Predicting Cow's Milk Oral Food Challenge Outcomes in Pediatric Patients and Assessing the Impact of Prehospital Epinephrine, Antihistamines, and Corticosteroids in Managing Anaphylaxis.

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Abstract

This thesis assesses two important aspects affecting the care of patients with food allergies: the diagnosis and management of anaphylaxis. Oral food challenges (OFCs) are considered the gold standard for diagnosis and determining reactivity thresholds. However, they are resource-intensive and carry a high risk of reaction. There is limited data on factors that increase the likelihood of positive OFC outcome, particularly among children with a history of anaphylaxis. The first objective is to assess factors associated with positive OFC and generate models that can better predict CM OFC outcomes.

Children aged 5-18 years old, being considered for oral immunotherapy (OIT) to milk underwent a single-blind placebo-controlled food challenge to CM, with either positive (reaction) or negative (tolerance) outcomes. Initial factors recorded included sex, age, history of asthma, eczema, and allergic rhinitis, prior epinephrine use for CM induced reactions, skin prick test size (SPT), and serum levels of total sIgE and sIgE antibodies to α-lactalbumin (ALA), β-lactoglobulin (BLG), and casein (CAS). Log-transformed total sIgE, ALA, BLG, and CAS levels were also evaluated. Stepwise backward multivariate Firth bias-reduced logistic regression analysis was used to create the final model. Despite limitations, my model has identified two factors; log transformed BLG and previous epinephrine use, that can be used to accurately identify true CMA without the need for an OFC.

Secondly, despite strict avoidance, individuals with CMA may encounter CM unknowingly due to cross-contamination or mislabeled food items, resulting in anaphylaxis. Epinephrine is the first-line treatment for anaphylaxis, but H1 - antihistamines or corticosteroids are sometimes used instead. There is limited data on the effects of prehospital administration of epinephrine compared

to H1 - antihistamines and steroids. The second objective is to assess the impact of prehospital treatment with epinephrine, H1 - antihistamines, and/or corticosteroids in anaphylaxis management.

Patients presenting with anaphylaxis were recruited prospectively and retrospectively in 11 emergency departments (ED) between April 2011 and August 2022, as part of the Cross-Canada Anaphylaxis Registry. Data on anaphylaxis cases were collected using a standardized form and factors collected were assessed using a multivariate logistic regression. Patients treated with prehospital epinephrine were less likely to have uncontrolled reactions, receive IV fluids in ED, and to be hospitalized. Patients treated with prehospital H1 - antihistamines were less likely to have uncontrolled reactions, and to be hospitalized. Patients who received prehospital corticosteroids were more likely to require IV fluids in ED and be hospitalized. The findings of this study highlight the importance of prioritizing epinephrine as first-line treatment, suggest potential advantages of prehospital antihistamine administration, and highlight the necessity to reassess current anaphylaxis management guidelines, particularly with regard to corticosteroid usage.

Résumé

Cette thèse évalue deux aspects importants des soins offerts aux patients souffrant d'allergies alimentaires : le diagnostic et la gestion de l'anaphylaxie. Les défis alimentaires oraux (DAO) sont considérés comme l'étalon-or pour le diagnostic et la détermination des seuils de réactivité. Cependant, elles nécessitent beaucoup de ressources et comportent un risque élevé de réaction. Il existe peu de données sur les facteurs qui augmentent la probabilité d'un résultat positif de DAO, en particulier chez les enfants ayant des antécédents d'anaphylaxie. Le premier objectif

est d'évaluer les facteurs associés à un DAO positif et de générer des modèles permettant de mieux prédire les résultats de DAO au LV.

Des enfants de 5 à 18 ans ont passé un DAO à simple aveugle, placebo, avant la désensibilisation au LV, dont les résultats ont été soit positifs (réaction), ou négatifs (tolérance). Les facteurs initiaux enregistrés comprenaient le sexe, l'âge, les antécédents d'asthme, d'eczéma et de rhinite allergique, l'utilisation antérieure d'adrénaline pour des réactions induites par le LV, la taille du test cutané et les taux sériques de sIgE totaux et d'anticorps sIgE dirigés contre l'α-lactalbumine (ALA), la β-lactoglobuline (BLG) et la caséine (CAS). Les niveaux de sIgE totaux, d'ALA, de BLG et de CAS log-transformés ont également été évalués. Une régression logistique multivariée progressive rétrograde à biais de Firth réduit a créé le modèle final. Malgré ses limites, notre modèle a identifié deux facteurs, le BLG transformé en logarithme et l'utilisation antérieure d'épinéphrine, qui peuvent être utilisés pour identifier avec précision une véritable allergie au LV sans avoir besoin d'un DAO.

Deuxièmement, malgré un évitement strict, les personnes atteintes d'allergies au LV peuvent entrer en contact, sans le savoir, du aux contaminations croisées ou des aliments mal étiquetés, entraînant une anaphylaxie. L'épinéphrine est le traitement de première ligne de l'anaphylaxie, mais les antihistaminiques ou les corticostéroïdes sont parfois utilisés à la place. Il existe peu de données sur les effets de l'administration préhospitalière d'épinéphrine en comparaison avec les antihistaminiques et les corticostéroïdes. Le deuxième objectif est d'évaluer l'impact du traitement préhospitalier à l'épinéphrine, aux antihistaminiques et/ou aux corticostéroïdes dans la gestion de l'anaphylaxie.

Des patients ayant eu une réaction anaphylactique ont été recrutés de manière prospective et rétrospective dans 11 services d'urgence entre avril 2011 et août 2022, dans le cadre du Cross-

Canada Anaphylaxis Registry (registre pancanadien de l'anaphylaxie). Les données sur les cas d'anaphylaxie ont été recueillies à l'aide d'un formulaire standardisé et les facteurs recueillis ont été évalués à l'aide d'une régression logistique multivariée. Les patients traités avec de l'épinéphrine préhospitalière étaient moins susceptibles d'avoir des réactions non contrôlées, de recevoir des fluides IV à l'urgence et d'être hospitalisés. Les patients traités avec des antihistaminiques préhospitaliers étaient moins susceptibles d'avoir des réactions non contrôlées et d'être hospitalisés. Les patients ayant reçu des corticostéroïdes préhospitaliers étaient plus susceptibles d'avoir besoin de fluides IV aux urgences et d'être hospitalisés. Les résultats de cette étude soulignent l'importance de donner la priorité à l'épinéphrine comme traitement de première ligne, suggèrent les avantages potentiels de l'administration préhospitalière d'antihistaminiques et soulignent la nécessité de réévaluer les directives actuelles de gestion de l'anaphylaxie, en particulier en ce qui concerne l'utilisation des corticostéroïdes.

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Contributions of Authors

The idea for the thesis was developed by Dr. Moshe Ben – Shoshan. The thesis introduction, including the literature review and study objectives, was conducted, and written by Luca Delli Colli, and subsequently reviewed and approved by Dr. Moshe Ben – Shoshan.

The milk oral immunotherapy study (manuscript one) design and protocol were developed by Dr. Moshe Ben – Shoshan. Data collection and curation for this study was conducted by Luca Delli Colli, in which Liane Beaudette, Vera Laboccetta, and Danbing Ke provided assistance with patient recruitment and sample collection. Luca Delli Colli, Casey Cohen, and Diana Toscano-Rivero performed the laboratory analysis on the collected samples. The data analysis for manuscript one was conducted by Luca Delli Colli and Dr. Joshua Yu. The introduction, methods, and discussion were written by Luca Delli Colli. The results section, tables and figures in manuscript one were written and created by Luca Delli Colli and Dr. Joshua Yu. All manuscript coauthors were involved in the revision and editorial process of the manuscript prior to submission to the journal. The manuscript was submitted by Luca Delli Colli to the *Journal of Allergy and Clinical Immunology: In Practice* and is currently under review.

The preamble to manuscript two was written by Luca Delli Colli and reviewed and approved by Dr. Moshe Ben – Shoshan.

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Abbreviations

ALA:	α-lactalbumin
BLG:	β-lactoglobulin
CAS:	Casein
CM:	Cow's milk
CMA:	Cow's milk allergy
ELISA:	Enzyme-linked immunosorbent assay
OFC:	Oral food challenge
OIT:	Oral immunotherapy
sIgE:	Specific immunoglobulin E
SPT:	Skin prick test
SBPCFC:	Single-blind, placebo-controlled food challenge

DBPCFC:	Double-blind, placebo-controlled food challenge
C-CARE:	Cross-Canadian Anaphylaxis Registry
ED:	Emergency department
ICU:	Intensive care unit
ICD-10:	International Classification of Diseases, Tenth Edition
IQR:	Interquartile range
IV	Intravenous
OR	Odds ratio
USA	United States of America
95%CI	95th percent confidence intervals

Introduction

Allergy diagnostic tests play a crucial role in identifying specific allergens and guiding appropriate management strategies. However, traditional diagnostic methods such as skin prick tests and serum-specific IgE measurements have limitations that can impede accurate diagnoses. These tests often lack specificity, leading to false positive results and unnecessary dietary restrictions.[1-6] Recognizing the need for improved diagnostic accuracy, recent advancements in component specific IgE testing have emerged as a promising alternative for diagnosing food allergies without the need for an oral food challenge.[7-10] This innovative approach allows clinicians to pinpoint individual allergenic components, aiding in the identification of allergen sensitization and facilitating personalized treatment plans for patients.

Despite advances in allergy diagnosis, the underutilization of epinephrine in the management of anaphylaxis remains a concerning issue.[11, 12] Anaphylaxis is a severe and potentially life-threatening allergic reaction that requires immediate intervention.[11, 12] Unfortunately, inadequate awareness and understanding among both patients and healthcare providers often result in the incorrect substitution of epinephrine by H1 - antihistamines and

corticosteroids.[13] Prompt administration of epinephrine is critical in preventing the progression of anaphylaxis and reducing the risk of fatalities.[13, 14] Emphasizing the importance of timely epinephrine use and understanding the role of H1 - antihistamines and corticosteroids is crucial to improving patient outcomes and ensuring optimal management of anaphylactic reactions. Efforts to educate healthcare professionals and the public on the proper use of epinephrine are essential in addressing this gap in anaphylaxis management.

There is limited data on factors associated with an increased likelihood of positive OFC, particularly among children with a history of anaphylaxis. Data on the effects of prehospital administration of epinephrine compared to H1 - antihistamines and corticosteroids in the management of anaphylaxis is also lacking. Therefore, I assessed factors associated with a positive OFC and developed a model to better predict CM OFC outcome. I also assessed the role of epinephrine, H1 - antihistamines, and corticosteroids in the management of anaphylaxis.

