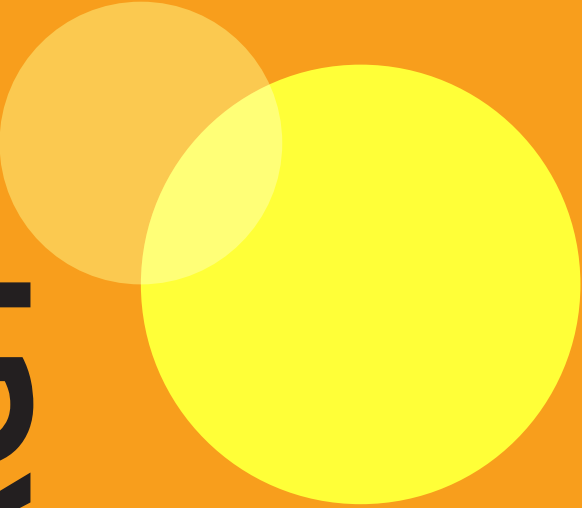




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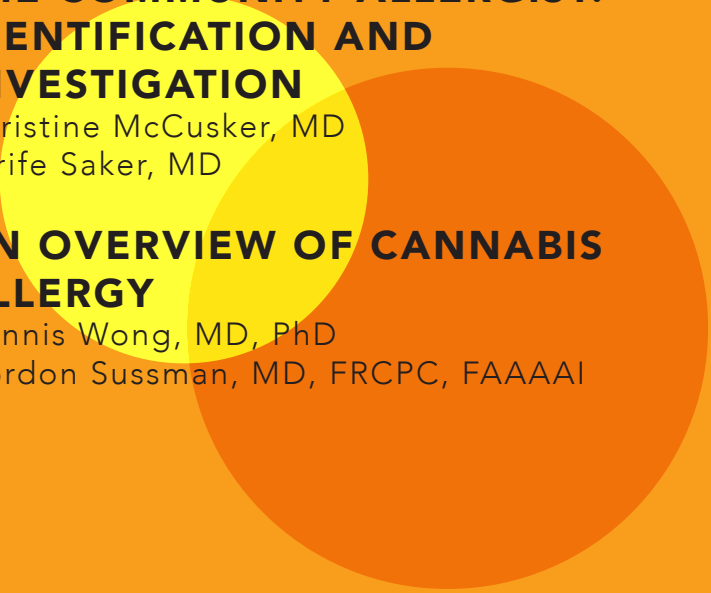
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AN OVERVIEW OF CANNABIS ALLERGY

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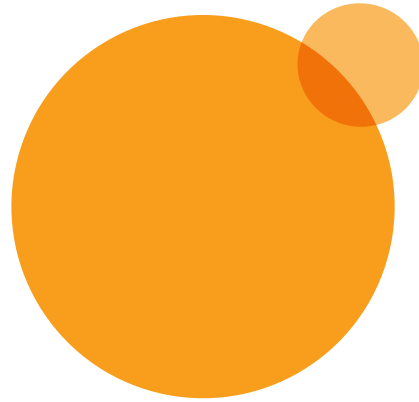
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EDITOR'S WELCOME

Dear Canadian Allergy & Immunology Community,

Welcome to our second issue of Canadian Allergy & Immunology Today in 2022! We hope this issue finds everyone enjoying a safe and healthy summer. We are actively recruiting for manuscript submissions for 2023, so please do drop a note with a topic of interest to info@catalytichealth.com.

In our newest issue, we discuss an overview of cannabis allergy, we review the identification and investigation of errors of immunity for the community allergist, we present Part 1 in a three part series around understanding, preventing and managing food allergy in pre-school children, we provide a guideline for the clinical workup of hypereosinophilia in the allergy community and we share a perspective on the use of biologics in food therapy.

As always, we hope you find these articles informative and helpful. We are grateful to our advertising partners for their ongoing support in 2022, to our authors for their commitment to sharing best practices and to our readers for their continued readership!

Please let us know how we are doing by suggesting topics and feel free to share our registration link at canadianallergyandimmunologytoday.ca with your peers so that, they too, can subscribe to future issues!

Best wishes,



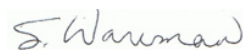
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UNDERSTANDING, PREVENTING AND MANAGING FOOD ALLERGY IN PRESCHOOL CHILDREN: PART 1

This 3-part series will discuss food allergy prevention in preschool children through early nutrition and eczema management. In Part 1, the prevalence of food allergy along with factors contributing to its rise and its impact on quality of life are presented. Part 2 in this series will examine the topic of food allergy prevention through early infant nutrition and early management of eczema. Finally, in Part 3, we will focus on adverse food reactions in children, symptoms of immunoglobulin E (IgE)-mediated food allergy in preschool children, food-specific IgE testing and management of food allergy.

PREVALENCE OF FOOD ALLERGY IN CHILDREN

The incidence of allergic diseases has increased, especially in children.¹ Food allergy is one of the most common chronic and potentially life-threatening conditions during childhood. While food-related anaphylaxis remains an uncommon cause of death and its prevalence varies according to the definition used, a significant proportion of these deaths are preventable.²

Food allergy is a public health concern, especially in an urbanized world, with increasing emergency department visits and hospitalizations for food-induced anaphylaxis events (**Figure 1**).^{1,2}

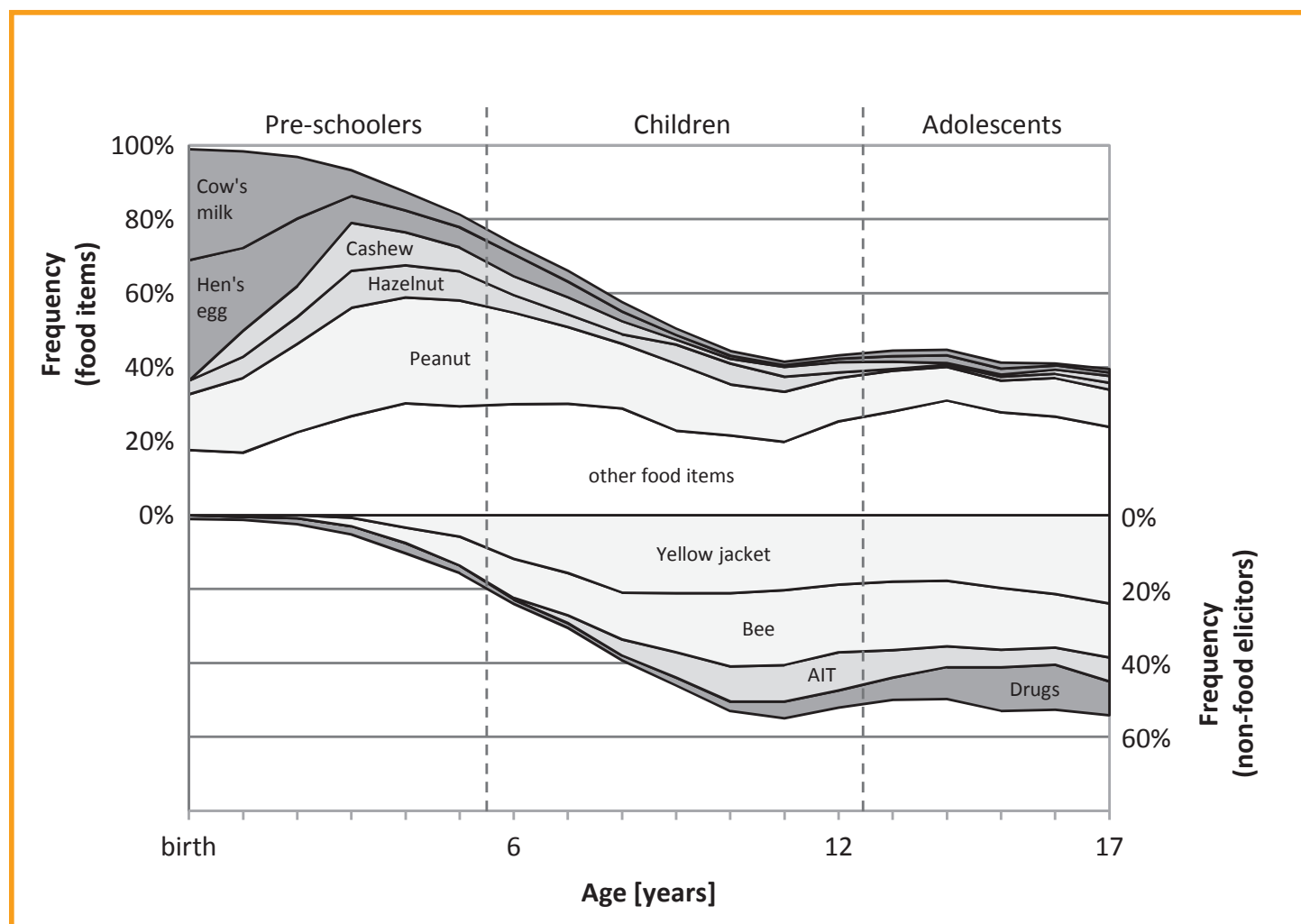


Figure 1. Elicitors of anaphylaxis by age (only for known common elicitors) in Europe. AIT, Allergen immunotherapy (from Grabenhenrich et al. 2016)¹

World-wide, food allergy affects about 10% of children in industrialized countries from North America (most common being peanut), Europe (most common being cow's milk) and Australia (most common being shellfish) and seems to be rising in other parts of the world, such as Asia (United Arab Emirates, China, Vietnam) and South Africa. Interestingly, children of East Asian or African descent born in a Western environment are at higher risk of food allergy compared to Caucasian children.

In the USA, the estimated prevalence of food allergy in children was 7.6%, after excluding 4% of children whose parent-reported food reactions were inconsistent with IgE-mediated food allergy. About 1 in 13 children have food allergies, affecting about 2 school-aged kids per classroom. Of these, approximately 40% were reported to be allergic to multiple foods and 42% had experienced at least one severe reaction requiring an emergency visit, and 40.7% had a current epinephrine autoinjector prescription.²

In Canada, the estimated prevalence was 6.7%, when excluding 2% of children with unconfirmed reports of IgE-mediated food allergy (e.g., lactose intolerance, gluten sensitivity). There has been an increase in probable milk allergy in children and adults and in probable wheat allergy in adults, which may relate to increasing dietary trends for the elimination of milk-based formula, dairy, and gluten in the general population.³ Based on physician-reported data from 2018, only 33.7% of children diagnosed with food allergy had an epinephrine autoinjector prescription.⁴

About 50% of Canadian households are impacted by food allergy, directly or indirectly. Self-reported rates of food allergy are much higher than the true prevalence, and the perceived disease burden may have significant economic and psychosocial impact on young patients/families who may have not yet seen an allergist.

WHY IS FOOD ALLERGY ON THE RISE?

Numerous genetic and environmental factors contribute to the development or loss of food allergy. The immune system is supported in mounting strong and long-lasting adaptive immune responses by many environmental triggers, including bacteria, particularly the colonizing bacteria of normal gut, skin, and airway. These benign microorganisms act to shape and train a person's immunological and metabolic functions. While the causal link between microbial 'exposome' (in utero, ex utero) and development of chronic inflammatory diseases later in life continues to be explored, evidence suggests that the risk for developing food allergy is multifactorial.⁵

Increasing industrialization, urbanization and pollution appear to affect the microbial exposome and contribute globally to increased prevalence of food allergies, eczema, and asthma.

One established risk factor for food allergy is early onset eczema. Dry, cold climate, filaggrin gene mutations, elevated skin pH caused by frequent washing and soaps, are contributing factors to xerosis.

