg) experienced sustained olfactory improvement (*P*=.0470) compared with placebo (Table) after 90 days of follow-up. A first-in-human study with 30 patients reported a statistical improvement in the CCCRC test (Table) between baseline and 24 weeks with 0.1% tretinoin added to intranasal budesonide compared to the latter alone [26]. Poletti et al [27] compared the efficacy of a specific device for endonasal aerosol delivery of corticosteroid (AMSA®) with that of a conventional nasal spray. The clinically relevant olfactory improvement was limited, and this device was not superior to the conventional spray according to the Sniffin' Sticks test results (Table). Lastly, a prospective randomized open-label trial compared the efficacy of montelukast as an add-on treatment to INCS in postoperative CRSwNP patients (n=72) with INCS alone. The mean change in BAST-24 and VAS scores after 1 year was similar between the 2 treatment groups (Table). Therefore, the addition of montelukast to INCS in the treatment of postoperative CRSwNP patients is not recommended [28].

## Discussion

Among the cardinal symptoms of CRSwNP, olfactory impairment is usually described by patients as one of the most bothersome, severely impacting on their quality of life [5]. This is the first systematic review to evaluate the 3 currently available therapeutic approaches to CRSwNP (biologics, surgery, and conventional medical treatment). In general, very few studies compare these approaches directly. Significant olfactory improvements were detected with all the interventions assessed. In terms of conventional medical treatment, better olfactory outcomes were achieved in more than 1 study with the combination of OCS and INCS than with the latter alone [23,24], although evidence is very limited and inconclusive for other combinations [26-28]. Comparisons between the outcomes retrieved with different biologics when using the same measurement tool reveal dupilumab to be the most beneficial in terms of recovery of olfaction [1,12,17,29,30]. In all these studies, the comparator was placebo or the medical standard of care. The findings are supported by the network meta-analysis [2,13-15], although they are based on indirect treatment comparisons. As a result, samples may not be comparable, and results may be subject to selection bias; hence the need for head-to-head comparisons between biologics with longer follow-up times and real-world evidence to draw more reliable conclusions. With respect to surgery, most publications included also reported a significant response to ESS and better olfactory function based on both subjective and objective measurements. However, there is some disagreement in this regard: Lind et al [31] stated that

certain patients are less likely to benefit from surgery and that 7%-10% of the patients may experience deterioration in their sense of smell after surgery.

Although the outcomes of medical interventions (mostly corticosteroids) show an improvement in olfaction, the results do not seem as clear as those obtained with surgery or biologics. According to DeConde et al [22], patients with CRSwNP were significantly more likely to report complete resolution of smell following surgery than following medical treatment. However, it is difficult to compare the 3 interventions globally owing to the heterogeneity of the olfactory tests applied and the characteristics of the study population. In general terms, the methodological design of studies assessing biologics or medical treatment is more robust, as most are RCTs with large samples, whereas studies analyzing surgery tend to be observational and include significantly fewer patients. Besides, in some of the studies included in this review, the authors performed subgroup analyses within the CRSwNP population. These revealed significant improvements in olfaction for dupilumab regardless of prior sinonasal surgery or prior systemic corticosteroids [1]. Furthermore, among patients with anosmia at baseline, the proportions of patients who regained some sense of smell (UPSIT >18) at week 24 with dupilumab were comparable among those with and without N-ERD [30]. However, patients with N-ERD are more frequently anosmic and have more severe and difficult-to-treat disease, which might result in better outcomes in this subpopulation than in patients without N-ERD.

It is important to emphasize that most studies to date only include information on olfactory outcomes expressed as objectively measured and/or patient-reported scores, and do not include data on the percentage of anosmic or hyposmic patients who recover their sense of smell, a variable that reflects the clinically relevant change. Only a few studies incorporate these qualitative criteria. According to these results, biological treatments, including dupilumab, seem to be the most effective intervention in terms of improved olfaction, suggesting that the design of studies should include more qualitative parameters for measuring recovery of the sense of smell.

Measurement of olfaction must be standardized in order to establish common criteria for studies that compare treatments in terms of efficacy or effectiveness. Nevertheless, the phenotyping of respiratory diseases with an underlying pathophysiologic mechanism, such as T2 inflammation, is becoming the cornerstone of accurate patient management [32], helping patients to benefit from the best treatment option depending on the primary goal of therapy. This classification is often omitted in current clinical practice, thus hindering the choice of the most suitable first-line therapeutic option to achieve the desired outcomes.

This work is limited by the broadly based research question, which gave rise to considerable heterogeneity between studies, including patient cohorts that differ in severity, number of previous surgeries, and type and location of polyps. Additionally, the severity of olfactory impairment may vary depending on the endotype, which was not assessed in all studies. Likewise, in patients with the same endotype, the degree of improvement in olfaction may vary according to the degree of impairment, which is not well defined in many publications. Therefore, while these samples are not always easily comparable, our results may guide the design of future studies. In contrast, a major strength of our work is that the studies selected are high-quality and recent and used validated measurement tools. Another asset of this review is that it brings together all the evidence on the effect of the 3 current therapeutic interventions in olfactory loss in CRSwNP.

In conclusion, this review of the literature reveals that treatments targeting CRSwNP, such as OCS, biologics, and ESS, improve not only other markers and symptoms of the disease, but also the loss of smell. However, given that the currently available evidence is highly diverse due to the variability in outcome measurements, establishing standardized criteria would be desirable. Further research with real-world data that include results on clinically relevant changes measured by qualitative parameters is needed to gain in-depth knowledge on the optimal management of olfactory impairment.

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### *Conflicts of Interest*

IA declares receiving consulting fees and honoraria for lectures, presentations, speakers bureaus, educational events, and expert testimony, as well as support for attending meetings and/or travel from Menarini, Metronic, Olympus, Salvat, Novartis, Sanofi, GSK, AstraZeneca, Galenus Health, and Viatris. BB declares receiving honoraria for lectures, presentations, speakers bureaus, educational events for Chiesi and Roxall, as well as support for attending meetings and/or travel from Sanofi, GSK, Allergopharma, Allergy, and Roxall. CC declares receiving consulting fees from Forwardontics, honoraria for lectures, presentations, speakers bureaus, educational events from Sanofi, GSK, Mylan, Forwardontics, and Cinfa and for expert testimony from Audifon, as well as support for attending meetings and/or travel from Sanofi, GSK, Mylan, Forwardontics, and Cinfa. MGF is an employee of Medical Statistics Consulting, SL and declares no other conflicts of interest. JS declares receiving grants from Sanofi paid to the Fundación Jimenez Diaz, consulting fees from Sanofi, AbbVie, and Novartis, honoraria for lectures, presentations, speakers bureaus, or educational events from Sanofi, GSK, FAES Farma, as well as support for attending meetings and/or travel from Sanofi. JS also declares having an unpaid leadership or fiduciary role in the Spanish Society of Allergology and Clinical Immunology (SEAIC), the European Academy of Allergy and Clinical Immunology (EAACI) and the American Academy of Allergy, Asthma & Immunology (AAAAI).

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ORIGINAL ARTICLE

# Prevalence of Atopic Dermatitis in the Adult Population of Catalonia, Spain: A Large-Scale, Retrospective, Population-Based Study

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## Abstract

*Background:* Studies on the prevalence of atopic dermatitis (AD) in adults in general populations are scarce worldwide. We performed a retrospective population-based observational cohort study of 537 098 adult patients diagnosed with AD in Catalonia, Spain, a larger population than in previous studies.
*Objectives:* To study the prevalence of AD by age, sex, disease severity, multimorbidity, serum total immunoglobin E (tIgE), and appropriate medical treatment (AMT) for the population of Catalonia.
*Methodology:* The study population comprised adult individuals (≥18 years) diagnosed with AD according to medical records at different health care levels (primary, hospital, emergency) in the Catalan Health System. Statistical analyses were conducted to evaluate sociodemographic characteristics, prevalence, multimorbidity, serum tIgE, and AMT.
*Results:* The prevalence of AD in the adult Catalan population was 8.7%, being higher for nonsevere disease (8.5%) than for severe disease (0.2%) and in females (10.1%) than in males (7.3%). Topical corticosteroids were the most prescribed drug (66.5%), and treatment was prescribed more frequently in severe AD patients, especially systemic corticosteroids (63.8%) and immunosuppressants (60.7%). More than half of severe AD patients (52.2%) had serum tIgE ≥100 kU/L, and higher values were observed for those with multimorbidity. The most frequent comorbid respiratory diseases were acute bronchitis (13.7%), allergic rhinitis (12.1%), and asthma (8.6%).
*Conclusions:* We provide new and robust evidence of the prevalence of AD and related characteristics in adults using a large-scale population-based study and a more significant cohort of individuals.

Key words: Atopic dermatitis. Epidemiological study. Population-based. Prevalence. Severity. Multimorbidity. Total serum IgE.

## Resumen

*Antecedentes*: Existen pocos estudios de prevalencia de dermatitis atópica (AD) con cohortes de población adulta a nivel mundial. Realizamos un estudio poblacional de cohortes retrospectivo observacional con 537.098 pacientes adultos diagnosticados de AD en Cataluña (España), una población mayor que en estudios previos.
*Objetivos:* Estudiar la prevalencia de la AD por edad, sexo, gravedad de la enfermedad, comorbilidades, inmunoglobina E total sérica (tIgE) y con un uso adecuado del tratamiento médico (ATM) en la población catalana.
*Metodología:* Se incluyeron personas adultas (≥18 años) diagnosticadas de AD por historia clínica en los diferentes niveles asistenciales (primaria, hospitalario, urgencias) del Sistema Catalán de la Salud. Se realizaron análisis estadísticos para evaluar características sociodemográficas, prevalencia, comorbilidades, tIgE sérica y ATM.
*Resultados:* La prevalencia global de AD diagnosticada en la población adulta catalana fue del 8,7%, siendo mayor la AD no grave (8,5%) que la AD grave (0,2%) y en el sexo femenino (10,1%) con respecto al masculino (7,3%). Los corticoides tópicos fueron el fármaco más prescrito (66,5%), y el uso de todos los tratamientos prescritos fue mayor en pacientes con AD grave, especialmente corticoides sistémicos (63,8%) e inmunosupresores (60,7%). Más de la mitad (52,2%) de los pacientes con AD grave presentaron tIgE sérica ≥100 kU/L, y se observaron valores más altos en aquellos con múltiples comorbilidades. La bronquitis aguda (13,7%), la rinitis alérgica (12,1%) y el asma (8,6%) fueron las enfermedades respiratorias concomitantes más frecuentes.
*Conclusiones:* Nuestro estudio proporciona evidencia nueva y sólida de la prevalencia de la AD y las características relacionadas en adultos utilizando un estudio poblacional a gran escala y una cohorte de individuos más significativa que los estudios previamente publicados.

Palabras clave: Dermatitis atópica. Estudio epidemiológico. Poblacional. Prevalencia. Gravedad. Comorbilidades. IgE sérica total.

**Summary box**

- **What do we know about this topic?**
  Atopic dermatitis (AD) has an estimated global prevalence of 2% to 8% in adults, with a high impact on an individual's quality of life. AD is the most common cutaneous disease in children. Severe cases may persist over time and are more common during adulthood.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  To our knowledge, this is the first population-based epidemiological study performed in a large population of AD patients. We provide more robust prevalence results for Spanish adults overall and by age group, as well as data on disease severity, multimorbidity, medical treatments, and biomarkers.

## Introduction

Atopic dermatitis (AD) and its related states (atopic eczema, eczema, neurodermatitis) is a noncontagious, pruritic, inflammatory skin condition characterized by defects in the epidermal barrier. It is a chronic relapsing condition, often occurring in families with atopic diseases, namely, AD, bronchial asthma, and/or allergic rhinoconjunctivitis [1]. According to the European Academy of Allergy and Clinical Immunology, atopy is defined as a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, and eczema/dermatitis [2].

The pathogenesis of AD is multifactorial [3]. The genetic component plays a significant role, as do the skin microbiome and environmental factors. Onset is usually during childhood. AD is the most common cutaneous disease in children, with a high impact on an individual's quality of life. Infants with AD may develop the atopic march, which comprises simultaneous development of atopic disorders, including food allergy, allergic rhinitis, and asthma [3-5]. Severe cases may persist over time and are more common during adulthood.

Reports on the epidemiology of AD have estimated the global prevalence to be about 2% to 8% in adults [1]. In Europe, 4.4% of the population is estimated to have AD. In the USA, numbers vary between 4.9% [6] and 10.2% [7]. Evidence of the estimated prevalence for Spanish adults is scarce, and results vary between 1.9% [8,9] and 7.2% [6], with vast differences between geographical regions. The prevalence estimates for severe AD range between and 0.07% [9] and 0.09% [10].

We performed a retrospective epidemiological study using a large-scale population-based database for the period 2013-2017. We aimed to investigate the prevalence of AD, overall and by age and sex in a cohort of adults with AD from Catalonia, Spain. We also assessed disease severity, multimorbidity, total serum IgE levels, and medical treatments. To our knowledge, this is the first population-based epidemiological study to be performed in a large population of AD patients and to provide richer patient information than previously reported in the literature. Therefore, we provide more robust prevalence results for Spanish adults overall and by age groups, as well as data on disease severity, multimorbidity, medical treatments, and biomarkers for Spain.

## Materials and Methods

### *Study Population*

We analyzed all residents of Catalonia, the second most populated region in Spain, with coverage in the National Health Service (NHS) and included in the Agency for Health Quality and Assessment of Catalonia (AQuAS) database. The inclusion criteria were age ≥18 years and a diagnosis of AD established by medical records at any care level covered by the NHS (primary, hospital, outpatient, and emergency care) at any point in time from January 2013 until December 2017 (different follow-up period for everyone in the dataset). The exclusion criteria were transfer to other regions in Spain and permanent institutionalization (ie, patients in nursing homes, psychiatric care, and other care facilities). The study was based on 537 098 patients diagnosed with AD during 2013-2017.

The data obtained were confidential, anonymous, and dissociated according to the Spanish Organic Law on Data Protection (Law 15/1999 of December 13). The Spanish Agency for Medicines and Medical Devices classified the study as No-EPA (ie, no drug postauthorization), as this is a retrospective observational study of the epidemiological characteristics of AD. It was approved by the Clinical Research Ethics Committee, the International University of Catalonia (Barcelona), and the Ethics Committee of Hospital Clínic de Barcelona.

### *Study Design*

The database was provided by AQuAS and contains details of all administrative medical registers on available admissions to primary care, hospital care, outpatient care, and emergency care at the individual-patient level of residents in Catalonia with coverage in the NHS.

AD was recorded in the database from records based on medically certified diagnoses coded according to the *International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM)*. The *ICD-9-CM* codes considered were as follows: 691.8 - Atopic dermatitis and related states - Other atopic dermatitis and associated conditions: AD, eczema, neurodermatitis; 692.9 - Contact dermatitis and another eczema, unknown cause. Including the second code might have led us to overestimate the prevalence, because it comprises irritant and allergic contact dermatitis and other nonatopic dermatoses. Nevertheless, not considering that code would lead to the exclusion of a consistent number of AD registered as such.

The type of AD therapies prescribed (topical corticosteroids, antihistamines, topical and systemic immunosuppressants, and systemic corticosteroids) available in the database for the period under study can be found in the supplementary material.

See supplementary material for further description of the database and prescribed treatment codes.

### *Outcomes*

#### Demographic characteristics

The socioeconomic and demographic characteristics obtained were sex, age, and annual income levels (adjusted for household size).

#### Epidemiology

The overall prevalence of AD in the general adult population was calculated based on all individuals from the study population diagnosed with AD over the total adult population in Catalonia (6 155 980 residents in 2017). Since the database encompasses the entire population of Catalonia, prevalence results do not represent estimated values. Instead, data should be interpreted as the diagnosed prevalence in Catalonia during 2013-2017.

#### Disease severity

Since data on symptoms were not available in the dataset, no information was available for the SCORAD scale [1,11], and disease severity (nonsevere, severe) was classified based on drug prescription following the existing literature [9,10]. The degree of severity was based on drug prescription over the previous 2 years. Individuals were classified as presenting severe AD if they had been prescribed immunosuppressants (cyclosporine, azathioprine, cyclophosphamide, methotrexate, alitretinoin, mycophenolic acid, interferon α-2a, interferon α-2b) at least once during the previous 2 years or if they had been hospitalized/attended the emergency department during the previous 2 years with AD as a first diagnosis. Disease was considered nonsevere in all other situations.

#### Total serum IgE biomarker

Atopy and its associated allergic responses correlate with increased serum total IgE (tIgE) production. Therefore, tIgE was also provided in the AQuAS dataset and used to calculate the median (confidence interval) and number of individuals above and below the cut-off value (≥100 kU/L was considered high) for the total adult population by disease severity and by comorbid condition. The maximum value reported during 2016-2017 was recorded.

#### Multimorbidity

Comorbid conditions of AD, including respiratory disease/allergy and systemic/general conditions, were also analyzed (supplementary material).

### *Statistical Analysis*

We performed an observational, multicenter, longitudinal, retrospective study based on a review of all available medical records related to AD in Catalonia (from 2013 to 2017) using computerized databases with dissociated data.

The statistical analyses were conducted using the statistical package Stata 16. A descriptive study reported frequencies and proportions of individuals in the overall population and by disease severity for confounders, comorbid conditions, treatment characteristics, and biomarkers. The Pearson $\chi^2$ test was performed to assess independence between categorical variables, as well as mean differences by disease severity. OR (95%CI) and *P* values were reported for the multivariate logistic regression analysis performed to determine the probability of having severe AD against multimorbidity and confounders. The overall prevalence of AD was reported, as was the prevalence by disease severity, by sex, and by age groups. A *P* value <.05 was considered statistically significant.

## Results

### *Descriptive Characteristics*

Even though the population under study comprises adults, it is worth noting that most AD cases were recorded during childhood, up to age 15 years (Figure 1 online supplementary figures). After that, the population shrank, with fewer AD cases among adults, although sufficient to be the object of study.

AD was diagnosed in 537 098 adults out of 6 155 980 Catalan residents in 2017. Of these, 2.4% (12 860 individuals) were classified as having severe AD, and 97.6% (524 238 individuals) as having nonsevere AD (Figure 2 online supplementary figures).

More women than men had a diagnosis of AD for both nonsevere disease (1.46:1) and severe disease (1.52:1). The diagnosis of AD is more frequent (65%) in young adults (18-59) than in patients aged ≥60 years, and the same is true among the nonsevere and severe subgroups. More than half of the adult AD cohort (70%) had annual incomes <€18 000€ (Table 1).

### *Prevalence of AD*

The diagnosed prevalence of AD was 8.7%. Prevalence was higher in the nonsevere than in the severe group (8.5% vs 0.2%) (Figure 3 online supplementary figures). By sex, the overall prevalence was higher for females than males (10.1% vs 7.3%, *P*<.0001). The prevalence was highest for women aged 18-29 years (11.2%). The prevalence increased over time for both sexes, especially in men, where it was highest (9.6%) for those aged ≥60 years (Figure 4A online supplementary figures). Differences in prevalence between males and females were statistically significant (*P*<.0001) from age 30 years onward. A similar pattern for both sexes was observed for nonsevere AD (Figure 4B online supplementary figures). However, the prevalence for females with severe AD increased slightly with age.

### *Treatment Prescribed*

During 2013-2017, topical corticosteroids were the most prescribed drugs in the population as a whole (66.5%) and in each severity group (66.3% for nonsevere and 77.9% for severe). These were followed by antihistamines (53.2%)

**Table 1.** Sociodemographic Characteristics of the Adult Atopic Dermatitis Cohort.[a]

| | Overall cohort | Cohort by severity | |
|---|---|---|---|
| **Sociodemographic characteristics** | **N=537 098 (100%)** | **Nonsevere n=524 238 (97.6%)** | **Severe n=12 860 (2.4%)** |
| Sex, No. (%) | | | |
| Males | 217 999 (40.6) | 212 895 (40.6) | 5104 (39.7) |
| Females | 319 099 (59.4) | 311 343 (59.4) | 7756 (60.3) |
| $\chi^2$ (4.42; *P*=.036) | | | |
| Age, y, No. (%) | | | |
| 18-59 | 349 335 (65) | 341 503 (65.1) | 7832 (60.9) |
| ≥60 | 187 763 (35) | 182 735 (34.9) | 5028 (39.1) |
| $\chi^2$ (99.28; *P*<.0001) | | | |
| Income, €, No. (%) | | | |
| Exempt | 26 071 (4.9) | 25 359 (4.8) | 712 (5.5) |
| <18 000 | 380 118 (70.8) | 370 852 (70.7) | 9266 (72.1) |
| 18 000-100 000 | 129 273 (24.1) | 126 420 (24.1) | 2853 (22.2) |
| >100 000 | 1636 (0.3) | 1607 (0.3) | 29 (0.2) |
| $\chi^2$ (37.83; *P*<.0001) | | | |

[a]Number of individuals in each phenotype (total, nonsevere, and severe). The proportion of individuals over the total adult population in each phenotype is in parenthesis. *P* values are for the Pearson $\chi^2$ test of independence between categorical variables. Data on income were only available for 2017.

and systemic corticosteroids (24.9%). All medications were more frequently prescribed in patients with severe disease than in those with nonsevere AD; this difference is especially significant for systemic corticosteroids (63.8% vs 24%, respectively) and immunosuppressants (60.7% vs 4.5%). No AD treatment was prescribed in 16% of individuals, whose disease was considered mild (Table 2).

### *Total Serum IgE*

During the last 2-year period (2016-2017), information on serum tIgE was available for 14 841 individuals (Table 3). Of these, 6320 (42.6%) had serum tIgE values ≥100 kU/L. This proportion was higher in severe than in nonsevere disease (52.2% vs 42.1%, *P*<.0001), and for those AD patients with comorbid conditions (asthma, 60.8%; nasal polyps [NP], 45.9%; and both asthma and NP, 60.7%) than those without (38.4%, *P*<.0001) (Figure 5 online supplementary figures).

Serum tIgE values were significantly higher (*P*<.05) in severe than in nonsevere AD (110 kU/L vs 72.3 kU/L, respectively). Concerning multimorbidity, patients with asthma, NP, or both had higher levels of serum tIgE than those without multimorbidity (Table 3).

### *Multimorbidity*

The most frequent respiratory/allergic comorbid conditions were acute bronchitis (13.7%), allergic rhinitis (12.1%), and asthma (8.6%). The most prevalent nonrespiratory comorbid conditions were hypertension (28.2%), anxiety (20.9%), and overweight (19.2%) (Table 4). The proportion of all comorbid conditions was higher in severe than in nonsevere AD, with a significant difference between those with severe AD and asthma (15%) for nonsevere AD (8.4%) and those with allergies and severe AD (9.2%) than in those with nonsevere AD (4.8%) and acute bronchitis.

Along the same lines, asthma, nonspecified allergies, and food allergy should be highlighted among the remaining respiratory and allergic comorbid conditions for having the strongest associations with severe AD (OR, 1.83, 1.95, and

**Table 2.** Adult Atopic Dermatitis Cohort According to Disease Severity by Treatment Prescribed.[a]

| | Overall cohort | Cohort by disease | | |
|---|---|---|---|---|
| **Treatment prescribed No. (%)** | **N=537 098 (100%)** | **Nonsevere n=524 238 (97.6%)** | **Severe n=12 860 (2.4%)** | ***P* Value** |
| Drugs | | | | |
| Topical CS | 357 362 (66.5) | 347 349 (66.3) | 10,013 (77.9) | <.0001 |
| Systemic CS | 133 766 (24.9) | 125 565 (24.0) | 8201 (63.8) | <.0001 |
| Antihistamines | 285 591 (53.2) | 276 215 (52.7) | 9376 (72.9) | <.0001 |
| Immunosuppressants | 31 650 (5.9) | 23 849 (4.5) | 7801 (60.7) | <.0001 |
| No drugs | 87 533 (16.3) | 87 533 (16.7) | - | - |

Abbreviation: CS, corticosteroids.

[a]In parenthesis, the proportion of individuals over the total adult population in each phenotype (total, nonsevere, and severe). *P* values are based on the test of differences in means between the degrees of severity for each treatment under study at a 95%CI of significance (null hypothesis [Ho], ie, no statistically significant differences between degrees of severity. Reject Ho if *P*<.05). No drug data were available for 87 533 individuals, who were assumed to have nonsevere AD. Drugs are not mutually exclusive, as 1 individual can be simultaneously prescribed more than 1 group of medications.