1.0 Literature Review

1.1 Epidemiology of Food Allergy

Food allergies affect a significant portion of the global population, are associated with substantial morbidity and mortality, and affect the quality of life. With a growing number of individuals experiencing allergic reactions to certain foods—approximately 1 in 10 adults and 1 in 12 children in the United States according to a 2018 population-based cross-sectional prevalence survey—it is crucial to correctly diagnose food allergy and to appropriately manage allergic reactions. [15-17] Additionally, a recent study has found that the relative frequency of anaphylaxis cases in the emergency department (ED) has increased from 0.22% from 2011-2015 to 0.42% in March 2020. [17] The increase in the number of individuals affected by food allergies has prompted

further inquiry into the factors responsible for this trend and the methods that can be employed to address the issues faced by those living with food allergies.[16, 17]

To understand the increasing prevalence of food allergies, it is necessary to consider the interaction between genetic factors, environmental factors, and lifestyle habits, as these play a pivotal role in the pathogenesis of food allergy. A study conducted on a registry of twins with allergist confirmed food allergies and positive confirmatory tests results found a general trend of higher concordance rates for all food allergies among monozygotic twins, as compared to dizygotic twins (0.58 vs 0.49, 95%CI 0.05-0.46).[18] Monozygotic twin concordance rates were higher compared to dizygotic twin concordance rates for fish, peanut, sesame, and walnut.[18] However, this study also found that atopic dermatitis is a significant risk factor for food allergy, even when controlling for genetic factors.[18] These findings were echoed by another study which found that patients with a history of asthma, eczema, or hay fever were at higher risk of developing food allergies.[19] This study also found that patients with a parent or sibling with food allergy were at increased risk of developing food allergies.[19] While these studies provide important insight on the impact of genetic risk factors for food allergies, future cohort and randomized controlled trials further investigating the interplay between genetic factors and other determinants are needed to better understand the multiple factors mediating food allergy development.

While genetics can predispose individuals to food allergies, it is also evident that gene - environment interactions also play a significant role.[18] Several hypotheses have been advanced in order to explain the increase in food allergy prevalence. The hygiene hypothesis suggests that decreased exposure to microbes in early life due to cleaner environments (smaller family size, decreased exposure to live stock, increased use of antibiotics and vaccinations, and improved sanitation), may lead to an overactive immune response to otherwise innocuous substances, like

allergens.[19, 20] The early introduction hypothesis proposes that early cutaneous exposure to an allergen can lead to allergic sensitization.[21] While the early introduction hypothesis puts forward a reason for increases in allergy prevalence, it also states that early oral consumption of foods may lead to tolerance.[21]

Microbiome related changes resulting from different lifestyle habits have also been proposed to play a role in the increased prevalence of food allergy. The typical Western diet, often characterized by high levels of processed foods and a lack of essential nutrients, has been suggested as a potentially significant contributor to the rise in food allergies.[22] This is particularly due to this diet's effects on intestinal microbiota, as consumption of foods high in fats and sugar while low in fiber, as well as excessive use of antibiotics, has a detrimental impact on host-microbe interactions.[22] In turn, recent findings have shown that changes to this interaction may negatively impact the regulation of immunity in the long term.[22] A comprehensive understanding of the epidemiology of food allergies is crucial for informing public health policies and interventions aimed at reducing their prevalence and severity. By exploring the complex interplay of factors contributing to food allergies, it is possible to work towards creating a more inclusive and health-conscious society, empowering those affected to live their lives without the constant fear of an allergic reaction.

1.2 Pathophysiology of Allergies

The pathophysiology of allergies is a complex process and involve reactions to otherwise seemingly harmless substances. Allergies occur when the immune system identifies a foreign substance, known as an allergen, as harmful and mounts an inappropriate response to neutralize it. The initiation of an allergic response begins with sensitization, a process in which the immune system encounters an allergen and generates a heightened response.[23] Dendritic cells, specialized

cells that play a crucial role in the immune system, capture allergens, process, and present them to T cells.[23] The T cells then differentiate into a specific subtype, known as T helper 2 (Th2) cells. Th2 cells release cytokines, that promote an immune response.[23] In the presence of interleukin-4 (IL-4), B cells are stimulated to produce Immunoglobulin E (IgE) antibodies specific to the allergen.[23] Immunoglobulin E antibodies are central to the development of an allergic response to a food.

IgE antibodies are produced by the immune system in response to exposure to an allergen. The IgE antibodies bind to mast cells and basophils, in turn causing these cells to release a variety of chemical mediators, when IgE are cross linked.[23] One of the primary mediators is histamine, which is responsible for the dilation of blood vessels, leading to redness and swelling, as well as the stimulation of nerve endings, causing itching and pain.[23] The release of histamine and other mediators such as prostaglandins and leukotrienes lead to the activation of additional immune cells and the production of more cytokines, perpetuating the allergic response.[23] The severity of an allergic reaction can vary widely among individuals and can range from mild symptoms, such as itching and sneezing, to severe and potentially life-threatening reactions, known as anaphylaxis. Anaphylaxis is characterized by a rapid onset of symptoms, including but not limited to difficulty breathing, a drop in blood pressure, and even loss of consciousness, which can be fatal if not promptly treated.[14] Several acceptable definitions have been put forward to diagnose anaphylaxis.[24-27] The definition put forward by the World Allergy Organization (WAO) and the European Academy of Allergy and Clinical Immunology (EAACI) describe anaphylaxis similarly, as a severe or serious life-threatening generalized or systemic hypersensitivity reaction. The WAO further defines anaphylaxis as a serious allergic reaction that is rapid in onset and might cause death, while the EAACI further defines anaphylaxis as an acute potentially fatal, multi-organ

system, allergic reaction. Definitions by the WAO and EAACI are more general than the definition put forward by the National Institute of Allergy and Infectious Disease (NIAID).[26] The NIAID defines anaphylaxis as a serious allergic reaction that involves two or more organ systems (ex: skin, respiratory tract, cardiovascular system and/or gastrointestinal tract). It can begin very rapidly and symptoms may be severe or life-threatening.[14, 26] Seeing as this definition has been associated with less ascertainment bias, the NIAID definition is the one selected to diagnose anaphylaxis in my studies.[14, 27, 28]

Understanding the pathophysiology of allergies and the definition of anaphylaxis is crucial for developing effective treatments and interventions to manage allergic reactions and improve the quality of life for those affected by allergies.

1.3 Diagnosis

The history of diagnosing and testing food allergies has evolved significantly over the years, reflecting advancements in scientific understanding and technology. In the early stages, food allergies were not well understood, and the diagnosis process was predominantly based on observation and elimination diets. Patients who experienced adverse reactions to certain foods were advised to eliminate the suspected food from their diet and gradually reintroduce it to observe any subsequent allergic reactions. However, the use of elimination diets is far less prominent today as it has been identified as a contributing factor to the development of food allergies.[29]

1.3.1 Skin Prick Test

The introduction of skin prick tests in the mid-20th century marked a turning point in food allergy diagnosis. This method involved placing a drop of allergen extract on the skin and pricking the skin through the drop to introduce the allergen to the immune system.[30] A reaction is identified by the presence of a raised, red bump called a wheal, surrounded by redness (also known as flare).[30]

While skin prick tests are still commonly used today, they are not always conclusive and can be difficult to interpret when test results are below the positive predictive values.[1, 31, 32]

1.3.2 Specific IgE Testing

As diagnostic methods continued to advance, blood testing emerged as an additional tool for diagnosing food allergies. The radioallergosorbent test (RAST) was developed to measure the levels of specific immunoglobulin E (IgE) antibodies in the blood, which are indicative of sensitization.[3, 33] RAST was predominantly considered a qualitative test and is now considered obsolete as it has been replaced by the more prominently utilized and accurate enzyme-linked immunosorbent assay (ELISA) and the ImmunoCAP test, which remain widely used today.[33] High sIgE levels are associated with allergen sensitization but is not diagnostic of clinical allergy.[3] While sIgE tests may be useful in determining the subsequent need for an OFC in patients with high sIgE levels, results are difficult to interpret for patients with sIgE levels below positive predictive values.[3, 32]

1.3.3 Specific IgE Component Testing

Component testing is the measurement of individual components of a single food sIgE allergen. Advancement in recombinant technology have made the production of specific protein components possible.[34, 35] Component testing can be used to identify the most problematic allergens in patients with specific sensitization patters.[35] This method has show promise in the diagnosis of true peanut allergy where utilizing the Ara h 2 component as part of a diagnostic process has resulted in a 2.5-4 times reduction in the need for OFC to diagnose true peanut allergy.[8, 34] Recent studies have also been exploring the use of sIgE component testing in cashew allergy, egg allergy, and milk allergy.[9, 10, 36] While sIgE component testing and its implications in

diagnosing true food allergy without an OFC are increasingly researched, sIgE component testing remains a tool and is not diagnostic on its own.

1.3.4 Oral Food Challenge

Despite these advancements, the "gold standard" for diagnosing food allergies is still the double-blind, placebo-controlled food challenge (DBPCFC). This test involves administering increasing amounts of the suspected allergen and a placebo in a controlled setting, with neither the patient nor the administering clinician aware of which is which.[37] If a reaction occurs, the allergen is identified, and a definitive diagnosis can be made.

1.3.5 Diagnostic Test Limitations

Each of the confirmatory tests has limitations. The skin prick test may be associated with high false positives rates, especially in the absence of supporting history, as the test may detect sensitization to an allergen without a true allergic reaction occurring.[2, 38] Moreover, it may not be suitable for individuals with severe skin conditions, such as eczema, or those who utilize H1 - antihistamines.[39, 40] While sIgE testing is not influenced by the presence of eczema or H1 - antihistamines, it is expensive and results or not obtained immediately.[40] Additionally, patients may have detectable sIgE level to a specific allergen without developing a clinical allergy, leading to a false positive result. [3, 32, 40] Furthermore, a study estimated that 10% to 25% of patients with undetectable sIgE may still have a reaction when challenged.[5] Although sIgE component testing has been found to be a better predictor of true food allergy compared traditional sIgE testing, it is limited by the lack of clinically relevant cutoff values currently established.[3] Specific IgE Component testing also shares limitations with traditional sIgE testing; notably, the relatively high cost and delay in obtaining a result.[3, 40] Lastly, the double-blind, placebo-controlled food challenge can be labor-intensive and time-consuming despite its rigorous design, as it requires meticulous

preparation of the food samples and close monitoring of the patient.[41] Furthermore, the risk of inducing severe allergic reactions, including anaphylaxis, cannot be overlooked, necessitating the availability of emergency medical intervention during the procedure.[41] The diagnosis should be stablished by corroborating a suggestive history of an IgE mediated reactions with the available confirmatory tests. However, given that food challenges are often required to establish the diagnosis it is crucial to identify factors associated with positive challenges in high-risk individuals to reduce the need and burden of an OFC.

1.4 Treatment of Food Allergies

1.4.1 Epinephrine

Epinephrine is the first line medication in the management of food allergies, particularly in cases of severe reactions known as anaphylaxis. Epinephrine is a naturally occurring hormone that plays a vital role in the body's fight-or-flight response by stimulating various physiological changes to help the individual respond to stress or danger.[42] Epinephrine exerts its effects through several mechanisms, including the constriction of blood vessels, which helps to counteract the drop in blood pressure often seen during anaphylaxis, and the relaxation of smooth muscles in the airways, which can help to alleviate breathing difficulties.[43] Anaphylaxis is a potentially life-threatening allergic reaction that can occur rapidly following exposure to an allergen. It manifests as a severe, systemic response involving multiple organ systems, with symptoms like difficulty breathing, rapid heartbeat, swelling, and low blood pressure.[44] In such cases, the prompt administration of epinephrine is crucial, as it can reverse the symptoms and ultimately save lives.[14]

For individuals with a known history of severe food allergies, carrying an auto-injector containing a pre-measured dose of epinephrine, such as an EpiPen®, is essential for immediate use in the event of an allergic reaction.[45] Despite its life-saving potential, epinephrine is not without

side effects. Some of the more common adverse reactions include palpitations, anxiety, tremors, headaches, and dizziness.[46] These side effects are generally mild and short-lived, and the benefits of using epinephrine in an emergency situation far outweigh the risks associated with these temporary discomforts. It is important to note, however, that individuals with certain medical conditions, such as uncontrolled high blood pressure, heart disease, or hyperthyroidism, may be at a higher risk for experiencing more severe side effects.[47] These individuals should consult with their healthcare provider to discuss the appropriate use of epinephrine for the management of anaphylaxis.