Delaying introduction of allergenic foods inhibits development of early oral tolerance, promoting development of food allergy. A likely contributor to the current food allergy epidemic was the 2000 North American recommendations to delay food allergen introduction in infants until after age 3 years and avoid food allergens in maternal diet during pregnancy.⁶ These recommendations were revised in 2008, but practice did not effectively change until 2015, when it was found that proactive early and weekly exposure to peanut during infancy fosters the development of peanut tolerance at school age.⁷

Currently, there is no evidence supporting dietary restrictions in breastfeeding mothers for the prevention of childhood food allergies. A diverse balanced diet containing dairy, vegetables, fruit, and whole grains is recommended for both mother and infant to promote a healthy microbiome. Gut colonization and the diversity and intensity of microbial exposure may play a role in inducing food tolerance to dairy. Studies supporting a preventive role with early, mostly between 2 and 4 weeks of age, regular cow's milk formula ingestion, suggest the possibility of a different mechanism of sensitization to cow's milk protein than for other food allergens (**Figure 2**).⁸

Exposure to the specific maternal microbiome and outside pollutants starts in utero. The microbiome of formula-fed infants born via caesarian section is different compared to breastfed vaginally delivered infants, but the impact on future food allergy is unclear. Complete avoidance of exposure to regular cow's milk protein in the first months of life through exclusive breastfeeding, use of hydrolyzed formulas, and avoidance of regular cow's milk formula does not appear to reduce the risk of cow's milk protein allergy. Concurrently, temporary supplementation with regular cow's milk formula in breastfed infants in the first week of life may actually increase the risk of cow's milk allergy^{9,10}, while starting ingestion in the second week of life could be the most protective. Additional studies to establish optimal timing for cow's milk protein introduction are needed.

Other factors contributing to the unique pattern of bacterial exposures include housing and living

conditions, the presence of pets in the household, urbanization versus living rurally, and use of antibiotics. Later in life, microbial colonization is heavily influenced by the type, structure, and composition/diversity of the food.⁵

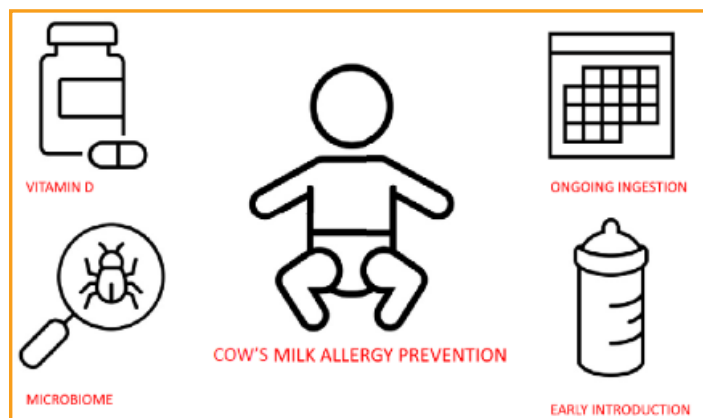


Figure 2. Possible factors involved in the pathogenesis of cow's milk allergy (from Abrams MA, Sicherer SH. 2021)⁸

These multiple contributing factors have been summarized as the “5Ds” and integrate three hypotheses of how food allergy develops: dual allergen exposure hypothesis (**d**ry skin and **d**iet, where skin exposure to food allergen precedes allergenic food ingestion), hygiene hypothesis (lack of exposure to **d**og, **d**ribble) and vitamin D hypothesis (both too low and too high^{11,12} levels of vitamin **D** appear to be unfavorable)(**Figure 3**).⁵

The dual allergen exposure hypothesis suggests that non-ingestion exposures to allergenic foods via the skin, especially on inflamed skin of allergy-prone infants with early-life atopic dermatitis, combined with a lack of early oral exposure, can result in early allergic food sensitization.¹³ Mounting evidence supporting this has resulted in recent changes to clinical practice and public health policy for food allergy prevention.

While beneficial for preventing eczema, starting infant

skin moisturization right after birth does not on its own prevent food allergy. Ongoing trials of exposure to allergenic solids and vitamin D supplementation are anticipated to inform further preventative strategies.

QUALITY OF LIFE IN CHILDREN WITH FOOD ALLERGY/ANAPHYLAXIS

Food allergy is not a lifestyle choice and should be viewed as a public health concern.¹⁴

There is no definite curative treatment for food allergy. The risk of near-fatal or fatal anaphylaxis is unpredictable and difficult to study, as it usually occurs outside the home or hospital environment. Uncontrolled asthma and delayed adrenaline injection are associated with fatal outcomes, but timely adrenaline alone may be insufficient at times, possibly due to insufficient dose/needle length or limited administration technique. This uncertainty about accurate prediction of future severe reactions and overall prognostic outcome substantially impairs the quality of life for young patients with food allergies¹⁵ and for their families, comparable to children/families living with type-1-diabetes.

Food allergies bring a complex pattern of requirements and emotions throughout a person's life, with early and strict eating rules, diligent reading of food labels and avoidance of triggering food(s). As the most common food allergens are often staple foods and highly prevalent in the Western diet (including trace amounts in packaged foods labelled as “May contain allergenic foods”), patients and families, but also daycares and schools, must remain vigilant. This may lead to maladaptive coping strategies such as maximisation (extreme levels of food avoidance, and hypervigilance) or minimisation behaviours (denial, risk taking behaviour) in patients and their families.¹⁶

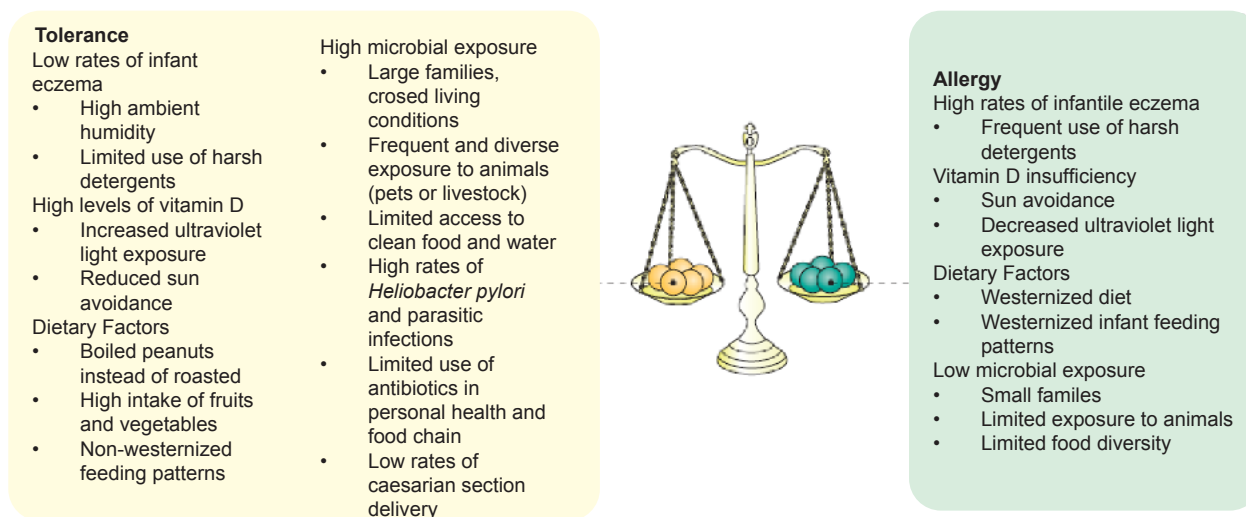


Figure 3. Integrated hypotheses of food allergy (from Renz et al. 2018)⁵

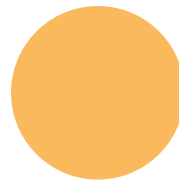
Living with uncertainty, strict food-related rules, and a feeling of being different from peers, together with an ever-present risk of accidental exposures, can lead to constant stress, fear of reactions, embarrassment, relational difficulties, anxiety and depression.¹⁷ Since 2020, the coronavirus pandemic has added an additional negative layer to the quality of life, with increasing difficulty accessing “safe” foods and food allergy-related health services.¹⁸

Children with food allergies and their families are in constant pursuit of normalcy and control over personal safety. Given this, educating new parents about food allergy prevention measures, such as infant skin care and early nutrition, by family physicians is equally important as an accurate diagnosis by an allergist to avoid unnecessary food restrictions, nutritional deprivation, and anxiety.

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THE USE OF BIOLOGICS IN FOOD THERAPY

INTRODUCTION

In the last decade, the advent of biologic medications has transformed the practice of allergy, allowing clinicians to address unmet needs in the treatment of asthma, chronic spontaneous urticaria, atopic dermatitis and nasal polyposis.^{1,2} Emerging and novel therapeutic agents in food allergy have however been slower to develop, with no biologic currently approved for this indication.

One factor has been that the low direct cost associated with food allergy created a poor incentive for pharmaceutical investment in research and development. However, the recent availability of health economic tools to quantify intangible costs and the recognition of oral immunotherapy as a valid treatment alternative has helped better define the unmet need.^{3,4} This may partly explain the renewed interest in developing medications for food allergy, with ongoing trials at various stages.

In practice, clinicians are confronted with severe and/or complex cases of food allergy that could potentially benefit from treatment with biologic therapy, but there are no published studies demonstrating their proper use in these clinical scenarios and patient populations. There are various review articles available summarizing the evidence from the literature.^{2,5} The objective of this article is to focus on practical knowledge regarding the off-label use of biologics in food allergies.

ANTI-IgE MONOTHERAPY TALIZUMAB

The first biologic used for the treatment of food allergy was talizumab. This anti-IgE monoclonal antibody, which is highly similar to omalizumab, was studied in the context of peanut allergy and was shown to significantly increase the patient's reactivity threshold in a dose-dependent manner.⁶ The development of talizumab was abandoned after Tanox was acquired by Genentech, which produced omalizumab.

OMALIZUMAB

Following the success of talizumab, trials were conducted on peanut allergy using omalizumab.⁷ A phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial was conducted to assess the efficacy of omalizumab in reducing the risk of peanut-induced allergic reactions. The study was designed to compare changes in peanut tolerability thresholds in subjects with proven peanut allergy who were treated with either omalizumab or placebo. Although the study intended to randomize 150 subjects, it was stopped early on the recommendation of the Data Safety Monitoring Committee because of the severity of 2 anaphylactic reactions that occurred during the qualifying oral food challenges (OFCs) before the administration of the study drug. Consequently, only 14 subjects reached the study's primary endpoint before the discontinuation of the trial. Despite these small numbers, some interesting trends were observed including the 80-fold increase in reactivity threshold for patients receiving omalizumab compared to patients in the placebo group (**Table 1**).

Placebo			Omalizumab				
Peanut Flour (mg)			Peanut flour (mg)			Free total IgE (kU/L)	
Subject Identification no.	Wk 1.	Wk 24.	Subject Identification no.	Wk 1	Wk 24	Wk 2	OFC 3
1107	15	50	1002	15	500	22	1.38
1419	15	50	1101	15	500	253	4.3
1502	50	50	1106	100	1500	40	2.44
1601	100	1000	1202	15	8000	406	3.04
1702	5	50	1407	50	500	216	11.35
Times increase from baseline: 4.07			1410	50	50	188	7.72**
			1501	100	1000	97	15.9
			1503	15	250	308	4.24
			1506	<5	1000	243	4.16
Times increase from baseline: 80.9							
*Value at week 20.							