**Table 3.** Total IgE Values for the Adult Atopic Dermatitis Cohort Over the 2016-2017 Period.[a]

| Biomarkers, median and mean values (95%CI) | Total population | By disease severity | | Cohort by disease | | | |
|---|---|---|---|---|---|---|---|
| | | Nonsevere | Severe | AD alone | AD + asthma | AD + NP | AD + asthma + NP |
| Serum total IgE | N=14 841 | n=14 172 | n=669 | n=11 887 | n=2472 | n=268 | n=214 |
| Median, kU/L | 73.4 (71.09-76.1) | 72.3 (70-74.9) | 110[b] (94.8-132.4) | 62.8 (60.2-64.8) | 153[b] (143-166) | 86[b] (62.15-106) | 132.6[b] (112-171.9) |
| Mean, kU/L | 275.4 (0.1-1513.1) | 259.5 (0.1-1405.6) | 582.1 (0.1-2903.1) | 227.8 (0.1-1286.9) | 474.9[b] (0.8-2246.9) | 270.0[b] (0.2-1327.1) | 441.4[b] (2.2-2039.4) |

Abbreviations: AD, atopic dermatitis; NP, nasal polyposis.

[a]For IgE, median and mean values are calculated and reported across the maximum value reported for everyone with available information on the biomarker during 2016-2017. The 95%CIs are reported in parenthesis for the median value and the test of statistically significant differences in means across degrees of severity and among multimorbidity phenotypes. Median values were preferred above average, as the kernel distribution for each biomarker was very asymmetric with extremely high skewness and kurtosis.

[b]$P<.05$.

1.54, respectively), as should rheumatoid arthritis among systemic conditions (OR, 22.63; $P<.0001$) (Table 4).

## Discussion

This is the first retrospective population-based epidemiological study of a large population of adult AD patients. Its main strength is that it offers abundant information for Spain, based on a total sample of 6.1 million residents. The main findings were as follows. First, the overall diagnosed prevalence of AD for the adult population of Catalonia was 8.7%, being higher for nonsevere disease (8.5%) than for severe disease (0.2%). Second, AD was more frequent among females than among males for the overall AD population and irrespective of disease severity and age range. Third, the prevalence of AD decreased during the lifespan for females and increased for males overall and by severity, except for females with severe AD, where it increased slightly with age. Fourth, in general, drug prescription was more frequent in severe than in nonsevere AD for all treatments, with more frequent prescription of systemic corticosteroids and immunosuppressants. Fifth, serum tIgE values were higher for severe than for nonsevere disease (52.2% vs 42.1%, $P<.0001$), and for AD patients with comorbid conditions (asthma, 60.8%; NP, 45.9%; and asthma and NP, 60.7%). Sixth, a higher proportion of individuals with severe AD had respiratory and allergic comorbid conditions, as well as systemic conditions, especially anxiety (20.9%) and hypertension (28.2%). There was also a strong association between rheumatoid arthritis and the probability of severe AD (OR, 22.63; $P<.0001$).

Our study is based on medical records from the Catalan health care system at the primary, hospital, and emergency care levels; this made it possible to identify a cohort of 537 098 adults with a diagnosis of AD established by medical records covered by the whole NHS, ie, 8.7% of the study population in Catalonia. This prevalence is very similar to recently reported data (2018) [6] for Spanish adults aged up to 65 years (7.2%), which were recorded using questionnaires on the diagnoses of AD in a sample of 9924 individuals.

Prevalence rates in this study are higher than those reported in the literature for Spain [9,10]. One cause of overestimation in the present study is the inclusion of the *ICD-9-CM* code 692.9. However, in our case, not including that code would have led the overall prevalence to be underestimated at 1.61% (99 062 individuals), possibly because of methodological differences between the studies cited and ours. The previous studies relied on a smaller population from 7 regions in Spain, whereas the current study is based on the Catalan adult population.

Moreover, the inclusion criteria for previous studies were more restrictive than for the present study. A recent report from Spain [10] used medical register data for adults aged >18 years, including only those individuals who met all the following inclusion criteria: prescription of any medication for AD with a minimum of 2 drugs during the follow-up period and ≥2 health records including 1 dermatology visit (38 475 individuals). These criteria considerably restricted the population and generated a lower prevalence result (1.9%) and a possible sample bias. Another difference in this result is the age group classification compared to previous studies [10], which is >18 years (ie, not including patients aged ≥18 years, thus potentially increasing prevalence). Yet, to date, the present population-based study is the first to analyze the prevalence of AD in the adult population using a much larger database and including all public medical registers of individuals with a diagnosis of AD during the study period in Catalonia, Spain, thus constituting a much larger sample of individuals with severe AD.

On the other hand, the prevalence of severe AD (0.21%) was more significant than reported in the literature, that is, 0.08% [8] and 0.07% [10], for the same reasons explained above. In addition, given the nature of the disease, the severity of AD is based on the medication prescribed instead of on the medical diagnosis. In the case of Spain [8-11], the medication used was directly associated with treatment of AD. In contrast, it was impossible in the present study to distinguish whether the drugs were explicitly prescribed to treat AD or other concomitant diseases.

In line with earlier studies [6,7], we found significant sex differences, with prevalence being higher in females (10.1%)

**Table 4.** Comorbid Conditions in the Adult Atopic Dermatitis Cohort.

| AD-related comorbid conditions | Total population N=537 098 | Population by disease severity[c] | | | | |
|---|---|---|---|---|---|---|
| | | Nonsevere n=524 238 (97.61%) | Severe n=12 860 (2.39%) | *P* Value | Logit regression Pr (severe) | |
| | | | | | OR (95% CI) | *P* Value[d] |
| Respiratory and allergy, No. (%) | | | | | | |
| Asthma | 45 934 (8.6) | 44 009 (8.4) | 1925 (15.0) | <.0001 | 1.83 (1.74-1.94) | <.0001 |
| Allergic rhinitis | 64 993 (12.1) | 63 498 (12.1) | 1495 (11.6) | .047 | 0.85 (0.80 -0.90) | <.0001 |
| Acute bronchitis | 73 608 (13.7) | 71 225 (13.6) | 2383 (18.5) | <.0001 | 1.12 (1.07-1.19) | <.0001 |
| Nasal polyposis | 4849 (0.9) | 4689 (0.9) | 160 (1.2) | <.0001 | 1.16 (0.98-1.37) | .085 |
| COPD | 8318 (1.5) | 7985 (1.5) | 333 (2.6) | <.0001 | 1.04 (0.92-1.18) | .536 |
| Nonspecified allergy | 26 295 (4.9) | 25 113 (4.8) | 1182 (9.2) | <.0001 | 1.95 (1.83-2.08) | <.0001 |
| Food allergy | 1747 (0.3) | 1661 (0.3) | 86 (0.7) | <.0001 | 1.54 (1.22-1.93) | <.0001 |
| Systemic and general, No. (%) | | | | | | |
| Hypertension | 151 486 (28.2) | 147 021 (28.0) | 4465 (34.7) | <.0001 | 1.16 (1.1-1.22) | <.0001 |
| Overweight | 103 080 (19.2) | 100 375 (19.1) | 2705 (21.0) | <.0001 | 0.93 (0.89-0.98) | .005 |
| Dyslipidemia | 57 399 (10.7) | 55 467 (10.6) | 1932 (15.0) | <.0001 | 1.18 (1.12-1.25) | <.0001 |
| Diabetes | 58 871 (11.0) | 57 027 (10.9) | 1844 (14.3) | <.0001 | 1.15 (1.08-1.22) | <.0001 |
| Ischemic heart disease | 21 087 (3.9) | 20 384 (3.9) | 703 (5.5) | <.0001 | 1.06 (0.97-1.16) | .191 |
| Alcohol-related disease[a] | 16 721 (3.1) | 16 105 (3.1) | 616 (4.8) | <.0001 | 1.36 (1.24 -1.49) | <.0001 |
| Smoking-related diseases[b] | 75 756 (14.1) | 73 577 (14.0) | 2179 (16.9) | <.0001 | 1.16 (1.10-1.22) | <.0001 |
| IBD | 6943 (1.3) | 6758 (1.3) | 185 (1.4) | .07 | 0.98 (0.84-1.14) | .775 |
| Rheumatoid arthritis | 4418 (0.8) | 2913 (0.6) | 1505 (11.7) | <.0001 | 22.63 (21.12-24.24) | <.0001 |
| Heart/liver/kidney failure | 6108 (1.1) | 5839 (1.1) | 269 (2.1) | <.0001 | 1.58 (1.38-1.80) | <.0001 |
| Anxiety | 112 084 (20.9) | 109 207 (20.8) | 2877 (22.4) | <.0001 | 1 (0.95-1.04) | .944 |
| Depression | 25 679 (4.8) | 24 829 (4.7) | 850 (6.6) | <.0001 | 1.12 (1.03-1.21) | .005 |

Abbreviations: COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease.

[a]Alcohol-related diseases include alcohol-induced mental disorders, alcohol dependence and abuse, alcoholic polyneuropathy, alcoholic cardiomyopathy, alcoholic fatty liver, acute alcoholic hepatitis, alcoholic cirrhosis of the liver, excessive blood level of alcohol, toxic (acute) effect of alcohol, alcoholic gastritis with or without bleeding, fetal alcohol syndrome.

[b]Smoking-related diseases include chronic pharyngitis, uncomplicated chronic bronchitis, and leukoplakia of the oral mucosa, including the tongue.

[c]The number of AD individuals in each phenotype (total, nonsevere, and severe) by related comorbid conditions. The proportion of individuals over the total adult population in each phenotype is in parenthesis.

[d]*P* values are for the test of differences in means between degrees of severity (nonsevere vs severe) for each comorbid condition under study at a 95%CI of significance (null hypothesis [Ho]: No statistically significant differences between severity degrees. Reject Ho if *P* value < .005). Logistic regression analysis for the probability of severe disease. The model includes sociodemographic characteristics as control variables.

than in males (7.3%), and differences in severity and over the lifespan, where prevalence tends to decrease for both sexes, except for females with severe AD.

Following the European Guidelines on Treatment for Atopic Dermatitis [1], we recorded more frequent prescription for severe AD than for nonsevere AD, especially in the case of systemic corticosteroids (63.8% vs 24%, respectively) and immunosuppressants (60.7% vs 4.5%).

Higher values for serum tIgE were found for severe AD and individuals presenting with comorbid conditions (asthma and/or NP). Serum tIgE values were ≥100 kU/L in 42.6% of individuals despite the lack of consensus on the use of serum tIgE for the diagnoses of AD [3] and the fact that it might be age-dependent, with tIgE levels decreasing with age [12,13]. This proportion was higher for severe AD (52.2%), as reported elsewhere [14], and for those with comorbid conditions [15,16].

Respiratory and allergy were the most frequent comorbid conditions, especially for those with severe AD compared to those with nonsevere AD. In line with earlier studies [8,10,17,18], the most prevalent comorbid conditions for the total adult AD population were acute bronchitis (13.7%), allergic rhinitis (12.1%), and asthma (8.6%). Among adults with severe disease, the most prevalent were acute bronchitis (18.5%), allergic rhinitis (11.6%), and asthma (15%). However, there are differences in the percentage of

multimorbidity reported in the severe AD population from other studies [10], which is higher (allergic rhinitis, 22.9%; and asthma, 20.8%).

Furthermore, a recent study [18] showed that 82% of patients with moderate-to-severe AD have ≥1 atopic comorbid condition, including allergic rhinitis (63%), asthma (54%), allergic conjunctivitis (44%), food allergy (40%), chronic rhinosinusitis (14%), atopic keratoconjunctivitis (8%), and NP (5%).

It can be concluded that there is a strong correlation between AD and type 2 diseases, which are even more frequent in severe AD patients, and that the proportion of comorbid conditions in this study could be lower owing to the restrictive inclusion criteria.

The most frequent systemic comorbid conditions were hypertension (28.2%), anxiety (20.9%), and overweight (19.2%), which were found in lower proportions, as reported elsewhere [10,20,21]. Moreover, although the frequency of patients with rheumatoid arthritis (0.8%) was lower than that of other systemic comorbid conditions, it had one of the strongest associations with severe AD (OR, 22.63), in line with previous studies [22].

Our study is subject to a series of limitations. First, the design was retrospective, and the severity of treatment was based on the medication prescribed instead of the medical diagnosis. Prescribed medication is understood as prescribed and purchased by the individual. However, information on whether this is taken is not available. Note that ruling out oral corticosteroids as a criterion of severity could have introduced bias between the severe and nonsevere clusters. Biological treatment was not considered, given its low implementation during the study period and because the reasons for this treatment were unknown. Second, we do not know whether some drugs, such as oral corticosteroids, were prescribed to treat AD or other, concomitant diseases specifically. Therefore, oral corticosteroid intake was not used as a severity criterion. On the other hand, the prevalence for severe AD patients could also be underestimated, by assuming that patients with no drug information present nonsevere AD, which might or might not always be the case. And third, the absence of individuals diagnosed and treated outside the NHS in private hospitals or medical centers could lead us to underestimate prevalence and disease severity.

In summary, evidence regarding the prevalence of AD in children is more plentiful than in adults. This paper aimed to contribute to the literature by providing new evidence based on a larger number of AD patients than in previous studies, including richer information on most of the patients diagnosed with AD from the general adult population of Catalonia. Our findings show an overall prevalence of 8.72%, with higher values for females than males overall and irrespective of severity and age range. The prevalence for severe AD was 0.21%, and this tended to increase slightly across age groups for females and decrease for males. Serum tIgE ≥100 was found in 42.6% of individuals and was more frequent in patients with severe AD and patients with comorbid conditions. Finally, the most frequent allergic and respiratory comorbid conditions were acute bronchitis and allergic rhinitis, and the most frequent systemic and nonallergic conditions were hypertension, anxiety, and obesity.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

# Noninfectious Complications in B-Lymphopenic Common Variable Immunodeficiency

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## Abstract

*Background:* Common variable immunodeficiency (CVID) is considered the most symptomatic type of inborn errors of immunity in humans. Along with infectious complications, which have numerous consequences, noninfectious complications are a major challenge among CVID patients.
*Methods:* All CVID patients registered in the national database were included in this retrospective cohort study. Patients were divided into 2 groups based on the presence of B-cell lymphopenia. Demographic characteristics, laboratory findings, noninfectious organ involvement, autoimmunity, and lymphoproliferative diseases were evaluated.
*Results:* Among 387 enrolled patients, 66.4% were diagnosed with noninfectious complications and 33.6% with isolated infectious presentations. Enteropathy, autoimmunity, and lymphoproliferative disorders were reported in 35.1%, 24.3%, and 21.4% of patients, respectively. Some complications, including autoimmunity and hepatosplenomegaly, were reported to be significantly more frequent among patients with B-cell lymphopenia. As for organ involvement, the dermatologic, endocrine, and musculoskeletal systems were predominantly affected in CVID patients with B-cell lymphopenia. Among autoimmune manifestations, the frequency of rheumatologic, hematologic, and gastrointestinal autoimmunity was reported to be higher than that of other types of autoimmunity not associated with B cell-lymphopenia. Furthermore, hematological cancers, particularly lymphoma, were the most common type of malignancy. The mortality rate was 24.5%, and respiratory failure and malignancies were the most common causes of death, with no significant differences between the 2 groups.

*Conclusion:* Considering that some of the noninfectious complications might be associated with B-cell lymphopenia, regular patient monitoring and follow-up with proper medication (in addition to immunoglobulin replacement therapy) are highly recommended to prevent sequelae and increase patient quality of life.

**Key words:** Primary immunodeficiency. Inborn errors of immunity. Common variable immunodeficiency. Autoimmunity. Malignancy. Immune dysregulation.

## Resumen

*Antecedentes*: La inmunodeficiencia común variable (IDCV) se considera el más sintomático error innato de la inmunidad en humanos. Las complicaciones infecciosas (que tienen numerosas consecuencias clínicas) son, junto a las complicaciones no infecciosas, un reto importante entre los pacientes con IDCV.
*Métodos:* Todos los pacientes con IDCV registrados en nuestra base de datos nacional se incluyeron en este estudio de cohortes retrospectivo. Los pacientes se dividieron en dos grupos en función de la presencia o ausencia de linfopenia de células B. Se evaluaron las características demográficas, los resultados de laboratorio, la afectación no infecciosa de diferentes órganos, la autoinmunidad y las enfermedades linfoproliferativas.
*Resultados:* De los 387 pacientes incluidos, el 66,4% fueron diagnosticados de complicaciones no infecciosas y el 33,6% solo presentaron cuadros infecciosos. La enteropatía, la autoinmunidad y los trastornos linfoproliferativos se registraron en el 35,1%, el 24,3% y el 21,4% de los pacientes, respectivamente. Algunas complicaciones, como la autoinmunidad y la hepatoesplenomegalia, se reportaron de forma significativamente superior entre los pacientes con linfopenia de células B. En cuanto a la afectación de órganos, los sistemas dermatológico, endocrino y musculoesquelético se vieron afectados predominantemente en los pacientes con IDCV con linfopenia de células B. Entre las manifestaciones autoinmunes, se observó que la frecuencia de autoinmunidad con afectación reumatológica, hematológica y gastrointestinal era superior en comparación con otros tipos de autoinmunidad independientes de la linfopenia de células B. Además, los tumores hematológicos, en particular el linfoma, fue ligeramente el tipo más común de neoplasia maligna. A su vez, la tasa de mortalidad global fue del 24,5%. La insuficiencia respiratoria y los tumores malignos fueron reportados como la causa más común de muerte en nuestros pacientes sin diferencias significativas entre los dos grupos.
*Conclusiones:* Teniendo en cuenta que algunas de las complicaciones no infecciosas podrían estar asociadas con la linfopenia de células B, la monitorización y el seguimiento regular del paciente junto con el tratamiento adecuado (además de la terapia de reemplazo de inmunoglobulinas) son recomendables para prevenir secuelas posteriores y aumentar la calidad de vida de los pacientes.

**Palabras clave:** Inmunodeficiencia primaria. Errores innatos de la inmunidad. Inmunodeficiencia común variable. Autoinmunidad. Malignidad. Disregulación inmune.

### Summary box

- **What do we know about this topic?**
  Peripheral B cells in CVID patients range from normal counts in most cases to B lymphopenia in a minority, indicating defects of early B-cell development in the latter.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  Our findings indicated that noninfectious complications including autoimmunity and lymphoproliferative manifestations affecting the dermatologic, endocrine, and musculoskeletal systems are associated with B-cell lymphopenia in CVID patients.

## Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic form of inborn errors of immunity (IEI) [1] and was first reported in 1945 by Sanford et al [2]. A heterogeneous immune defect, CVID is characterized by decreased serum immunoglobulin levels [3], reduced or absence of specific antibody production, and normal or low B-lymphocyte counts [4-6]. The prevalence of CVID is estimated at 1:50 000 to 1:25 000 [7]. Although CVID can occur at any age, this rare disease frequently appears in childhood or early adulthood [1,8]. Furthermore, men and women are equally affected [9]. Of note, given the gradual development of humoral immunity and the probability of transient hypogammaglobulinemia in infancy, a diagnosis of CVID should not be considered in patients aged <4 years [5]. The fact that CVID patients experience failure in B-cell differentiation into functional Ig-secreting plasma cells means that it is categorized mainly as comprising intrinsic B-cell defects. However, some patients experience a defect in other types of lymphocytes and immune components that play a significant role in the normal antibody response [7].

CVID has a wide spectrum of clinical presentations, including recurrent infections and noninfectious complications

such as autoimmunity, gastrointestinal inflammatory disease, liver disease, lymphoid hyperplasia, granulomatous disease, cytopenia, progressive lung disease, and cancer [10-12]. Noninfectious manifestations may be the first or predominant clinical presentation of CVID [13]. Approximately 20%-30% of CVID patients develop different forms of autoimmunity (as the most commonly reported form of noninfectious complications), such as juvenile rheumatoid arthritis, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus (SLE), alopecia areata, vitiligo, pernicious anemia, and autoimmune thyroiditis [14]. The risk of death is 11 times higher in CVID patients with noninfectious complications [11,12,15]. In addition, immunoglobulin replacement therapy, which is the standard treatment approach for CVID patients, cannot prevent or diminish most noninfectious manifestations [10,11]. Therefore, mortality and morbidity are major concerns amongst CVID patients with noninfectious complications [5,12,16].

CVID is a heterogeneous group of antibody defects in which the B-lymphocyte populations are mostly dysregulated. Although B-cell count as a key diagnostic marker has been discussed in detail, few studies have evaluated in depth CVID patients with low total B-cell counts and expected early B-cell developmental defects. Here, an updated clinical spectrum of noninfectious complications was compared between 2 groups, namely, CVID patients with B-cell lymphopenia and without B-cell lymphopenia. This study compares the diverse characteristics of CVID cases with early B-cell defects and those of patients with abnormalities in late B-cell developmental stages. Both types are currently labeled with the same diagnosis.

## Material and Methods

### *Patients*

This retrospective cohort study was based on the records of all registered CVID patients [17-29] who were referred to and diagnosed and treated in the research center for immunodeficiencies at the Children's Medical Center, which is affiliated to Tehran University of Medical Sciences, Iran. CVID patients were identified and managed (immunoglobulin replacement therapy, prophylactic antibiotics, targeted treatments) according to signs and symptoms linked with the syndrome based on the national IEI consensus [20,30], the European Society for Immunodeficiencies (ESID) diagnostic criteria [31], and The Middle East and North Africa Diagnosis and Management Guidelines for IEI [32]. Symptomatic patients with reduced IgG and IgA and/or low serum IgM levels were included. Other causes of hypogammaglobulinemia were considered exclusion criteria in patients aged >4 years with a weak antibody response to vaccines or low switched memory B cells and no evidence of profound T-cell deficiency. Patients with normal responses to vaccines who had low switched memory B cells and lack of isohemagglutinin were also considered to have CVID. The present study was approved by the Ethics Committee of the Tehran University of Medical Sciences, and written informed consent was obtained from the patients and/or their parents.

### *Clinical Evaluation and Classification*

We designed a comprehensive questionnaire, which was completed by the patients. The data recorded included age at clinical presentation, age at diagnosis, family history, consanguinity, autoimmunity, enteropathy, lymphoproliferation, malignancy, medications, last follow-up, and laboratory findings. Clinical phenotyping was performed using a standard method of phenotype subdivision that has been shown to correlate with quality of life and morbidity in patients with infections only and noninfectious phenotypes [12,33]. The evaluation also involved a complete blood count, lymphocyte subpopulations, serum Ig levels, specific antibody response, pulmonary function test (PFT), and high-resolution computed tomography (HRCT) scan, as previously described [28,34-40]. Immunologic tests were repeated for each patient every 6 months during routine follow-up visits after diagnosis to evaluate progression of their antibody deficiency. Patients were classified according to the absolute count of total peripheral B cells at the time of diagnosis and before initiation of treatment as having B lymphopenia (Group 1, <2 standard deviations of their normal age and >2% of circulating lymphocytes cells) and normal B-cell counts (Group 2) based on the age-standard ranges at the Research Center for Immunodeficiencies as the tertiary referral center using the previously described method [41,42]. Age-matched B-cell reference levels were as follows: 4-8 years, 300-1000/μL; 8-12 years, 200-500/μL; 12-18 years, 150-500/μL; and >18 years, 150-500/μL.

### *Statistical Analysis*

The statistical analysis of this retroactive cohort study was performed using IBM SPSS Statistics for Windows (version 22.0, IBM Corp.) and R studio (version 4.1.3). The statistical analysis was based on parametric and nonparametric assumptions. The Kolmogorov-Smirnov test was conducted to determine the normality of the distribution. The $\chi^2$ test and Fisher exact test were used to compare categorical variables based on a 2 × 2 table. The numerical variables were compared using the Mann-Whitney test or Kruskal-Wallis test and their parametric equivalents.

## Results

### *Demographic Characteristics*

The cohort comprised 387 patients diagnosed with CVID (222 male and 165 female, Table 1) in the Iranian national IEI registry. The median (IQR) ages of the patients at disease onset and at the time of the study were 2.0 (0.5-8.0) years and 25.0 (14.0-35.0) years, respectively. The median age of the patients at the time of the diagnosis was 10.0 (3.0-21.0) years. Overall, 70% of patients were diagnosed before age 18 years. Among the 387 patients, 215 (55.6%) were born to consanguineous families. Based on the B-cell count at the time of diagnosis, 168 patients were classified in Group 1, with low B-cell lymphopenia. Despite a similar age at onset and diagnosis, CVID patients with B lymphopenia (Group 1) had a significantly higher median age at the time of the study (27.0

**Table 1.** Demographic Data of the 387 CVID Patients With B Lymphopenia and Normal B Cells.

| Parameter | All CVID patients (N=387) | CVID with B lymphopenia (n=168) | CVID with normal B cells (n=219) | *P* Value[a] |
|---|---|---|---|---|
| Sex ratio (male/female) | 222/165 | 98/70 | 124/95 | .73 |
| Consanguinity, No. (%) | 215 (55.6%) | 102 (60.7%) | 113 (51.6%) | .07 |
| Family history of IEI, No. (%) | 47 (12.1%) | 19 (11.3%) | 28 (12.8%) | .65 |
| Median (IQR) age of patients at baseline, y | 25.0 (14.0-35.0) | 27 (18.0-38.0) | 22 (12.2-32.7) | .02 |
| Median (IQR) age at onset of symptoms, y | 12.0 (0.5-8.0) | 2.0 (0.5-9.0) | 2.0 (0.5-8.0) | .89 |
| Median (IQR) age at diagnosis, y | 10.0 (3.0-21.0) | 10.0 (4.0-26.2) | 9.0 (2.6-19.0) | .13 |
| Dead/Alive, No. | 82/252 | 42/103 | 40/149 | .10 |

Abbreviation: CVID, common variable immunodeficiency; IEI, inborn errors of immunity.
*$P<.05$, statistically significant.