1.4.2 H1 – Antihistamines

H1 - antihistamines have also been included in the management of anaphylaxis. H1 - antihistamines are inverse agonists which produce the opposite effect on the H1 – receptor, compared to histamine.[48] The speed of onset of action for H1 - antihistamines varies by medication and age. Maximum clinical effect has been found to occur nearly two hours after administration in children and approximately four hours after administration in adults.[48] By reducing the effects of histamine, H1 - antihistamines can help alleviate these symptoms and provide relief to individuals experiencing mild to moderate allergic reactions. While H1 - antihistamines can be effective in treating symptoms associated with food allergies, it is essential to note that they are not sufficient for managing anaphylaxis, which requires the prompt administration of epinephrine, a medication that acts more rapidly and broadly on the body's physiological systems to counteract the rapid and severe symptoms of a systemic allergic reaction.[49] Additionally, H1 - antihistamines should therefore never be used as a substitute for epinephrine, as they are incapable of providing the immediate and comprehensive relief necessary in such situations.

H1 - antihistamines can, however, be useful in controlling certain symptoms, such as rhinitis and urticaria.[49] The use of H1 - antihistamines for the treatment of food allergies is generally well-tolerated, with many individuals experiencing only mild and transient side effects. Some of the most common side effects associated with H1 - antihistamines include drowsiness, dizziness, and dry mouth.[49] These symptoms tend to be more pronounced with first-generation H1 - antihistamines, such as diphenhydramine, while second-generation H1 - antihistamines, like cetirizine or loratadine, are less likely to cause drowsiness.[49] It is important for individuals taking H1 - antihistamines to be aware of these potential side effects, as they may affect their ability to perform tasks that require alertness, such as driving or operating heavy machinery. While they are not suitable for treating anaphylaxis, H1 - antihistamines can be a valuable component of an individual's allergy management plan.

1.4.3 Corticosteroids

The third class of medications used ion anaphylaxis management are corticosteroids. Corticosteroids are a class of steroid hormones that play a crucial role in managing food allergies. These compounds work by suppressing the immune system's response to allergens, thereby reducing inflammation and alleviating allergy symptoms.[50] The mechanism of action of corticosteroids involves binding to the glucocorticoid receptor, which in turn modulates the transcription of genes responsible for the production of inflammatory mediators like cytokines and chemokines.[50] By doing so, corticosteroids control the allergic cascade, mitigating the severity of symptoms and preventing further complications. While epinephrine is typically administered as the first-line therapy for anaphylaxis, corticosteroids are considered adjunct therapy and hypothesized to prevent the recurrence of symptoms or to treat refractory cases.[51] It is essential to

note that corticosteroids are incapable of replacing epinephrine in the management of anaphylaxis, and instead may function as a supplementary intervention to enhance overall treatment efficacy.

Corticosteroids carry the potential for side effects, particularly with long-term use or high doses. Even short-term use carries risk of experiencing various unpleasant and sometimes even severe side effects, which may include fluid retention, hypertension, mood swings, and increased susceptibility to infections.[52] Long-term use can result in serious complications including osteoporosis, impaired wound healing, and adrenal suppression, which can lead to a decreased ability to respond to stress or illness.[53]

1.5 Living with Food Allergies

Living with food allergies requires a proactive approach to ensure the safety and well-being of individuals affected by these conditions. Food allergies can significantly impact a person's daily life, necessitating heightened vigilance to avoid contact with food allergens. Strategies for avoiding exposure to allergens include careful meal planning, thorough label reading, effective communication with others, and awareness of cross-contamination risks.

Meal planning is an especially important aspect of managing food allergies, as it allows individuals to have greater control over the ingredients in their meals. It also becomes more time-consuming and a greater challenge when food allergies are involved than otherwise.[54] Still, by effectively preparing meals at home, one can ensure that allergen-free ingredients are used, reducing the risk of accidental exposure. Additionally, planning meals in advance can help individuals with food allergies to maintain a balanced and nutritious diet, despite any dietary restrictions. Reading food labels is also particularly crucial for individuals with food allergies, as it enables them to easily identify and avoid products containing allergens.[55] However, it is also important to familiarize oneself with different forms of allergens and to be aware that allergenic

ingredients can sometimes be hidden in food products, as food labeling and precautionary statements (such as “may contain”) regulations remain imperfect and are often ignored by consumers.[55, 56]

Effective communication also plays a vital role in managing food allergies, particularly in social situations or when dining out. Informing friends, family, and restaurant staff about one's allergies and the potential consequences of exposure can help ensure that appropriate precautions are taken. When visiting restaurants, individuals with food allergies should not hesitate to ask detailed questions about menu items and food preparation methods to minimize the risk of cross-contamination and accidental exposure.[57] While these measures may be socially burdensome, neglecting the responsibility to establish a dialogue with others may place one at risk of an unnecessary—and certainly far more burdensome—allergic reaction. Cross-contamination, the unintentional transfer of allergens from one food item to another, can occur during food preparation, storage, or handling.[58] To actively avoid cross-contamination, individuals with food allergies should maintain separate utensils, cutting boards, and storage containers for allergen-free foods whenever possible.[58] Additionally, one should be mindful of potential cross-contamination risks when shopping for groceries or eating at shared facilities, such as buffets or potlucks. By implementing these measures, individuals with food allergies can significantly reduce the likelihood of coming into contact with allergens, ensuring their safety and well-being while navigating the challenges of living with food allergies.

Desensitization mainly through oral immunotherapy (OIT) is an emerging approach aimed at protecting patients and improving the quality of life for individuals with food allergies. Desensitization involves controlled exposure to small, gradually increasing amounts of the allergenic food with the goal of increasing the patient's tolerance to the allergen, thereby reducing

the risk of severe reactions upon accidental exposure.[59] Various protocols have been developed to guide the administration of desensitization and OIT, with variations in dosing regimens, treatment durations, and allergen forms. Often OIT involves an initial escalation phase, during which the patient consumes minuscule amounts of the allergen under medical supervision, followed by a build-up phase with incremental dose increases over weeks or months.[59] Finally, the maintenance phase is reached, where a stable, daily dose of the allergen is consumed to maintain desensitization.[59] The specific dosing and duration of each phase may vary depending on factors such as the individual's age, medical history, and the overall severity of the allergy.

Safety and efficacy are critical considerations when evaluating desensitization protocols. While these approaches have shown promise in increasing allergen tolerance for some individuals, they are not without risks. Potential side effects may include mild to moderate allergic reactions during the treatment process, ranging from oral itching to gastrointestinal symptoms.[60, 61] In rare cases, severe reactions, such as anaphylaxis, can occur, necessitating prompt medical intervention.[60, 61] As a result, desensitization and OIT should only be conducted under the supervision of experienced healthcare professionals in a controlled setting. It is important to note that the degree of desensitization achieved through these protocols varies among patients, with some individuals experiencing significant improvements in allergen tolerance, while others may achieve only modest gains.[60, 61] Additionally, long-term maintenance of desensitization may require continued consumption of the allergen, which can be challenging for some patients.[62] Nevertheless OIT represent promising avenues for improving the management of food allergies, offering hope for a future in which individuals with these conditions can experience greater freedom and safety in their daily lives.[62]

2.0 Study Objectives

There is currently a lack of studies exploring the role of OFC in the diagnosis of food allergy, studies assessing the role of different prehospital medications in the management of anaphylaxis. Therefore, my thesis has two aims: First, to assess factors associated with a positive OFM to CM. Second, to assess the role of epinephrine, H1 - antihistamines, and corticosteroids in the management of anaphylaxis.

2.1 First Objective:

To assess factors associated with a positive OFC and to create a model to predict milk OFC outcome.

2.2 Second Objective:

To assess factors associated with uncontrolled reactions (defined as the use of two or more doses of epinephrine in the emergency department [ED]), no prehospital epinephrine use, the use of intravenous (IV) fluids in the ED, and intensive care unit (ICU) or hospital ward admission.

Manuscript 1: Predicting Milk Oral Food Challenge Outcomes in Pediatric Patients: An Exploratory Multivariate Analysis of Clinical Predictors

Predicting Cow's Milk Oral Food Challenge Outcomes in Pediatric Patients Prior to Oral Immunotherapy: An Exploratory Multivariate Analysis of Clinical Predictors

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Highlights:

What is already known about this topic?

Oral food challenges are the gold standard for diagnosis of food allergies. However, they are resource intensive and have a high risk of reaction.

What does this article add to our knowledge?

We have found strong predictors of true CMA and have developed an exploratory predictive model that may serve as a tool to establish the presence of true CMA without an oral food challenge.

How does this study impact current management guidelines?

Reduce the need for oral food challenges for the diagnosis of CMA, especially for patients with limited access to an allergist.

Abbreviations:

ALA: α-lactalbumin

BLG: β-lactoglobulin

CAS: Casein

CMA: Cow's milk allergy

ELISA: Enzyme-linked immunosorbent assay

OFC: Oral food challenge

OIT: Oral immunotherapy

sIgE: Specific immunoglobulin E

SPT: Skin prick test

SBPCFC: Single-blind, placebo-controlled food challenge

ABSTRACT

Background:

Oral food challenges (OFC) are the gold standard for diagnosis and reactivity thresholds but are resource-intensive and high-risk for reactions. Limited data on factors associated with increased risk of positive OFC exist.

Objective:

Assess factors associated with positive OFC and create a model to predict CM OFC outcomes.

Methods:

Children aged 5-18 being considered for CM-OIT underwent a SBPCFC to CM, with either positive (reaction) or negative (tolerance) outcomes. Initial factors recorded included sex, age, history of asthma, eczema, and allergic rhinitis, prior epinephrine use for CM-induced reactions, SPT size, serum levels of IgE antibodies to ALA, BLG, and CAS and log-transformed values. Univariate logistic regression analysis followed by stepwise backward multivariate Firth bias-reduced logistic regression analysis was used to create the final model.

Results:

111 children underwent an OFC, 103 patients reacted, and 8 tolerated the challenge. Univariate analysis showed previous epinephrine use, history of asthma, and log-transformed ALA, BLG, and CAS were significantly associated with positive OFC. A multivariate model included two significant factors: log-transformed BLG (aOR 2.5;95%CI 1.4-6.5;$p<0.001$) and previous epinephrine use (aOR 7.6;95%CI 1.4-79.5;$p=0.02$). The final model showed good

discriminatory performance (AUC 0.92, 95%CI: 0.78-0.99); at a threshold of 0.804, the sensitivity and specificity were 0.913 and 0.875, respectively.

Conclusion:

The study suggests that multivariate models, including log-transformed BLG and previous epinephrine use, may help predict OFC outcomes in pediatric patients.