Table 1. Change in peanut dose tolerability, per-protocol analysis, and change in free IgE in omalizumab-treated group ; adapted from Sampson et al, 2011

LIGELIZUMAB

Ligelizumab is a new anti-IgE monoclonal antibody that has demonstrated higher potency compared with omalizumab at suppressing skin prick tests in early asthma studies.⁸ While the molecule didn't show clinical superiority in asthma, it has shown efficacy in chronic spontaneous urticaria and it is now being studied in clinical trials as a monotherapy in food allergy.⁹

Currently, omalizumab is the only anti-IgE biologic that is Health Canada approved for chronic spontaneous urticaria, asthma and nasal polyps. It can be used off-label in food allergy to increase a patient's reactivity threshold and reduce the risk of accidental reactions. However, this approach is rarely used because of the associated cost, which is hard to justify given the total direct and indirect healthcare costs associated with treatment. However, there have been instances when public payers have provided reimbursement on compassionate grounds in patients with recurrent episodes of severe food allergic reactions despite appropriate precautions.

With monotherapy, treatment duration is indefinite, which can be costly. The usual dosing strategy has been to follow asthma dosing regimens based on total IgE. This approach was recently shown to be inadequate in food allergy, where omalizumab dosages should be adjusted for body weight alone, independent of total IgE levels.¹⁰ Therefore, rather than using the asthma dosing table, one approach could be to aim for a dose of 12mg/kg, which was the average dosage used in that cohort. Given the cost of the medication, the aim should be to start with the lowest effective dose, knowing that higher dosages will increase reactivity threshold in a linear fashion.

Oral food challenges in the clinic can be useful to help determine dosing. The use of omalizumab in food challenges reveals a dual mechanism of action. The main mechanism of action is that the molecule disarms mast cells, both by directly displacing IgE from its receptor and

by preventing free IgE from binding to it.¹¹ The expected effect of this is a dose-dependent increase in reactivity threshold. The second mechanism is that the molecule creates food specific IgE-IgG complexes that can neutralize the allergen upon entry into the circulation, similar to IgG4.¹² This offers a protection against systemic reactions but not against local reactions. Contrary to the first mechanism, IgE-IgG complexes are formed at relatively low dosages.¹⁰ In practice, low dosages tend to provide a significant increase in the reactivity threshold, but the symptoms are often localized to the oral and gastro-intestinal tract. If challenge is pursued despite local symptoms, the neutralizing capacity of IgE-IgG complexes appears to saturate and systemic reactions eventually occur.

ANTI-IgE TO SUPPORT ORAL IMMUNOTHERAPY

A more common use of omalizumab in food therapy has been to use it to enable otherwise difficult oral immunotherapy treatments. This approach was first described by researchers in 2010 and has since been extended to other foods.¹³⁻¹⁹ The main advantage of this approach is that omalizumab is used for a limited duration, and therefore is potentially more cost-effective.

Protocols involving omalizumab-enabled oral immunotherapy generally include a pre-treatment phase of two to three months, which is the time required to reach a plateau effect for reactivity threshold reduction. The medication is typically continued during the oral immunotherapy up-dosing phase and discontinued when the patient reaches maintenance.

When combined with a standard "slow" oral immunotherapy-to-milk algorithm, this approach has been shown to decrease dosing reactions by half and markedly reduce the incidence of severe reactions.²⁰ However, this involves prolonged use of the medication, which may not be affordable. When combined with an accelerated oral immunotherapy protocol, a short treatment with

omalizumab has been shown to allow patients to reach maintenance doses in a few weeks, making it much more cost-effective.^{21,22}

Clinical trials are ongoing to further establish the potential for omalizumab in oral food therapy and to help elucidate the optimal dosage.

PATIENT SELECTION AND DOSAGE

The Canadian guidelines on oral immunotherapy suggest that omalizumab may be warranted for complex cases of oral immunotherapy.²³ However, the guidelines do not offer a firm definition of what constitutes a complex case.

In practice, omalizumab will usually be considered as an adjunct to oral immunotherapy in the following situations:

- ✓ Previous failure of regular oral immunotherapy
- ✓ Patients desensitized to multiple foods simultaneously
- ✓ Patients with low baseline reactivity thresholds or very high IgE levels
- ✓ Patients with a history of severe reactions
- ✓ Patients in rural areas where reducing the number of up-dosing visits can offset the cost of medication
- ✓ Patients willing to pay out-of-pocket despite their case not being “challenging”

OMALIZUMAB DISCONTINUATION ONCE ON MAINTENANCE

About 40% of patients experience dosing reactions approximately 6 to 8 weeks following the discontinuation of omalizumab.¹⁰ Due to ineluctable rise in free IgE, it is important that patients keep dosing regularly to prevent rapid loss of protection when this happens. One strategy is to pre-medicate with anti-histamines, proton-pump inhibitors or disodium cromoglycate during this transition period. Another, potentially more effective approach has been to take the full allergen twice a day during this period, but it is often difficult for active patients to avoid co-factors twice every day.

The risk of reaction or OIT failure upon discontinuation of omalizumab appears significantly higher in patients with a high specific-to-total IgE ratio.¹⁰ For these patients, one option can be to wean omalizumab progressively. Importantly, the specific-to-total IgE ratio should be considered when OIT is an option and potentially treated as a relative contra-indication.

Clinicians should be extra cautious when treating patients with asthma who may have discontinued controller medication during omalizumab treatment due to improved response with omalizumab. If controller medication is not re-initiated these patients can be at a risk of a severe asthma attack when omalizumab is eventually discontinued.

LOW-DOSE OMALIZUMAB TO PREVENT CO-FACTOR INDUCED ANAPHYLAXIS

Co-factor induced anaphylaxis is a frequent cause of systemic reactions during oral immunotherapy. These reactions usually become less frequent after the first year of therapy, likely owing to the development of neutralizing IgG4 antibodies. However, some patients may experience anaphylaxis on their maintenance dose with minor co-factors. An effective approach has been to administer a low dose of omalizumab (150 mg or 75 mg every 4 weeks) to gain protection from food-specific IgE-IgG complexes without incurring the cost of a full dose. Currently, this strategy is limited by the poor access to allergen-specific IgG4 outside the research setting.

DUPILUMAB MONOTHERAPY

Dupilumab is monoclonal antibody that blocks the IL-4 and IL-13 signaling pathways, two of the sources of Type 2 inflammation.

Because specific IgE decreases by up to 70% in patients receiving dupilumab for the treatment of atopic dermatitis²⁴, it has been suggested that it could potentially help improve IgE-mediated allergy in a similar fashion to omalizumab. However, in practice, patients receiving dupilumab for atopic dermatitis do not appear to significantly increase their tolerance threshold to their food. One explanation could be that this effect is canceled out by a proportional decrease in total IgE and loss of the associated protection.

On the other hand, dupilumab appears highly effective at suppressing cellular-mediated food allergy. Phase 2 and preliminary phase 3 trial results are promising for eosinophilic oesophagitis, which is likely the next indication for this biologic medication.²⁵

In practice, dupilumab has been used successfully for the off-label treatment of patients with primary severe eosinophilic gastrointestinal disease, where omalizumab has not demonstrated efficacy. Reimbursement for this indication is often difficult but is generally approved in pediatric patients when the impact on growth and development is demonstrated.

The following criteria have been used to justify the off-label use of dupilumab in patients with primary eosinophilic gastrointestinal disease:

- ✓ Growth and developmental delay
- ✓ Delayed puberty
- ✓ Low bone density
- ✓ Hypoalbuminemia
- ✓ Vitamin deficiencies
- ✓ Active inflammation on biopsies despite previous treatments
- ✓ Disabling symptoms despite other treatments
- ✓ Impact on quality of life

The dosage for patients with primary eosinophilic gastrointestinal disease is the same dosage as is used for asthma or atopic dermatitis. Symptoms generally improve progressively and usually quite dramatically over the initial months following initiation of treatment. Treatment response can be measured by following the various parameters, including albumin, bone density and endoscopy. Clinically, parents will usually report an improvement in appetite and energy, a growth spurt and the successful reintroduction of multiple foods that had been previously restricted from the diet. The optimal duration of therapy is unknown, but therapy should ideally be maintained until puberty is completed.

DUPILUMAB AND ORAL IMMUNOTHERAPY

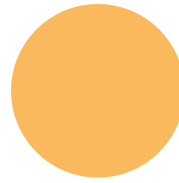
In patients with IgE-mediated food allergy, oral immunotherapy will sometimes uncover an unknown cellular-mediated allergy to the food that had been avoided to date. In patients desensitized for multiple foods simultaneously, atopy patch testing can be helpful to identify the culprit.²⁶ Omalizumab is not effective in preventing these symptoms. In fact, patients with severe eosinophilic gastrointestinal disease in the course of omalizumab-enabled OIT have successfully transitioned to dupilumab, allowing them to reintroduce the culprit allergen.¹⁰ However, dupilumab therapy must be continued over the long term in order to maintain tolerance. It is possible that cellular tolerance may develop over many years, but it is rare for this to occur in the short term.

In addition to omalizumab, ligelizumab and dupilumab, there has been positive proof-of-concept trial results with etokimab, an anti-IL-33 monoclonal antibody²⁷, and there is an ongoing trial with abatacept, a CTLA-4 fusion protein (NCT04872218). In the next decade, new indications for food allergy will likely be established for some of these, and potentially other, biologic drugs, which could transform the way clinicians currently manage food allergy. In the meantime, the use of biologics in food allergy should be reserved for challenging cases, in which they have the potential to greatly improve patient health and quality of life.

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HYPEREOSINOPHILIA: A GUIDELINE FOR WORKUP IN THE ALLERGY COMMUNITY

INTRODUCTION

Determining the etiology of eosinophilia, defined as an absolute eosinophil count (AEC) greater than 500 cells/ μ L, is often difficult given the many potential causes spanning several medical specialties. The diagnosis becomes more urgent in the case of hypereosinophilia ($\geq 1,500$ cells/ μ L)¹ due to concerns over potential end organ damage. The goal of this review is to educate physicians about the biology, etiologies, and workup of hypereosinophilia.

BIOLOGY

Eosinophils represent up to 6% of total bone marrow nucleated cells where cytokine-mediated growth and maturation is regulated by interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-5². IL-5 is essential for eosinophil survival and mobilization from the marrow. Mature eosinophils are recruited to tissues where they mediate numerous immune responses through cytokine interactions with other immune response cells, including mast cells and T-lymphocytes².

Eosinophils also regulate immune responses and direct systemic reactions via intracellular primary and secondary granules. Primary granules contain a protein that forms Charcot Leyden crystals, regulate T-cells, and have lysophospholipase activity. Specific granules contain proteins, such as major basic protein, that are directly cytotoxic towards cells and regulate mast cell degranulation. These proteins can damage host tissue through infiltration, fibrosis, thrombosis, and allergic inflammation³. The most commonly damaged organ systems include gastrointestinal (eosinophilic gastrointestinal disorders), pulmonary (eosinophilic asthma/pneumonia), upper airways/sinuses (eosinophilic chronic rhinosinusitis), cardiac, and neurologic (hypereosinophilic syndromes) tissues³.

CAUSES OF EOSINOPHILIA

Most cases of eosinophilia are benign^{4,5} and can vary based on age or geography. In pediatric patients, secondary hypereosinophilia ($\geq 1,500$ cells/ μ L) from atopic dermatitis, graft-vs-host disease, sickle cell disease, and parasitic infections are most common⁶. In adults with mild eosinophilia (1,000 cells/ μ L), common causes include bacterial infections, asthma, chronic lymphocytic leukemia (CLL), and multiple myeloma (MM)⁷. Researchers have demonstrated that 86% of outpatient hematology patients presenting with eosinophilia had an allergic etiology including eosinophilic chronic rhinosinusitis (ECRS), eosinophilic granulomatosis and polyangiitis (EGPA), and severe asthma^{5,8,9}. Beyond age differences, geographic location may also provide insight into the common causes of eosinophilia. Patients from tropical areas are at increased risk of developing eosinophilia through infection, highlighting the importance of obtaining a thorough travel history¹⁰. Common infectious causes for reactive

eosinophilia include helminths (e.g., strongyloidiasis, trichinellosis, schistosomiasis), parasites (e.g., scabies), protozoans (e.g., isosporiasis), fungi (e.g., coccidiomycosis), and viruses (e.g., human immunodeficiency virus)¹¹.