**Table 2.** Immunologic Profile and Laboratory Data Comparison Between CVID Patients With B-Cell Lymphopenia and Patients With Normal B Cell Values.[a]

| Laboratory finding | All CVID patients (N=387) | CVID with B lymphopenia (n=168) | CVID with normal B cells (n=219) | *P* value[b] |
|---|---|---|---|---|
| IgG, mg/dL | 220 (50-470) | 198.0 (29.5-493.0) | 229.5 (71.2-440.5) | .40 |
| IgA, mg/dL | 9.5 (1.8-37.0) | 9.0 (0.7-36.0) | 10.5 (2.0-40.0) | .17 |
| IgM, mg/dL | 25.0 (8.5-50) | 24.0 (8.0-53.0) | 25.0 (9.0-50.5) | .96 |
| Neutrophils, % | 54.0 (41.0-67.0) | 57.0 (44.0-68.0) | 53.0 (37.2-65.0) | .03 |
| Neutrophils/μL | 3848.0 (2475.0-6014.0) | 3914.3 (2377.0-6545.3) | 3776.0 (2720.0-5636.4) | .83 |
| Lymphocytes, % | 35.0 (24.3-50.0) | 30.5 (21.0-46.0) | 38.0 (27.0-52.0) | .002 |
| Lymphocytes/μL | 2498.4 (1704.0-4221.5) | 2224.8 (1555.0-3503.2) | 2717.2 (1907.5-4694.0) | .003 |
| $CD3^+$ T cells, % | 74.0 (64.0-83.0) | 82.0 (71.0-88.0) | 69.0 (60.0-77.0) | <.001 |
| $CD3^+$ T cells/μL | 1778.4 (1244.8-2998.3) | 1632.3 (930.0-2839.0) | 1971.8 (1299-3073.6) | .04 |
| $CD4^+$ T cells, % | 32.0 (23.0-42.0) | 32.0 (19.0-44.0) | 33.5 (25.0-42.0) | .18 |
| $CD4^+$ T cells/μL | 771.8 (433.7-1349.0) | 626.5 (339.7-1101.2) | 954.6 (600.7-1647.7) | <.001 |
| $CD8^+$ T cells, % | 35.0 (25.0-50.0) | 41.0 (31.0-56.0) | 30.0 (22.2-42.0) | <.001 |
| $CD8^+$ T cells/μL | 889.4 (549.9-1425.2) | 890.8 (483.6-1567.2) | 888.0 (564.8-1317.0) | .95 |
| CD19 lymphocytes, % | 9.0 (4.0-17.0) | 4.0 (2.0-6.0) | 16.0 (11.5-23.0) | <.001 |
| CD19 lymphocytes/μL | 210.9 (79.3-493.6) | 73.4 (36.1-144.0) | 445.7 (258.8-915.8) | <.001 |
| CD16 lymphocytes, % | 7.0 (5.0-11.0) | 7.0 (4.0-10.4) | 7.4 (5.0-12.0) | .54 |
| CD16 lymphocytes/μL | 182.2 (105.4-345.3) | 154.4 (64.4-292.4) | 217.8(140.0-418.7) | .02 |
| CD21 lymphocytes, % | 4.7 (2.07-8.7) | 2.9 (0.4-6.5) | 5.7(2.1-12.7) | .08 |
| CD21 lymphocytes/μL | 3.6 (0.7-9.8) | 1.05 (0.1-4.6) | 7.6(2.1-12.8) | .03 |

[a]Values are shown as median (IQR).
[b]$P<.05$, statistically significant.

[18.0-38.0] years) than those with normal B cells (Group-2, 22.0 [12.2-32.7] years, $P=.02$).

### *Laboratory Features*

The immunological profile of CVID patients is summarized in Table 2. Compared to patients with normal B-cell values, CVID patients with B lymphopenia had significantly increased percentages of neutrophils (53.0% [37.2%-65.0%] vs 57.0% [44.0%-68.0%], $P=.03$), $CD3^+$ T cells (69.0% [60.0%-77.0%] vs 82.0% [71.0%-88.0%], $P<.001$), and $CD8^+$ T cells (30.0% [22.2%-42.0%] vs 41.0% [31.0%-56.0%], $P<.001$). In contrast, in patients with B-cell lymphopenia, significantly lower values were recorded for absolute counts of $CD3^+$ T cells (1971.8 [1299-3073.6] vs 1632.3 [930.0-2839.0]/μL, $P=.04$), $CD4^+$ T cells (954.6 [600.7-1647.7] vs 626.5 [509.7-1101.2]/μL, $P<.001$), and $CD16^+$ lymphocytes (217.8 [140.0-418.7] vs

154.4 [64.4-292.4]/μL, *P*=0.02) than in patients with normal B-cell counts. Similarly, significantly lower values were also recorded among patients with B-cell lymphopenia for total lymphocyte percentages (38.0% [27.0%-52.0%] vs 30.5% [21.0%-46.0%], *P*<.001) and absolute counts (2717.2 [1907.5-4694.0] vs 2224.8 [1555.0-3503.2]/μL, *P*<.001). Although absolute counts of $CD21^+$ lymphocytes were lower in Group 1 than in the group with normal B-cell values (1.05 [0.1-4.6] vs 7.6 [2.1-12.8]/μL, *P*=.03), surprisingly, the percentage of this B-cell subpopulation did not differ significantly between the 2 groups (*P*=.08, Table 2).

### *Clinical Phenotyping and Organ Involvement*

Among 387 CVID patients, 130 cases (33.6%) developed only infectious complications and 257 patients (66.4%) developed noninfectious complications. The most common noninfectious presentations were enteropathy (35.1%, n=136), autoimmunity (24.3%, n=94), and lymphoproliferation (21.4%, n=83). Of note, in patients with B lymphopenia, higher frequencies were recorded for all noninfectious phenotypes (73.8% vs 60.7%, *P*<.001) and autoimmune phenotypes (31.5% vs 18.7%, *P*=.003) than in patients with a normal B-cell count (Table 3). Considering the affected organ according to clinical phenotype, gastrointestinal complications (58.9%, n=228) were predominant in all CVID patients. Expectedly, hematologic complications (25% vs 13.7%, *P*<.001), musculoskeletal complications (10.7% vs 5%, *P*=.03), dermatologic complications (39.3% vs 24.2 %, *P*<.001), and endocrine complications (11.3% vs 5.5%, *P*=.03) were more frequent in Group 1. The frequency of cardiovascular complications (11.3% vs 5.9%, *P*=.05) was also higher in patients with B lymphopenia, although this difference was not significant (Table 4).

### *Autoimmune and Lymphoproliferative Complications*

The clinical spectrum of autoimmune disease among selected 94 CVID patients was wide and included both hematologic autoimmunity (30.8%, n=29) and organ-specific autoimmunity (87.2%, n=82). Of note, some patients presented more than 1 type of autoimmunity. In this regard, organ-specific autoimmunity included rheumatologic autoimmunity (32%, n=30), gastrointestinal autoimmunity (24%, n=23), dermatologic autoimmunity (19.1%, n=18),

**Table 3.** Comparison of Clinical Phenotypes Between CVID Patients With B Lymphopenia and Patients With Normal B Cell Values.

| Parameter | All CVID patients (N=387) | CVID with B lymphopenia (n=168) | CVID with normal B cells (n=219) | *P* Value[a] |
|---|---|---|---|---|
| Infection only | 130 (33.6) | 44 (26.2) | 86 (39.3) | <.001 |
| Noninfectious complications | 257 (66.4) | 124 (73.8) | 133 (60.7) | <.001 |
| Autoimmunity | 94 (24.3) | 53 (31.5) | 41 (18.7) | .003 |
| Lymphoproliferation | 83 (21.4) | 39 (23.2) | 44(20.1) | .45 |
| Enteropathy | 136 (35.1) | 61 (36.3) | 75 (34.2) | .67 |
| Allergy | 73 (18.9) | 39 (23.2) | 34 (15.5) | .05 |
| Malignancy | 28 (7.2) | 14 (8.3) | 13 (5.9) | .35 |

**P*<.05, statistically significant.

**Table 4.** Specific Organ Involvement Between CVID Patients With B Lymphopenia and Patients With Normal B Cell Values.

| Parameter | All CVID patients (N=387) | CVID with B lymphopenia (n=168) | CVID with normal B cells (n=219) | *P* Value[a] |
|---|---|---|---|---|
| Cardiovascular complications | 32 (8.3%) | 19 (11.3%) | 13 (5.9%) | .05 |
| Hematologic complications | 72 (18.6%) | 42 (25%) | 30 (13.7%) | .004 |
| Musculoskeletal complications | 29 (7.5%) | 18 (10.7%) | 11 (5%) | .03 |
| Neurologic complications | 71 (18.3%) | 31 (18.5%) | 40 (18.3%) | .96 |
| Dermatologic complications | 119 (30.7%) | 66 (39.3%) | 53 (24.2%) | .001 |
| Endocrine complications | 31 (8.0%) | 19 (11.3%) | 12 (5.5%) | .03 |
| Noninfectious gastrointestinal complications | 228 (58.9%) | 100 (59.5%) | 128 (58.4%) | .83 |
| Rheumatoid complications | 67 (17.3%) | 34 (20.2%) | 33 (15.1%) | .18 |
| Complications affecting multiple sites | 259 (67%) | 120 (71.4%) | 139 (63.5%) | .09 |

**P*<.05, statistically significant.

endocrine autoimmunity (6.3%, n=6), and neurological autoimmunity (5.3%, n=5). ITP and AIHA were the most common types of hematologic autoimmunity in CVID patients (n=25 and n=15, respectively). Surprisingly, there were no sex differences in the prevalence of autoimmunity (Figure 1). However, the frequency of hematologic autoimmunity, especially AIHA, was higher in patients with B-cell lymphopenia than in those with normal B cells, although these differences were not significant. Moreover, autoimmune neutropenia (n=1), SLE (n=3), autoimmune vasculitis (n=3), juvenile dermatomyositis (n=1), and growth hormone deficiency (n=1) were only documented in Group 1 patients with B-cell lymphopenia (Table S1).

Splenomegaly, hepatomegaly, and granulomas were found in 26.1% (n=101), 17.3% (n=67), and 2.06% (n=8) of the cohort, respectively. The most common sites of granulomas identified by biopsies included the skin (n=3, 37.5%), liver (n=2, 25%), and lung (n=2, 25%). Other locations included the brain, lymph nodes, and spleen. There were no significant differences between granulomatous diseases in the 2 groups of patients in this study; however, hepatomegaly and splenomegaly (25.6% vs 11% [*P*<.001] and 36.9% vs 17.8% [*P*<.001], respectively) were significantly more frequent in Group 1 than in Group 2 (Table S2), indicating paradoxical lymphopenia in the periphery of these patients, together with lymphoid hyperplasia in their secondary lymphoid organs.

### *Other Clinical Manifestations*

In our cohort, the prevalence of hematological diseases was 18.6% (n=72) overall. The most common hematological complications during course of the disease were anemia (68.0%, n=49), thrombocytopenia (52.7%, n=38), and neutropenia (41.6%, n=30). The frequency of hematological disease in patients with B-cell lymphopenia (25%, n=42) was significantly higher than in patients with normal B-cell values (13.7%, n=30, *P*=.004). Of note, higher values were recorded in Group 1 for bronchiectasis (33.9% vs 19.2%, *P*<.001), clubbing (24.4% vs 13.2%, *P*=.004), and sterile conjunctivitis (14.9% vs 7.8%, *P*=.02) (Table S3).

Twenty-eight patients (7.2%) had malignancies. The clinical spectrum was wide and included both hematologic cancers (85.7%, n=24) and solid tumors (14.3%, n=4). There were no sex differences in the prevalence of malignancy. The most common type of malignancy was non-Hodgkin lymphoma (50%, n=14), followed by Hodgkin lymphoma (28.5%, n=8). Two patients had leukemia. Gastric, breast, ovarian, and brain mass cancer were also observed (Table S4). There were no significant differences in the malignancy detected between the 2 groups of patients in this study.


**Figure 1.** Age distribution of noninfectious complications among patients with common variable immunodeficiency showing B lymphopenia and normal B-cell values.

### *Lung Function and Radiological Assessment*

The results of PFTs were available for 65 patients at the time of diagnosis (age >6 years) and before therapy. The results were abnormal in slightly more than half of them (n=35, 53.8%), with an almost equal proportion of respiratory patterns: obstructive ($FEV_1$/FVC <70% in 12 patients, 18.4%), restrictive (defined as FVC% <80%, in 14 patients, 21.5%), and mixed (restrictive/obstructive, in 9 patients, 13.8%). Of note, all defective PFT patterns were slightly more frequent in Group 1 patients with B lymphopenia than in other CVID patients (Table S5).

HRCT indicated for 70 patients revealed abnormal findings in 52 (74.2%). The severity of bronchiectasis was as follows: severe in 9 patients (25.7%), moderate in 7 patients (20%), and mild in 19 patients (54.3%). The extent of bronchiectasis was as follows: 1-5 segments in 19 patients (54.4%), 6-9 segments in 8 cases (22.9%), and >9 segments in 8 cases (22.9%). The median Bhalla score was 19 (15-23). The Bhalla score was excellent in 43.2% of patients, and none of the patients were classified as having severe bronchiectasis. In the mild category, 66% of patients had moderate bronchiectasis, and 77.7% had 6 to 9 bronchopulmonary segments affected. Similar to the observed PFT result, abnormal HRCT findings were more common in patients in Group 1 (81.2% vs 68.4%, *P*=0.22, Table S5), as was a higher Bhalla score (20.2 vs 18.5, *P*=0.42). However, these differences were not statistically significant between the 2 CVID groups.

### *Mortality*

A total of 82 patients (24.5%) died during follow-up (Table 1), and 53 (13.0%) patients did not attend their final visit. Respiratory failure was the most common cause of death among the deceased patients, accounting for 18.2% of cases (n=15). Other common causes of death were malignancy (n=7), neurological complications (n=5), gastrointestinal complications (n=3), and meningitis (n=3). The median age at onset of the disease among nonsurvivors was significantly lower than among survivors (1.0 [0.4-4.5] vs 2.0 [0.5-9.0] years, *P*=.02, respectively). The median diagnostic delay among nonsurvivors and survivors was 4.0 years (1.3-7.5) and 4.0 years (1.0-10.5), respectively. In nonsurvivors, the median duration of follow-up was 16 years (11.0-18.6). Moreover, among nonsurvivors, consanguinity was observed in 49 patients (59.8%). Despite differences in the B-cell count, the mortality rate was similar for Group 1 and Group 2 (28.9% vs 21.1%; *P*=0.1, Table 1). Although the Kaplan-Meier analysis revealed nonsignificant differences in the cumulative survival of the 2 groups (*P*=.36), we observed slightly more frequent earlier death in patients with normal B-cell counts (mainly in

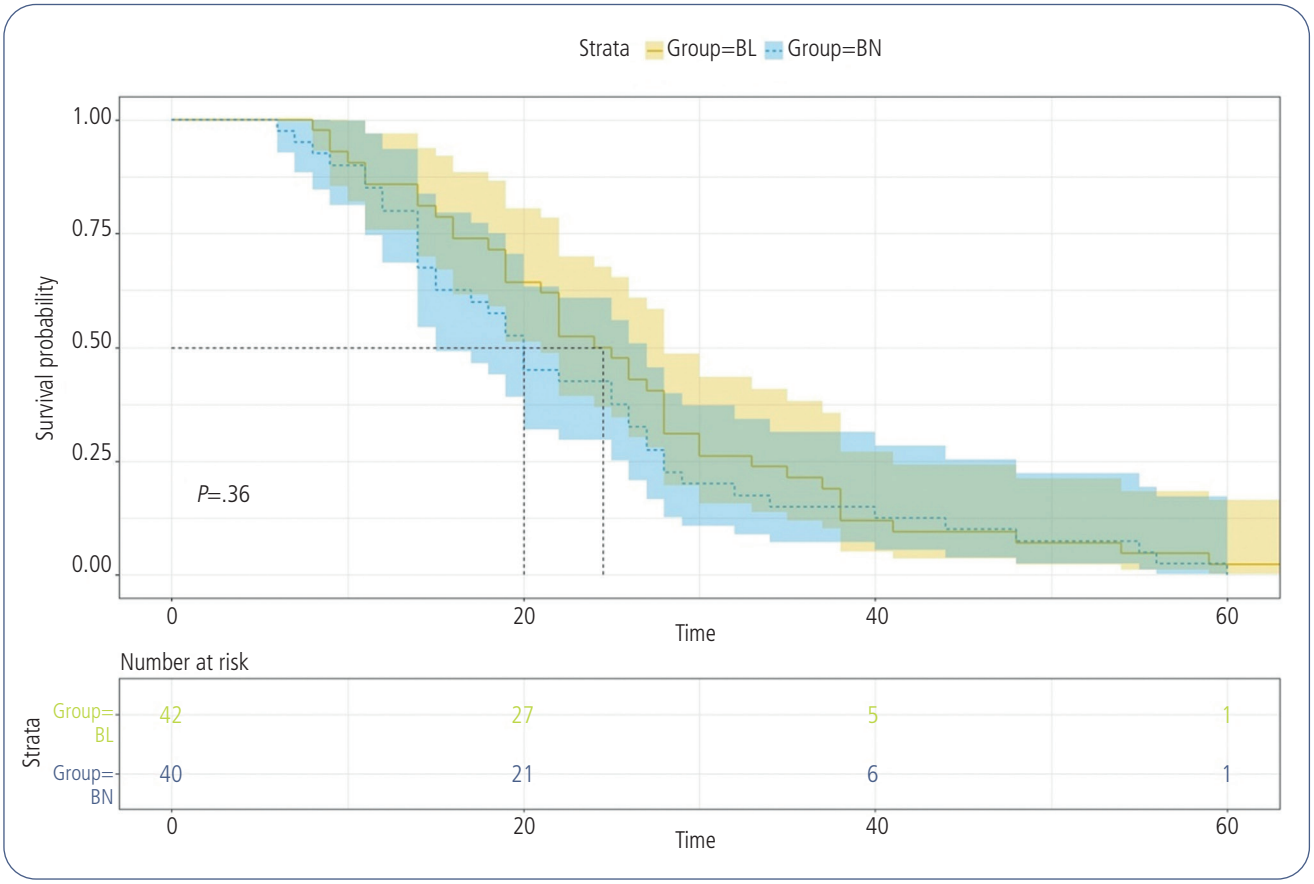


**Figure 2.** Kaplan-Meier graph depicting patient survival between common variable immunodeficiency patients with B-cell lymphopenia (BL) and with normal B-cell counts (BN).

patients aged 15-40 years, Figure 2); consequently, patients in group 1 were older at the time of the study, despite mortality rates being similar.

## Discussion

CVID is defined as a heterogeneous type of IEI with a broad spectrum of immunological and clinical presentations. In the present study, we evaluated noninfectious complications in 387 enrolled CVID patients. CVID patients are characterized by a lower proportion of total and switched memory B cells than healthy controls [43]. Interestingly, the absence or reduced values of these B-cell subpopulations has been associated with specific clinical features, including splenomegaly, granulomatous disease, lymphadenopathy, and autoimmune cytopenia [44]. In our CVID cohort, noninfectious complications were recorded in 66.4% of the patients, a finding that is consistent with those of previous studies [45,46].

Autoimmune disorders are common in antibody defects, particularly CVID, affecting more than 20%-30% [47]. In the present study, the prevalence rate of autoimmune complications was 24.3%, the second most prevalent noninfectious clinical phenotype in our highly consanguineous, early-onset CVID cohort. Resnick et al [11] observed noninfectious complications in 68% of 473 CVID individuals, and 28.6% of these patients had hematologic or organ-specific autoimmune manifestations similar to those of the patients in our study. In contrast, Azizi et al [48] reported autoimmunity in 42.4% of patients diagnosed with a CVID-like disorder and monogenic defects, nearly double the autoimmunity reported in the present study. The prevalence of autoimmune cytopenia, or at least 1 type of autoimmune hematologic disease in 31 studies was 4.2%-44.7% [49]. Furthermore, ITP and AIHA have been reported to be the most common CVID-associated autoimmune disorders [49], a finding that is consistent with those of the current study, namely, that the prevalence of ITP and AIHA in B-lymphopenic CVID cases was 6.4% and 3.8%, respectively. Another report from the USIDNET registry demonstrated the prevalence of ITP and AIHA in CVID patients to be 7.4% and 4.5%, respectively [50], again, similar to the results of the present study. In this regard, in patients with B lymphopenia, the frequency of the autoimmune phenotype was higher than in patients with a normal B-cell count (31.5% vs 18.7%, $P$=.003).

Several studies have confirmed the association between rheumatologic disease and CVID [1,25]. It has been reported that rheumatologic disease affects up to 13% of patients with CVID [51-53]. Similarly, our data indicated that 17% of patients experienced rheumatologic complications; of these, 7.7% were diagnosed with different types of rheumatologic-related autoimmunity. RA, JIA, Sjögren syndrome, and SLE are the most frequent types of autoimmunity among these patients, as observed in our study [54]. Although the exact mechanisms of rheumatologic presentations in CVID patients are not completely clear, various autoimmune patterns have been suggested in affected patients, including the presence of autoantibodies, diminished number of regulatory T cells, elevated number of autoreactive B cells, reduced number of regulatory B cells producing IL-10, and cytokine production [55-57]. In contrast, Barsotti et al [58] could not find any correlation between the frequency of these immune cells and autoimmunity in CVID patients.

The reason for such a high rate of autoimmunity in B-lymphopenic CVID cases is still a matter for speculation. It has been proposed that specific autoreactivity checkpoints interrupt the expansion of self-reactive antibodies before the onset of somatic hypermutation and during B-cell maturation [59]. In fact, the most frequent type of immune dysregulation that renders CVID patients susceptible to autoimmune disorders is B-cell defects, as central tolerance can be disturbed owing to intrinsic defects and abolished B-cell receptor signaling, together with increased B cell–activating factor levels and, possibly, altered Toll-like receptor signaling, as well as defects in genes affecting multiple lymphoid subsets [60,63]. Above all, expanded $CD21^{low}$ B cells were repeatedly documented in mixed genetic CVID patients with autoimmunity [64-66]. In this regard, in the present study, absolute counts of $CD21^{+}$ lymphocytes were lower in patients with B-cell lymphopenia than in the normal B-cell group. Therefore, autoimmune diseases in B-lymphopenic CVID patients deserve special consideration, because dysfunctions of the immune system and immune dysregulation, along with continuous inflammation, can extend the procedure of recognition and treatment. Reduced central T-cell tolerance with the same molecular defects in negative selection and defective regulatory T-cell development can also accelerate the process of autoimmunity in affected patients. Other autoimmune manifestations in B-lymphopenic CVID in the present study affected the skin (4.6%), endocrine system (1.5%), and nervous system (1.2%) and were found to be uncommon yet limited to this group of patients, consistent with a previous systematic review [49]. Although most CVID patients have abnormalities in switched memory B cells and plasma cells, patients with B-cell lymphopenia may have other specific subpopulation defects. However, since the B-cell subpopulation can be affected by therapeutic modalities and was not available for deceased patients and for many patients at diagnosis, this analysis of the remaining few patients may be subject to bias, since we only focused on the main immune markers, which were investigated homogenously in all patients at diagnosis. Higher proportions of neutrophils and T cells, but lower counts of helper T cells, were among the significant phenotypes in CVID patients with B-cell lymphopenia. However, deeper investigation of B-cell subsets for newly diagnosed patients before initiation of treatment in future studies may elucidate the main disturbed developmental stage in these specific patients.