INTRODUCTION

The burden of food allergies is growing with a self-reported food allergy prevalence of 9.3% and a physician-diagnosed food allergy prevalence of 2.5%.[1, 2] Cow's milk allergy (CMA) is one of the most common food allergies affecting 0.4% of children, and Cow's milk (CM) is the main culprit of accidental allergic reactions and a major cause of food-induced anaphylaxis-related fatalities.[1] The major protein allergens in cow's milk are α-lactalbumin (ALA), β-lactoglobulin (BLG), and casein (CAS).

Skin prick tests (SPT), food-specific immunoglobulin E (IgE) testing, and oral food challenges are often used to establish the diagnosis of food allergy. However, these tests have substantial limitations. The SPT relies on a liquid allergen extract applied onto the surface of the skin where it activates IgE antibodies on mast cells, resulting in degranulation and measurable wheal and flare formation.[3, 4] Despite being highly sensitive, SPTs have low specificity.[3, 4] Additionally, they can be affected by allergen extract quality, recent anaphylaxis, recent use of antihistamine medication, or young age, all of which can be associated with false negative tests.[3, 4] Specific IgE (sIgE) measures are generated by detecting circulating sIgE levels for a given food allergen in a blood sample and similar to SPTs, this type of testing is highly sensitive, yet has low specificity.[3, 4]

Oral food challenges (OFC) are considered the gold standard for food allergy diagnosis and assessing reactivity threshold, especially in the context of oral immunotherapy (OIT), through the administering of the food allergen in incremental doses.[3, 4] Despite being the gold standard, the OFC is time consuming, resource intensive, and exposes patients to a potentially severe allergic reaction.[4, 5] Data on factors associated with positive OFC outcomes are sparse, especially in patients with a history of physician diagnosed anaphylaxis. We aimed to assess factors associated with a positive OFC and to generate a model to predict CM OFC outcome.

METHODS

Patient recruitment:

Between April 2013 and December 2022, 111 children aged 6 to 18 years old followed for physician-diagnosed IgE-mediated CMA were recruited at the allergy clinics of the Montreal Children's Hospital, Hôpital Sainte-Justine, British Columbia Children's Hospital, and the Hospital for Sick Children to participate in cow's milk (CM) desensitization. This was accomplished through an OIT protocol with a randomized, controlled study with a crossover design.[6] Ethics approval was granted from all participating sites and informed consent was obtained from all participants.

Prior to beginning OIT, patients underwent an initial screening by single-blind placebo-controlled food challenge, with either positive (reaction) or negative (tolerance) outcomes. Eligible patients had a suggestive clinical history of IgE-mediated CMA, positive skin prick test, defined by a wheal with diameter ≥3mm as compared to saline, and/or CM-sIgE level >0.35kU/L, and a positive OFC. The CM used for challenge consisted of 40mg of protein per milliliter of milk. Patients who tolerated a cumulative dose of CM greater than 150mL (6,000mg protein) or baked forms of milk were not eligible to participate in the study. Children with uncontrolled asthma,

cardiovascular disease, severe hypertension, malignancies, autoimmune diseases and/or severe primary and/or secondary immune deficiencies, and on treatment with β-blockers were excluded.

Data collection & quantification of specific IgE:

Initial factors recorded included sex, age, history of asthma, eczema, allergic rhinitis, prior history of allergic reactions, history of anaphylaxis to CM, epinephrine use for CM-induced reactions, SPT wheal size, and serum sIgE levels to total CM and components ALA, BLG, and CAS. Both age and SPT size were treated as binary variables.

Total serum CM-sIgE was quantified using ImmunoCAP (Phadia 250, Thermo Fisher Scientific, Uppsala, Sweden) for 100 of the 111 subjects. Enzyme-linked immunosorbent assay (ELISA) was used to quantify ALA, BLG, and CAS-sIgE antibodies as previously described.[7] To prepare the 96-well polystyrene plates, 20μg/mL solutions of each protein were coated overnight at 4˚C. Subsequently, plates were washed with 0.1% Tween 20 in phosphate-buffered saline and blocked with 100μL per well of 1% bovine serum albumin for 1 hour at room temperature.

Patient serum samples obtained at baseline prior to OFC served as the primary antibody and were added to each well at a range of dilutions in 1% bovine serum albumin (50μL/well) for 2 hours at room temperature.[7] For detection, biotinylated polyclonal goat anti-human IgE antibody (1:20,000, 50μL/well, 1 hour at room temperature; Bethyl Laboratories, Inc. Montgomery, Texas) was added, followed by incubation with horseradish peroxidase-streptavidin (1:3,000, 50μL, 1 hour at room temperature, Bio-Legend, San Diego, Calif).[7] The optical density values were measured at 450 nm with reference at 570 nm after incubation with 3,3’,5,5’-tetramethylbenzidine substrate (Bio-Legend).

A serial dilution of recombinant human IgE antibody starting at 50ng/mL (ELISA Ready-SET-Go! Kit, Thermo Fisher Scientific, Ottawa, Ontario, Canada) binding to goat anti-human IgE capture antibody (1:1,000; Bethyl Laboratories, Inc) coated to the plate was used to construct a standard curve by plotting known concentrations versus optical density values at 450 nm.[7] Values were converted from nanograms per milliliter to kilo units per liter by dividing by a factor of 2.4.[8]

Statistics:

Data pre-processing:

Predictors with low variance or with more than 30% missing data from either positive or negative OFC subgroups were excluded. Continuous variables were assessed for normality using QQ plots and log-transformed if non-normal. If continuous variables with a correlation >0.75 were found, only one was used for logistic regression modelling. Finally, the Random Forest algorithm was used for data imputation for remaining missing values.

Descriptive statistics:

Means and standard deviations are presented for quantitative data, while percentages are presented for categorical data. Differences between positive and negative challenges by each predictor were assessed using Fisher's exact test for categorical data and unpaired Student's t-test for quantitative data.

Model generation and evaluation:

Univariate logistic regression analysis was performed to identify promising predictors ($p < 0.2$), before stepwise backward multivariate Firth bias-reduced logistic regression analysis was used to create the final model. Firth bias-reduced logistic regression was chosen instead of traditional logistic regression due to its better performance with relatively smaller sample sizes and imbalanced outcomes.[9] We present odds ratios (OR) for each variable alongside 95%

confidence intervals (CI). For continuous variables, the OR represents the increase in the odds for a positive challenge for each unit increase of that predictor. Age and SPT were each analyzed as both dichotomous and continuous variables. Age was dichotomized so that patients aged 7 years or older were considered in the high age group, while those below 7 years old were placed in the low age group. SPT was dichotomized so that patients with SPT measures of 8mm or greater were defined as in the high SPT group. These cut-off values were selected based on previous studies.[10] For continuous variables identified in the final model, cut-off threshold values for maximum specificity were also determined.

Discriminatory performance of the final model was evaluated via its receiver operator characteristics (ROC) curve. Further validation and generation of a 95% CI was also performed with 3-fold cross-validation with 10,000 bootstrap replicates. All analyses were performed using the open-source software R (R Core Team, 2022) and RStudio (Rstudio Team, 2022).

RESULTS

Only 73 (5.2%) observations were missing from the dataset: asthma (4 cases), eczema (6), allergic rhinitis (4), previous epinephrine use (22), ALA-sIgE (5), BLG-sIgE (5), CAS-sIgE (5), total CM-sIgE (11). Of these, total CM-sIgE had more than 30% missing values for negative OFC patients and was thus discarded.

Clinical and demographic profiles of patients are listed in Table 1. Among the 111 children who underwent an OFC, 103 patients had a positive reaction, while 8 tolerated the challenge. Specific IgE levels for ALA, BLG, and CAS were highly right-skewed and non-normal and were therefore log-transformed (Figure 1, Supplementary Figure 1).[12] There was moderate correlation between the log-transformed values (Supplementary Figure 2). Descriptive analysis revealed significant differences between positive and negative OFC groups for asthma, previous

epinephrine use, and log-transformed ALA-, BLG-, and CAS-sIgE (Table 1). While not significant, the difference in SPT tests ≥ 8mm between groups had a p-value of 0.075.

Univariate logistic regression analysis revealed that a history of asthma, previous epinephrine use, and log-transformed ALA-, BLG-, and CAS-sIgE were significantly associated with positive OFC (Table 2). These results were not observed with non-transformed sIgE components. The final multivariate model included two significant factors: log-transformed BLG (aOR 2.5; 95% CI 1.4 - 6.5; $p < 0.001$) and previous epinephrine use (aOR 7.6; 95% CI 1.4 – 79.5; $p = 0.02$). The final multivariate model itself showed good discriminatory performance (AUC 0.92, 95% CI: 0.78 - 0.99); at its optimal threshold of 0.804, the sensitivity and specificity were 0.922 and 0.875, respectively. By itself, a threshold log-transformed BLG value of 1.9 had a specificity and sensitivity of 1.00 and 0.738, respectively. While the total ImmunoCap values were excluded from analysis, even if their missing data was imputed and included in the model, it was removed during the stepwise backward multivariate Firth bias-reduced logistic regression.

Table 1: Patient Characteristics and Comparison Between Negative and Positive Challenge Groups

Variable, n (%)	**Total (N = 111)**	**Negative Challenge (N = 8)**	**Positive Challenge (N=103)**	**P-Value†**
Sex (male), n (%)	64 (57.7)	5 (62.5)	59 (57.3)	1
High age (≥ 7 years old)	95 (85.6)	8 (100)	87 (84.5)	0.6
Age **Mean (S.D.)** **Median (Min, Max)**	 10.8 (3.6) 10.0 (5.0, 18.0)	 13.1 (4.1) 14.5 (7.0, 17.0)	 10.6 (3.6) 10 (5.0, 18.0)	0.131
History of anaphylaxis to milk	60 (54.1)	3 (37.5)	55 (53.4)	0.476
Previous use of epinephrine	48 (43.2)	1 (12.5)	47 (45.6)	0.091

Asthma	91 (82.0)	4 (50.0)	87 (84.5)	0.066
Eczema	64 (57.7)	4 (50.0)	60 (58.3)	1
Seasonal allergy	69 (62.1)	4 (50.0)	65 (63.1)	1
Low skin prick test (≤ 8 mm)	62 (55.9)	7 (87.5)	55 (53.4)	0.075
Skin prick test (mm) **Mean (S.D.)** **Median (IQR)**	 7.6 (4.2) 7.0 (0.0, 25.0)	 3.6 (2.9) 3.5 (0.0, 8.0)	 7.9 (4.2) 7.0 (0.0, 25.0)	0.004*
ImmunoCAP sIgE (kU/L) **Mean (S.D.)** **Median (IQR)**	 47.7 (38.7) 34.2 (0.11, 101)	 5.9 (7.5) 3.4 (0.11, 16.9)	 49.4 (38.5) 38.5 (0.21, 101)	<0.001*
Log-transformed ALA-sIgE (kU/L) **Mean (S.D.)** **Median (IQR)**	 3.2 (1.8) 3.5 (0, 7.6)	 1.1 (1.4) 0.48 (0.0, 3.5)	 3.4 (1.7) 3.6 (0.0, 7.6)	0.005*
Log-transformed BLG-sIgE (kU/L) **Mean (S.D.)** **Median (IQR)**	 3.3 (2.3) 3.1 (0.0, 8.5)	 0.50 (0.73) 0 (0.0, 1.9)	 3.5 (2.3) 3.5 (0.0, 8.5)	<0.001*
Log-transformed CAS-sIgE (kU/L) **Mean (S.D.)** **Median (IQR)**	 3.6 (2.3) 3.6 (0.0, 8.5)	 0.62 (0.99) 0.19 (0.0, 2.7)	 3.8 (2.2) 3.97 (0.0, 8.5)	<0.001*

* Indicates statistical significance ($p<0.05$)
† Differences between positive and negative challenges were assessed using Fisher's exact test for categorical data and unpaired Student's *t*-test for quantitative data.
Note: All values reported in this table are based on data without imputations.