WORKUP FOR EOSINOPHILIA

A vital step in the diagnosis, treatment, and management of hypereosinophilia involves determining the urgency of the patient's presenting symptoms. Hypereosinophilia (cell counts exceeding 50,000 cells/ μ L) often presents as severe illness requiring emergent hospitalization to expedite treatment¹⁰. Symptomatic but clinically stable patients warrant further investigation within days¹⁰. Asymptomatic patients or incidental eosinophilia requires follow-up depending on the AEC. Eosinophilia greater than 1,500 cells/ μ L, regardless of symptoms, should be reassessed within two weeks to ensure that the AEC is not increasing^{10,12} (Figure 1). A thorough drug, travel, allergy, and medical history along with specific physical examination of all major organ systems is essential¹⁰.

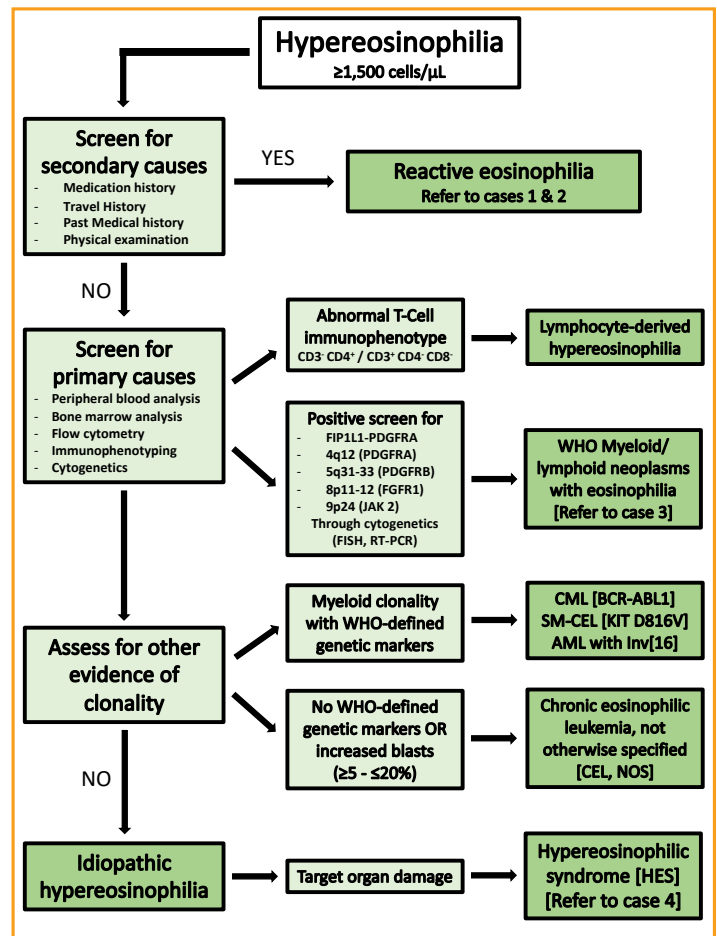


Figure 1.: FIP1L1 = Factor interacting with PAPOLA and CPSF1; PDGFRA = Platelet-derived growth factor receptor alpha; PDGFRB = Platelet-derived growth factor receptor beta; FGFR1 = Fibroblast growth factor receptor 1; JAK 2 = Janus kinase 2; FISH = Fluorescence in situ hybridization; RT-PCR = Reverse-transcription polychain reaction; CML = Chronic myeloid leukemia; SM-CEL = Systemic mastocytosis chronic eosinophilic leukemia; AML = Acute myeloid leukemia; courtesy of O'Connell and Sussman, 2022

Non-specialized tests such as complete blood count and differential, erythrocyte sedimentation rate, c-reactive protein, and proteinuria can provide diagnostic information to rule out benign causes of eosinophilia⁴. Specialized tests to assess for allergic causes include measuring IgE levels, specific antibodies to allergens (e.g., *Aspergillus*), or assessing for parasitic causes through serology and/or stool microscopy (i.e., antibodies or eggs)⁴. If there is no clear secondary cause for eosinophilia, referral to a specialist(s) should be considered (Figure 1).

CASES

Case 1: Infectious case of eosinophilia

A 48-year-old male (originally from Somalia; sheep farmer) presented with a cough, generalized abdominal pain, and fever (40.1°C) after returning from Somalia 9-months earlier. Initial imaging demonstrated bilateral lower lobe infiltrates, and CT imaging detected ill-defined hypodense liver lesions. The patient had elevated eosinophils (8,200 cells/ μ L) and normal transaminases. A liver biopsy identified necrotic areas and eosinophilic infiltrates suspicious for echinococcal abscess. Infectious disease experts felt further testing was warranted. Serological testing for *Strongyloides stercoralis*, *Entamoeba histolytica*, *Toxocara* species, and *Schistosoma* species were negative. Examination of a stool specimen detected *Blastocystis hominis* and *Fasciola hepatica* eggs. The patient was diagnosed with fascioliasis secondary to a liver fluke and was treated with triclabendazole; the liver lesions and eosinophilia resolved.

Comment: This case underscores the importance of a patient's travel history and the coordination of care with infectious disease experts. The original pathology suggested echinococcal disease whereas the consultant physician felt additional lab tests were needed, leading to the correct diagnosis and treatment. Clinicians should always consider parasitic and infectious causes of eosinophilia prior to initiating treatment. Glucocorticoids are appropriate first line treatment for reactive eosinophilia. If strongyloidiasis is suspected, concomitant steroid and ivermectin treatment is strongly recommended as steroids alone can lead to hyper-infection¹³.

Case 2: Eosinophilic asthma resistant to chronic oral glucocorticoids

A 40-year-old man was diagnosed with eosinophilic asthma and was steroid-dependent. Treatment with mepolizumab during a clinical trial resulted in an excellent response, however at the end of the clinical trial, further anti-IL-5 antibody treatment could not be accessed for the patient. As a result, the patient's worsening asthma symptoms required resumption of oral prednisone (20 mg daily). The patient was subsequently enrolled in another clinical trial evaluating benralizumab, a humanized monoclonal antibody targeting the alpha subunit of the IL-5 receptor. The patient responded to benralizumab treatment and was able to reduce his prednisone dose to 2.5 mg daily.

Comment: A small subset of asthma patients have eosinophilic asthma. The pivotal role of IL-5 in eosinophil differentiation, survival, and migration makes this cytokine an ideal target for treatment. This treatment involving the targeting of the IL-5 pathway significantly reduces exacerbations of asthma, as well as blood and sputum eosinophils, even under reduced steroid usage^{14,15}. Research has shown that mepolizumab is effective in reducing steroid dosing in patients with eosinophilic asthma.⁵

Case 3: Eosinophilia presenting as a clonal myeloproliferative disorder

A 58-year-old previously healthy male presented with drenching night sweats, headaches, and fatigue of two weeks duration. His AEC was markedly elevated (45,600 cells/ μ L) with hemoglobin and platelet counts mildly decreased. Lab tests revealed normal serum creatinine, transaminases, and total bilirubin counts, while troponin was elevated to 9995 ng/L. An echocardiogram showed multiple wall motion abnormalities and an MRI of the brain detected multiple areas of bilateral subacute ischemia in watershed distribution. Nerve conduction studies confirmed axonal motor polyneuropathy. Treatments with oral steroids and hydroxyurea were initiated. A bone marrow exam demonstrated increased eosinophilic precursors and the blast count was <5% (Figure 2). Cytogenetics confirmed a male karyotype and interphase fluorescence *in situ* hybridisation (FISH) testing confirmed a platelet-derived growth factor receptor alpha (PDGFRA) gene rearrangement. The patient was treated with 100 mg/day of imatinib; the eosinophilia resolved within 6 weeks.

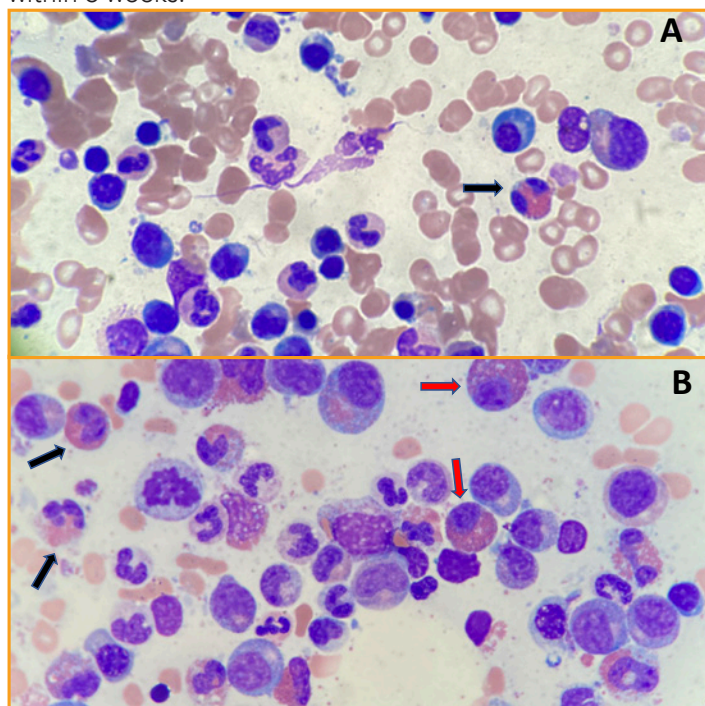


Figure 2. A. Normal bone marrow aspirate. A single mature eosinophil (dark arrow) noted surrounded by a plasma cell and maturing granulocytes. Wright-Giemsa stain, 1000X. B. Bone marrow aspirate from case 3 (Platelet-derived growth factor receptor alpha positive myeloproliferative disorder). Granulocytic hyperplasia and increased mature eosinophils (dark arrows) and eosinophil precursors (red arrows) present. Wright-Giemsa stain, 1000X.

Comment: This presentation underscores how target organ damage can rapidly occur in extreme eosinophilia. This patient presented with a tyrosine kinase inhibitor (TKI)-responsive myeloproliferative disorder (MPD) resulting from an interstitial deletion on chromosome 4 that resulted in the expression of the FIP1L1-PDGFR α fusion oncoprotein. The constitutively active PDGFR α is sensitive to TKI's at much lower doses than treating chronic myeloid leukemia. The new category designated by the World Health Organization of "myeloid and lymphoid neoplasms with eosinophilia and abnormalities of platelet-derived growth factor α (PDGFR α), platelet-derived growth factor β (PDGFR β), fibroblast growth factor receptor 1 (FGFR1) or PCML1-JAK2" describes a number of diseases characterized by recurrent genetic translocation that result in constitutively activated tyrosine kinases and eosinophilia. Rearrangements involving PDGFR α and PDGFR β are considered to be sensitive to TKI's^{9,16-21}.

Case 4: Eosinophilia presenting as a hypereosinophilic syndrome (HES)

A 23-year-old woman was diagnosed with a HES after presenting with wheezing, cough, chest pain, diarrhea, elevated troponins with an associated ST-elevation, and eosinophilia (5,100cells/ μ L). There were no precipitating causes²². Diagnostic criteria for EGPA were not met²³. A bone marrow exam did not suggest a MPD, and both molecular and genetic testing did not confirm clonality. Flow cytometry was unable to detect CD3⁺, CD4⁺ lymphocytes. Other investigations confirmed eosinophilic bronchitis; colonoscopy confirmed eosinophilic infiltration, and cardiac MRI confirmed endocardial apical thickening and possible subendocardial involvement, suspicious for HES. Sinusitis was noted and nasal polyps were removed that were confirmed by histological exam to be infiltrated by eosinophils without vasculitis. Treatment with hydroxyurea and imatinib were discontinued due to intolerance. Mepolizumab was initiated and symptoms were controlled.