The prevalence of CVID-associated complications, including lymphoproliferative disease, has been shown to vary between countries [67]. Lymphoproliferative disease was reported in 21.4% of the 387 CVID patients included in this study, that is, more than in a recent adult-onset study [68]. In addition, the prevalence of splenomegaly as the most frequent type of lymphoproliferation in the present study (26.1%) was approximately half that of the outbreak reported in the EUROclass trial (40.5%) [69]. Furthermore, there was a significant difference between the prevalence of hepatosplenomegaly in B-lymphopenic cases and patients with a normal B-cell count. This might indicate a developmental defect leading to the arrest of B-cell maturation within the

secondary lymphoid organ, particularly during germinal center formation, thus also explaining the reduced level of B cells in the peripheral blood of these selected groups of patients. In our cohort, the prevalence of malignancy was 7.2% (28 cases in 387 CVID patients), which is consistent with a previous systematic review and meta-analysis showing that 48 studies assessed malignancy and reported 790 cases among 8123 cases of CVID (9.7%). Five of these patients had 2 types of malignancy [70,71]. The incidence of malignant lymphoma around the world has been increasing at a rate of 3%-4% over the last 4 decades [72]. Malignant lymphoma has been reported to comprise 3.37% of all malignancies worldwide [72,73]. In contrast, the results of the current study indicated that lymphoma accounts for 78.5% of all malignancies in CVID patients (22 cases in 28 patients with cancer), with equal findings in B-lymphopenic individuals and patients with a normal B-cell count (11 cases each). In contrast, another study reported a 40.5% prevalence of lymphoma in sporadic CVID patients with mostly adult-onset disease [74]. Although early recognition and medical treatment of CVID have improved in recent years, epidemiological findings show a high frequency of fatal malignancy in these cases [75,76]. In this regard, the mortality rate among patients with malignancy was high in the present study (40.7%). To date, the exact pathological mechanisms underlying this high frequency are not fully specified; however, various mechanisms, including impaired clearance of oncogenic viruses, genetic predisposition, immune dysregulation, impaired genetic stability, and iatrogenic causes, are thought to contribute to the development of malignancies in CVID [74]. Nevertheless, our study suggested that this is independent of B-cell lymphopenia.

The gut is the largest lymphoid organ, containing the highest percentage of lymphocytes, which, along with other immune cells (including macrophages and dendritic cells), manage the balance of the mucosal immune system. This, in turn, is in close contact with antigens of microorganisms such as viruses, bacteria, and parasites. Any related dysfunctions regarding the regulatory mechanisms might result in inflammation and gastrointestinal diseases. Therefore, in IEI patients with dysfunction of cellular or humoral immunity, observing gastrointestinal complications is not beyond expectation. It has been reported that 5%-50% of IEI patients are diagnosed with some gastrointestinal complications [77]. Various clinical immunologists have reported a high incidence of gastrointestinal presentations in patients diagnosed with CVID, ranging from 20% to 60%. Most patients have transient or chronic diarrhea, and some are also diagnosed with malabsorption and weight loss [78]. Following previous data [79-82], the frequency of gastrointestinal complications in our study was about 58.9%, which was slightly higher among patients with B-cell lymphopenia. Some CVID patients might be diagnosed with inflammatory and/or autoimmune gastrointestinal disease, which is considered a major cause of both mortality and morbidity. It has been reported that the mortality risk among CVID patients who develop gastrointestinal complications is 2.8 times higher than in patients without these complications [83]. In accordance with this statement, 3.6% of patients in our study died from various types of gastrointestinal complications. CVID-related enteropathy is characterized by the absence of plasma cells, follicular lymphoid hyperplasia, prominent intraepithelial lymphocytosis, and villous blunting. Besides, immunoglobulin replacement therapy does not improve the manifestations of enteropathy, potentially explaining why the rate of gastrointestinal complications remains high despite regular immunoglobulin replacement therapy [84].

Immunoglobulin replacement therapy and long-acting antibiotics are usually prescribed for CVID patients at specific intervals during their lifetime. Although the above-mentioned treatment reduced the frequency of infections and increased the survival rate among affected patients, it does not seem to have had any protective effect on some noninfectious complications, including autoimmunity, malignancies, structural and functional lung disease, and gastrointestinal presentations. These complications should be considered important, since inflammatory and autoimmune conditions might increase the mortality and morbidity rate. The mortality rate in our study was about 24.5%. In line with previous studies, respiratory complications and lung failure were the most common causes of death in CVID patients. In this regard, bronchiectasis is a common respiratory problem that might result in serious medical complications. In our study, 25.6% of patients were diagnosed with bronchiectasis using HRCT. However, the incidence rate might vary between studies. In a recent study by Ho et al [7], bronchiectasis affected 32.3% of patients. In another study conducted by Busse et al [85], 42% of CVID patients who had recurrent pneumonia were diagnosed with bronchiectasis. It should also be noted that, although most patients are receiving immunoglobulin replacement therapy, some CVID patients still develop bronchiectasis, and the condition might be the consequence of decreased switched memory B cells and deteriorated antibody production [86], potentially explaining why bronchiectasis is significantly more common among patients with B-cell lymphopenia than among patients with normal B cells. Furthermore, various malignancies, especially lymphoma, increase the mortality rate in patients diagnosed with CVID [83]. In our study, 7 patients died from cancer, which was the second most common cause of death.

Cultural diversity in various regions of the world means that determinants such as the consanguinity rate are different. In this regard, a higher rate of certain inheritance patterns, for example, autosomal disease, can be predicted. Moreover, the different genetic backgrounds of the CVID cohort studied may be dependent on founder mutations associated with geographical distribution. On the other hand, in countries that implement more complete diagnostic protocols and have access to advanced laboratory equipment, CVID may be diagnosed earlier, thus leading to a reduction in diagnostic delay and proper management without complications. All these parameters should be considered before generalizing the findings of this study; therefore, B-cell lymphopenia should be further studied in other CVID cohorts worldwide in the future. One of the limitations of the current study was the lack of a genetics-based diagnosis. The evaluation of genetic findings may enable evaluation of B-cell lymphopenia in patients with normal B cells and can potentially suggest targeted treatment for a selected group of patients in future studies.

## Conclusion

B-cell defects, cellular abnormalities, and immune dysregulation have been detected in CVID patients. As a result, a broad spectrum of clinical presentations—both infectious and noninfectious—are expected in a considerable proportion of patients diagnosed with this disease. As mentioned earlier, some of these complications, such as respiratory complications and autoimmunity, are correlated with an increased mortality rate, which is also more common in CVID patients with B-cell lymphopenia, and might be challenging for physicians to manage. In addition, these early B-cell developmental defects in CVID patients may significantly increase the chance of dermatologic, endocrine, and musculoskeletal complications, with negative consequences for patients' quality of life. Our findings can serve as a prognostic guide for physicians who suspect CVID in patients with a history of noninfectious complications. These findings can lead clinicians to consider CVID and request additional tests to improve diagnosis, thus reducing diagnostic delay, preventing progression to severe disease, and enabling better therapeutic approaches.

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### *Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

# Respiratory Microbiome Profiles Are Associated With Distinct Inflammatory Phenotype and Lung Function in Children With Asthma

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## Abstract

*Background:* Respiratory microbiome studies have improved our understanding of the various phenotypes and endotypes in heterogeneous asthma. However, the relationship between the respiratory microbiome and clinical phenotypes in children with asthma remains unclear. We aimed to identify microbiome-driven clusters reflecting the clinical features of asthma and their dominant microbiotas in children with asthma.
*Methods:* Induced sputum was collected from children with asthma, and microbiome profiles were generated via sequencing of the V3-V4 region of the *16S rRNA* gene. Cluster analysis was performed using the partitioning around medoid clustering method. The dominant microbiota in each cluster was determined using linear discriminant effect size analysis. Each cluster was analyzed to identify associations between the dominant microbiota, clinical phenotype, and inflammatory cytokines.
*Results:* We evaluated 83 children diagnosed with asthma. Among 4 clusters reflecting the clinical characteristics of asthma, cluster 1, dominated by the genera *Haemophilus* and *Neisseria*, demonstrated lower postbronchodilator (BD) forced expiratory volume in 1 second ($FEV_1$)/forced vital capacity (FVC) than the other clusters and more mixed granulocytic asthma. *Neisseria* correlated negatively with pre-BD and post-BD $FEV_1$/FVC. *Haemophilus* and *Neisseria* correlated positively with programmed death-ligand (PD-L) 1.
*Conclusion:* To our knowledge, this study is the first to analyze the relationship between an unbiased microbiome-driven cluster and clinical phenotype in children with asthma. The cluster dominated by *Haemophilus* and *Neisseria* was characterized by fixed airflow obstruction and mixed granulocytic asthma, which correlated with PD-L1 levels. Thus, unbiased microbiome-driven clustering can help identify new asthma phenotypes related to endotypes in childhood asthma.

Key words: Asthma. Children. Cluster analysis. Cytokines. Microbiota. Phenotype.

## Resumen

*Antecedentes*: Los estudios del microbioma respiratorio han favorecido nuestra comprensión de diversos fenotipos y endotipos del asma. Sin embargo, la relación entre el microbioma respiratorio y los fenotipos clínicos en niños con asma sigue sin estar clara. Nuestro objetivo fue identificar, en niños con asma, agrupaciones (clúster) de microbiomas que identifiquen las características clínicas del asma y sus microbiotas dominantes.
*Métodos:* Se recogió esputo inducido de niños con asma y se generaron perfiles de microbioma mediante secuenciación de la región V3-V4 del gen *16S rRNA*. El análisis de clúster se realizó usando el algoritmo PAM (*Partitioning Around Medoids*). El microbiota dominante en cada clúster se determinó mediante el análisis lineal discriminante. En cada conglomerado se analizó la asociación entre la microbiota dominante, el fenotipo clínico y la citocina inflamatoria.
*Resultados:* Se evaluaron 83 niños diagnosticados de asma. Entre los cuatro clústeres que reflejaban las características clínicas del asma, el clúster 1, dominado por *Haemophilus* y *Neisseria*, se caracterizaba por tener un volumen espiratorio forzado en 1 segundo ($FEV_1$) y la capacidad vital forzada (FVC), posbroncodilatador (BD) inferior al de los demás clúster y un asma granulocítica más mixta. *Neisseria* se correlacionó negativamente con el $VEF_1$/CVF pre y post-BD. *Haemophilus* y *Neisseria* se correlacionaron positivamente con el ligando de muerte programada (PD-L) 1.
*Conclusiones:* Hasta donde sabemos, este estudio es el primero en analizar la relación entre un clúster no sesgado de microbioma y el fenotipo clínico en niños con asma. El clúster dominado por *Haemophilus* y *Neisseria* mostró obstrucción fija del flujo aéreo y asma granulocítica mixta, que se correlacionó con los niveles de PD-L1. Así pues, la agrupación no sesgada derivada del análisis del microbioma puede ayudar a identificar nuevos fenotipos de asma relacionados con los endotipos en el asma infantil.

Palabras clave: Asma. Niños. Análisis clúster. Citocinas. Microbiota. Fenotipo.

**Summary box**

- **What do we know about this topic?**
  Unbiased microbiome-driven clustering analysis in childhood asthma suggests that a cluster composed primarily of *Haemophilus* and *Neisseria* displayed fixed airflow obstruction and mixed granulocytic asthma. This observation points to a possible association with programmed death-ligand 1.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  New, microbiome-driven asthma endotyping provides valuable information for an elaborate classification of clinically heterogenous asthma. This approach could enable us to refine management and predict prognosis in children with asthma.

## Introduction

Identifying asthma phenotypes and endotypes facilitates a more systematic and differentiated approach for efficient and personalized treatment of asthma, which is heterogeneous and constitutes a syndrome, as opposed to a simple, single disease [1]. The phenotype characterizes the outward clinical features, including the inflammatory cell type and airway obstruction or reversibility; it can be applied intuitively in clinics [2]. Conversely, the endotype provides a comprehensive understanding of the underlying biological mechanisms at the molecular level, including cytokine and microbiome profiling, which can be used to identify disease-specific markers [3]. Identifying the relationship between phenotype and endotype helps to predict the prognosis of heterogeneous asthma and determine the course of treatment [1].

Sputum inflammatory markers, which are characteristic of the asthma phenotype, can be used as a representative tool to understand and explain the diversity and heterogeneity of asthma [4]. Eosinophilic inflammation induced by a heightened type 2 helper T-cell ($T_H2$) immune response has been suggested as a classical hypothesis for asthma, whereas neutrophil inflammation is characteristic of nonatopic asthma, which is resistant to corticosteroids [5,6]. The respiratory microbiome is an important tool for determining the asthma endotype and thus helping us to understand the mechanism underlying the development and exacerbation of asthma, which may be related to the sputum inflammatory phenotype [7,8]. Early asymptomatic colonization by *Streptococcus* was suggested as a strong predictor of onset of asthma [9]. Gram-negative microbes and airway microbiome composition and diversity could be related to asthma exacerbation [10]. Respiratory microbiome diversity is reduced in neutrophilic asthma, and opportunistic microbes, such as the genus *Haemophilus*, are replaced. These findings may be associated with severe asthma [11]. Unbiased clustering of the microbiome may reflect the clinical characteristics and severity of asthma [12].

Unlike adult asthma, childhood asthma is characterized by allergic comorbidities, including atopic dermatitis and food allergy associated with the allergic march, which, in turn, is thought to be associated mainly with the $T_H2$ immune response and eosinophilic inflammation [13]. However, neutrophilic asthma has recently been reported to be very frequent in children, possibly owing to bacterial and/or viral infection [14]. Given the limitation of sampling in children compared with adults, few studies have assessed the relationship between the respiratory microbiome and clinical phenotypes in children with asthma [15].

Therefore, we aimed to classify and characterize the respiratory microbiome in children with asthma using unbiased clustering methods and to evaluate the relationship between these microbiome features and clinical phenotypes, including sputum inflammatory phenotype, bronchial hyperresponsiveness (BHR), bronchodilator responsiveness (BDR), and airway obstruction. We also aimed to evaluate inflammatory cytokines to elucidate the mechanisms by which microbiome-driven inflammation can affect specific phenotypes.

## Methods

### *Participants*

We screened children who visited the Severance Children's Hospital for an asthma work-up or treatment from January 2015 to December 2018. The children underwent spirometry, sputum induction, and blood sampling at the first visit, followed by the challenge test at the second visit.

Children with typical asthma symptoms, such as recurrent cough or dyspnea, shortness of breath, and chest tightness, underwent spirometry with a bronchodilator (BD) and the bronchoprovocation test. Asthma was diagnosed based on the Global Initiative for Asthma guidelines if a 20% reduction in the forced expiratory volume in 1 second ($FEV_1$) occurred in response to a provocative concentration of inhaled methacholine (provocholine, $PC_{20}$ <10 mg/mL) or BD response, which was verified as a >12% increase in $FEV_1$ after inhalation of albuterol 200 μg [16]. We excluded children with the following symptoms: 1) fever, myalgia, purulent sputum, persistent wet cough, and runny and congested nose for 10 days, as found in differential diagnoses of asthma, including acute respiratory infection; and 2) cough when feeding or vomiting easily, as in cardiac murmur or aspiration [16]. Children with acute asthma exacerbation in the previous 4 weeks requiring systemic corticosteroids or increased use of inhaled corticosteroids were also excluded [17].

We used the Pharmacia CAP assay (Thermo Fisher Scientific) to measure serum specific immunoglobulin E (IgE) levels for the following common inhalant allergens in Korea: 2 types of dust mites (*Dermatophagoides pteronyssinus*

and *Dermatophagoides farinae*); cat and dog epithelium; cockroach; mold; and pollen allergens, including *Alternaria*, birch, mugwort, Japanese hop, and ragweed. Atopy was defined as specific IgE ≥0.35 $kU_A/L$ for more than 1 allergen.

### *Sputum Induction and Processing*

After washing their mouths thoroughly with water, all children inhaled 3% saline solution nebulized in an ultrasonic nebulizer (NE-U12, Omron Co.) at maximum output at room temperature and were encouraged to cough deeply at 3-minute intervals thereafter. For the cell count and microbiome analysis, sputum samples were stored at 4°C for no more than 2 hours before further processing. A fraction of the sample was diluted with phosphate-buffered saline (PBS) containing 10 mmol/L dithiothreitol (WAKO Pure Chemical Industries Ltd). For cytokine analysis, another fraction of the sample was vortexed gently at room temperature for 20 minutes after dilution with PBS containing dithiothreitol 10 mmol/L. Sputum aliquots for microbiome and cytokine analysis were stored at –20°C immediately after collection and then at –70°C within 12 hours to maintain acceptable quality for microbiome analysis [18].

Sputum samples were classified as eosinophilic (>2.5% eosinophils), neutrophilic (>54% neutrophils), mixed granulocytic (>2.5% eosinophils, >54% neutrophils), or paucigranulocytic (≤2.5% eosinophils, ≤54% neutrophils) [19].

This study was approved by the Institutional Review Board of Severance Hospital (protocol no. 4-2004-0036). Written informed consent was obtained from the participants and their parents.

### *DNA Extraction, Polymerase Chain Reaction Amplification, and Sequencing*

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing were performed concurrently for all samples stored at –70°C during the recruitment period (2015-2018). For microbiome analysis, total DNA was extracted from a fraction of the sputum sample using the FastDNA® SPIN Kit for Soil (MP Biomedicals) in accordance with the manufacturer's instructions. The ratio of absorbance was calculated at 260 nm and 280 nm (A260/A280) to assess the purity of DNA. The A260/A280 values of all samples were >2.0, indicating that the purity of DNA was acceptable [20]. Polymerase chain reaction (PCR) amplification was performed using fusion primers targeting the V3-V4 regions of the *16S rRNA* gene with the extracted DNA. For bacterial amplification, fusion primers of 341F (5′-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXXX-TCGTCGGCAGCGTC-AGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′; underlined sequence indicates the target region primer) and 805R (5′-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3′) were constructed in the following order: P5 (P7) graft binding, i5 (i7) index, Nextera consensus, Sequencing adaptor, and Target region sequence.

Amplifications were performed under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and final elongation at 72°C for 5 minutes.

The PCR product was confirmed using 1% agarose gel electrophoresis and visualized using a Gel Doc system (Bio-Rad). The amplified products were purified with Clean PCR (CleanNA). Equal concentrations of purified products were pooled, and short fragments (nontarget products) were removed using Clean PCR (CleanNA). The quality and product size were assessed using the Agilent 2100 Bioanalyzer system (Agilent) with a DNA 7500 chip. Mixed amplicons were pooled and sequenced at Chunlab, Inc. (Seoul, Korea), with the Illumina MiSeq Sequencing system (Illumina), according to the manufacturer's instructions.

### *Microbiome Data Analysis*

Raw reads were processed by performing a quality check and filtering low-quality (<Q25) reads using Trimmomatic ver. 0.32 [21]. Once the quality check was complete, paired-end sequence data were merged using the fastq_mergepairs command of VSEARCH version 2.13.4 [22] with default parameters. Primers were trimmed using the alignment algorithm of Myers and Miller [23] at a similarity cut-off of 0.8. Nonspecific amplicons that did not encode *16S rRNA* were detected using nhmmer [24] in the HMMER software package ver. 3.2.1 with hmm profiles. Unique reads were extracted, and redundant reads were clustered with unique reads using the derep_full length command of VSEARCH [22]. The EzBioCloud *16S rRNA* database [25] was used for the taxonomic assignment based on the usearch_global command of VSEARCH [22], followed by more precise pairwise alignment [23]. Chimeric reads were filtered based on <97% similarity by reference-based chimeric detection using the UCHIME algorithm [26] and the nonchimeric *16S rRNA* database from EzBioCloud. After chimeric filtering, reads that were not identified to the species level (with <97% similarity) in the EzBioCloud database were compiled, and de novo clustering was performed using the cluster_fast command [22] to generate additional operational taxonomic units (OTUs). OTUs with single reads (singletons) were omitted from further analysis.

### *Cytokine Analysis*

Cytokine analysis of sputum was performed using a human fixed immunotherapy discovery magnetic panel-24 plex kit (Magnetic Luminex® Performance Assay multiplex kit, R&D Systems). This kit was used to analyze CD40, granulocyte-macrophage colony-stimulating factor, granzyme B, interferon α, interferon γ, interleukin (IL) 1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-33, C-X-C motif chemokine 10, monocyte chemoattractant protein-1, macrophage inflammatory proteins (MIP)-1α, MIP-1β, programmed death-ligand (PD-L) 1, and tumor necrosis factor α.

### *Microbiome Data Analysis for the Clustered Groups*

Samples were clustered using species-level abundance data with partitioning around medoid (PAM) clustering based on

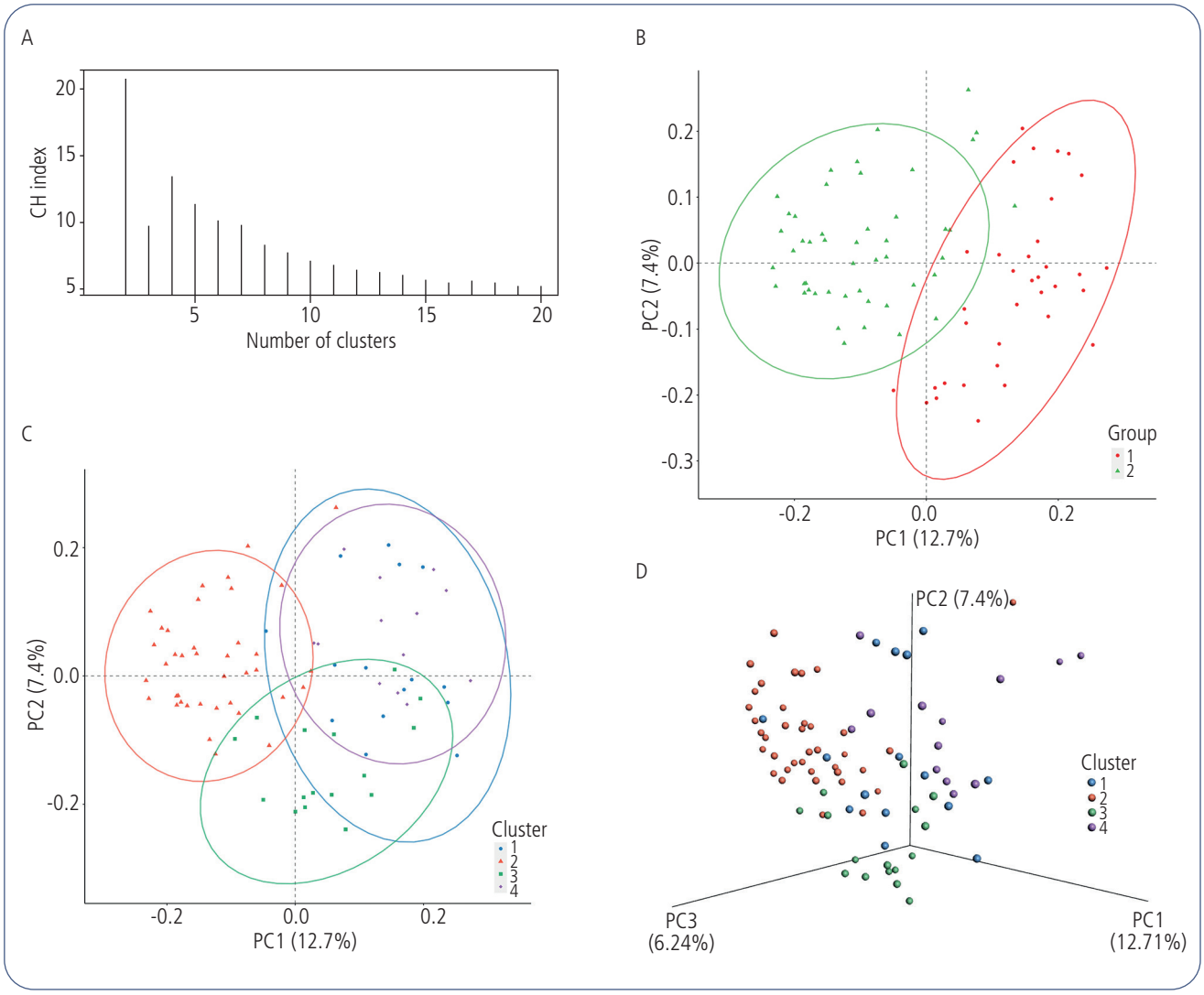


**Figure 1.** A, Calinski–Harabasz (CH) index according to cluster number using the partitioning around medoid clustering method based on Jensen–Shannon divergence at the species level. B, Two-dimensional (2D) principal coordinate analysis (PCoA) plot for cluster 2. C, 2D PCoA plot for cluster 4. D, Three-dimensional PCoA plot for cluster 4.

the Jensen-Shannon divergence [27]. The Calinski–Harabasz (CH) index was calculated according to the number of clusters and used to determine the optimal number of clusters [28]. The resulting clusters were visualized using R with package ade4 for principal coordinate analysis (PCoA) based on the Jensen-Shannon divergence [29].