Table 2: Univariate Logistic Regression Model Assessing Factors Associated with a Positive Oral Food Challenge to Cow's Milk

Factors	**OR (95%CI)**	**P-Value**
Sex (Male)	0.805 (0.211, 2.73)	0.774
Asthma	6.923 (1.917, 25.20)	0.011*
Eczema	1.64 (0.475, 5.671)	0.501
Seasonal allergy	1.861 (0.538, 6.44)	0.400
Previous anaphylaxis to CM	1.910 (0.564, 7.273)	0.392
Previous epinephrine use	9.022 (1.968, 92.36)	0.043*
Low SPT measurement (<8mm)	0.164 (0.016, 0.750)	0.096
Log-transformed ALA-sIgE	2.396 (1.549, 4.131)	0.003*
Log-transformed BLG-sIgE	2.930 (1.691, 6.945)	0.009*

Log-transformed CAS-sIgE	3.000 (1.748, 6.578)	0.005*
* Indicates statistical significance (p<0.05)		

Table 3: Adjusted Odds Ratios for Final Model After Stepwise, Backward, Multivariate Firth Bias-Reduced Logistic Regression Analysis

Factors	aOR (95%CI)	P-Value
Previous Epinephrine Use	7.627 (1.408, 79.54)	0.02*
Log-transformed BLG-sIgE	2.498 (1.442, 6.472)	<0.001*
* Indicates statistical significance (p<0.05)		

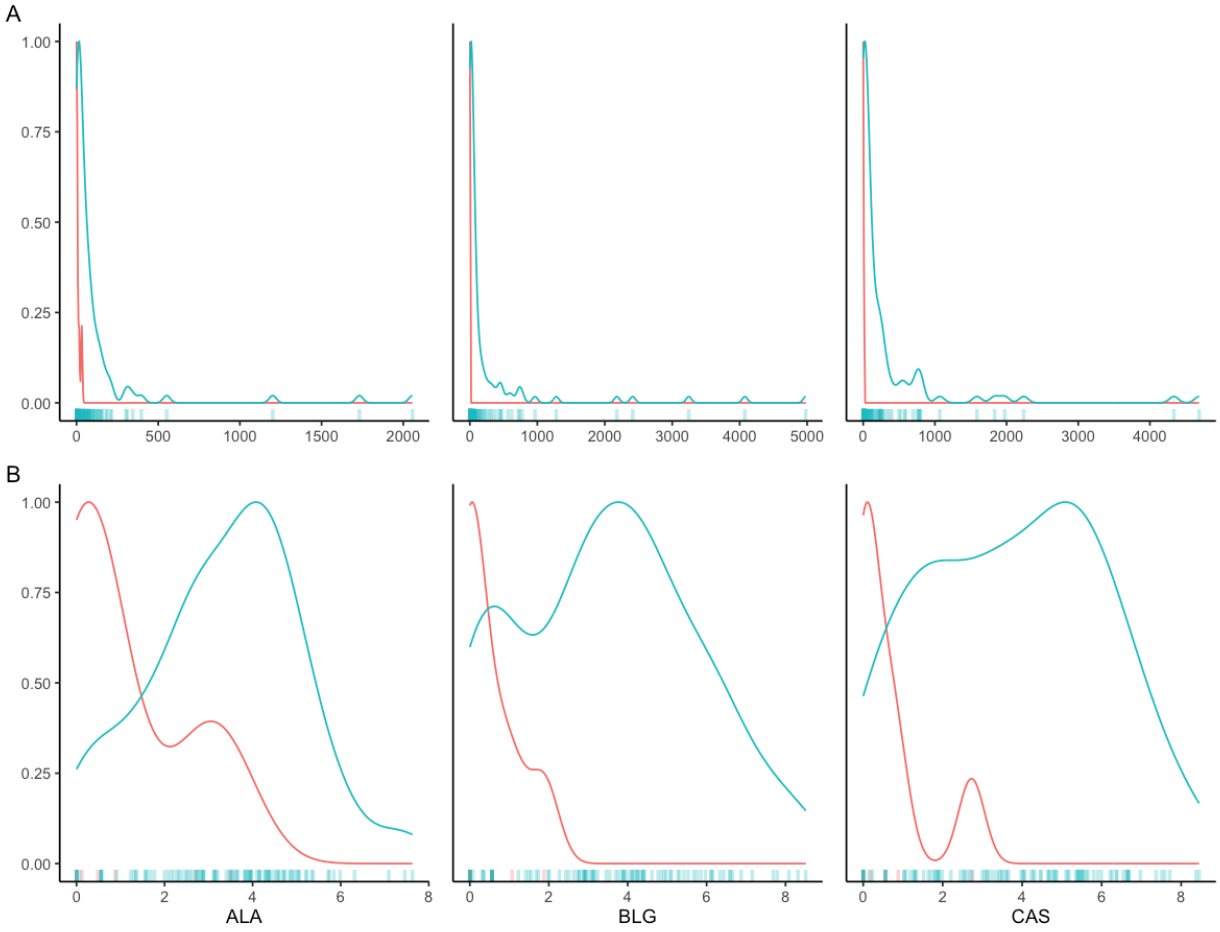


FIGURE 1. Scaled density plots with overlaying rug plots of ALA-, BLG-, and CAS-specific IgE levels. Negative OFC outcomes are in red and positive OFC outcomes are in blue. Original data is displayed in A, while log-transformed data is in B.

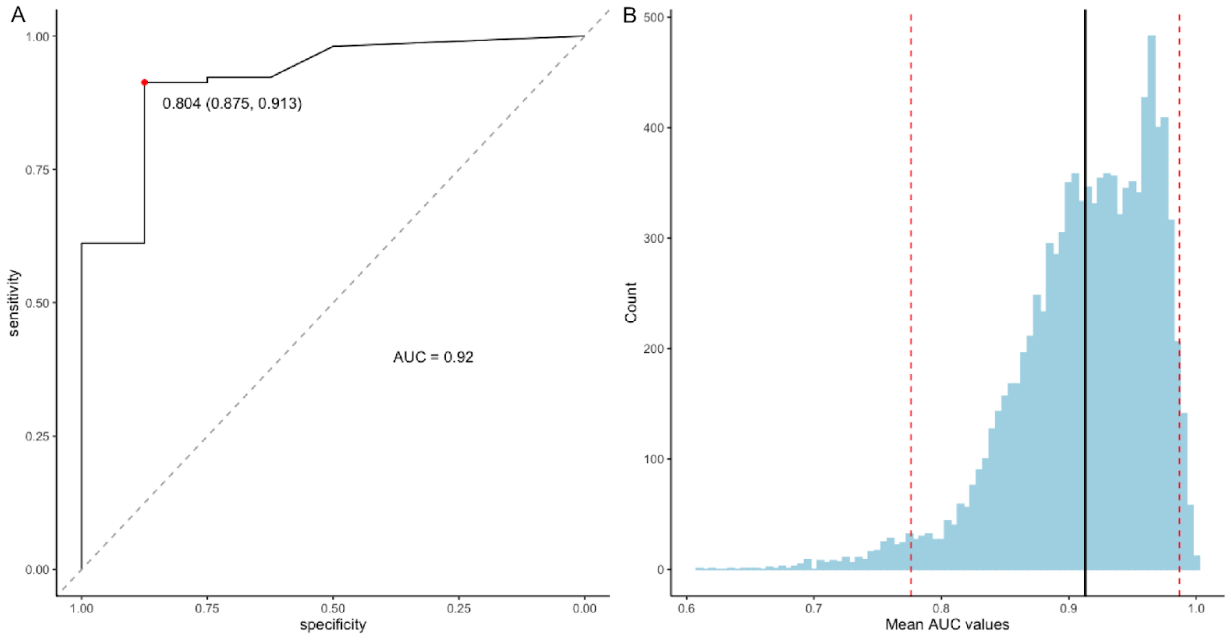


FIGURE 2. A) ROC curve for in-sample multivariate Firth bias-reduced logistic regression, with previous epinephrine use and log-transformed BLG-specific IgE as predictors. B) Mean AUC values after 3-fold cross validation of 10,000 bootstrap replicates, with the 95% confidence interval indicated by dashed red lines. Overall mean AUC was 0.92 (95% CI 0.78 - 0.99).

DISCUSSION

Data regarding predictors for positive oral food challenges are sparse. We have conducted the largest Canadian study assessing predictive models for positive OFCs in children with severe CM allergy. Our study reveals that log-transformed BLG-sIgE and previous epinephrine use are useful in predicting OFC outcomes in pediatric patients. We have developed a model to predict true CM allergy with high specificity (0.875) and sensitivity (0.913). A threshold log-transformed BLG value of 1.9 was found to have a specificity and sensitivity of 1.00 and 0.738, respectively, for predicting positive OFC results. Log transformed components seem more accurate to diagnose true CMA as compared to traditional diagnostic tests.

A smaller, retrospective study that aimed to determine values of CM-sIgE and ratios of CM-sIgE and its components to total IgE developed a model for prediction of OFC outcome.[11] A

cut-off value of CAS-sIgE >0.95kU/L was found to be 88.9% sensitive and 90.9% specific, and when combined with a compatible history of CMA, could accurately diagnose CMA without need for an OFC.[11] This study differs from ours in that we performed a logarithmic transformation on the data to eliminate any potential confounding effect by the lack of normality present in our data set. Previous studies have found that the utilization of logarithmic transformation enables accurate statistical inference for immunologic data with a positive skew.[12] However, this has not yet been done with ALA, BLG, or CAS sIgE values. A consequence of employing this technique is the determination of the geometric mean, which proves to be a more reliable measure of central tendency for this type of data compared to the typical sample mean.[12] Apart from use in allergy diagnostics, associations have been found between CM-sIgE component levels and milk OIT outcomes. Previous studies by our group found that high sIgE levels to total CM and its components are associated with a decreased likelihood of reaching the maintenance dose during OIT.[7]

Not only has component testing been found to be highly predictive of the presence of CMA in the absence of OFC but it has also been found to be useful in the diagnosis of other food allergies. Recent studies underlined the deficits in allergy diagnostics, especially for peanut-allergic patients who have intermediate positive results on first-line diagnostic tests, such as the SPT or sIgE testing.[13] Increased sIgE levels for the peanut protein component Ara h 2 have been associated with increased likelihood of true peanut allergy.[13, 14] Therefore, Ara h 2 component testing might be a useful second-line test to accurately identify patients with true peanut allergy without the need for an OFC.[13, 14] High ratios of IgE/IgG_4 to ovalbumin and ovomucoid in egg-allergic patients have been found to be associated with the need for treatment with epinephrine during OFC to baked and raw egg.[15] This group also developed an accurate logistic regression model that predicts baked egg

reactivity and includes the interactions between IgE and IgG_4 to ovalbumin and ovomucoid.[15] Allergen component testing has proven to be useful in the identification of multiple different food allergies and provides an additional measure that can serve to decrease the need for OFCs by improving accuracy of allergy diagnostic tests and models.[10, 13-15]