Comment: This case demonstrates the complexity of patients who present with eosinophilia and the need for care coordination among numerous specialties in order to establish a diagnosis. The patient had a number of diagnostic criteria for EGPA (sinusitis, pulmonary infiltrates, eosinophilia) but no evidence of vasculitis on biopsy and minimal evidence to support a diagnosis of asthma. Lymphoid-variant HES and myeloid causes were excluded. Infectious causes were excluded. Eosinophilic chronic rhinosinusitis (ECRS) is associated with the formation of nasal polyps, elevated IgE levels and eosinophilia through Th2-mediated mechanisms but not associated with tissue damage outside the upper airways⁸. A diagnosis of HES was made via persistent eosinophilia and target organ damage. The patient's response to mepolizumab is consistent with the published literature²⁴.

CONCLUSIONS

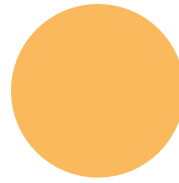
Eosinophilia can be a manifestation of an underlying complex disease. The history, medication profile, travel history and physical exam are important first steps in establishing diagnoses. Patients often require referral to

specialists for further evaluation. In our center, we have established an interdisciplinary clinic to evaluate complex patients, allowing us to gain insight into the biology of these diseases. As an example, we have recently identified a group of patients presenting with eosinophilia and similar lung manifestations after noxious exposures where the patients also harbor identical exon 8 mutations in the KIT gene²⁵. By working together with a multidisciplinary team to better understand the underlying etiology and complex biology involved with eosinophilia, we hope our patients will benefit and achieve optimal outcomes in disease management.

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ERRORS OF IMMUNITY FOR THE COMMUNITY ALLERGIST: IDENTIFICATION AND INVESTIGATION

Clinical vignette

An 18-year-old male presented with eczema at the age of 6 months. During infancy he also developed multiple food allergies, and progressed with asthma, and allergic rhinitis over time. He had significant dermatitis, frequent acute otitis media, and upper/lower respiratory tract infections often requiring antibiotics. At 3 years of age, he had an Influenza A related pericardial effusion. He was noted to have eosinophilia of $12.5 \times 10^9/L$ (30%) and elevated IgE (4320 ug/L).

At 4 years of age, he was admitted with a left lobar pneumonia, pansinusitis and was positive for adenovirus. IgE was elevated (9600 ug/L) and he was noted to have persistent eosinophilia $26.9 \times 10^9/L$ (60%), poor specific antibody response to vaccines, normal levels of IgG, IgA and IgM and normal lymphocyte subsets but with decreased T-cell proliferation. He was diagnosed with combined immunodeficiency and treated with intravenous immunoglobulin (IVIg) replacement and antibiotic prophylaxis.

Over the next few years, he developed eosinophilic dermatitis, esophagitis and pneumonitis, severe asthma, recurrent otitis media and sinusitis, and generalized flat warts. He had dysgammaglobulinemia with low IgA and IgM and high IgE levels (up to 25000 ug/L). Profound lymphopenia developed with very low T-cells; normal B-cell counts but poor antibody responses.

He was ultimately diagnosed with hyper-IgE syndrome secondary to DOCK8 deficiency. While waiting for a matched donor for hematopoietic stem cell transplant (HSCT), he developed squamous cell carcinoma of the skin and died at the age of 21 years. This case highlights how the presentation with common allergic conditions may be early signs of important inborn errors of immunity.

DEFINITION

Human inborn errors of immunity (IEI), referred to as primary immunodeficiency disorders (PID), are a heterogeneous group of disorders, characterized by an increased susceptibility to infection, autoimmune, autoinflammatory, allergic and/or malignant diseases¹. To date, more than 400 disorders have been genetically identified². Most identified IEI are monogenic variants which result in loss of expression, loss-of-function (LOF; amorphic/hypomorphic), or gain-of-function (GOF; hypermorphic) of the encoded protein^{3,4}.

PREVALENCE

IEI are common. There is an estimated prevalence of 1 in 1000 to 1 in 5000 live births⁷ as illustrated in **Figure 1**⁹, with the exception of IgA deficiency (prevalence of 1 in 500 in Caucasians⁸). These conditions may present at any age, although children between the ages of 5 to 19 have the highest prevalence rate (**Figure 2**^{10,11}). There is a 1:1 ratio in the gender disposition of IEI per a recent study in the US.

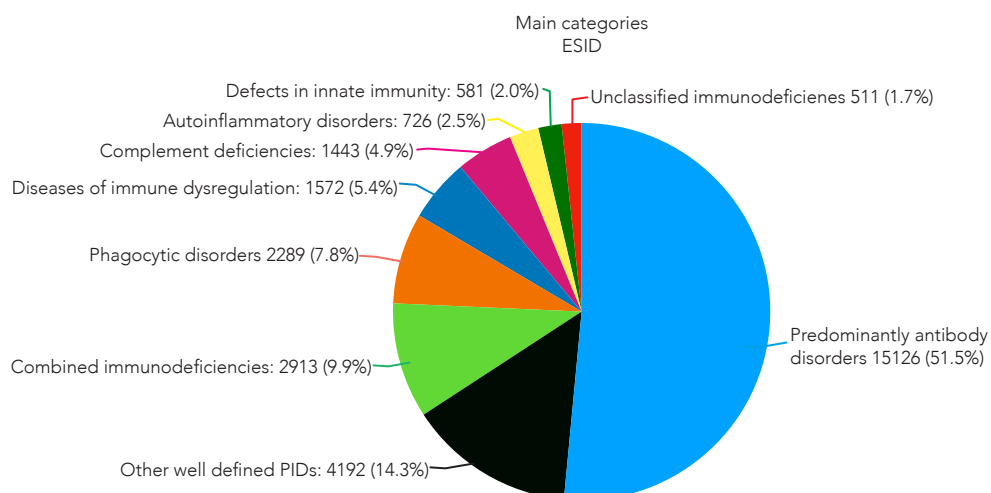


Figure 1. Distribution of major PID groups; Adapted from the Manual of Allergy and Clinical Immunology, 2021

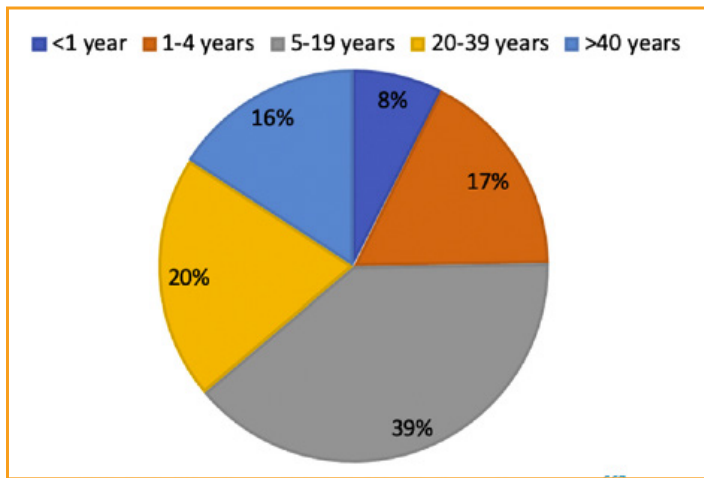


Figure 2. Prevalence of PID by age groups; Adapted from Stiehm's Immune Deficiencies Inborn Errors of Immunity, 2020

CLINICAL APPROACH TO IEI

The immunologic variations of IEI is increasingly complex and a systematic approach for patients with suspected IEI, as described in the questionnaire below¹⁷ is a useful tool for the clinician (**Figure 3**).

Investigations into IEI, should be initiated based on clinical presentations of recurrent infections or evidence of immune dysregulation. Unexplained lymphopenia or persistently abnormal levels of other leukocytes should also prompt investigations. Secondary causes of potential immunodeficiency, including infection, immunosuppressive therapies or malignancy, are also considerations for evaluation of immune function^{18,19}.

IEI CLASSIFICATION

There are eight categories of IEI per the Expert Committee of the International Union of Immunological Societies (IUIS) (**Table 1**^{2,23}). Recently, an IEI classification of two major categories has been proposed: Primary Immune Deficiency Disorders (PID) and Primary Immune Regulatory Disorders (PIRD)²⁴. Seventy percent of identified disorders are classified as PID²⁵, and are infection dominant conditions. PIRD are dominated by immune-mediated pathologies (autoimmunity, lymphoproliferation, autoinflammation/hyperinflammation, malignancy and severe atopy)²⁴. Defining the precise nature of the PIRD is key to directing clinical management and selecting targeted therapies^{26,27}.

CLINICAL PRESENTATION

Infections with atypical severity or pathogens, and/or increased frequency are often the first manifestations of IEI. Unusual or early onset autoimmunity, lymphoproliferation or autoinflammation¹ are also suggestive features of IEI. The main categories of IEI, as well as their clinical presentation, are described in **Table 1**^{2,23}.

IEI may mimic common childhood diseases, including eczematous dermatitis and food allergies. Atopic conditions may result from an underlying immunodeficiency or immune dysregulation²⁸. Mechanistically, the development of atopy includes skin barrier disruption, mast cell dysregulation, tolerance failure and impaired T-cell receptor signaling²⁹. The classic triad of eczema, elevated serum IgE and eosinophilia are hallmarks of conditions including atopy, hyper-IgE syndromes (HIES), Omenn syndrome and Wiskott-Aldrich syndrome³⁰. The differentiation of these conditions in patients with severe atopic dermatitis is still challenging. Findings directing investigations include increased frequency of infection, severity at presentation and comorbidities such as thrombocytopenia. A high index of suspicion is key³¹.

Clinical approach to evaluate IEI

1. Is the main concern infection or immune dysregulation?
 - If infection: Are the main infections bacterial, viral, fungal, mycobacterial, or mixed? Obtain information about sites and severity of infection, opportunistic/unusual organisms and therapies required to treat the infections.
 - If immune dysregulation: Are the features autoantibody-driven (i.e., cytopenias, colitis, arthritis), granulomatous or lymphoproliferation?
2. Are there any somatic/dysmorphic features (i.e., short stature, microcephaly, poor wound healing)?
3. Are the exposures unusual?
4. Is there any severe atopy?
5. In children, are there any developmental concerns or reactions to live vaccines?
6. Is there any cause of secondary immunodeficiency (i.e., infections, medications, disease-causing protein loss, malnutrition, hypercatabolic states, chronic illnesses, splenectomy, or malignancy)?
7. Is there a family history of PID, recurrent/severe infection, early/unexplained deaths, autoimmunity, malignancy or autoinflammation? Any history of consanguinity?
8. Are there any clues on physical exam (failure to thrive, thrush, absence of lymph nodes including tonsils, heart/lung disease, delayed separation of umbilical cord, hepatosplenomegaly, or bone/joint/skin abnormalities)?
9. Are there any laboratory studies already pointing toward a category of deficits or dysregulation?