Linear discriminant analysis (LDA) effect size was used to discover microbiota as a biomarker related to each cluster. The clusters showed a significant difference in the analysis using the Kruskal-Wallis H test. Significant biomarkers were obtained with an LDA score >4.0 and $P$ value <.05 in the pairwise comparison using the Mann-Whitney test and Bonferroni adjustment [30]. The resulting biomarkers were visualized using GraPhlAn for cladogram and the statistical package R (R version 3.2.5.; Institute for Statistics and Mathematics; www.R-project.org) with the package *ggplot* for boxplot using the Kruskal-Wallis H test [31].

### *Statistical Analysis*

The clusters were defined using PAM clustering and the CH index. In order to evaluate clinical characteristics across the clusters, we compared the participants' demographics, the pulmonary function parameters, such as the airway obstruction index ($FEV_1$, $FEV_1$/forced vital capacity [FVC]), fixed airway obstruction index (post-BD $FEV_1$, post-BD $FEV_1$/FVC), airway hyperresponsiveness, and BD response, and sputum inflammatory phenotype across the clusters. Continuous variables were analyzed using the t test, Mann-Whitney test, 1-way analysis of variance, or Kruskal-Wallis test. Categorical variables were analyzed using the $\chi^2$ or Fisher exact test. Post hoc analysis with the Bonferroni correction was performed if a significant difference was observed between the 4 clusters. The Spearman rank correlation was used to assess the relationship between the microbiota as a biomarker

for the clusters compared with inflammatory cytokines and pulmonary function parameters. *P* values <.05 were considered statistically significant. The analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp.) and R (R version 3.2.5; Institute for Statistics and Mathematics; www.R-project.org).

## Results

### *Clinical Characteristics Across the Clusters*

We evaluated 83 children diagnosed with asthma (median age, 7.5 years; 31.3% boys). Most children (approximately 83%) were atopic.

According to the number of clusters, defined using the PAM clustering method, a higher CH index was obtained in 2 and 4 clusters than in other numbers of clusters (Figure 1A), which were well separated in the PCoA plots (Figures 1B-D).

We compared the clinical characteristics of the participants across the 2 and the 4 clusters. The 4 clusters showed significantly different clinical characteristics, including inflammatory phenotype (*P*=.007) and pulmonary function parameters (Table), whereas the 2 clusters did not show any significantly different clinical characteristics (Supplementary Table 1). Post-BD $FEV_1$/FVCs (*P*=.020) differed significantly across the 4 clusters; however, the difference in pre-BD $FEV_1$/FVC was not statistically significant (*P*=.060). Therefore, we comprehensively evaluated the clinical characteristics and microbiome profile in the 4 clusters to identify microbiotas as meaningful biomarkers related to clinical characteristics, such as inflammatory phenotype and pulmonary function parameters.

### *Sputum Inflammatory Phenotype and Pulmonary Function Parameters Across the Clusters*

We performed a post hoc analysis to identify the clusters that differed significantly in inflammatory phenotype. Only clusters 1 and 2 exhibited a significant difference with multiple corrections (Figure 2A). A post hoc analysis was also performed to identify which inflammatory phenotype differed significantly in clusters 1 and 2. Given the absence of a significantly different inflammatory phenotype that could explain the difference between clusters 1 and 2, the difference in inflammatory phenotype between these 2 clusters was evaluated without a Bonferroni correction (Figure 2B). This explorative investigation revealed differences in the mixed granulocytic and paucigranulocytic types in clusters 1 and 2 (Figure 2B).

Cluster 1 had a lower post-BD $FEV_1$/FVC than the other clusters (Figure 2C). In a pairwise comparison between 2 clusters, the post-BD $FEV_1$/FVC of cluster 1 was significantly lower than that of cluster 2 (*P*=.031) after the Bonferroni correction.

In summary, cluster 1 had a lower post-BD $FEV_1$/FVC, indicating fixed airflow obstruction and more mixed granulocytic and paucigranulocytic asthma.

### *Dominant Microbiotas in the Clusters*

The *16S rRNA* analytic method has limitations in identifying an individual microbe at the species level when applied with only partial amplicons [8]. Therefore, the abundance of the microbiotas was analyzed up to the genus level (Supplementary Fig. 1) and compared at the genus level between the clusters (Figure 3) at *P*<.05 using the Kruskal-Wallis H test to identify

**Table.** Patient Characteristics Across the 4 Clusters (N=83).

| | Total (N=83) | Cluster 1 (n=15) | Cluster 2 (n=39) | Cluster 3 (n=16) | Cluster 4 (n=13) | *P* value |
|---|---|---|---|---|---|---|
| Age, y | 7.5 (6.5-9.7) | 8.2 (6.5-10.4) | 7.5 (6.3-9.7) | 8.9 (7.5-10.7) | 6.5 (5.7-7.9) | .020 |
| Male sex, No. (%) | 26 (31.3) | 14 (93.3) | 24 (61.5) | 9 (56.3) | 10 (76.9) | .081 |
| Atopy, No. (%) | 29 (82.9) | 10 (66.7) | 31 (79.5) | 15 (93.8) | 11 (84.6) | .282 |
| Sputum inflammatory phenotype, No. (%) | | | | | | |
| Eosinophilic | 27 (32.5) | 3 (20.0) | 18 (46.2) | 1 (6.3) | 5 (38.5) | .007 |
| Neutrophilic | 32 (38.6) | 2 (13.3) | 15 (38.5) | 10 (62.5) | 5 (38.5) | |
| Mixed | 13 (15.7) | 6 (40.0) | 4 (10.3) | 2 (12.5) | 1 (7.7) | |
| Paucigranulocytic | 11 (13.3) | 4 (26.7) | 2 (5.1) | 3 (18.8) | 2 (15.4) | |
| Pulmonary function parameters | | | | | | |
| Mean (SD) $FEV_1$, % predicted | 96.8 (16.2) | 92.4 (16.0) | 98.4 (16.2) | 98.5 (15.5) | 94.8 (17.8) | .610 |
| $FEV_1$/FVC | 81.4 (74.1-85.5) | 0.76 (0.70-0.81) | 0.83 (0.74-0.86) | 0.84 (0.80-0.88) | 0.80 (0.71-0.84) | .060 |
| Mean (SD) post-BD $FEV_1$, % predicted | 105.7 (15.8) | 101.3 (18.5) | 108.1 (15.1) | 105.1 (14.6) | 104.3 (16.2) | .540 |
| Post-BD $FEV_1$/FVC | 85.3 (80.8-91.1) | 80.5 (75.8-86.3) | 88.0 (83.0-91.3) | 86.5 (83.0-91.8) | 84.5 (77.3-89.0) | .020 |
| BDR to assess Δ $FEV_1$ | 29 (34.9) | 7 (46.7) | 14 (35.9) | 4 (25.0) | 4 (30.8) | .633 |
| BHR to assess challenge test | 62 (74.7) | 8 (57.1) | 32 (84.2) | 12 (75.0) | 10 (83.3) | .204 |

Abbreviations: BD, bronchodilator; BDR, bronchodilator response; BHR, bronchial hyperresponsiveness; Δ, change before and after bronchodilator; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity.

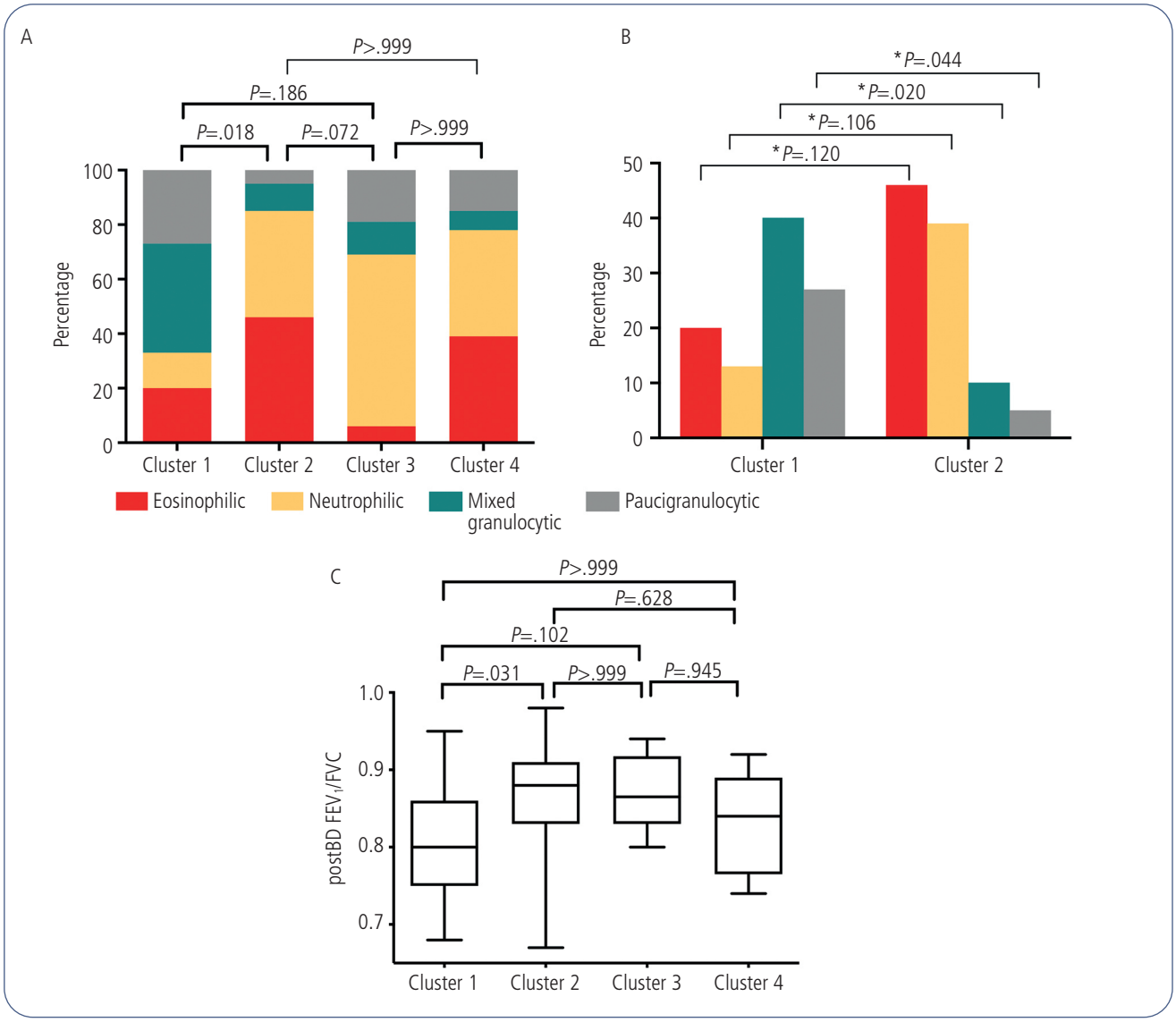


**Figure 2.** A, Comparison of the sputum inflammatory phenotype across the clusters. B, Comparison of the sputum inflammatory phenotype in clusters 1 and 2. C, Post-bronchodilator (BD) forced expiratory volume in 1 second ($FEV_1$)/forced vital capacity (FVC) across the clusters. The *P* value was calculated using post hoc analysis with Bonferroni correction. **P* was calculated using post hoc analysis without a Bonferroni correction for exploratory purposes.

the dominant microbiotas related to each cluster. Microbiotas were selected at the genus level with an LDA score >4.0, as seen in the LDA histogram and cladogram in Figure 4. The predominance was as follows: *Neisseria* and *Haemophilus* in cluster 1; *Prevotella*, *Veillonella*, and *Actinomyces* in cluster 2; *Streptococcus* and *Granulicatella* in cluster 3; and *Ralstonia* in cluster 4.

### *Correlation Between Microbiota and Inflammatory Cytokines and Pulmonary Function*

The correlation between the prominent genera and inflammatory cytokines was analyzed (Supplementary Table 2). Samples from 63 of the 83 participants were available for analysis of inflammatory cytokines. Since cluster 1 had a more mixed granulocytic type and fixed airway obstruction, and *Neisseria* and *Haemophilus* were predominant in cluster 1, we focused on the cytokine that correlated significantly with these 2 genera. Only PD-L1 had a meaningful correlation with both microbes (r=0.445, *P*=.016 for *Neisseria*; r=0.450, *P*=.014 for *Haemophilus*).

We analyzed the correlation between the predominant genera, including *Neisseria* and *Haemophilus*, and the less abundant genera, including *Streptococcus*, in cluster 1 and compared the results with the pre-BD and post-BD $FEV_1$/FVC indices (Figure 5). Only *Neisseria* correlated negatively with pre-BD $FEV_1$/FVC (r=–0.227, *P*=.039) and post-BD $FEV_1$/FVC (r=–0.227, *P*=.039), whereas no significant correlation was detected between the other microbiotas and the pre-BD and post-BD $FEV_1$/FVC indices.

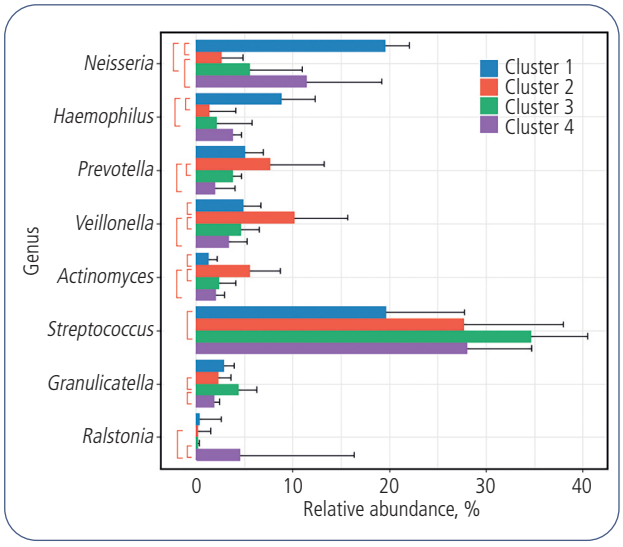


**Figure 3.** Comparison of microbiota at the genus level among the clusters with *P*<.001 in the Kruskal–Wallis H test. The red lines represent *P*<.05 in the pairwise comparison using the Mann-Whitney test and Bonferroni correction.

## Discussion

An unbiased microbiome profile clustering method used in children with asthma revealed that the cluster with abundant *Neisseria* and *Haemophilus* demonstrated fixed airflow obstruction based on the post-BD $FEV_1$/FVC index and a more mixed granulocytic phenotype. The pre-BD and post-BD $FEV_1$/FVC indices decreased, with an increase in the relative abundance of *Neisseria*, indicating that *Neisseria* could be related to airway obstruction in childhood asthma. *Neisseria* and *Haemophilus* correlated positively with PD-L1 levels, suggesting that they could affect fixed airflow obstruction and mixed granulocytic phenotype in relation to PD-L1 in childhood asthma.

Microbiome study is used for asthma endotyping, which defines the subtypes of heterogenous asthma based on the underlying pathologic mechanisms [7]. Previous studies on microbiome data are limited to a supervised approach using known clinical phenotypes and do not address independent microbiome-driven subtyping [11,32,33]. A recent microbiome study in adult asthma suggested the clinical significance of unbiased clustering based on microbiome profiles alone [12]. We applied this unbiased clustering method in children with asthma, and the cluster showed a significant association with clinical characteristics, including fixed airflow obstruction and the mixed granulocytic type. Thus, the unbiased cluster analysis of the airway microbiome was clinically meaningful in childhood asthma.

*Haemophilus*, a pathogenic microbe found in airway dysbiosis, is considered a major pathogenic microorganism in asthma attacks [10]. It is highly abundant in the neutrophilic phenotype of severe asthma [11] and is prominent in eosinophilic asthma [33]. The relevance of *Neisseria* in eosinophilic asthma is debatable [32,33]. As both neutrophilic and eosinophilic inflammatory processes play a role in asthma related to the $T_H1$ and $T_H2$ immune responses and many debatable results have been reported [13,14], it is reasonably acceptable that cluster 1 is characterized by more mixed granulocytic asthma.

PD-L1, which correlated significantly and positively with *Neisseria* and *Haemophilus* in our study, may strengthen $T_H2$ inflammation and increase airway hyperresponsiveness in asthma; however, it can suppress CD8 T-cell immunity, preventing the clearance of infected pathogens from the perspective of acute infection [34,35]. The dual roles of PD-L1 in asthma, including strengthening $T_H2$ inflammation and weakening innate immunity from infected pathogens, can explain its contribution to asthma exacerbation [10]. These dual roles can also contribute to eosinophilic inflammation through a $T_H2$ immune response and to neutrophilic inflammation

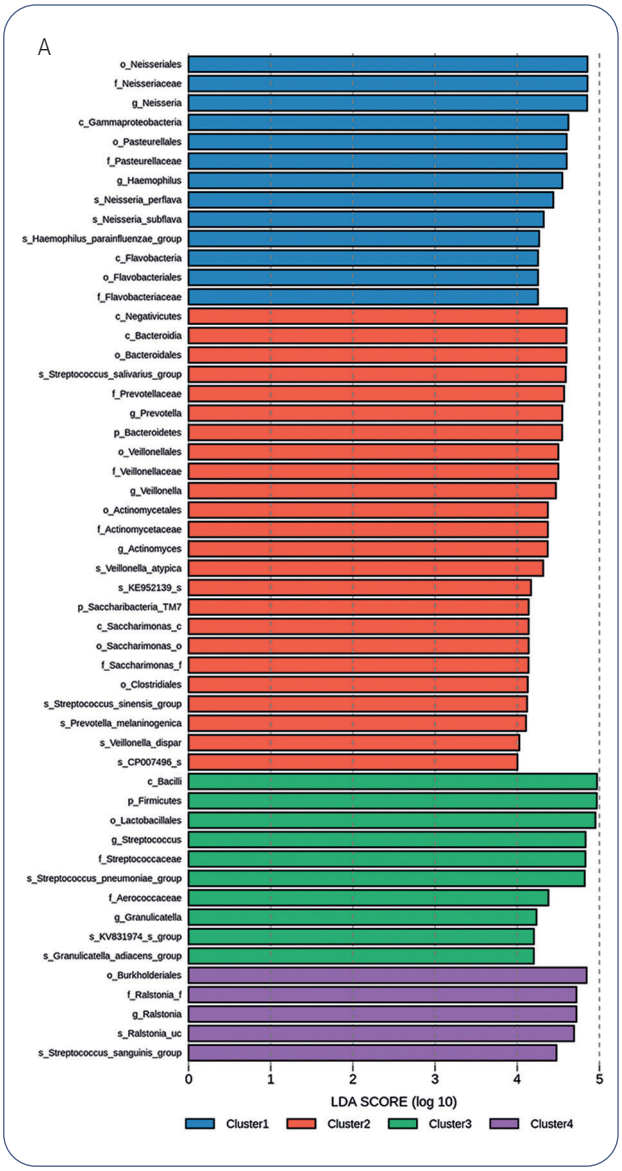


**Figure 4.** A, Linear discriminant analysis (LDA) effect size analysis across the 4 clusters with *P*<.05 and an LDA score >4.0.

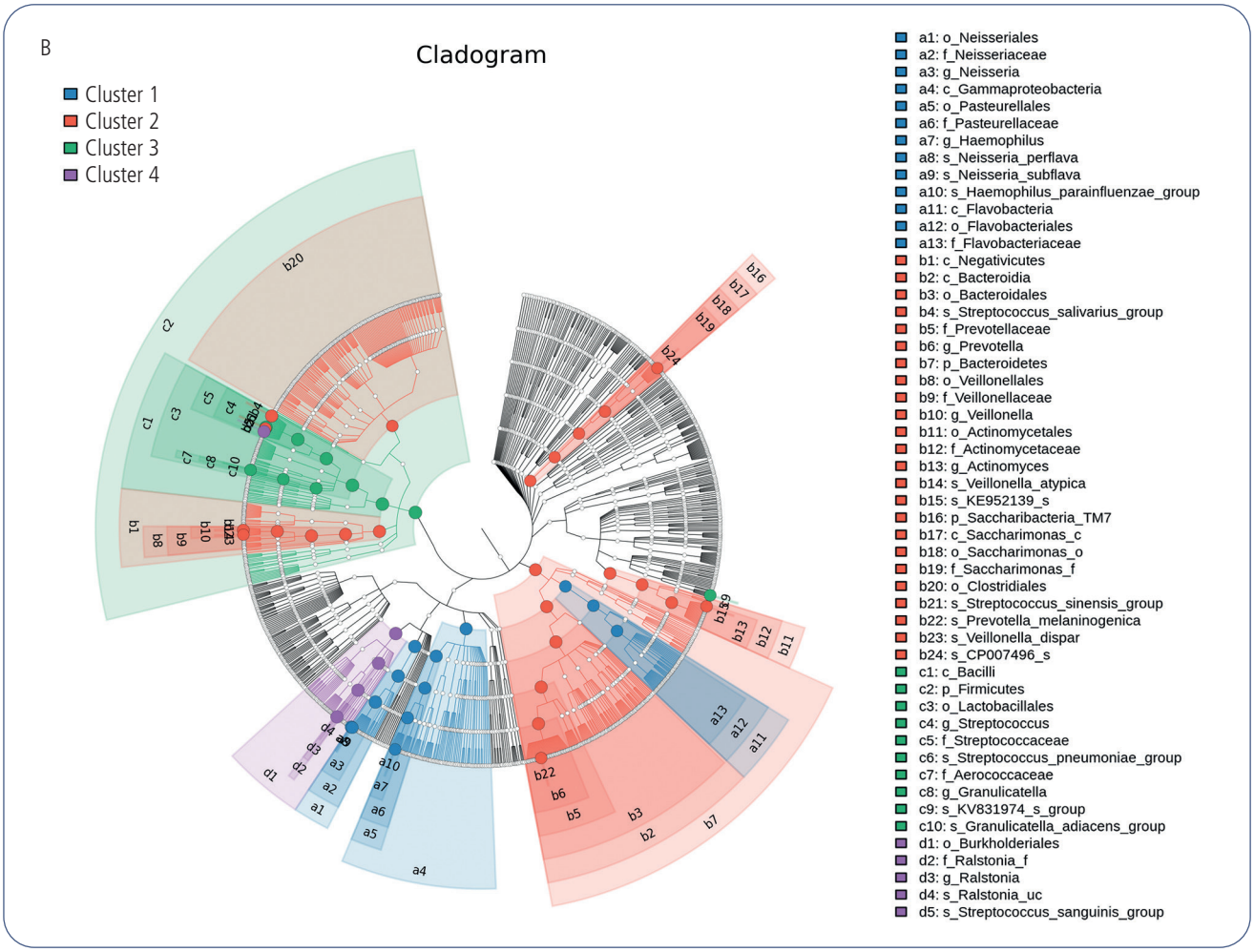


**Figure 4.** B, Cladogram showing differentially abundant taxa according to each cluster. *Haemophilus parainfluenzae* group (*Haemophilus influenzae*, *Haemophilus aegyptius*, and unclassified microbes), *Streptococcus salivarius* group (*Streptococcus salivarius* subsp. *salivarius*, *Streptococcus thermophilus*, *Streptococcus vestibularis*, and unclassified microbes), *Streptococcus sinensis* group (*Streptococcus sinensis* and unclassified microbes), *Streptococcus pneumoniae* group (*Streptococcus pneumonia*, *Streptococcus oralis* subsp. *oralis*, *Streptococcus oralis* subsp. *tigurinus*, *Streptococcus oralis* subsp. *dentisani*, *Streptococcus mitis*, *Streptococcus infantis*, *Streptococcus pseudopneumoniae*, *Streptococcus timonensis*, and unclassified microbes), *Streptococcus sanguinis* group (*Streptococcus sanguinis* and unclassified microbes), and *Granulicatella adiacens* group (*Granulicatella adiacens* and unclassified microbes).

through recurrent infection, leading to a more mixed phenotype in the cluster in which the genera *Neisseria* and *Haemophilus* were dominant in our study.

There are few studies on fixed airflow obstruction in children, a characteristic of chronic obstructive pulmonary disease (COPD), which can be an index of severe asthma when accompanied by asthma in adults [36-38]. It generally develops owing to airway remodeling driven by chronic inflammation [36,39]. Frequent asthma exacerbation can be a risk factor for fixed airflow obstruction in children with asthma [40], and infections are the leading cause of asthma exacerbations in children [41,42]. In this study, cluster 1 was characterized by a mixed granulocytic phenotype, causing fixed airflow obstruction owing to increased inflammatory reactions triggered by eosinophilic and neutrophilic inflammatory responses [43]. This finding is supported by previous reports revealing that overlapping inflammatory pathways, which present as elevated eosinophil and neutrophil values, might be detrimental to lung function [44].