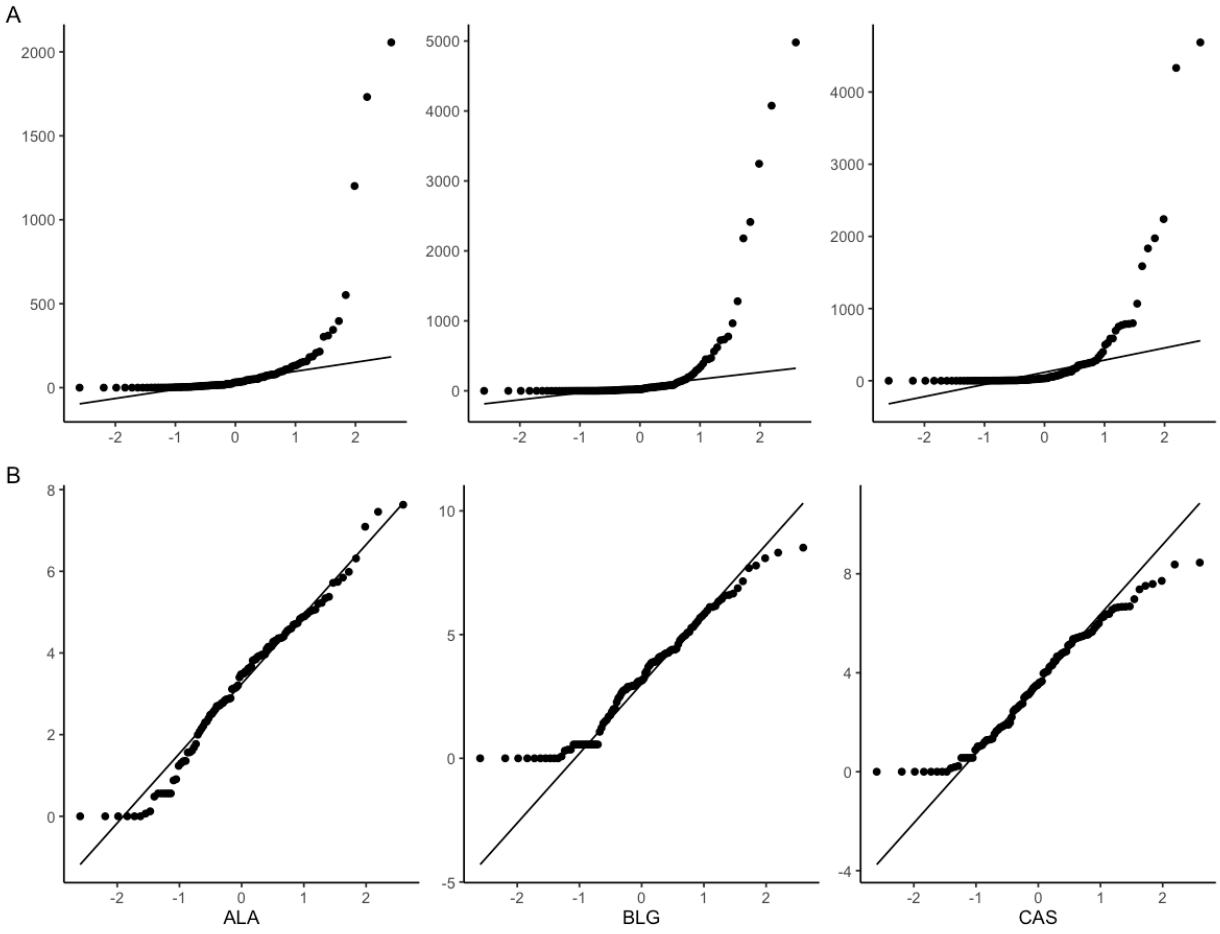
A recent study reported that individuals experience reproducibly stereotypic allergic reactions over time.[16] Hence, it is likely that those who experienced anaphylaxis in the past and require epinephrine are more likely to react during challenge. Indeed, it was suggested in other studies that previous use of epinephrine is a predictor of severe reactions.[16, 17] However, no study thus far has incorporated this factor into a model including component-sIgE.[16, 17]

The hesitancy of some patients to undergo an OFC and lack of access to an allergist are barriers to establish true food allergy.[18] Previous use of machine learning in various clinical domains has successfully predicted patient outcomes, yet few attempts have been made to utilize it in predicting OFC outcomes.[18] The machine learning method with the highest performance in terms of AUC was learning using concave and convex kernels for CMA and peanut allergy with an AUC of 0.94 and 0.91, respectively. The Random Forest machine learning method was reported to have the highest predictive performance for egg OFCs.[18] Machine learning predictions were based on patient demographics, comorbidities, sIgE levels, SPT results, and clinical rationale for administering an OFC.[18] Nevertheless, this study highlights the importance of incorporating other factors, such as log-transformed component-sIgE levels, to improve the accuracy and performance of predictive models. Therefore, adjusting machine learning and predictive modeling based on emerging research remains critical in the development of an accurate tool for the diagnosis of true food allergy, without the need for an OFC.

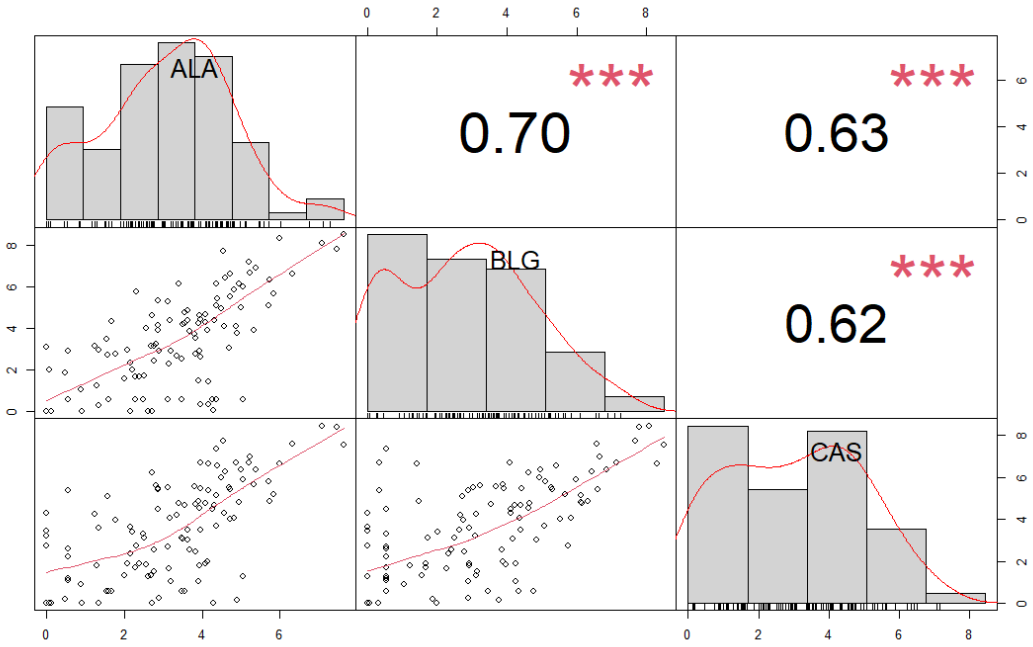
Our study has some potential limitations. Firstly, we had a relatively small sample size with imbalanced outcomes. Differently skewed variables and a small sample size may affect the associations evaluated and the generalizability of findings. However, we still expect the results to be robust given the statistical measures used, and similar performance after cross validation. Secondly, data imputations were made to account for missing values using a Random Forest algorithm. While multiple data imputations increase the risk of statistical bias, we minimized this bias by excluding variables with over 30% missing data from either positive or negative subgroups.

In conclusion, we conducted the largest study in Canada and the first study to utilize log-transformed data in evaluating predictive models for OFC outcomes in patients with CMA. Our findings suggest that components, such as log-transformed BLG-sIgE levels, and previous epinephrine use may be useful in predicting OFC outcomes in pediatric patients. Although not without its limitations, we believe our model may serve as a tool to establish the presence of true CMA and reduce the need for OFC. Future studies should attempt to use modelling for other food allergies and use larger sample sizes in order to accurately and reliably detect true food allergy without the need for OFCs.

Supplementary Figures



Supplementary Figure 1: QQ-plots of ALA-, BLG-, and CAS-sIgE levels before (A) and after (B) log-transformation.



Supplementary Figure 2: Correlation plot between log-transformed ALA-, BLG-, and CAS-sIgE predictors, using Pearson product-moment correlation coefficient. ***: p<0.001.

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Preamble to Manuscript 2

Following a diagnosis of food allergy, patients are educated on the risks associated with being exposed to their allergen and how to treat a potential reaction following exposure. Despite practicing strict avoidance, accidental or unintentional exposure remains the main culprit for triggering allergic reactions in patients with diagnosed food allergy. In a large, American, national registry surveying 4075 patients with food allergies, half of patients (50.5%) reported experiencing at least one food related allergic reaction per year.[63] Of those that had allergic reactions, over 80% were as a result of unintentional exposure, of which more than half cited cross-contamination as the reason for unintentional exposure.[63] This registry reported that 70.2% of patients who had

reactions treated their reaction with H1 - antihistamines, as compared to 22.8% for epinephrine.[63] Therefore, despite strict avoidance, accidental exposure to a food allergens is common, yet it is often not treated with epinephrine. These findings are consistent with previous reports on the underutilization of epinephrine in the management of anaphylaxis.[11, 64] My second manuscript assesses the role of epinephrine, H1 - antihistamines, and corticosteroids in the management of anaphylaxis, to further inform on the importance of prompt epinephrine administration in cases where anaphylaxis is suspected.

Manuscript 2: Managing Anaphylaxis – Epinephrine, H1 - antihistamines, and Corticosteroids: Over 10 years of C-CARE Registry Data

Managing Anaphylaxis – Epinephrine, H1 - antihistamines, and Corticosteroids: Over 10 years of C-CARE Registry Data

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Abbreviations

C-CARE	Cross-Canada Anaphylaxis REgistry
ED	Emergency department
ICU	Intensive care unit
ICD-10	International Classification of Diseases, Tenth Edition

IQR	Interquartile range
IV	Intravenous
OR	Odds ratio
USA	United States of America
95%CI	95th percent confidence intervals

Competing interests

Moshe Ben-Shoshan reports consultant fees from Pfizer, ALK Abello, Bausch Health, Kaleo, Sanofi, Food Allergy Canada, all outside the submitted work. Julia Upton reports research support/grants from Novartis, Regeneron, ALK Abello, DBV Therapeutics, CIHR, SickKids Food Allergy and Anaphylaxis Program, and fees from Pfizer, ALK Abello, Bausch Health, Kaleo, Astra Zeneca and Food Allergy Canada, all outside the submitted work. Elissa Abrams is an employee of public health agency of Canada (PHAC); views expressed are her own and not those of PHAC. Jennifer Gerdts is an employee of Food Allergy Canada. Food Allergy Canada receives a portion of its funding from Pfizer, Bausch Health and the American Peanut Council. Ann. E. Clarke has received research funds from GlaxoSmithKline and honoraria from AstraZeneca, BristolMyersSquibb, and GlaxoSmithKline, all outside the submitted work. Jennifer LP Protudjer is Section Head of Allied Health, Canadian Society of Allergy and Clinical Immunology; sits on the steering committee for Canada's National Food Allergy Action Plan, and reports consultancy for Novartis, Nutricia and ALK-Abelló, all outside the submitted work. Christine McCusker has served on scientific advisory boards for Sanofi Adventis and Bausch Health Canada, all outside the submitted work. Edmond. S. Chan has received research support from DBV Technologies; has been a member of advisory boards for Pfizer, Miravo, Medexus, Leo Pharma, Kaleo, DBV, AllerGenis, Sanofi Genzyme, Bausch Health, Avir Pharma, AstraZeneca, ALK; is on the Executive of the CSACI (Canadian Society of Allergy and Clinical Immunology); is on the

Executive of the CPS (Canadian Paediatric Society) Allergy Section; and is a member of the healthcare advisory board for Food Allergy Canada, all outside the submitted work. Andrew O'Keefe has received consultant fees from Sanofi, Astra-Zeneca, Novartis, PediaPharm, Innomar, ALK, Takeda, all outside of the submitted work. All other authors have no competing interests to declare.