Figure 3. Questionnaire adapted from Stiehm's Immune Deficiencies, Inborn Errors of Immunity, 2020

Classification and examples	Clinical presentation
1. Immunodeficiencies affecting cellular and humoral immunity	
T-B+ severe combined immune deficiency (SCID)	
yc deficiency (common gamma chain SCID, CD132 deficiency)	
JAK3 deficiency	Severe, recurrent opportunistic infections; failure to thrive; diarrhea; rash
T-B- SCID	
RAG 1/2 deficiency	
ADA deficiency	Bone defects, may have pulmonary alveolar proteinosis, cognitive defects
Combined immunodeficiencies (generally less profound than SCID)	
CD40 ligand deficiency	Severe and opportunistic infections, idiopathic neutropenia; hepatitis and cholangitis, Cryptosporidium infections
CD40 deficiency	Neutropenia, opportunistic infections, gastrointestinal and biliary tract and liver disease, Cryptosporidium infections
ICOS deficiency	Recurrent infections, autoimmunity, gastroenteritis, granulomas
2. Combined immunodeficiencies with associated or syndromic features	
Wiskott-Aldrich syndrome	Thrombocytopenia with bleeding and bruising; eczema; recurrent bacterial and viral infections; autoimmune disease
Ataxia telangiectasia	Chronic sinopulmonary disease; cerebellar ataxia; small, dilated blood vessels of the eyes and skin; malignancy
DiGeorge syndrome	Hypoparathyroidism; seizures; cardiac abnormalities; abnormal facies; infection
Hyper IgE syndrome	Chronic dermatitis; recurrent, severe lung infections; skin infections; bone fragility; failure to shed primary teeth
3. Predominantly antibody deficiencies	
XLA	Severe bacterial infections
CVID	Clinical phenotypes vary: recurrent infections, lymphoproliferation, autoimmune cytopenias and/or granulomatous disease
Selective IgA deficiency	Recurrent sinopulmonary infections with encapsulated bacteria
Specific antibody deficiency	Autoimmune disease and increased risk of malignancy in CVID
IgG subclass deficiency	
4. Diseases of immune dysregulation	
HLH	Fever, splenomegaly, cytopenia, rash
ALPS	Splenomegaly, adenopathy
IPEX	Autoimmune enteritis, early onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema
APECED	Autoimmunity affecting parathyroid, adrenal, other endocrine organs; candidiasis; dental enamel hypoplasia
5. Congenital defects of phagocyte number or function	
Congenital neutropenias	
Chronic granulomatous disease	Severe infection; abscesses with granuloma formation
Leukocyte adhesion deficiency	Recurrent, severe bacterial infections; poor wound healing; delayed separation of the umbilical cord
6. Defects in intrinsic and innate immunity	
Mendelian susceptibility to mycobacterial disease (MSMD)	
IL-12 and IL-23 receptor β 1 chain deficiency	Susceptibility to mycobacteria and salmonella infections
IFN γ receptor 1 deficiency	
Predisposition to severe viral infection	
STAT 1/2 deficiency	Severe viral infections (disseminated vaccine-strain measles), mycobacterial infection
Herpes simplex encephalitis (HSE)	
TLR3 deficiency	Herpes simplex virus (HSV) 1 encephalitis; severe pulmonary influenza; varicella-zoster virus infection
Predisposition to invasive fungal diseases	
CARD9 deficiency	Invasive candidiasis infection, deep dermatophytosis, other invasive fungal infections
Predisposition to mucocutaneous candidiasis	
STAT1 GOF	CMC, various fungal, bacterial and viral (HSV) infections, auto-immunity (thyroiditis, diabetes, cytopenias), enteropathy
TLR signaling pathway deficiency with bacterial susceptibility	
IRAK4 deficiency	Bacterial infections (pyogens)
Other inborn errors of immunity related to non-hematopoietic tissues	
Isolated congenital asplenia	Bacteremia (encapsulated bacteria)
7. Autoinflammatory disorders	
Type 1 interferonopathies	
STING-associated vasculopathy, infantile onset (SAVI)	Skin vasculopathy, inflammatory lung disease, systemic autoinflammation
Aicardi-Goutieres syndrome	
Defects affecting the inflammasome	
Familial Mediterranean fever (FMF)	Recurrent fever, serositis and inflammation responsive to colchicine
Non-inflammasome-related conditions	
TNF receptor-associated periodic syndrome (TRAPS)	Recurrent fever, serositis, rash, and ocular or joint inflammation
8. Complement deficiencies	
Deficiency in early complement pathway components (C1q, C1r, C2, C4)	SLE-like syndrome, rheumatoid disease, multiple autoimmune diseases, infections
Deficiency in late complement pathway components (C5, C6, C7, C8, C9)	Neisserial infections, SLE-like syndrome
C3 and regulatory components	Recurrent infections with encapsulated bacteria

JAK3 Janus kinase 3, RAG recombination activating gene, ADA adenosine deaminase, ICOS inducible costimulator gene, XLA X-linked agammaglobulinemia, CVID common variable immunodeficiency, HLH hemophagocytic lymphohistiocytosis, ALPS autoimmune lymphoproliferative syndrome, IPEX immunodysregulation polyendocrinopathy enteropathy X-linked, APECED autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy, IL interleukin, IFN interferon-, STAT signal transducer and activator of transcription, TLR Toll-like receptor, CARD9 caspase recruitment domain family 9, GOF gain of function, IRAK4 interleukin 1 receptor associated kinase 4, STING stimulator of interferon genes, CMC chronic mucocutaneous candidiasis, SLE systemic lupus erythematosus

Table 1. Simplify classification of IEL; adapted from the International Union of Immunological Societies: 2019 Expert Committee on IEL

Clinical vignette

The clinical case of early onset of severe atopy with frequent sinopulmonary infections, unusual severe viral illnesses, eosinophilia, and high serum IgE are key elements suggestive of a combined immunodeficiency, particularly hyper-IgE syndromes. HIES is a multi-systemic syndrome characterized by recurrent skin abscesses, pneumonia with pneumatocele formation, eczematous dermatitis, and elevated IgE levels. However, autosomal dominant and autosomal recessive forms of the disease differ significantly in their clinical features, as shown in **Table 2**.

Disease	Genetic Defect	Inheritance	Main Features	Distinguishing features from common allergic disorders
AD-HIES STAT3 deficiency (Job syndrome)	STAT3	AD LOF	Eczema, skin abscesses, CMC, recurrent pneumonias leading to pneumatoceles, and skeletal and connective tissue abnormalities	Early-onset eczema; peculiar thickened texture of the facial skin, retroauricular fissures, and severe folliculitis of the axillae and groin; cold abscesses; distinctive facial and skeletal features, low frequency of allergy
DOCK8 deficiency	DOCK8	AR	Severe eczema, severe allergies, immunodeficiency with increased susceptibility to bacterial and viral infections, autoimmunity, and increased risk for malignancies	Severe eczema associated with warts, severe skin and sinopulmonary infections
PGM3 deficiency	PGM3	AR	Skeletal dysplasia, immunodeficiency and tendency to bone marrow failure, severe atopy, neurodevelopmental delay; some patients display renal, intestinal, and heart defects.	Complex syndromic phenotype associated with atopy
ILG signal transducer (ILGST) deficiency	IL6ST	AR or AD LOF	Largely overlapping with AD-HIES: eczema, recurrent skin and pulmonary infections, craniosynostosis, neurodevelopmental delay	Severe eczema, recurrent cutaneous and pulmonary infections, distinctive skeletal features
Comel-Netherton syndrome	SPINK5	AR	Congenital ichthyosis, bamboo hair, atopic diathesis; increased bacterial infections; enteropathy, failure to thrive	Congenital ichthyosis
TYK2 deficiency	TYK2	AR	Susceptibility to intracellular bacteria (mycobacteria, Salmonella) and viruses; dermatitis	Peculiar susceptibility to infections

Table 2. Clinical features of HIES; Adapted from Castagnoli et al. World Allergy Organization Journal (2021)

INVESTIGATING IEI

Early diagnosis of IEI is critical for prevention of disease-associated morbidity and mortality. This requires a high index of clinical suspicion and early treatment dramatically improves life expectancy and quality of life^{1,32}.

Laboratory investigations, prompted by the clinical phenotype, are described in **Table 3**. Commonly available

tests include complete blood count (CBC) and smear, which may reveal signs of lymphopenia or neutropenia. Additionally, both levels of cells and proteins, as well as their function should be evaluated. For example, a patient may have normal levels of IgG, but additional evaluation of specific antibodies post-vaccination may demonstrate a functional failure (**Table 4**).

Affected immunity arm	Typical site of infection	Common pathogens	Screening tests
B cells/ antibody	Sinopulmonary tract, GI tract, joints, CNS	Pyogenic bacteria: Streptococci, staphylococci, <i>Haemophilus influenzae</i> Enteroviruses: ECHO, polio Mycoplasma	IgG IgA IgM Vaccine responses (titers)
T cells	Sepsis, lung, GI tract, skin	Viruses: CMV, adenovirus, measles, molluscum Fungi: <i>Candida</i> , <i>Aspergillus</i> , <i>Pneumocystis jirovecii</i> Protozoa: <i>Cryptosporidium</i>	CBC with differential Flow cytometry for T cells and subsets T cell proliferation to mitogens
NK cells	Skin, lung GI tract, disseminated infections	Viruses: EBV, CMV, VZV, HSV, HPV	Flow cytometry for NK cells CD107a surface expression NK cytotoxicity assays
Phagocytes	Skin infections, lymphadenitis, liver, lung, bone, GI tract, gingivitis/ periodontitis	Bacteria: Staphylococci, <i>Serratia marcescens</i> , <i>Burkholderia cepacia</i> , <i>Klebsiella</i> , <i>E. coli</i> , <i>Salmonella</i> , <i>Proteus</i> Fungi: <i>Candida</i> , <i>Aspergillus</i> , <i>Nocardia</i>	Neutrophil count DHR Flow cytometry for CD11/CD18
Complement	Systemic infections, meningitis	Pyogenic bacteria: Streptococci, <i>Haemophilus influenzae</i> , <i>Neisseria</i>	CH50

Table 3. Laboratory testing according to the IEI clinical phenotype; courtesy of McCusker, MD and Saker, MD

EVALUATING ANTIBODY RESPONSES

Clinical manifestations such as recurrent sinopulmonary infections and those involving encapsulated bacteria should prompt an evaluation for both primary B cell defects and combined immune disorders^{34,35}. Initial screening tests should include a CBC with differential and immunoglobulin quantification such as total IgG, IgA, IgM, and IgE. There are no strict standards for pathologically low immunoglobulin levels, although a serum IgG below 3 g/L in an adolescent or adult and values below the age-matched range in children warrant further evaluation¹. Albumin levels will rule out protein loss as the underlying cause of hypogammaglobulinemia.

If serum immunoglobulins are detectable, specific antibody response to protein antigens (diphtheria and tetanus vaccines) and polysaccharide antigens (23-valent polysaccharide vaccine) and/or presence of

isohemagglutinins should be assessed. Ideally, specific antibody levels are measured pre-immunization and 3 to 4-weeks post-vaccination. Guidelines for normal responses are available to guide clinicians³⁶. There are 2 methods defining sufficient response to pneumococcal vaccination. Protection against infection and colonization is associated with antibody concentrations of 1.3 mg/mL or greater. Most patients can mount a 2-4-fold increase over baseline titers post-vaccination if the preimmunization levels are <4. For the diagnosis of immunodeficiency, the general standards of normal responses are at least a 4-fold increase or a level $\geq$ to 1.3 μ g/ml of at least 4 pneumococcal serotypes postimmunization. Vaccine response cannot be reliably evaluated in patients who have received immunoglobulin replacement therapy within the past 4 to 6 months. Other useful tests to evaluate the B cell compartment are listed in **Table 4**.