*Neisseria* and *Haemophilus* were the predominant genera in cluster 1, which was associated with fixed airflow obstruction; in contrast, *Prevotella, Veillonella*, and *Actinomyces* were the predominant genera in cluster 2, which was associated with favorable lung function. This finding is in line with that of previous reports showing that airway microbial dysbiosis with overgrowth of opportunistic pathogens and lower normal airway microbes can develop simultaneously and aggravate asthma [11]. *Neisseria* correlated independently with airflow limitation parameters, consistent with previous findings, and the increased prevalence of *Neisseria* due to rhinovirus infection can induce the immunomodulatory properties of dendritic cells and proinflammatory cytokines [45,46], which might affect pulmonary function. This possible explanation is justified in children, who are increasingly exposed to respiratory virus infections [47].

Cluster 2, with favorable lung function, was characterized by a predominance of *Prevotella, Veillonella*, and *Actinomyces*. *Prevotella* is more predominant in controls and infants

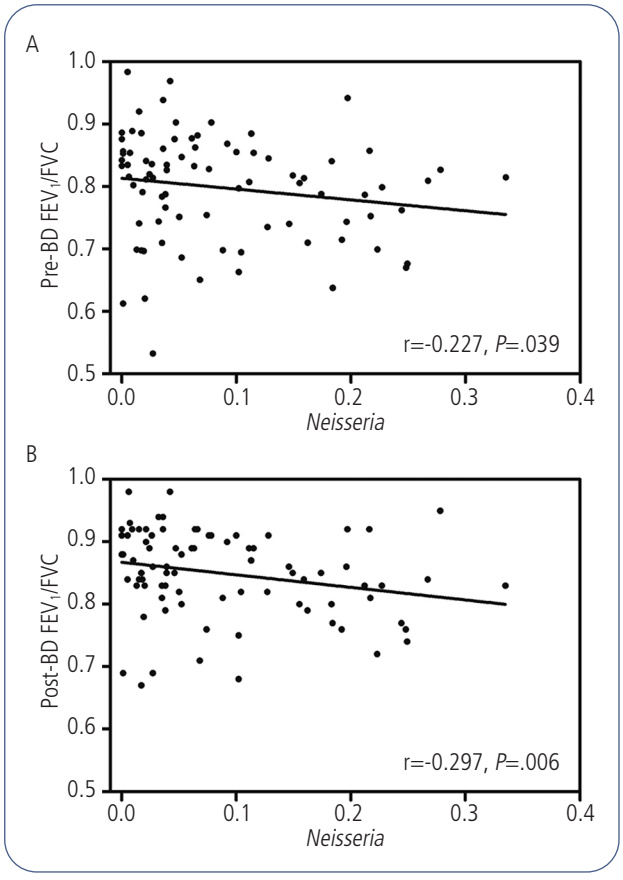


**Figure 5.** Correlation between pre-BD and post-BD $FEV_1$/FVC vs *Neisseria*
BD indicates bronchodilator; FEV*1*, forced expiratory volume in 1 second; FVC, forced vital capacity.

without wheezing than in patients and infants with asthma or COPD and wheezing [48]. *Prevotella* was thought to reduce pathologic *Haemophilus influenzae*–induced IL-12p70 [49] and neutrophilic airway inflammation [50]. However, the presence of *Prevotella* and *Veillonella* at 1 month of age was associated with the incidence of asthma at 6 years of age [51]. *Actinomyces* was less abundant in acute asthma exacerbation than in stable asthma [10]. It was also less abundant in neutrophilic asthma, which is considered a severe type of asthma [11].

Cluster 3 included older patients and more women than the other clusters. *Streptococcus* and *Granulicatella* were predominant in cluster 3. *Streptococcus* was the most abundant genus in our study, as reported elsewhere [52], and is an early marker for predicting asthma during later childhood in infants [9]. In contrast, cluster 4 included younger patients, and *Ralstonia* was predominant in cluster 3. *Ralstonia*, classified as pathologic *Pseudomonas* until recently, was reported to be positively correlated with pyruvic acid, which has a crucial protective role in IgE production in response to allergens [53]. Airway microbiomes exhibit distinct features according to age and sex; however, this finding has not been adequately addressed [52,54,55].

Our study has several limitations. First, the number of patients for evaluation of the 4 clusters was small. Second, we could not evaluate the 2 clusters with the optimal CH index, as these clusters could not account for the clinical characteristics. Third, we could not collect detailed clinical information, including the degree of control of asthma, asthma duration, drug usage, and the frequency of acute exacerbation. Despite these limitations, to our knowledge, this study is the first to analyze the relationship between an unbiased microbiome-driven cluster and clinical phenotype in children with asthma. In addition, it is significant that the characteristics of fixed airflow obstruction and mixed granulocytic asthma in children, which were sporadically reported, were assessed through the relationship between the microbiome and inflammatory cytokines. The findings of this study provide insights into the effect of the airway microbiome on lung function, which has not been addressed [42].

In conclusion, the microbiome-driven unbiased clustering method can help to identify new endotype-related asthma phenotypes in childhood asthma. Our findings suggested that the cluster dominated by *Haemophilus* and *Neisseria* found through this method is characterized by fixed airflow obstruction and mixed granulocytic asthma, which can be related to PD-L1. Thus, new asthma endotyping driven by the airway microbiome can provide valuable information for determining precise management modalities and predicting prognosis in children with asthma.

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### *Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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## Use of Triple Therapy in Asthma: The GEMA-FORUM V Task Force

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Palabras clave: Dosis. ABAP. AMAP. Inhalador. Triple terapia.

According to the Spanish Asthma Management Guidelines (GEMA) and the Global Initiative for Asthma (GINA) guidelines, the preferred treatment for steps 4 and 5 is the combination of inhaled corticoids (ICS) at medium or high doses, respectively, and long-acting ß2-agonists (LABAs) [1,2]. In patients with uncontrolled asthma despite medium- or high-dose ICS/LABA, triple therapy including ICS (medium or high doses), LABAs, and long-acting muscarinic antagonists (LAMAs) can be considered. This approach has been shown to improve lung function and reduce exacerbations, albeit with no clinically significant changes in symptoms or quality of life [3-12]. A meta-analysis showed that medium- or high-dose ICS/LABA/LAMA achieved a 17% reduction in severe exacerbations [9]. However, another study reported that the severe exacerbation rate was lower in patients receiving high-dose ICS/LABA than in those receiving low/medium-dose ICS/LABA/LAMA [11]. In fact, guidelines recommend increasing the dose of ICS before considering adding LAMAs. Therefore, the position of triple therapy in these therapeutic steps is not clear. For this reason, the GEMAFORUM task force proposed a Delphi consensus to know the opinion of experts on areas in which there is no or scarce evidence for the use of LAMAs and triple therapy in clinical practice.

After reviewing the most recent literature and 13 discussion meetings, a scientific committee of 3 coordinators and 13 experts in pulmonology and allergology proposed a questionnaire comprising 62 items grouped into 3 topics: *1*) The role of LAMAs in asthma; *2*) Triple therapy at medium doses of ICS as an early indication; and *3*) Triple therapy at high doses of ICS as a late indication. Following the Delphi methodology described above [13] and explained in the supplementary material, the items were sent to a panel of 85 experts in asthma from all over Spain (53 pulmonologists and 32 allergists) to determine their degree of agreement. It is important to note that the Delphi consensus is an indirect observation of the real prescribing situation and does not include the patient's perspective or the position of the general practitioner.

After 2 rounds, a consensus was reached on 45 items: 41 in agreement (66.1%) and 4 in disagreement (6.5%). The Table shows the items with the highest degree of agreement. The results of the 62 items are shown in the supplementary material.

Regarding the role of LAMAs in asthma, the panelists agreed that a LAMA can replace the LABA in combination with ICS when the LABA is poorly tolerated or contraindicated, but they disagreed, stating that a LAMA cannot replace a LABA in combinations where the ICS is only an additional drug. The panelists also agreed that LAMAs have a good safety profile and a better cardiovascular safety profile than LABAs. However, they also agreed that LAMAs should be administered with caution in patients with narrow-angle

**Table.** Items With the Highest Degree of Agreement Achieved After the 2 Rounds.

| Topic 1. Role of LAMAs in asthma | Agreement, % |
|---|---|
| Experience with the use of LAMAs in COPD confirms that adverse effects are infrequent and mild in most cases and that, therefore, they have a good safety profile in the treatment of asthma. | 96.5 |
| LAMAs are especially indicated in asthma patients with chronic airflow obstruction. | 91.8 |
| Combined ICS/LABA/LAMA treatment in a single device improves adherence. | 95.3 |
| Combined ICS/LABA/LAMA treatment in a single device minimizes the risk of poor technique with respect to the use of multiple devices. | 91.8 |
| **Topic 2. Early indication: ICS/LABA/LAMA at medium doses of ICS** | **Agreement, %** |
| In patients treated with ICS/LABA at medium doses of ICS, adding LAMAs is preferable to stepping up ICS in patients with osteoporosis. | 74.1 |
| In patients treated with ICS/LABA at medium doses of ICS, adding LAMAs is preferable to stepping up ICS in patients with a history of oropharyngeal mycosis. | 74.1 |
| Triple therapy is effective in preventing exacerbations when treatment is planned for the long term. | 73.3 |
| Before adding LAMAs to the treatment of asthma, it is recommended to assess the patient's inflammatory profile. | 91.8 |
| **Topic 3. Late indication: ICS/LABA/LAMAs at high doses of ICS** | **Agreement, %** |
| The priority criterion for response to triple therapy is a decrease in exacerbations. | 88.4 |
| Studies comparing triple therapy with ICS/LABA and MART are needed. | 86.0 |
| Triple therapy is not recommended in MART owing to the possible adverse effects of medication overuse. | 83.5 |
| Triple therapy can be considered, in most cases, as a step prior to the use of a biologic drug. | 95.4 |

Abbreviations: COPD, chronic obstructive pulmonary disease; ICS, inhaled corticosteroids; LABA, long-acting ß2-agonist; LAMA, long-acting muscarinic antagonist; MART, maintenance and reliever therapy.

glaucoma, prostatic disease, or urinary retention. The panelists agreed that LAMAs are especially indicated in patients with asthma and bronchiectasis, chronic airflow obstruction, frequent coughing, and mucosal hypersecretion. Indeed, to choose the best treatment, the panelists agreed to determine the phenotype of asthma patients, regardless of severity, as the neutrophilic phenotype is associated with a better response to LAMAs. Accordingly, they disagreed, refusing to identify patients responding to LAMA without phenotyping. Of note, they did not reach a consensus with some response criteria, such as bronchial hyperresponsiveness in the methacholine challenge test, obesity-associated asthma, and reversibility in the bronchodilator test. With a high rate of consensus, the panelists agreed that combining ICS/LABA/LAMA in a single device improves adherence and efficacy (by ensuring synergy between drugs), is cost-effective, brings ecological benefits (by reducing materials and energy in manufacturing and reducing waste), and even makes it possible to modify the ICS dose. On the other hand, the administration of LAMAs in a separate device enables the response to this drug to be assessed and LAMAs to be added transiently without modifying the base treatment. Of note, no consensus was reached with respect to the possible transient use of LAMAs in clinical practice.

Regarding the use of medium-dose ICS/LABA/LAMA, in accordance with guidelines, the panelists agreed that stepping up ICS is more effective for symptom control than adding LAMAs. However, they agreed that adding LAMAs to ICS/LABA is preferable to stepping up ICS in patients with airflow obstruction, osteoporosis, or a history of oropharyngeal mycosis. Of note, they agreed that stepping up to high-dose ICS is preferable to switching to triple therapy for prevention of exacerbations, although they did not reach a consensus on the item stating that stepping up ICS is preferable to switching to triple therapy. In contrast, panelists did not reach a consensus on items that stated that ICS/LABA/LAMA is equally effective as high-dose ICS in preventing exacerbations (regardless of severity). Finally, the panelists agreed that it was necessary to assess the patient's inflammatory profile before adding LAMAs and that triple therapy is effective in preventing exacerbations when long-term treatment is planned. However, the panelists disagreed with respect to the statements "Triple therapy in a single device should be administered after testing the response to LAMAs in a separate device" and "LAMAs should be administered in a separate device in elderly patients to avoid having to change the previous inhaler".

Concerning the use of high-dose-ICS/LABA/LAMA, the panelists agreed that this treatment is particularly useful in patients with non-T2 asthma or noneosinophilic asthma. They considered that the priority criteria for response to triple therapy are symptom control, improvement in quality of life, and decrease in exacerbations, although they did not reach a consensus on improving pulmonary function. They also agreed that triple therapy is not recommended in maintenance and reliever therapy (MART) because of the potential adverse events of overuse and the lack of clinical trials. However, they considered that more comparative studies between ICS/LABA/LAMA and ICS/LABA with MART are needed. Regarding the stepping-down of ICS in triple therapy, the panelists agreed that a single device does not constitute an obstacle in patients

 

with controlled disease. They also agreed that withdrawal of LAMAs or reduction of ICS dosage should be based on the patient's inflammatory profile. Other important agreements were that triple therapy is indicated in smokers and patients who have previously received biologics.

Most of the replies given by the panelists were consistent with the published literature. A relevant point of this consensus was the need to characterize patients before prescribing treatment. However, it is noteworthy that the panelists did not consider one of the best predictors of response to LAMA, such as airflow obstruction, and took into account others with less evidence, such as the inflammatory profile. Although triple therapy is included in clinical guidelines, further studies are still needed to draw solid conclusions and compare long-term use with alternatives.

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*Conflicts of Interest*

In the last 3 years, Vicente Plaza has received the following: honoraria for speaking at sponsored meetings from AstraZeneca, Chiesi, GlaxoSmithKline, and Novartis; travel assistance from Chiesi and Novartis; fees for consultancy from ALK, AstraZeneca, Boehringer Ingelheim, Mundipharma, and Sanofi; and funding/grant support for research projects from a variety of government agencies and not-for-profit foundations, as well as from AstraZeneca, Chiesi, and Menarini.

In the last 3 years, Juan Antonio Trigueros has received honoraria for speaking at sponsored meetings from Chiesi, GlaxoSmithKline, AstraZeneca, Mundipharma, Boehringer Ingelheim, Menarini, and Gebro Pharma.

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## Real-life Experience of Subcutaneous Plasma Derived C1-Inhibitor as Long-term Prophylaxis in HAE-C1INH

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Palabras clave: Angioedema hereditario por déficit de C1 inhibidor. Calidad de vida relacionada con la salud. AEQoL. HAE-QoL. Derivado plasmático de C1 inhibidor subcutáneo.

Hereditary angioedema (HAE) due to C1-inhibitor deficiency (HAE-C1INH) is an incurable and life-threatening disease [1,2]. Angioedema attacks interfere with the patient's daily and work activities and decrease health-related quality of life (HRQOL), even during angioedema-free intervals [3].

Replacement therapy with subcutaneous (SC) plasma-derived C1-inhibitor (pdC1INH) (Berinert, CSL Behring) was shown to be effective as long-term prophylaxis (LTP) in patients with HAE-C1INH [4,5] and is currently one of the first-choice treatments for LTP in this disease [1,2]. It is easier to self-administer and more efficacious than intravenous (IV) pdC1INH [6]. It has recently become available in Spain [7,8].

The aim of our study was to assess efficacy and changes in HRQOL in HAE-C1INH patients treated with SC pdC1INH (Berinert) as LTP under real-world conditions.

We performed a retrospective, cross-sectional study (Ethics Committee approval, PI-4598). All the patients diagnosed with HAE-C1INH who had received SC pdC1INH for at least 6 months until December 2021 were included. Demographic and clinical data were collected retrospectively. Patients prospectively completed a symptom diary, 2 HRQOL questionnaires, and a treatment satisfaction questionnaire at their follow-up visits as part of their routine health care. HRQOL was assessed using the Angioedema Quality of Life questionnaire (AE-QoL), a specific questionnaire for angioedema as a symptom, and the HAE-QoL, which is specific for HAE-C1INH [3] at 2 time points: prior to starting SC pdC1INH LTP and 6 months later. Disease activity was measured as the monthly attack rate (number of attacks/mo) during the 6 months prior to starting SC pdC1INH LTP and during the 6 months after starting this treatment. The degree of satisfaction with SC pdC1INH LTP was assessed using the specific Treatment Satisfaction Questionnaire for Medication (TSQM, v1.4) [9] 6 months after starting the treatment. Statistical analysis was performed using IBM SPSS for Windows, Version 24. 0 (IBM Corp.).

We included 8 patients (5 women [62.5%]) diagnosed with type I or II HAE-C1INH treated with SC pdC1INH LTP. Mean (SD) age was 47.1 (14.7) years and mean weight 83.1 (17.1) kg (Table S1). Six patients had previously undergone LTP (3 with attenuated androgens [AAs] and 3 with IV pdC1INH) (Table S1). The reasons for switching treatment were adverse effects of AA, the lack of effectiveness of previous LTP, and the high emotional burden of IV treatment. Two patients started LTP with SC pdC1INH because of inability to self-administer IV medication.

The protocol followed that suggested by the Spanish Group for the Study of Bradykinin-mediated Angioedema (GEAB) of the Spanish Society of Allergy and Clinical Immunology (SEAIC). SC pdC1INH LTP was initiated with 2000 IU twice per week in most patients (Figure) [10].

The initial SC pdC1INH doses can be seen in Table S1. Six patients received 2000 IU twice weekly. Patient 3 started at a higher dose (4000 IU twice weekly) owing to high disease activity (prior treatment with IV pdC1INH 1000 IU every 2 days). Patient 1 started with a lower dose (1500 IU twice weekly) because the treatment was initiated in 2018, before SC pdC1INH came onto the market in Spain (the patient signed a consent form). Over the first 6 months, the SC pdC1INH dose had to be increased in 3 patients (Patients 4, 6, and 7), reduced in 4 (Patients 1, 2, 3, and 8), and maintained in Patient 5. The initial median (IQR) SC pdC1INH dose was 37.8% (35.19%-54.29%) of the corresponding dose in the summary of product characteristics (SmPC); at 6 months this was 54.9% (53.425-61.95) (Table S1). All the patients received doses lower than that indicated in the SmPC (60 IU/kg) [8] throughout the study.

The median number of attacks per month prior to SC pdC1INH LTP was 1.93 (1.53-2.94), and a nearly significant reduction was observed 6 months after initiation of SC pdC1INH LTP (median, 0.3 [0-1.69]; P=.069) (Table S1 and Table S5). The clinical condition improved in 6 patients, 3 of whom, remarkably, became asymptomatic (0 attacks/mo). Only 2 patients experienced a worsening of their HAE activity 6 months after the beginning of SC pdC1INH: patient 6 achieved a reduction in attacks 3 months after the dose increase (8 months after initiation of SC pdC1INH LTP), and patient 8 did not complete the dose increase as prescribed.

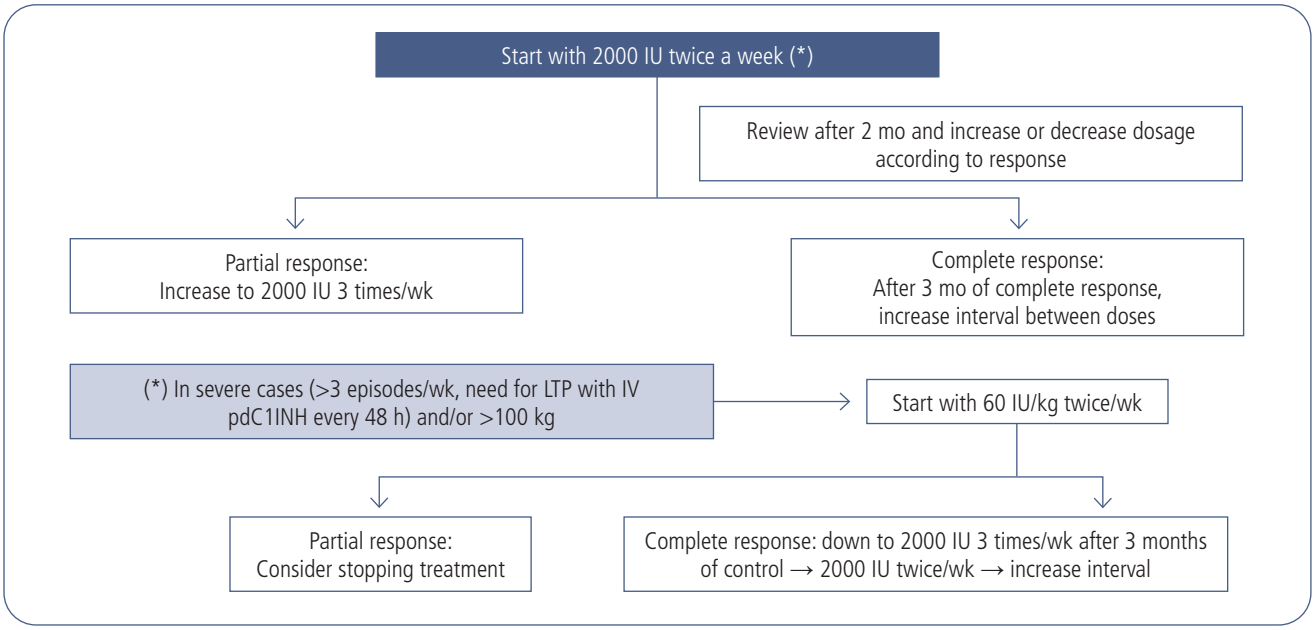


**Figure.** Subcutaneous pdC1INH LTP protocol of the Spanish Group for the Study of Bradykinin-mediated Angioedema.

HRQOL improved 6 months after initiation of treatment according to both the AE-QoL and HAE-QoL.

The total AE-QoL score improved, and the difference was higher than the minimal clinically important difference (6 points) [3], although it was not statistically significant (Table S5). There was also a nonsignificant improvement in all the dimension scores (Table S2), except in the Fatigue/Mood domain. Individual scores are shown in Table S5.

The total HAE-QoL score also improved (nearly significant, *P*=.093) (Table S5), as did all the dimension scores, except for the Disease-related Stigma domain (Table S3). Statistical significance was achieved in 2 dimensions: Perceived Control over Illness (*P*=.031) and Mental Health (*P*=.020). Individual scores are shown in Table S5.

According to the TSQM questionnaire, the mean satisfaction rate was 77.7% (Table S4). Only 2 patients had adverse effects, mainly local discomfort at the injection site (itching and stinging).

In our series, the use of lower doses of SC pdC1INH than those approved in the SmPC and even lower than those proposed in the GEAB protocol proved to be effective, even in patients with high body weight and decreased HAE activity, increased HRQOL, and high overall satisfaction. Other authors also used SC pdC1INH as LTP at doses lower than those approved in the SmPC (42.86-65.22 IU/kg/wk) in real life, with good results [11]. The lower SC pdC1INH doses imply a reduction in direct costs and the possibility of prescribing this treatment to more patients.

In conclusion, the GEAB protocol for starting LTP with SC pdC1INH in HAE-C1INH proved useful for individualizing treatment in our case series.

Since the present study is limited by the small number of patients and the short observation period, further studies are needed.

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*Previous Presentations*

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## Gut Sphingolipid Metabolites in Infants With Atopic Dermatitis Are Associated With Food Allergy

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**Palabras clave:** Esfingolípido intestinal. Metabolito. Dermatitis atópica. Alergia alimentaria.

Food allergy (FA) can affect 20%-80% of patients with atopic dermatitis (AD) [1,2]. Sensitization to food through the skin due to damage to the skin barrier can cause FA, and failure to acquire tolerance to food allergens in the gut can also lead to FA [3]. Gut metabolites can influence the physical gut barrier and intestinal homeostasis [4]. Therefore, it is possible that gut metabolites related to gut immunity play an important role in the development of FA. Sphingolipids are key factors in cell inflammatory response and affect gut epithelial cells and skin barrier integrity and function [5]. Sphingolipid levels have been shown to be lower in FA than in controls [6]; they have also been shown to be significantly higher in patients with FA than in controls [7]. Therefore, the issue of sphingolipid levels in FA remains unresolved. The lipid messenger gut diacylglycerol (DAG), a product of the metabolic reaction between ceramides and sphingomyelins, was increased in FA [8]. In asthma, one of the main signaling pathways associated with the activation of T lymphocytes involves the generation of DAG [9]. However, targeted metabolomics has not shown FA-associated gut sphingolipid in infants. In our previous study, we showed that when FA is present in various phenotypes of AD in early life, it might be associated with subsequent development of asthma [10]. The discovery of a biomarker that can distinguish the phenotypes of AD and FA from other AD phenotypes is therefore expedient. Consequently, we used targeted metabolomics to investigate whether FA in infants with AD is associated with gut sphingolipid metabolites.