The lead author (LDC) affirms that the manuscript is honest, accurate, and a transparent account of the study being reported. No important aspects of the study have been omitted.

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Trial Registration

Trial registration: not applicable.

INTRODUCTION

Anaphylaxis is defined as an acute, hypersensitivity reaction, often but not always allergic in nature, that can be severe and may be fatal if not treated appropriately.[1-3] It is estimated that at least 1.6% of the United States of America (USA) population have experienced anaphylaxis.[4] In the USA it is reported that there are 63 to 99 deaths per year ($0.8/10^6$ population) from anaphylaxis.[5] Fatality for food induced anaphylaxis, the most common cause of anaphylaxis, is estimated to occur in approximately 0.03 to 0.3 deaths per million person years.[4, 5, 6]

Management guidelines highlight the importance of prompt epinephrine administration as the first-line treatment for anaphylaxis.[1, 2, 7] In light of no known contraindications to the use of epinephrine, it should be administered to all patients with suspected anaphylaxis.[1] Nevertheless, H1 - antihistamines and corticosteroids, which may also play a role in anaphylaxis management, often replace epinephrine in the prehospital setting, despite little evidence supporting their use.[2, 7, 8] Although the occurrence of fatal anaphylaxis is rare, delayed epinephrine administration and severe anaphylaxis are significant risk factors for both fatal anaphylaxis and biphasic anaphylaxis.[4, 9] Despite numerous reports attempting to evaluate the role of H1 - antihistamines and corticosteroids in the management of anaphylaxis, there is currently little convincing evidence to establish the effectiveness of these medications in the treatment of acute anaphylaxis and prevention of biphasic anaphylaxis.[10]

Due to the ethical, financial, and clinical implications of assessing this through a randomized controlled trial, anaphylaxis management is examined through large cohort studies collecting early treatment data. To this end, we aimed to determine the impact of prehospital treatment of anaphylaxis with epinephrine, H1 - antihistamines, and/or corticosteroids on primary outcomes including uncontrolled reactions (defined as 2 or more doses of epinephrine in the emergency department (ED)), need for IV fluids in the ED, and hospital admission.

METHODS

Study Design

This is a multicenter, international, observational study. Data on patients presenting to the emergency department (ED) of one Israeli and 10 Canadian hospitals with anaphylaxis were collected. Data were collected prospectively at the time of presentation or retrospectively through chart review between April 2011 and August 2022, as part of the Cross-Canada Anaphylaxis

REgistry (C-CARE). Prior to approaching patients, a trained research assistant first confirmed the anaphylaxis diagnosis with the treating physician, at which point informed consent was obtained from patients and families (for those < 15 years). Upon consent, a standardized survey documenting symptoms, triggers, and anaphylaxis management was completed. Information was collected from patients, their medical record, and the treating team. Data on cases that were not recruited at time of presentation to the ED were collected retrospectively. Retrospective cases were identified through a previously validated algorithm based on the International Classification of Disease (Tenth Revision; ICD-10) codes related to allergic reaction/anaphylaxis.[2, 11] Anaphylaxis was defined as the involvement of two or more organ systems after exposure to a possible allergen, or hypotension after exposure to a known allergen.[2, 11, 12] Patients who did not meet the definition of anaphylaxis according to their treating physician and by two authors (LDC and SG) did not satisfy the inclusion criteria and were thus not eligible for recruitment into the registry. Uncontrolled reactions were defined as reactions needing two or more doses of epinephrine in the ED. Data collected from centers in other provinces (Ontario, British Columbia, Alberta, Newfoundland, and Labrador), as well as in Israel, were initially sent to the Quebec center, and were verified based on the diagnostic algorithm. Only cases fulfilling the definition of anaphylaxis were included. The C-CARE study was developed in conjunction with Food Allergy Canada, the largest food allergy patient organization in Canada.

Setting

The Montreal Children's Hospital, Hôpital Sainte-Justine, the Royal Victoria Hospital, the Montreal General Hospital, and Hôpital Sacré-Coeur constituted the five Quebec EDs contributing patients presenting with anaphylaxis to the C-CARE study. Additionally, patients were recruited from The Hospital for Sick Children in Toronto, Ontario, London Health Sciences Center in

Western Ontario, British Columbia Children's Hospital in British Columbia, the Foothills Medical Center in Alberta, Janeway Children's Health and Rehabilitation Center in Newfoundland and Labrador, and the Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, Israel.

Patient and Public Involvement

The research, preparation, and submission of this manuscript did not involve any patients or members of the public.

Participants

Children and adults presenting to the previously mentioned EDs with anaphylaxis were recruited for the C-CARE cohort study. Participants who did not meet the definition of anaphylaxis were excluded. Criteria were assessed by two authors (LDC and SG), and in cases of controversy a third reviewer (MBS) confirmed the presence of anaphylaxis.

Outcome

The primary outcomes were uncontrolled reactions (defined as the use of two or more doses of epinephrine in the ED), no epinephrine use prior to arrival at the ED, the use of intravenous (IV) fluids in the ED, and hospital admission (either intensive care unit (ICU) or hospital ward admission).

Independent Variables

Assessed variables included demographics factors (age and sex), clinical characteristics of the reaction (trigger, symptoms, and severity), comorbidities (presence of physician diagnosed asthma and physician diagnosed food allergies, and daily use of medication, including beta blockers, angiotensin-converting-enzyme (ACE) inhibitors, monoamine oxidase inhibitors, angiotensin receptor antagonists, tricyclic antidepressants, and non-steroidal anti-inflammatory

drugs (NSAIDs)), location of the reaction (home, school, work, restaurant, and other), cofactors for anaphylaxis (exercise within 1 hour of reaction, cold exposure, alcohol consumption, and other), administration of epinephrine, corticosteroids, and H1 - antihistamines both in ED and in the prehospital setting. Furthermore, we attempted to classify anaphylaxis severity using a modified grading system described by Muraro et al., which was independent of the assessment made by the treating physician.[13] Mild reactions were characterized as generalized pruritis, urticaria, flushing, angioedema, nausea or vomiting, mild abdominal pain, sneezing and/or nasal congestion, throat tightness, rhinorrhea, mild wheezing, anxiety, and tachycardia. Moderate reactions were defined as crampy abdominal pain, recurrent vomiting, diarrhea, "barky" cough, hoarse voice, difficulty swallowing, dyspnea, moderate wheezing, and light headedness. Finally, severe reactions were defined as loss of bowel control, cyanosis, respiratory arrest, hypotension and/or circulatory collapse, dysrhythmia, severe bradycardia and/or cardiac arrest, confusion, and loss of consciousness.[2, 13] Reaction severity was scored based on clinical symptoms reported by the patient or their parent collected by the study questionnaire.

Statistical Analysis

Patients' demographics and reaction characteristics were displayed as percentages for categorical variables and by median (interquartile range [IQR]) for continuous variables. Associations between primary outcomes and age, sex, food trigger, clinical characteristics of the reaction (severity as defined by symptoms), prehospital epinephrine administration, prehospital antihistamine administration, prehospital corticosteroid administration, reported history of physician diagnosed food allergy, reported history of physician diagnosed asthma, reactions taking place at home, and reactions taking place in Israel were examined via multivariate regression, reported as odds ratios (OR) and 95% confidence intervals (95%CI). All statistical analysis was

performed using the R version 4.2.2, statistical software (R Core Team [2022]; R Foundation for Statistical Computing, Vienna, Austria).

Ethics

This study was granted ethical approval by the Ethics Committees of all participating hospitals (ethics code: 2011-933-10-203 GEN).

RESULTS

Between April 2011 and August 2022, 5364 cases of anaphylaxis were identified across all involved EDs, with 2098 (39.1%) collected prospectively. Age, sex of subjects, and severe reactions were similarly distributed between prospective and retrospective patients (eTable 1). The median age was 8.8 (IQR, 3.78, 16.9) years, (Table 1) with a maximum age of 89.2 years and a minimum of 0.1 years. More than half of patients (54.9%) were male, half of patients (52.5%) reported a history of physician diagnosed food allergy, and 14.7% had a history of physician diagnosed asthma. The most common anaphylaxis treatment prior to arriving at the ED was H1 - antihistamines, followed by intramuscular epinephrine and short – acting inhaled β – agonists, representing 44.3%, 37.9%, and 6.8% respectively. In the prehospital setting, treatment with epinephrine alone was administered in 19.0% of moderate or severe reactions and 14.2% of mild reactions. Treatment with H1 - antihistamines exclusively was administered in 22.9% of moderate or severe reactions and 30.4% of mild reactions. Combined treatment of epinephrine and H1 - antihistamines prior to arrival at the ED was administered in 13.6% and 12.4% of moderate or severe and mild reactions, respectively.

In the ED, reactions were treated with H1 - antihistamines (51.8%), epinephrine (46.3%), and corticosteroids (40.1%). Nearly half of patients having moderate and severe reactions (46.9%)

and nearly half of patients having mild reactions (44.4%) received epinephrine. When combining both prehospital treatment and treatment given in the ED, a total of 4116 (76.7%) patients were treated with epinephrine for their reaction. Regardless of reaction severity, 250 (4.66%) patients required two or more doses of epinephrine in the ED, corresponding to an uncontrolled reaction, while 5114 patients had a controlled reaction, requiring less than 2 doses of epinephrine in the ED. Nearly one quarter (23.0%) of reactions were not treated with epinephrine at all for their reaction. A total of 286 (5.34%) patients out of 5364 were admitted following their reaction, 47 (0.88%) to the intensive care unit, and 239 (4.46%) to a ward. No fatalities occurred among the 5364 reactions.

Reactions requiring two or more doses of epinephrine in the ED were more likely in older patients [Adjusted odds ratio (aOR) 1.0005 (95% CI, 1.0001, 1.0008)], or males [aOR 1.0263 (1.0147, 1.0381)], or if triggered by peanut [aOR 1.0220 (1.0054, 1.0390)], or if severe [aOR 1.0974 (1.0742, 1.1210)] (Table 2). Reactions were less likely to require two or more doses of epinephrine in the ED if treated with prehospital epinephrine [aOR 0.9552 (0.9434, 0.9670)] or prehospital H1 - antihistamines [aOR 0.9786 (0.9675, 0.9898)], while adjusting for different food triggers, prehospital corticosteroid administration, prehospital beta-agonist administration, reported history of physician diagnosed food allergy, reported history of physician diagnosed asthma, and reactions occurring at home.