Assessment	Quantitative	Functional	Advanced Test
Humoral (B-cells)	- Ig levels: IgG, IgA, IgM and IgE - B cell immunophenotyping by flow cytometry (CD19, CD20, CD10, CD21, CD23, CD27, CD38, CD40, CD81, CD138, surface Igs, κ chain, λ chain)	- Natural antibodies (e.g., isohemagglutinins-IgM antibody to blood group A and/or B) - Specific antibody levels - Random, pre/post immunization antibody levels to protein (e.g., tetanus toxoid, diphtheria toxoid) and polysaccharide antigens (pneumococcal vaccine)	- IgG subclasses (restricted utility) - Antibody response to vaccination with a neoantigen (Phi X174, rabies, Salmonella typhi) - Class switching - In vitro Ig production (antibody secreting cell generation, ELISPOT for specific Ig production) - Mutation analysis (e.g., BTK, AID, IGDM,...)
Cellular (T-cells)	- CBC with differential - T cell immunophenotyping by flow cytometry (CD3, CD4, CD8, CD45RA/RO, TCR $\alpha\beta/\gamma\delta$)	- In vitro lymphocyte proliferation in response to mitogens (PHA, ConA, PWM, PMA+I), CD3/CD28, and antigens (including alloantigens and recall antigens). - TRECs: SCID newborn screening - T-cell receptor Vbeta repertoire (by immunophenotype or spectratyping)	- Extended T cell immunophenotyping (CD3 chains, CD62L, CD31, CCR7, CXCR5, CD40L, CD127, CD132; MHC-I, MHC-II) - In vitro cytokine production - Adenosine deaminase, purine nucleoside phosphorylase levels and % daxP accumulation - Radiosensitivity testing - Mutation analysis (e.g., IL2RG, RAG1/2, DCLRE1C,...)
Phagocytic	- CBC with differential - Morphology: Smear evaluation	- DHR flow cytometry assay (alternative NBT test) for chronic granulomatous disease - Adhesion molecule evaluation: β2 integrins (CD18, CD11a.,b,c), CD15 for leukocyte adhesion defect	- Phagocyte cell i.e. (APC, monocytes) phenotyping (CD14, CD68, CD86, HLA-DR, 7DA, IFNGR1, IL12RB1) - Chemotaxis - Bactericidal activity - STAT1/STAT4 phosphorylation in response to IFNγ/IL12 - IL-12 production in response to IFNγ - Mutation analysis (e.g., CYBB, CYBA, NCF1, NCF2, NCF4, IFNGR1, IL12RB1,...)
Complement	- C3, C4 - C1 esterase inhibitor levels	- Total hemolytic complement (CH50): Classical pathway - Alternative pathway (AH50) - MBL - C1 esterase inhibitor function (for hereditary angioedema)	- Individual complement components - C3 nephritic factor - Mutation analysis (e.g., C1QA, CFB, CFD, MASP2,...)
NK cells	- CBC and differential - NK/NKT cell immunophenotyping by flow cytometry (CD3, CD16, CD56)	- NK cytotoxic activity on K562 cells	- NK cytotoxic activity on other cells (Raji, 721.221, SKBR3) - NK ADCC - NK cytokine production (ELISPOT) - Mutation analysis (e.g., GATA2, IRF8, MCM4, GINS1,...)

Table 4. Laboratory testing to evaluate IEL; courtesy of McCusker, MD and Saker, MD

EVALUATING T CELLS

A history of prolonged viral infections, opportunistic infections, autoimmunity and failure to thrive (in the setting of an affected infant or young child) suggest a possible T cell defect. Initial evaluation includes a CBC focusing on the white blood cell count and absolute lymphocyte count as up to 75% of circulating lymphocytes are T cells.

Particularly in infants, lymphopenia may suggest a T cell developmental defect or marked T cell destruction and should prompt immediate immunological evaluation for potentially life-threatening conditions, such as a severe combined immune deficiency (SCID). Low lymphocyte count in isolation, as a screen for SCID, is not adequate as this will fail to identify infants with "leaky (hypomorphic) SCID"³⁷, who may have normal or even elevated T cell numbers yet profound deficiency in T cell function. Other causes of T cell lymphopenia, including HIV infection or mechanical loss of lymphocytes (e.g., intestinal lymphangiectasia) also should be rapidly ruled out.

NEWBORN SCREENING FOR SEVERE COMBINED IMMUNE DEFICIENCY (SCID)

The evaluation for SCID may be initiated by an abnormal newborn screening test which measures the number of copies of T cell restriction excision circles (TRECs), formed during T cell development⁴². TREC screening alone is not diagnostic for SCID, but requires immediate additional evaluation, including lymphocyte subset immunophenotyping to confirm a failure of T cell development. This is typically followed by lymphocyte proliferation, testing for maternal chimerism, and ultimately genotyping⁴³.

Importantly, TREC screening identifies classic forms of SCID characterized by <300 T cells/mm³ at birth, but it fails to capture atypical SCID due to hypomorphic mutations in known SCID genes, as shown in **Table 5**⁴⁴.

EVALUATION OF COMPLEMENT SYSTEMS

Specific clinical presentations prompt evaluation of complement defects including encapsulated bacterial infection and angioedema⁴⁴. The complement system is activated by three pathways: the classical, the alternative, and the lectin pathways, all of which converge at C3 to activate a common final pathway (the membrane attack complex). The three complement pathways should be functionally assessed.

Defects of C3 result in susceptibility to encapsulated bacterial infections, whereas defects of C5 to C9 are associated specifically with *Neisseria sp.* infections. C1, C2 or C4 complement deficiency are associated with infection and autoimmunity, such as systemic lupus erythematosus⁴⁷. Functional tests of individual components are available in specialized laboratories (**Table 4**).

EVALUATION OF PHAGOCYTES

Recurrent bacterial and/or fungal infections involving the skin and deep organs are suggestive of a neutrophil defect⁴⁴. Assessment should begin with a CBC for the absolute neutrophil count and a peripheral blood smear for cellular morphology. Several genetic IEI have been identified leading to neutropenia.

Assays of neutrophil function should also be considered. Neutrophil migration to sites of infection and pus production are compromised in leukocyte adhesion deficiency (LAD) and neutrophil killing activity is affected in chronic granulomatous disease (CGD). Rapid screening for CGD can be achieved using the dihydrorhodamine 123 (DHR) assay⁵². Other tests to evaluate the phagocyte compartment are listed in **Table 4**.

EVALUATING NATURAL KILLER (NK) CELLS

Recurrent viral infections and primary hemophagocytic lymphohistiocytosis, suggest a possible NK cell defect^{44,53}. NK cell evaluation is done in specialized laboratories (**Table 4**). Classical NK deficiency results when both the number and function of the NK cells are profoundly reduced, whereas, in functional NK deficiency, only the cytotoxic capacity is abnormal in the setting of normal NK-cell counts.

GENETIC TESTING

Access to genetic testing has become an essential and indispensable tool. Gene discovery has accelerated with significant advances including whole exome and whole genome sequencing. As a result, there are currently more than 400 immune disorders genetically identified².

The contribution of genetic testing has significant impact on patient care with a shorter time to definitive diagnosis, the identification of asymptomatic family members and better family planning decisions. Genetic testing allows for the identification of molecular defects, allows the use of targeted therapies and increases our understanding of molecular pathways crucial for immune functions.

TREC results	Causes	Absolute T-cell counts and mitogen proliferation testing
TREC critically abnormal or absent/not detectable	Typical SCID or athymic conditions	Absence of or very low CD3 T-cell number ($<300/\mu\text{L}$) Low to absent naive T cells PHA proliferation $<10\%$ lower limit of normal
TREC abnormal or low	Atypical SCID	Low CD3 T-cell number ($300-1500/\mu\text{L}$) Low to absent naive T cells PHA proliferation $10\%-50\%$ lower limit of normal
TREC abnormal or low	Once SCID is ruled out, consider other conditions: • Preterm birth • Syndromes associated with T-cell lymphopenia • Secondary T-cell lymphopenia • Idiopathic T-cell lymphopenia	Reduced CD3 T-cell number

NBS, Newborn screen; TREC, T-cell receptor excision circle.

Table 5. Newborn screening for SCID: Test results and possible diagnosis; Adapted from Knight V. et al., 2021

Despite these benefits, the emergence of broad-based sequencing approaches, has also introduced new challenges. The vast amount of genetic information obtained through sequencing, particularly in the form of variants of unknown significance, is problematic due to the need for functional assessments, not always readily available, and the need of genetic counselling.

CONCLUSIONS

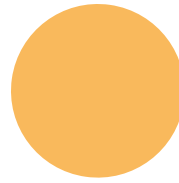
Investigation of immune function is essential for accurate diagnoses in patients with recurrent and/or unusual infections as well as those with features of immune dysregulation. Many new diagnostic tools have been added to our medical armamentarium in recent years yet the diagnosis of IEI still relies on the combination of clinical acumen to identify patients at risk, leading to appropriate laboratory and genetic tests. The early evaluation of immune function provides not only critical diagnostic information, but also guides clinical decisions regarding appropriate therapies and prevention of disease-associated morbidity and mortality.

As illustrated in this article and by the clinical vignette, infection may not be the significant presenting feature for IEI. Patients for whom there are clinical suspicions for IEI should be evaluated with screening tests followed by directed protein/cellular and genetic testing. As this remains an evolving field, patients may need to be re-evaluated as our understanding progresses.

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Dr. Sussman recognized and reported some of the first cases of latex allergy, which eventually lead to widespread declines in latex allergy





AN OVERVIEW OF CANNABIS ALLERGY

INTRODUCTION

Cannabis refers to a genus of annual, herbaceous, dioecious flowering plants that are members of the family *Cannabaceae*, which include about 102 plant species.¹ Although there is much debate, the most common taxonomy is that the genus *Cannabis* comprises one species, *Cannabis sativa* L. (*C. sativa*), which includes the highly polymorphic subspecies *sativa*, and *indica*.²²

Hemp and cannabis both refer to the same species *C. sativa*; however, there is important distinction between the two. Whereas hemp (fiber-type) is grown for its cellulose-rich fiber in the stem, cannabis (drug-type) is cultivated for its flowers where the glandular trichomes produce the psychoactive delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC provides the analgesic and relaxing effects of cannabis, whereas CBD produces other effects such as antiemetic and soporific properties. Researchers have used the THC content to define *C. sativa* subspecies *sativa* as containing less than 0.3% THC in dried flowering tops of female plants and *C. sativa* subspecies *indica* as containing $\geq 0.3\%$ THC.³ This threshold has been used by regulatory bodies to legally differentiate hemp plants ($<0.3\%$ THC) and cannabis plants ($\geq 0.3\%$ THC).

Canada legalized the production, distribution, sale and non-medical use of cannabis for adults in October 2018. Recent data from nation-wide surveys show that approximately 6.2 million people aged 15 or older, or 20% in this age group, reported using cannabis in the past 3 months.⁴ which represents an increase from 14% before legalization. Cannabis can be used and/or ingested in a variety of forms including capsules, oils, dried flower, vaporization and through the consumption of edibles. With the increased use of recreational cannabis in Canada, it is expected that there will be a concomitant increase in cases of cannabis hypersensitivity.