The study population consisted of 158 six-month-old infants (46 healthy infants, 30 with AD only, and 82 with combined AD and FA) from the Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) [11], which

was a general population–based birth cohort. The baseline characteristics of the participants are presented in Table S1. Detailed methods are provided in this article's Online Supplement.

DAG, ceramide, and sphingomyelin values were higher in the AD with FA group than in the controls and AD only group (Figures S1A-C). Sphingosine values were higher in the AD with FA group than in the AD only group (Figure S1D), whereas sphingosine-1-phosphate (S1P) levels were lower in the AD with FA group than in the controls (Figure S1E). There were no significant differences in sphinganine between the 3 groups (Figure S1F). DAG and sphingomyelin values were positively correlated with total IgE and specific IgE to food allergens (Figure S2). S1P was weakly and negatively correlated with specific IgE to milk (Figure S2C).

This study showed that gut sphingolipid metabolites can distinguish cases with FA among various AD phenotypes. The metabolites were associated with total and specific IgE levels to food allergens. Our results suggest that the difference in the composition of gut sphingolipid metabolites is associated with FA and food sensitization in infants with FA and AD. Sphingomyelin, ceramide, and sphingosine can be phosphorylated to S1P. The increase observed in sphingomyelin, ceramide, and sphingosine and the decrease in S1P in infants with AD and FA suggest that the sphingolipid mechanism finds it difficult to synthesize S1P. Milk-derived sphingomyelin promotes an iNKT cell–mediated TH2-type cytokine that boosts sensitization to food allergens [12]. Sphingomyelin and ceramide values have been shown to be significantly increased in patients with inflammatory bowel disease and in an animal colitis model [13]. Accumulated ceramide in tight junctions alters lipid composition, contributing to a disturbed barrier function. Moreover, in the colitis model and IL-10 knockout mice, sphingomyelin triggers apoptosis in intestinal epithelial cells and aggravates intestinal inflammation [13]. Therefore, in AD, changes in gut sphingolipids lead to the development of FA as a result of gut barrier damage and inflammation.

In our study, gut sphingolipid levels in the AD only group tended to decrease more than those of controls (Figure S1). In a previous study, a sphingolipid module comprising several metabolites involved in de novo sphingolipid metabolism was significantly more elevated in participants with food sensitization than in those with FA [8]. The major difference was that the changes in the sphingolipid module for feces were observed based on untargeted metabolomic profiling without considering AD [8], whereas, in the present study, single sphingolipid metabolite analysis was conducted through targeted metabolomics according to the AD phenotype.

In this study, of the many sphingolipids assessed, 15 were chosen because they contain major fatty acids and are commercially available. Over time, more sphingolipids have become commercially available. However, regrettably, these newly available sphingolipids were not included in the present study. Not all gut sphingolipids, including ceramides, DAG, and sphingomyelin, could be measured in this study. However, in most of the gut sphingolipids measured, differences were only observed between the AD only and AD with FA groups. Further studies are needed to evaluate other sphingolipids such as ceramide-1-phosphate, lactosylceramide, hexosylceramide, and dihydroceramide.

Studies on sphingolipid metabolites in FA and AD to date have been limited to serum and skin samples with untargeted metabolite profiling. To our knowledge, this is the first study to explore gut sphingolipid metabolite profiles using targeted metabolomics in AD according to the presence of FA.

Restricted diets in children with FA might eventually affect metabolic profiling. The alteration of gut sphingolipids may be the result of dietary restrictions to suppress FA symptoms. Therefore, it is unclear in this study whether this result is the cause of FA or a secondary phenomenon caused by a restricted diet in patients with FA. However, since milk and eggs are sphingolipid-rich foods, sphingolipids tended to increase in patients with milk or egg allergy, probably owing to a mechanism other than the effect of dietary restrictions. Further research related to dietary restrictions is needed in this regard. In conclusion, our results indicate that gut sphingolipid metabolites may play a role in the development of FA in infants with AD.

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*Conflicts of Interest*

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# Drug-Induced Enterocolitis Syndrome due to Acetaminophen in an Adult: A Call for Diagnostic Tools and Accurate Management

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Palabras clave: Paracetamol. Enterocolitis inducida por drogas. Patogénesis. Test de activación linfocitaria. Adulto.

Drug-induced enterocolitis syndrome (DIES) is an uncommon and poorly documented severe non–IgE-mediated hypersensitivity reaction caused by drugs and characterized by gastrointestinal symptoms. Even though DIES is a potentially severe condition, awareness is low and diagnostic tools and the pathogenic mechanisms involved are unexplored [1]. To our knowledge, this is the first report of a case of DIES due to acetaminophen in an adult that was confirmed with a positive lymphocyte transformation test (LTT) result. The patient gave his informed consent for the publication of this case report.

We report the case of a 45-year-old man with no previous allergy history who developed repetitive vomiting and diarrhea 2-3 hours after the intake of Argidol 650 mg (acetaminophen and codeine) as an antipyretic for an upper respiratory tract infection. The patient reported complete recovery in 24 hours. No medical care was requested. He also subsequently reported tolerance to celecoxib, although tolerance to other traditional nonsteroidal anti-inflammatory drugs (NSAIDs) was unknown.

We performed skin prick tests with acetaminophen (10 mg/mL) and intradermal tests (0.1 mg/mL), which yielded negative results. A drug provocation test (DPT) was carried out with acetaminophen until a cumulative dose of 1 g was reached. Some 3 hours after the last dose of acetaminophen (at home), the patient developed pallor, abdominal discomfort, nausea, and diarrhea, with no other symptoms (neither cutaneous nor respiratory). The symptoms resolved spontaneously in 24 hours. The study was considered inconclusive, and an underlying gastrointestinal infection was suspected. A DPT was repeated 1 month later. Two hours after the final dose of acetaminophen, the patient developed more severe symptoms including repetitive vomiting, abdominal pain, diarrhea, marked pallor, hypotension, and dizziness (again, without cutaneous or respiratory symptoms). He was treated initially with oral corticosteroids (deflazacort 60 mg) and an antihistamine (bilastine [Bilaxten] 20 mg), although his condition did not improve. Intravenous rehydration with saline 500 mL was prescribed, with complete recovery from symptoms 1 hour after onset.

Based on the results of the oral challenge, we considered that the patient met the major criteria for DIES due to acetaminophen (vomiting in the 1- to 4-hour period after ingestion and absence of classic IgE-mediated allergic skin or respiratory symptoms), together with more than 3 minor criteria (a second episode of repetitive vomiting after ingestion of the same drug, marked pallor, need for intravenous fluid support, diarrhea during the 24 hours after ingestion, and hypotension). In addition to the diagnostic criteria for patients presenting possible DIES, LTT was carried out in Hospital Universitario La Paz, Madrid and yielded a positive result. Briefly, peripheral blood mononuclear cells ($2\times10^5$ cells in 200 µL) were stimulated with 1, 10, 100, and 200 µg/mL of acetaminophen in triplicate for 6 days. For the final 18 hours of the incubation period, proliferation was determined by the addition of [3H] thymidine (0.5 µCi/well). Proliferative responses were calculated as the stimulation index (SI), defined as the ratio of mean values of counts per minute in culture with drug to those obtained without drug. The LTT result was considered positive if the SI was higher than 2. The SI was 2.4 for acetaminophen 200 mg/mL (Supplementary figure).

In order to rule out potential cross-reactivity with other NSAIDs, DPT was carried out with aspirin and showed good tolerance. Skin tests and DPT with codeine yielded negative results. The patient was recommended to avoid acetaminophen and other members of the para-aminophenol family. Alternative NSAIDs were allowed, along with codeine.

DIES due to acetaminophen confirmed by oral challenge test was reported by Pascal et al [2] in a 12-month-old child. The first publication on the topic dates from 2014; since then, a further 11 clinical cases of DIES have been reported (8 children and 3 adults). The drugs involved were amoxicillin or amoxicillin/clavulanate in 10 cases and pantoprazole in the remaining case [3-5].

Clinically, DIES resembles food protein-induced enterocolitis syndrome (FPIES). Specific criteria have been proposed for diagnosis, and the potential existence of atypical forms has been described [6,7]. It has also been postulated that FPIES and DIES share common pathogenic mechanisms, as they are both nonimmediate hypersensitivity reactions involving adaptive immunity [8]. However, the pathogenesis

 

of DIES remains unclear, the underlying immunologic mechanisms have not been verified, biomarkers have not been validated, and predisposing genetic factors are not known. Mori et al [9] recently documented involvement of T cells in the pathogenesis of DIES, reporting the first case of DIES with amoxicillin/clavulanate and a positive LTT result, findings suggestive of a T cell–mediated response. Given the high risk and potential severity of DPT, we propose LLT as a potentially useful and complementary tool for diagnosis of DIES when the clinical criteria are met and the suspicion is strong.

Epinephrine is not effective for the treatment of DIES. Favorable responses have been reported with antiemetics, intravenous rehydration, and corticosteroids [1,2]. In the present case, the patient did not respond to antihistamines or to corticosteroids, although he did respond well to intravenous fluids.

Because of the small number of cases reported, it is difficult to establish whether DIES is a transient or persistent condition. The symptoms seem to be more frequent in children, although no data are available on prognosis or natural history [6].

In conclusion, we present the first case of DIES due to acetaminophen in an adult confirmed by oral challenge and a positive LTT result. Potential cross-reactivity with other NSAIDs was ruled out. We highlight the need for a better understanding of the pathogenic mechanisms, natural history, and prevalence of this disorder. We propose this case report as a call for clinicians to recognize DIES as a potentially severe non–IgE-mediated hypersensitivity reaction requiring specific treatment. Further studies are needed to establish appropriate management of affected patients.

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## Kounis Syndrome During an Oxaliplatin Desensitization Protocol

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Palabras clave: Síndrome de Kounis. Alergia medicamentosa. Desensibilización medicamentosa. Quimioterapia.

Anaphylaxis is a clinical emergency and the most dangerous manifestation of hypersensitivity reaction owing to systemic involvement [1]. Kounis syndrome (KS) is the simultaneous occurrence of acute coronary syndrome during a hypersensitivity reaction [2,3]. Inflammatory mediators released during anaphylaxis extend to cardiac mast cells, which are directly involved in the pathophysiology of KS. This leads to coronary artery spasm and erosion or rupture of atheromatous plaque, followed by myocardial infarction [3,4]. Three variants of KS have been described based on coronary artery conditions (online supplementary table) [2-4]. We report a case of KS (type I) that developed during an oxaliplatin desensitization protocol. To our knowledge, this is the first reported case of KS during an oncology desensitization protocol. The patient gave her written informed consent for the publication of her medical data.

A 59-year-old woman with no history of atopy who had stage IV rectal carcinoma experienced dyspnea, epigastric pain, and hives on the chest and face within a few minutes of her seventh cycle of oxaliplatin. She was treated with antihistamines and corticosteroids. Oxaliplatin was discontinued, and successive lines of platin-free chemotherapy were administered. Two years later, owing to progression of her cancer, oxaliplatin was reintroduced. The patient was referred to our allergy unit. Prick tests performed with oxaliplatin at 5 mg/mL and intradermal tests at 0.5 and 5 mg/mL yielded negative results [5]. After a risk assessment and multidisciplinary team discussion, we considered rapid drug desensitization (RDD), as a drug challenge was deemed too risky. We follow the protocol of 3 bags and 10 steps established by the Ramon y Cajal University Hospital (RCUH) group [6]. RDD was carried out in an allergy-led recovery ward that was risk-assessed for these procedures under constant monitoring and supervision by an allergy nurse and clinician at the bedside, as per the guidelines of the World Allergy Organization [7]. The first RDD with oxaliplatin was uneventful. During the second desensitization cycle, at step 8 of the protocol, the patient developed hives on her head and neck, profuse sweating, dyspnea, and severe epigastric/chest pain. Her blood pressure was 70/40 mmHg, and her oxygen saturation was 97% in room air. Administration of oxaliplatin was stopped. The patient was immediately treated with intramuscular adrenaline (0.3 mg), intravenous methylprednisolone (80 mg), dexchlorpheniramine (5 mg), and fluid therapy (500 mL). Her condition improved rapidly. A 12-lead electrocardiogram showed a sinus rhythm with ST-segment depression in V3-V6 and D2-D3-AVF and ST elevation in V1-AVL (Figure). Serial measurements of ultrasensitive cardiac troponin were initially 19 ng/L, increasing to 335 ng/L at 2 hours after the onset of symptoms. Serum tryptase and IL-6 levels were measured half an hour after the initial symptoms (4.74 µg/L and 6.8 pg/mL, respectively [basal serum tryptase, 2.9 µg/L]). Given the unexpected clinical situation and the cardiac involvement, a second tryptase determination was not performed, and the patient was transferred immediately to the intensive care unit. Coronary angiography revealed normal coronary arteries. Six weeks after this event, an allergology study with oxaliplatin was repeated. Intradermal testing at a concentration of 0.05 mg/mL yielded a positive result, presumably showing an IgE-mediated mechanism of sensitization to oxaliplatin. Based on the clinical history and electrocardiographic, laboratory, and angiography results, the patient was diagnosed with KS type 1.

Anaphylactic reactions with concomitant cardiac involvement have received little attention in the scientific literature. In 1991, Kounis and Zavras described *allergic angina syndrome* as the occurrence of endothelial dysfunction or microvascular angina resulting in an allergic acute myocardial infarction [2-4].

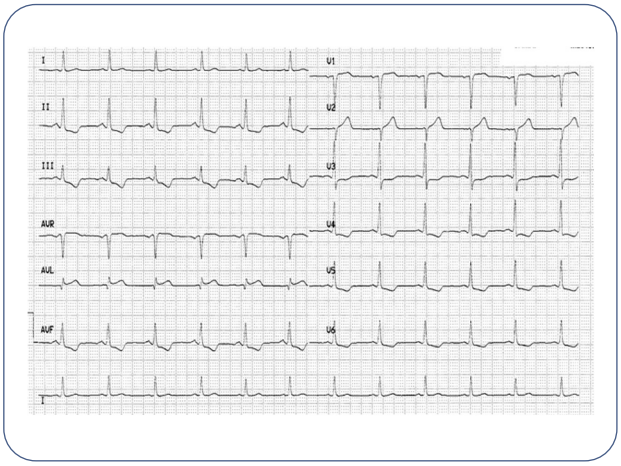


**Figure.** ST segment depression in V3-V6 and D2-D3-AVF and ST elevation in V1-AVL.

 

Laboratory evidence has demonstrated that cardiac mast cells, particularly in the vicinity of coronary plaques, play a key role in the pathophysiology of KS. Inflammatory mediators released by mast cells contribute to endothelial dysfunction, plaque erosion or rupture, and subsequent microvascular angina associated with cardiac insult and allergic reactions [2-4].

Oncology patients are particularly susceptible to cardiac complications, likely owing to the cardiotoxic effects of various chemotherapeutic agents. The risk of hypertension, dyslipidemia, early atherosclerosis, and coronary artery disease can vary depending on the specific antineoplastic agent used, potentially predisposing patients to coronary artery insults. Unlike cardiotoxicity, which refers to a dose-dependent cardiovascular adverse reaction that persists despite the discontinuation of the causative treatment, the term *cardiohypersensitivity* refers to a non–dose-dependent immunologic effect (IgE-mediated or non–IgE-mediated) that may occur at any time during treatment, even with a minimal drug dose, the main mechanism being associated with the coronary syndromes or cardiac insults that develop immediately after chemotherapy [8]. Cases of KS associated with antineoplastic agents have been reported in the literature. Chang et al [9] documented a case of KS induced by oxaliplatin that was diagnosed based on co-occurrence of anaphylaxis and cardiology symptoms, together with ECG abnormalities. However, neither cardiological assessments (troponin and angiographic tests) nor an allergology study (tryptase and skin testing) were performed to confirm the diagnosis of KS.

RDD for antineoplastic and biological agents enables temporary tolerance. It is a cost-effective procedure that makes it possible to ensure the most efficacious drug therapy for the affected patient, with the same life expectancy as for nonhypersensitive patients. RDDs are personalized procedures that are adapted to the high complexity of affected patients, requiring multidisciplinary collaboration led by an expert allergist whose role is fundamental in maximizing safety [5,7].

In a series of RDDs reported by the RCUH group, no breakthrough reactions were observed in 88% of the desensitizations, and in cases where reactions did occur, they were typically mild [6]. To our knowledge, no cases of KS have been reported in the major published series of RDD to antineoplastic agents [6,10].

Diagnosis of KS requires a high degree of suspicion, which should be supported by clinical symptoms and evidence from laboratory, electrocardiographic, and angiographic evaluations [2]. The treatment and management of KS is challenging owing to the convergence of 2 potentially life-threatening conditions, anaphylaxis and myocardial involvement [2,3], which make management of KS a challenging endeavour [1-3]. Clinicians must navigate the complex balance between treating anaphylaxis and the potential adverse effects of adrenaline [1,2]. However, prompt treatment of anaphylaxis must take precedence, as, otherwise, it could lead to persistent hypotension and end-organ failure, which could in turn cause ischemia and worsen coronary vasospasm [1].

In conclusion, we report the first case of KS resulting from a breakthrough reaction during the administration of an antineoplastic agent (oxaliplatin) via RDD. In this case, the diagnosis of KS type 1 is based on the clinical history and supported by laboratory, electrocardiographic, and angiographic findings. Positive intradermal test results suggest an IgE-mediated immunological mechanism.

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*Conflicts of Interest*

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## Occupational Asthma Caused by Exposure to Alumina

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Palabras clave: Exposición ocupacional. Prueba de provocación bronquial específica. Metales. Asma.

Alumina, also known as aluminum oxide ($Al_2O_3$), is a naturally occurring mineral with a high melting point (2072°C) and remarkable hardness. It is known for its versatility, featuring high porosity, the ability to absorb heavy metals and contaminants, and exceptional density, hardness, and wear resistance. This makes it invaluable as an electrical insulator, in the production of ball mills, and in equipment for water and gas purification. It is also used to obtain aluminum. At the respiratory level, exposure to aluminum or its derivatives by welders can cause pneumoconiosis (with or without progression to fibrosis [1]), occupational asthma (OA) due to potassium aluminum tetrafluoride [2], and potroom asthma [3]. Reported mainly in aluminum smelters, potroom asthma is characterized by respiratory symptoms similar to asthma in workers producing aluminum using electrolytic cells. It is unclear whether the cause is direct exposure to aluminum or exposure to fluorides associated with these industrial processes [3,4].

To our knowledge, there are no reports in the literature of asthma secondary to exposure to alumina dust. Here we present the first case of OA due to alumina demonstrated through specific inhalation challenge (SIC).

The patient was a 41-year-old man (smoker [40 packs/year]; body mass index, 37), with no other relevant history. He had worked for 15 years in the construction of outdoor swimming pools and for the last 3 years in a company that manufactured absorbent material for insulation using alumina as raw material. To produce this material, the patient mixed alumina powder with sodium bicarbonate and potassium permanganate for 8 hours a day, 5 days a week. He wore an FFP2 mask at work for respiratory protection. The patient gave his consent for publication.

Two months after starting the job, he developed rhinitis, conjunctivitis, dry cough, and dyspnea. The symptoms began early, approximately 1 hour after arriving at work, improved with inhaled ß-adrenergics, and were clearly work-related, as they abated on weekends and during vacation periods or periods of sick leave.

 

Respiratory auscultation was normal. A blood test showed an eosinophil level of 300/µL, IgE level of 902 kU/L, and a positive ImmunoCAP Phadiatop result (Phadia AB) (41.60 $kU_A$/L). Forced spirometry revealed $FEV_1$ of 3.59 L (75%; z-score, –1.92) and an $FEV_1$/FVC ratio of 71% (z-score, –1.28). The methacholine test result was positive, with a $PC_{20}$ of 6.6 mg/mL and FeNO of 7 ppm. Chest computed tomography showed nonspecific bronchial thickening.

Given the suspicion of OA due to alumina, an SIC was performed following the recommendations of the European Respiratory Society [5]. Briefly, the patient was exposed on successive days for increasing periods to a mixture of 20 g of alumina powder with 150 g of lactose in a 7-$m^3$ challenge cabinet and with the mixture being tipped from one tray to another at 30 cm from the face. $FEV_1$ was measured every day prior to exposure, at 10-minute intervals during the first hour after exposure, and every hour thereafter for a maximum of 12 hours. The day before the exposure, the patient was tested with placebo (powdered lactose). On the first day, after a 10-minute exposure to alumina, $FEV_1$ had dropped progressively at 7 hours after exposure; this reached a maximum of 19% between 10 and 12 hours after exposure (Figure). The decrease was accompanied by mild bronchospasm that required treatment with inhaled bronchodilators. At 24 hours after exposure, PC20 to methacholine was 4 mg/mL and FeNO 11 ppb.

OA is a disease characterized by variable airway obstruction and/or bronchial hyperresponsiveness due to causes and conditions that can be attributed exclusively to a specific work environment [6]. It is estimated that the average proportion of asthma cases in adults attributable to occupational exposure is between 10% and 15% and that this is currently the most common work-related respiratory disease in developed countries [7]. More than 400 agents have been associated with its pathogenesis. Metals are especially important, with platinum, nickel, chromium, cobalt, zinc, and manganese being the most frequently involved [8]. The present description suggests that aluminum should be added to this list.

The tests confirmed the relationship between alumina and the development of OA but did not enable us to establish the mechanism via which this disease develops. The high IgE levels, the presentation of atopy, and the high eosinophil rate may suggest an IgE-mediated mechanism; however, the late reaction in the SIC suggests that the immunological mechanism involved was independent of IgE. This last option seems to be supported by the observation of neutrophilia and elevated IL-8 in induced sputum recorded by Sikkeland et al [9] in 15 healthy individuals exposed to alumina through an SIC performed to assess its ability to generate inflammation in the airway.

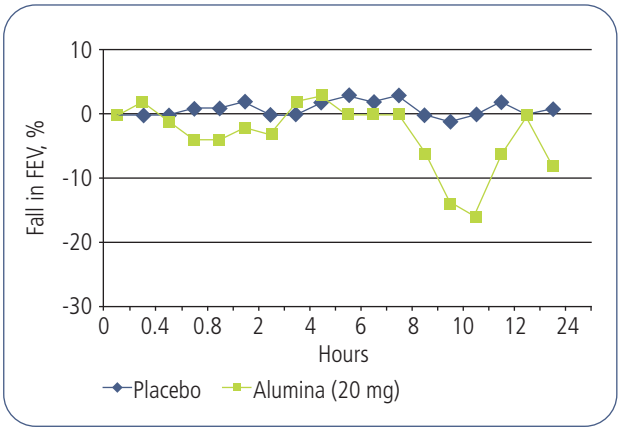


**Figure.** Result of specific inhalation challenge to alumina powder.

The mechanism involved in the pathogenesis of OA when the agent is a metal is not well defined. Among the possibilities proposed are exposure to platinum, in which the basic mechanism involved in the development of asthma is IgE-dependent, or exposure to other agents, such as chromium or nickel, in which the mechanism can be IgE-dependent or not [6].

The differential diagnosis of OA due to aluminum should include potroom asthma and OA due to other derivatives of aluminum or other metals. It is essential to conclusively determine the source of exposure and the type of industrial process involved in order to identify the agent. While potroom asthma has been related to exposure to agents released during the smelting process [3], other activities, such as aluminum welding, have been related to the development of OA, as demonstrated by exposure to potassium aluminum tetrafluoride [2].

In conclusion, given the increase in the use of aluminum, and especially alumina in multiple industrial processes, exposure to these elements should be acknowledged as a possible cause of respiratory disease (especially OA). This would make it possible to provide a rapid response to potentially affected patients and to implement primary prevention measures designed to avoid future exposures.

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## Successful Subcutaneous Desensitization to Certolizumab Pegol

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Key words: TNFα inhibitor. Certolizumab. Hypersensitivity. Desensitization. Allergy.

Palabras clave: Inhibidor TNFα. Certolizumab. Hipersensibilidad. Desensibilización. Alergia.

Certolizumab pegol (CZP) is a recombinant, humanized antibody Fab' fragment that targets tumor necrosis factor α (TNFα). It is conjugated to polyethylene glycol (PEG) and has an approximate molecular weight of 20 000 g/mol. CZP has been approved for chronic autoimmune and inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, axial spondyloarthritis, and plaque psoriasis. The recommended dose is 400 mg (2 subcutaneous injections of 200 mg each on 1 day) at weeks 0, 2, and 4, followed by a maintenance dose of 200 mg every 2 weeks [1]. Adverse effects, including paradoxical psoriasiform eruptions and hypocomplementemic urticaria-vasculitis have been reported [2,3]. Hypersensitivity reactions to CZP have been described, both to the anti-TNF portion of the antibody and to the PEG component [4,5].

We present the case of a 30-year-old woman with a medical history of spondyloarthritis treated for 2 years with CZP until its suspension due to severe pain at the injection site. A year later, CZP had to be reintroduced because of ineffective treatment with golimumab. Within 60-90 minutes of the first subcutaneous injection of 400 mg of CZP, the patient developed pruritic, erythematous papules on the neck, trunk, and extremities. A 4-day treatment with oral methylprednisolone 40 mg and ebastine 10 mg was indicated, with complete resolution of the lesions and no residual hyperpigmentation or desquamation. Two weeks later, she received 400 mg of subcutaneous CZP and presented a similar reaction; therefore, treatment was interrupted, and the patient was referred to the allergy department.

After providing her written informed consent, the patient underwent a skin prick test (SPT) with certolizumab (200 mg/mL) and an intradermal test (IDT) at 2, 20, and 200 mg/mL. A positive result (5-mm wheal) was obtained 20 minutes after the 200-mg/mL IDT. Four hours later, the patient developed several slightly pruritic erythematous papules on her right forearm (where the IDT was performed). The lesions resolved within 24 hours after application of a topical corticosteroid. These concentrations were nonirritant in 4 healthy controls. Furthermore, SPTs with strawberry flavor

 

**Table.** Desensitization Protocol for Certolizumab Pegol.

| Desensitization | | | | | | |
|---|---|---|---|---|---|---|
| **Step** | **Time, min** | **Concentration, mg/mL** | **Volume, mL** | **Dose administered with this step, mg** | **Cumulative dose, mg** | **Comment** |
| 1 | 0 | 2 | 1 | 2 | 2 | X |
| 2 | 30 | 20 | 0.2 | 4 | 6 | X |
| 3 | 60 | 20 | 0.4 | 8 | 14 | X |
| 4 | 90 | 20 | 0.8 | 16 | 30 | X |
| 5 | 120 | 200 | 0.16 | 32 | 62 | X |
| 6 | 150 | 200 | 0.32 | 64 | 126 | X |
| 7 | 180 | 200 | 0.64 | 128 | 254 | X |
| 8 | 210 | 200 | 0.73 | 146 | 400 | X |
| Observation: 60 min | | | | | | |
| Total time: 270 min | | | | | | |

chewable alginate antacid tablets (Gaviscon, containing PEG 20 000) were performed at 0.25, 2.5, 25, and 250 mg/mL. The results were negative, thus ruling out allergy to the excipient. Therefore, the patient was recommended to avoid TNFα inhibitors.

After 13 months of treatment with secukinumab (IgG1/κ monoclonal antibody), which proved to be ineffective, the patient was again referred to the allergy department to consider the possibility of prescribing etanercept, another TNFα inhibitor. An SPT (50 mg/mL) and IDT (0.5 mg/mL and 5 mg/mL) to etanercept yielded negative results, leading us to perform a drug challenge, which also yielded a negative result (cumulative dose of 50 mg). Unfortunately, this treatment, as well as subsequent treatment with ixekizumab (anti–IL-17A antibody), was unsuccessful. Therefore, both options were discontinued, and the patient was referred to the allergy department to undergo a desensitization procedure with certolizumab.

Cetirizine 10 mg and ranitidine 50 mg were administered 30 minutes before the procedure. CZP was administered in a 3-solution, 8-step regimen with an initial subcutaneous dose of 2 mg that was gradually increased every 30 minutes until a cumulative dose of 400 mg was reached (Table). Cetirizine 10 mg and famotidine 40 mg were prescribed daily for 3 days after the procedure. No incidents were reported. An identical procedure carried out 2 weeks later caused no adverse reactions. As the interval of 14 days did not exceed 2 half-lives of the medication, the third dose comprised the full dose of 400 mg divided between both arms, with no adverse reactions. The next dose was 200 mg, which the patient received in a single dose in a prefilled syringe with good tolerance (12 weeks) until its withdrawal due to loss of efficacy.

The positive skin test result with CZP proves that an immune mechanism could be involved in the reaction experienced by the patient. Among TNFα inhibitors, infliximab has caused the highest number of hypersensitivity reactions [6], although the literature contains few data on cross-reactivity between drugs in this group. Reactions to infliximab and subsequent tolerance to adalimumab have been reported [7,8]. However, there are no cross-reactivity studies comparing CZP and etanercept. Consequently, after confirming hypersensitivity to certolizumab, we assessed allergy to etanercept and confirmed that it was tolerated.

Desensitization procedures enable allergic patients to receive the best treatment option for their disease. Cases series of subcutaneous desensitization with biologic drugs have been described. In the case of TNFα inhibitors, desensitization protocols to adalimumab and etanercept are also available, although, to date, no desensitization protocols to golimumab or certolizumab have been published [9].

To our knowledge, we describe the first desensitization protocol to certolizumab, in a case of confirmed allergy to CZP with tolerance to another TNFα inhibitor, etanercept.

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*Conflicts of Interest*

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## Successful Desensitization to Oral Dasatinib in Immediate Hypersensitivity Reaction

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Palabras clave: Desensibilización oral. Dasatinib. Alergia a fármacos. Inhibidores de tirosin-kinasa. Test de activación de basófilos.

Chronic myeloid leukemia (CML) is caused by abnormal myeloid cell proliferation in bone marrow, resulting in the BCR-ABL fusion gene and a constitutively active tyrosine kinase [1]. Recent progress in CML treatment has led to the introduction of targeted tyrosine kinase inhibitors (TKIs) for long-term remission [1]. Imatinib, the first approved TKI for CML, is now joined by second-generation drugs, such as dasatinib, bosutinib, and nilotinib, as well as the third-generation ponatinib. While safety profiles vary, all TKIs are associated with common adverse reactions (ARs), such as nausea, myopathy, rash, diarrhea, and fatigue, as well as late-onset hematologic responses, such as myelosuppression.

Imatinib triggers muscle pain, headaches, and edema; dasatinib is linked to pleural effusion and pulmonary hypertension; bosutinib can impair liver function and cause rash; nilotinib is associated with cardiovascular abnormalities, QT interval prolongation, and hyperglycemia; and ponatinib poses risks for liver disorders and pancreatitis [2,3]. Consequently, close monitoring of patients' hematologic, metabolic, and cardiovascular profiles is imperative for effective disease management.

Desensitization is vital for IgE-mediated hypersensitivity reactions necessitating discontinuation of therapy when no equivalent treatments are available [4]. Here, we present the case of a patient with CML who experienced an immediate hypersensitivity reaction to dasatinib and successfully underwent desensitization to dasatinib.

The patient was a 53-year-old woman with no known drug allergy and a history of ischemic heart disease. She was diagnosed with CML in December 2019 and started first-line imatinib at 400 mg daily.

She tolerated imatinib well, with a good hematologic response, having reached a state of major molecular response [1]. During the first month, the only AR was eyelid edema, which improved with diuretics, until she reported that the skin on her hands had become increasingly fragile and prone to erosion. A dermatologist diagnosed the lesion by means of a skin biopsy, which was consistent with imatinib-induced pseudoporphyria, leading to discontinuation of treatment.

CML subsequently worsened, and the patient started treatment with dasatinib 100 mg every 24 hours. Less than 2 hours after the first dose, a nonpruritic maculopapular exanthema appeared on her arms. After the second dose of dasatinib, the exanthema became generalized and was accompanied by severe headache; therefore, the patient called the clinical team from home. Her vital signs remained unaltered. Neither tryptase nor IL-6 was measured. The symptoms improved gradually with oral antihistamines and corticosteroids over 3 days prescribed on an outpatient basis. Treatment was suspended, and the patient was referred to the allergy unit. A skin prick test (SPT) and basophil activation test (BAT) were performed with dasatinib and bosutinib, as a potential alternative.

In the SPT, a 50-mg dasatinib and a 500-mg bosutinib capsule were diluted with sterile water. For BAT, 10 mL of heparinized blood was obtained and taken immediately to the laboratory, where it was analyzed using the Flow2CAST kit. Basophils were identified by flow cytometry (FACS-Canto-II, BD-Biosciences). A minimum of 800 basophils was gated, and expression of $CD63^+$ and $CD203c^+CD63^+$ as markers of activation was assessed. A stimulation index (SI) above 2 was considered positive.

The result of the SPT with dasatinib was negative, and the BAT result was positive at all concentrations tested (SI<2) (see Figure and Figure S1); for bosutinib, all test results were negative. Considering these findings and the need to continue treatment of the patient's CML, it was decided to initiate bosutinib 500 mg/24 h and to monitor the patient closely for a response. Unfortunately, the patient reported nausea, abdominal pain, diarrhea, and headache. These symptoms gradually became incapacitating over the following days, with both regular and reduced doses of bosutinib 200 mg, which eventually led to discontinuation. The symptoms resolved completely after 24 hours.

Since the patient needed to continue TKI treatment, we proposed a rapid dasatinib desensitization protocol (Table S1), aiming for a 100-mg dose, which is the standard approach for effective treatment of CML.

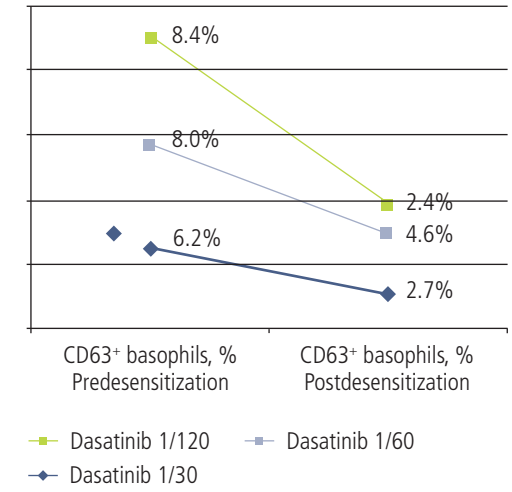
The dasatinib desensitization protocol was successful, and the patient continues to tolerate dasatinib 100 mg/24 h.

After more than a year of daily dasatinib, the BAT result became negative, indicating reduced basophil reactivity (Figure).

The patient provided her written informed consent for the SPT and desensitization procedure, as well as for the publication of this report.

Treatment of CML poses significant challenges, with inherent TKI-induced toxicity, and requires the contribution of a multidisciplinary team to assess drug safety and explore alternative treatments. Cutaneous effects have been reported, especially with imatinib, including facial edema, which affects most patients, pruritic rash that typically develops at 9 weeks of treatment and can in some cases be severe, and other inflammatory eruptions, which have also been observed with

A




B

**Table.**

| | Before desensitization | | After desensitization | |
|---|---|---|---|---|
| | Gated basophils | $CD63^+$ basophils, % | Gated basophils | $CD63^+$ basophils, % |
| C (–) (SB) | 1817 | 1.0 | 1076 | 1.0 |
| C (+) (anti-FcεRI) | 952 | 15.3 | 1000 | 59.0 |
| Dasatinib 1/30 | 1136 | 6.2 | 1026 | 2.7 |
| Dasatinib 1/60 | 1129 | 8.0 | 1036 | 4.6 |
| Dasatinib 1/120 | 1116 | 8.4 | 1038 | 2.4 |

Abbreviations: C (–), negative control; SB, buffer; C (+), positive control.

**Figure.** Basophil activation test with dasatinib. A, $CD63^+$ basophils (%) before and after desensitization; B, Positive and negative controls of the basophil activation test and number of gated basophils for each dasatinib dilution before and after desensitization.

dasatinib [2,5]. However, the underlying mechanisms have not been studied.

In this case, the patient had imatinib-associated pseudoporphyria, a rarely reported AR. Given the absence of cases of skin fragility in their report, Martínez-Mera et al [6] recommended switching to dasatinib or nilotinib for imatinib-associated pseudoporphyria. Dasatinib was chosen here owing to cardiovascular ARs linked to nilotinib (contraindicated in patients with a history of ischemic heart disease) [3].

Desensitization protocols for imatinib have been described. Nelson et al [7] conducted a 4-hour oral desensitization protocol for 10 patients with imatinib-induced rash, succeeding in 8 cases. Klaewsongkram et al [8] outlined a slow desensitization protocol for severe nonimmediate skin reactions, resulting in reduced $CD5^+$, $CD25^+$, and $CD135^+$ T cells.

Karaatmaca et al [9] reported 2 pediatric cases of delayed hypersensitivity to dasatinib that were successfully treated with a 1-day rapid desensitization protocol [9]. However, in many cases, allergology studies were not conducted or yielded negative results.

We present a case of successful desensitization to dasatinib in a patient with immediate hypersensitivity. The success of the protocol was confirmed by a positive BAT result. The patient could not tolerate other TKIs. While the literature hints at TKI cross-reactivity, the positive BAT result for dasatinib and the negative result for bosutinib suggest otherwise in the present case. Notably, more than 1 year after successful desensitization, the patient continued to tolerate daily dasatinib, and the BAT result turned negative.

In conclusion, desensitization with older-generation TKIs such as imatinib has shown promising results, although few cases have been published [7,10]. While a literature search revealed a dasatinib desensitization protocol for 2 pediatric patients with delayed reactions and negative allergy test results [9], this report highlights the first successful dasatinib desensitization protocol in an adult patient with immediate hypersensitivity reaction confirmed by a positive BAT and no viable alternative TKIs owing to severe adverse effects.

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The authors declare that they have no conflicts of interest.

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## Off-label Use of Mepolizumab: A Potential Therapeutic Option for Eosinophilic Cystitis

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Palabras clave: Cistitis eosinofílica. Mepolizumab.

*To the Editor:*

We read with interest the recent publication by Trefond et al [1] on the efficacy of mepolizumab for the treatment of eosinophilic cystitis. The authors first reported 2 patients with idiopathic eosinophilic cystitis whose condition improved after off-label treatment with mepolizumab [1]. We support and appreciate the authors' work and agree with their conclusions. Here, we would like to share a case of successful treatment of eosinophilic cystitis using mepolizumab in China. The patient gave his written informed consent for the publication of his case details.

A 77-year-old man came to the rheumatology and immunology department with a >2-year history of difficulty urinating. Questioning during the physical examination also revealed frequent urination. There was no tenderness in the kidney area, and he did not have an allergic rash. Two years previously, he had undergone a bladder biopsy in the urology department, which showed eosinophilic infiltration. He was diagnosed with eosinophilic cystitis and discharged after a week of treatment with antibiotics and hemostasis. Subsequently, he received long-term treatment with methylprednisolone 4 mg once daily and *Tripterygium wilfordii* 20 mg twice daily. During this period, he did not take any biological agents. Over a period of 1 month, his symptoms of difficulty urinating and frequent urination worsened. He experienced nocturia (3-4 times a night) but not fever or significant weight changes. Laboratory tests (reference values in parenthesis) showed an erythrocyte sedimentation rate of 25 mm/h (0-15 mm/h), C-reactive protein level of 40.3 mg/L (0-10 mg/L), urine white blood cell count of 53/μL (0-28/μL), urine red blood cell count of 20/μL (0-17/μL), white blood cell count of 9.8 × $10^9$/L (3.5-9.5 × $10^9$/L), eosinophil count of 0.85 × $10^9$/L (0.02-0.52 × $10^9$/L), and IgE level of 175 IU/mL. Values for tumor markers, prostate-specific antigen, and the tuberculosis T-cell spot test were within normal ranges. Urinary system ultrasound and abdominal CT did not reveal tumors. After excluding urinary tract infections, tuberculosis, and tumors based on the bladder biopsy results, we eventually diagnosed the patient with eosinophilic cystitis. Treatment was initiated with methylprednisolone 4 mg combined with *Tripterygium wilfordii* twice daily, although the symptoms of difficulty urinating, frequent urination, and nocturia did not improve, and IgE levels remained persistently elevated. Subsequently, the patient received mepolizumab (a humanized monoclonal antibody against interleukin [IL] 5), and after 1 course of injections (100 mg), both laboratory test results and symptoms improved. The results of tests repeated 1 month later revealed lower values than the previous ones (supplementary material 1-SM1). The patient was followed up for 6 months, and his irritative urinary tract symptoms improved considerably, with all laboratory indicators gradually returning to normal.

Eosinophilic cystitis is a rare inflammatory disease characterized by eosinophilic infiltration of the bladder wall [2]. It is associated with infections, drug therapy, bladder cancer, trauma, and allergy, although its exact cause remains unclear [2]. Irritative bladder symptoms are the main manifestations in most cases of eosinophilic cystitis and include frequency (67%), dysuria (62%), gross/microscopic hematuria (68%), suprapubic pain (49%), and urinary retention (10%) [2]. Approximately 43% of cases involve peripheral eosinophilia, while more than half do not have significantly elevated peripheral eosinophil counts [2]. There is no consensus on the treatment of eosinophilic cystitis, although initial treatment typically involves corticosteroids, antihistamines, and antibiotics. Medication-based treatment is associated with a recurrence rate of 17%; surgical treatment is associated with a recurrence rate of 2.6% [2]. Recent studies have shown that activated eosinophils release cytotoxic cationic proteins, which can induce tissue damage. In vitro studies have demonstrated that IL-5, a cytokine, can attract and activate eosinophils [3]. Mepolizumab blocks the binding of interleukin-5 to its receptor, thereby inhibiting eosinophil proliferation, differentiation, and activation [4]. In the case we report, the patient had a >2-year history of refractory eosinophilic cystitis, and his symptoms included frequent urination and difficulty urinating. Despite antibiotic treatment, his symptoms persisted. Subsequently, he underwent systemic treatment including prednisolone, although the results were not satisfactory. After starting low-dose mepolizumab, the patient responded well in terms of symptoms and laboratory indicators. Mepolizumab could be an effective treatment for eosinophilic cystitis. However, formal clinical trials are needed to standardize the treatment.

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### *Conflicts of Interest*

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## Mepolizumab for the Treatment of Eosinophilic Cystitis: Reply

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Palabras clave: Síndrome de hipereosinofílico. Cistitis eosinofílica. Mepolizumab.

*To the Editor:*

We read with interest the publication by Wang et al [1] on the off-label use of mepolizumab for the treatment of eosinophilic cystitis in a 77-year-old man. The authors presented a new case of idiopathic eosinophilic cystitis in a patient who was successfully treated with mepolizumab. We appreciate the authors' reference to our publication on the first 2 cases of eosinophilic cystitis (EC) successfully treated with mepolizumab [2].

In their report, Wang et al [1] highlighted an initial biological inflammatory syndrome, with C-reactive protein of 40 mg/L and an erythrocyte sedimentation rate of 25 mm/h, while in the literature review of 135 patients with EC in 2000 [3], only 7% of patients had an elevated erythrocyte sedimentation rate. The 2 patients we report [2] did not have inflammatory syndrome. We also lack details on the eosinophil infiltration rate in the biopsy in the case reported by Wang et al. In our case, the patients had counts of 200/HPF and 180/HPF, that is, significantly higher than the cutoff of 15/HPF in eosinophilic esophagitis [4]. Another surprising aspect is the absence of imaging abnormalities in the study of Wang et al, whereas in our cases, imaging facilitated diagnosis and provided clear evidence of improvement under treatment.

Nevertheless, the case is compelling and aligns with an organ-restricted presentation without hypereosinophilia, similar to patient 1 in our report [2].

The Table summarizes the key elements from these 3 cases [1,2].

Despite the limited follow-up in the report of Wang et al [1], there have been no reported relapses. This supports the notion, as we previously suggested, that mepolizumab is a promising option for the off-label treatment of idiopathic EC.

 

**Table.** Characteristics of 3 Cases of Eosinophilic Cystitis Successfully Treated With Off-Label Mepolizumab

| | Case 1[a] | Case 2[a] | Case 3[b] |
|---|---|---|---|
| Country | France | France | China |
| Age, y | 69 | 15 | 77 |
| Symptoms | Hematuria, dysuria, urinary frequency | Abdominal pain, pollakiuria, dysuria | Difficulty in urination, frequent urination |
| Eosinophilia, $\times 10^9$/L | 660 | 3000 | 850 |
| IgE | 1500 kU/L | 1800 kU/L | 175 IU/mL |
| CRP, mg/L | <5 | <5 | 40 |
| Urine eosinophils | 90/100 cells | | |
| Biopsy | 200/HPF | 180/HPF | Eosinophilic infiltration |
| Treatment before mepolizumab | Prednisone | Prednisone | Methylprednisolone and *Tripterygium wilfordii* |
| Other organ | - | Eosinophilic gastroenteritis | - |
| Type of HES[c] | Organ-restricted without HE | Multi-organ involvement with severe HE | Organ-restricted without HE |
| Relapse | - | - | - |
| Follow up | 1 y | 15 y | 6 mo |

Abbreviations: CRP, C-reactive protein; HE, hypereosinophilia; HES, hypereosinophilic syndrome; HPF, high-power field.
[a]Reference [2].
[b]Reference [1].
[c]Reference [5].

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*Conflicts of Interest*

J.-E. Kahn reports consulting fees for advisory boards from AstraZeneca and GSK, research funding from AstraZeneca and GSK, and participation in clinical trials sponsored by AstraZeneca. The remaining author declares that he has no conflicts of interest.

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## Highlighting the Need for Each Excipient to Appear Under a Single Name in All Products That Contain it to Guarantee Identification

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Palabras clave: Alergia. Excipiente. Hipersensibilidad. Nomenclatura. Excipientes farmacéuticos.

*To the Editor:*

Chemical compounds can be found under different names in product ingredient lists. This is especially relevant when it comes to molecules that act as excipients in the formulation of medicines and cosmetics or as food additives.

The aim of this letter is to draw attention to the need to unify the nomenclature for excipients and additives in such a way that they always appear under the same name in all the preparations containing them to ensure identification and prevent future reactions in sensitized patients.

Two clear examples are carboxymethylcellulose, which is found in the product ingredient list under various names (eg, carmellose, croscarmellose, colloresine, carboxymethylcellulose ether, and thylose), and aspartame, which in addition to being an excipient in pharmaceutical products, is found in diet soft drinks, fruit drinks, yogurts, and chewing gum and appears in the technical data sheet under names such as L-aspartame-L-phenylalanine methyl ester, E951, Canderel, or Nutrasweet. The complete list of names or synonyms related to a chemical compound can be accessed online via the PubChem database of the National Center for Biotechnology Information of the National Institutes of Health [1].

PubChem is an open archive that contains information on a wide range of chemical compounds. It provides a detailed description of individual molecules, offering information on identifiers, structure, synonyms, molecular weight, and chemical and physical properties. Additionally, since PubChem links its records to PubMed articles indexed with a Medical Subject Heading (MeSH), biomedical literature related to any PubChem record can be obtained [2].

In Table I (view online only supplementary table), we present the PubChem links to some of the excipients previously reported in the literature as being responsible for severe hypersensitivity reactions [3-6].

From an allergological point of view, information on the synonyms by which an excipient or additive can be found is relevant to ensure appropriate identification. This is of vital importance for sensitized patients. A patient sensitized to a molecule has to be able to recognize its presence in the composition of any medication, cosmetic, or food in order to avoid it and minimize the risk of reaction. Likewise, a clinician must also be able to clearly identify the molecule in order to choose a therapeutic alternative that does not contain it.

Additionally, given our focus on the safety of sensitized patients, it is worth commenting on groups of excipients that may be related by cross-reactivity. This is particularly important in patients sensitized to polyethylene glycol (PEG) and, through cross-reactivity, to other molecules derived from ethylene oxide. These include PEG sorbitans (polysorbates), PEG castor oils (eg, Cremophor), poloxamers, and PEG laureths [5,7], all of which have been implicated in severe hypersensitivity reactions and are contained in a wide number of medicines and cosmetics. Therefore, since patients sensitized to ethylene oxide derivatives are at great risk, they must ensure that these excipients are not found in any product they are going to use. However, this is not always easy, since molecules to avoid may be hidden if they are labelled with synonyms not known to the patient; hence the need to standardize the nomenclature used to declare the presence of PEGs or other related molecules, as proposed elsewhere [8]. In this sense, a label warning that the product contains ethylene oxide derivatives could strengthen safety and thus prevent reactions.

In conclusion, we suggest that, at least in the pharmaceutical and food industries, a consensus should be reached so that a chemical compound that acts as an excipient or additive appears with the same name in all products that contain it to ensure its identification and prevent reactions in sensitized patients.

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### *Conflicts of Interest*

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