CLINICAL MANIFESTATIONS OF CANNABIS ALLERGY

Since the first description of reactions to cannabis-containing cigarettes in 1971, there have been numerous reports of cannabis hypersensitivity associated with different routes of exposure and a wide-range of symptoms.⁵⁻¹¹ Most case reports described an immediate onset of upper airway symptoms such as rhinitis and conjunctivitis after smoking cannabis in recreational users.⁶⁻¹⁰ There has also been evidence suggesting that long-term use of cannabis can result in chronic airway inflammation and exacerbate existing asthma, despite its mild and short bronchodilator effect.¹² Indeed, there are several reports of patients experiencing the immediate onset of lower respiratory symptoms such as dyspnea, coughing, wheezing and chest tightness with recreational cannabis use. Gastrointestinal symptoms such as vomiting and abdominal cramps can also occur especially after ingestion of marijuana edibles.⁷ In regions where *C. sativa*

is cultivated or wild cannabis plants exist, environmental exposure to *C. sativa* pollen, which typically peak in summer months, has also been implicated in seasonal allergic rhinoconjunctivitis.¹¹ Contact urticaria has also been described in patients who have repeated direct contact with the plant.^{6,13,14} In general, the symptoms of cannabis allergy can be variable and are not limited by the route of exposure. Not surprisingly, anaphylactic reactions from recreational cannabis use have been reported.^{7,15} There is also one report of cannabis-dependent exercise-induced anaphylaxis where the patient reported allergic reaction only when he engaged in rigorous activity after smoking cannabis.⁹ Hemp seed ingestion can result in anaphylaxis in patients sensitized to cannabis from recreational cannabis use.¹⁶ It is important for patients who have a history of cannabis allergy to be educated on the risk of hemp seed as a potential food allergy.

Occupational exposure has also been recognized as a risk of sensitization to cannabis.^{17,18} There also have been several reports of workers in cannabis facilities and forensic laboratory personnel developing allergic symptoms including rhinitis, urticaria and angioedema from cannabis exposure in the work environment despite having no history of recreational cannabis use.^{13,14,19} A recent study involving law enforcement officers with a reported history of cutaneous or respiratory symptoms from work-related cannabis exposure was unable to establish a causal relationship between cannabis allergy and symptomology.²⁰ This potentially suggests that a non-immune mechanism exists for some of the symptoms experienced with cannabis exposure or that the relevant cannabis allergens implicated in occupational exposure remain yet to be identified.

CANNABIS ALLERGENS AND CROSS-REACTIVITY

Although cannabis allergy has long been recognized, it is only recently that investigations into the allergenic component have been reported. Can s 3, a non-specific lipid transfer protein (ns-LTP) that belongs to the pathogenesis-related (PR)-14 group, is the first IgE-binding allergenic protein identified (Table 1).^{6,8,21}

This protein is believed to be the major allergen in the European population. In a Spanish study, sensitization to Can s 3 was observed in 124 of 130 patients with primary cannabis allergy and a similar trend was also observed in another European study.^{22,23} Since ns-LTP is ubiquitous throughout the plant kingdom, sensitization to Can s 3 could lead to secondary plant-derived food allergies as reported in the literature. This pattern of cross-reactivity has been termed "cannabis-fruit/vegetable syndrome".^{23,24} The foods most commonly implicated are allergies to peach, banana, apple, nuts, grapes, cherry, and tomato (Figure 1).

The symptoms due to cannabis-fruit/vegetable syndrome

Allergen	WHO/IUIS allergen nomenclature	Examples of homologues	Reference Source
Profilin	Can s 2	Bet v 2, Pru p 4, Sola i 1	21
Non-specific lipid transfer protein	Can s 3	Pru p 3, Cor a 8, Hev b 12, Ara h 9, Sola i 3, Vit v 1	6, 8, 21
Oxygen-evolving enhancing protein 2	Can s 4	-	27
Pathogenesis-related protein 10	Can s 5	Bet v 1, Mal d 1, Ara h 8	26
Ribulose-1,5-bisphosphate carboxylase/oxygenase	-	-	27

Table 1. Cannabis allergens. Adapted from Decuyper et al.²⁴

are typically more severe as compared to those observed in Bet v 1-related pollen food syndrome. This is likely because ns-LTPs are resistant to gastroduodenal proteolysis and thermal processing. Cross-reactivity with Can s 3 has also been shown to extend to latex and tobacco (**Figure 1**).²⁵ The cannabis homologue of Bet v 1 and *C. sativa* profilin, now termed Can s 5 and Can s 2 respectively, have also been demonstrated to play a role in cannabis allergy.²⁶

Unlike the European investigations, the first study on *C. sativa* allergen in the North American population did not show the same pattern of sensitization.²⁷ Rather than Can s 3, the predominant IgE-binding allergens were found to be ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and oxygen evolving enhancer protein 2 (Can s 4), both of which are involved in photosynthesis.²⁷ Interestingly, in line with the different pattern of sensitization, there is also a lack of association between cannabis allergy and plant-food allergies in the North American population.^{7,27,28} Whether this is related to geographical differences in *C. sativa* resulting in different clinical phenotypes is unknown. A more recent study did detect Can s 3 sensitization in a

small number of Canadian patients with cannabis allergy although the exact prevalence remains to be elucidated.²⁹

DIAGNOSTICS AND MANAGEMENT

As with other allergies, the diagnosis of cannabis allergy relies mainly on an accurate history of the allergic reaction. However, several important factors can make this challenging. Although cannabis is now legalized in Canada, patients may not be forthcoming about their cannabis use due to social stigma and taboos. Another challenge is that cannabis smoking or ingestion can result in side effects such as conjunctival injection and panic attacks that can be misattributed as allergic reaction. For workers in cannabis facilities or law enforcement officers, occupational exposure to pesticide, organic dusts or fungi from handling or processing cannabis can also elicit or mimic allergic symptoms.

Currently, there is no standardized or commercially available diagnostic test for cannabis allergy. Even direct provocation testing, which is the gold standard in allergy diagnosis, has unclear reliability given the paradoxical short-term bronchodilator effect of cannabis. For skin prick

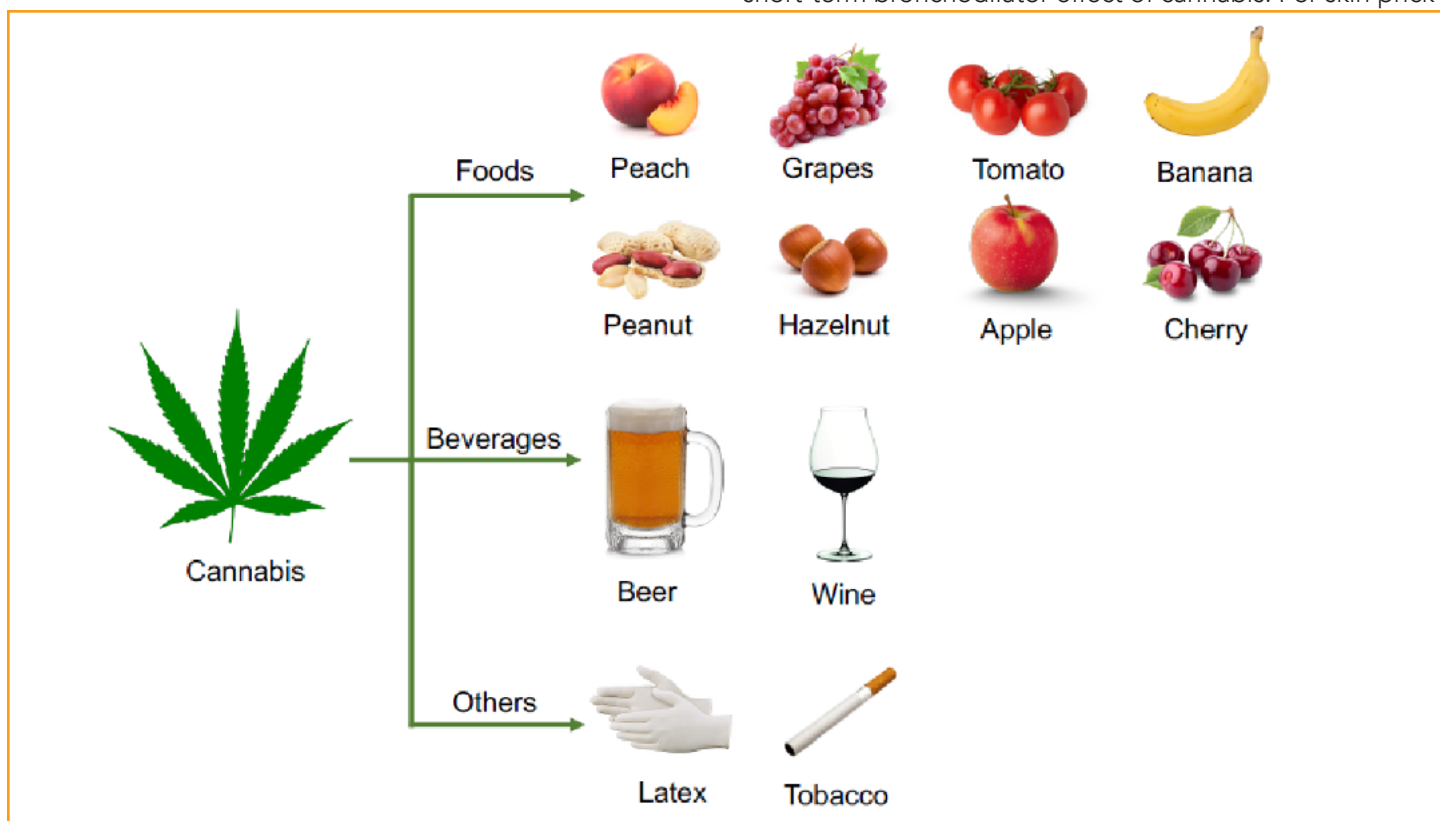


Figure 1. Sensitization to *C. sativa* can lead to cross-reactivity with a variety of foods, beverages, latex and tobacco due to the ubiquitous non-specific lipid transfer protein. This is termed "cannabis-fruit/vegetable syndrome". Adapted from Decuyper et al.²⁴

testing, most case reports in the literature use prick-prick tests with crude cannabis or non-standardized cannabis extracts for diagnosis.³⁰ This approach could potentially be confounded by variable results depending on the composition of the source material or varieties of *C. sativa* used. A recent European study showed that Can s 3-based testing is the most effective and reliable.³⁰ Although not commercially available, skin prick testing with a Can s 3 enriched extract and specific IgE testing to recombinant Can s 3 were both demonstrated to have positive and negative predictive value of around 80% and 60%, respectively.³⁰ However, whether these results apply to the North American population, which seems to have different pattern of sensitization, remains unknown. Specific IgE testing to hemp, which is commercially available, can be considered a proxy although it lacks specificity (32%).³⁰ Certainly, more research in the development of diagnostic testing, targeted towards the North American population is needed.

With regards to management of cannabis allergy, strict avoidance when feasible is the only available treatment. There is one case report in the literature describing the use of omalizumab for the treatment of cannabis allergy in a patient who had regular occupational exposure to cannabis resulting in anaphylactic reactions.³¹ After 4 months of therapy, the patient was able to tolerate exposure to large amounts of cannabis with only mild cutaneous symptoms. Successful immunotherapy treatment for cannabis allergy has also been previously reported.³² However, the lack of a standardized extract along with uncertain efficacy and safety data make it challenging to foresee broad application particularly for recreational cannabis users.

CONCLUSION

With the legalization of cannabis, there is likely to be a continuing trend of increased numbers of recreational users and an increased prevalence of cannabis allergy. Occupational exposure is also recognized as a risk for cannabis sensitization. Diagnosing cannabis allergy remains challenging due to a lack of standardized testing, however, there is hope that commercially available testing will be available in the near future with a better understanding of the allergenic components. An important question to answer concerns the role that geographical differences may play in cannabis allergy as shown by the distinct sensitization and clinical phenotypes between North American and European populations, which will most certainly impact the diagnosis and management of cannabis allergy. As there are numerous strains and varieties of *C. sativa*, it is unclear whether the various strains have different allergenicity and more research is needed in this regard. Component-specific cannabis extracts including Can s 3 are not yet available as allergen extracts. Research is ongoing using component-specific diagnostics.

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