Reactions not treated with prehospital epinephrine prior to arrival at the ED were more likely to be triggered by shellfish [aOR 0.9212 (0.8628, 0.9644)], or to occur at home [aOR 1.0376 (1.0119, 1.0639)], or to occur in Israel [aOR 1.2251 (1.1342, 1.3233)] (Table 3). Reactions were more likely to be treated with prehospital epinephrine if triggered by cows' milk [aOR 0.9212 (0.8628, 0.9644)], or if occurring in patients with a reported history of physician diagnosed food allergy [aOR 0.7282 (0.7097, 0.7471)], or if severe [aOR 0.8958 (0.8551, 0.9384)], while adjusting

for age, sex, different food triggers, prehospital antihistamine administration, and reported history of physician diagnosed asthma.

Reactions requiring IV fluid administration in the ED were more likely to occur in older patients [aOR 1.0052 (1.0046, 1.0057)], or if reaction was severe [aOR 1.2531 (1.2174, 1.2899)], or if prehospital corticosteroids were administered [aOR 1.0594 (1.0133, 1.1077)] (Table 4). Reactions were less likely to require IV fluid administration if prehospital epinephrine [aOR 0.9761 (0.9599, 0.9925)] was administered, while controlling for male sex, different foods as triggers, prehospital epinephrine administration, reported history of physician diagnosed food allergy, reported history of physician diagnosed asthma, and reactions taking place at home.

Reactions were more likely to result in hospital admission if occurring in older patients [aOR 1.0040 (1.0035, 1.0045)], if reaction was severe [aOR 1.0724 (1.0432, 1.1025)], or if prehospital corticosteroids [aOR 1.2328 (1.1814, 1.2864)] were administered (Table 5). Reactions were less likely to result in hospitalization if triggered by tree nuts [aOR 0.9607 (0.9374, 0.9845)] or shellfish [aOR 0.9447 (0.9041, 0.9870)], or if treated with prehospital epinephrine [aOR 0.9645 (0.9492, 0.9800)] or prehospital H1 - antihistamines [aOR 0.9634 (0.9493, 0.9777)], or if occurring at home [aOR 0.9519 (0.9379, 0.9660)], while adjusting for sex, different food triggers, reaction severity, prehospital administration of inhaled beta-agonists, reported history of physician diagnosed food allergy, and reported history of physician diagnosed asthma.

DISCUSSION

This is the largest study assessing the outcomes of prehospital treatment of anaphylaxis. Our study demonstrates important relationships between prehospital medications and anaphylaxis outcomes. Our study establishes the protective effects of prehospital epinephrine or prehospital H1 - antihistamines in the management of anaphylaxis and highlights, for the first time, the potential

harmful effects of prehospital corticosteroids. Prehospital epinephrine or prehospital H1 - antihistamines were both associated with a lower likelihood of requiring two or more doses of epinephrine in the ED, and a lower likelihood of hospitalization, and prehospital epinephrine was associated with a lower likelihood of IV fluids administration in the ED. In contrast, prehospital corticosteroids were associated with a higher likelihood IV fluid administration in the ED, and hospitalization.

Epinephrine is first-line treatment for anaphylaxis and there are no contraindications for its use.[1, 2, 7] Given the significant risk of severe anaphylaxis and biphasic anaphylaxis associated with delayed epinephrine administration, and the potential for fatal disease, prompt epinephrine administration, regardless of anaphylaxis severity, is crucial.[4, 9, 10, 14] Studies by our group and by others have shown that delayed epinephrine administration following anaphylaxis symptom onset has been associated with a higher risk of biphasic reactions, hospital admission, and mortality.[2, 4, 5, 6, 14, 15] Despite this, we found that only approximately one-third of patients presenting to the hospital with anaphylaxis received epinephrine prior to arriving at the ED. Of these patients, almost three-quarters reported a history of physician diagnosed food allergy, 1509 of which used prehospital epinephrine. In the emergency department, epinephrine was administered to less than half of the patients, almost half of whom reported a prior history of physician diagnosed food allergy. Notably, approximately one-quarter of the patients did not receive epinephrine at all, one third of which reported a history of physician diagnosed food allergy. These findings are consistent with previous studies and systematic reviews highlighting the underuse of epinephrine by patients, caregivers, and healthcare professionals.[1, 16-18]

Histamine has a role in anaphylaxis, and H1 - antihistamines are often utilized in anaphylaxis management.[7] However, histamine is not the only mediator of anaphylaxis, thus,

treating this type of reaction exclusively with H1 - antihistamines is likely to be inadequate.[7, 10] Earlier reviews suggest that there is lack of data supporting the benefit of H1 - antihistamines in the acute management of anaphylaxis.[7, 10, 19] However, results previously published by our group and by others suggest that treatment with H1 - antihistamines may serve a supportive role in reducing cutaneous symptoms but cannot replace epinephrine.[1, 2, 7] Given that the peak onset of action of H1 - antihistamines, such as cetirizine, fexofenadine, or rupatadine, range from one to two hours, and that they are associated with less side effects, such as drowsiness, compared to first-generation H1 - antihistamines, a combined therapy of epinephrine followed by a second-generation antihistamine may be an appropriate way to manage anaphylaxis.[2, 20, 21]

The role of corticosteroids in anaphylaxis management remains controversial.[22] Currently, corticosteroids are included in multiple anaphylaxis treatment algorithms, despite studies reporting that peak plasma concentrations are only reached one to two hours after administration.[22-26] Although the clinical utility of corticosteroids is clear for asthma and croup, there is a lack of evidence supporting their role in acute management of anaphylaxis.[24] In fact, there is mounting evidence against their use. A recent study revealed that treatment of anaphylaxis with corticosteroids may increase the length of hospital admission and total hospitalization costs.[27] Our findings suggest additional negative effects of corticosteroids in anaphylaxis including higher risk of ICU/hospital ward admission and need for IV fluids in the ED. There is one randomized controlled trial on the use of epinephrine, H1 - antihistamines, and corticosteroids to reduce the risk of allergic reactions to antivenom that revealed that epinephrine had the highest protective effect.[28] This study also found that the use of corticosteroids negated the beneficial effects of epinephrine and was associated with higher risk of fatality.[28] The pathogenic pathways underlying the negative effect of corticosteroids in anaphylaxis are not clear. Studies suggest that

corticosteroids activate the glucocorticoid receptors that interact with transcription factors, leading to increases in the production of Immunoglobulin E (IgE) from β lymphocytes, stimulated with Interleukin-4 (IL-4). In addition, corticosteroids may induce CD40L in T lymphocytes, which may also activate other CD40-expressing inflammatory cells, such as macrophages and eosinophils. This would paradoxically increase inflammation in the short-term.[29] However, future studies are required in order to elucidate the pathogenic pathways leading to the negative effect of corticosteroids in the acute management of anaphylaxis.

Our study is not without its limitations. First, this was an observational study and there is always a risk of treatment bias engendered by this design, as sicker patients would normally receive more treatment both in the prehospital setting and in the ED. However, this was not demonstrated in our result and patients who received less prehospital epinephrine were at greater risk of needing 2 or more doses of epinephrine in the ED. Given the limited amount of time in the ED, our 12-question questionnaire was designed for efficient administration. However, as a result, certain data on other potential cofactors for anaphylaxis and biphasic anaphylaxis were not recorded. Approximately 60% of cases were recruited retrospectively, meaning that data on reactions were limited to the information found in the patient's medical records (eTable 1). Although certain portions of the data on anaphylaxis location were missing, anaphylaxis management data were complete for all recorded cases. Secondly, a majority of patients (71.3%) were recruited from EDs in the province of Quebec, and a minority from Newfoundland and Labrador (0.88%), Alberta (4.34%), as well as Israel (2.82%). This made it more difficult to identify differences in treatment between provinces and countries. However, the country where anaphylaxis took place was controlled for through a multivariate logistic regression model. Thirdly, our study is limited to anaphylaxis cases presenting to the ED and did not capture cases that were treated and resolved

outside the hospital. Yet, all observational studies collecting data on anaphylaxis cases presenting to the ED are subject to this limitation. Fourthly, our sample was skewed towards a pediatric population as a result of the numerous collaborating pediatric centers. However, age of patients was controlled for through multivariate logistic regression model. It is also possible that certain symptoms used to define anaphylaxis grading were challenging to record (e.g. mild abdominal pain). Finally, as our study is not a randomized controlled trial, our results reveal different associations between selected factors and outcomes but do not imply any causative relationships. Despite this, our study remains the largest and most recent analysis examining prehospital management of anaphylaxis.

In conclusion, we conducted the largest study assessing the outcomes of prehospital treatment of anaphylaxis. Our study demonstrates decreased odds of negative outcomes to anaphylaxis when promptly treated with epinephrine or H1 - antihistamines in the prehospital setting, while also highlighting the increased odds of negative outcomes to anaphylaxis when corticosteroids were administered in the prehospital setting. Our findings, therefore, re-emphasize the importance of epinephrine as first line treatment for anaphylaxis and suggest potential protective effect of H1 - antihistamines when used in the prehospital setting. They also suggest that use of corticosteroids should not be part of the current guidelines for managing anaphylaxis.

TABLES

Table 1: Anaphylaxis Management Information (N = 5364) (n, %)	
Age in years, Median (IQR)	8.80 (3.78, 16.9)
Sex (% Male)	54.9
Reported History of Physician Diagnosed Food Allergy	2817 (52.5)
Reported History of Physician Diagnosed Asthma	791 (14.7)
Administered in prehospital setting	n (%)
Epinephrine IM	2034 (37.9)
H1 - antihistamines	2377 (44.3)
Anti – H2	46 (0.86)
Short-acting inhaled β-agonist	366 (6.82)
Corticosteroids	169 (3.15)
Epinephrine only	971 (18.1)
H1 - antihistamines only	1295 (24.1)
Both epinephrine and H1 - antihistamines	717 (13.4)
Moderate and severe reactions (N=4431)*	
Epinephrine only	840 (19.0)
H1 - antihistamines only	1015 (22.9)
Both epinephrine and H1 - antihistamines	603 (13.6)
Mild reactions (n=922)*	
Epinephrine only	131 (14.2)
H1 - antihistamines only	280 (30.4)
Both epinephrine and H1 - antihistamines	114 (12.4)
Administered by healthcare professional in ED	n (%)
Epinephrine IM	2482 (46.3)
Epinephrine IV	60 (1.12)
H1 - antihistamines	2778 (51.8)
Anti – H2	943 (17.6)
Short-acting inhaled β-agonist	542 (10.1)
Corticosteroids	2135 (40.1)
Moderate and severe reactions (n=4431)	
Received epinephrine in ED†	2083 (46.9)
Mild reactions (n=922)	
Received epinephrine in ED†	409 (44.4)
Did not receive epinephrine	
Mild	282 (30.6)
Moderate	876 (21.8)
Severe	77 (18.6)
All reaction severities†‡	
Received epinephrine	4121 (76.8)
Received 2 or more epinephrine doses	849 (15.8)
Received 3 or more epinephrine doses	181 (3.37)
Received 4 or more epinephrine doses	53 (0.99)

Number of doses of epinephrine administered in the ED for all reaction severities†	
Received epinephrine	2492 (46.5)
Received 2 or more epinephrine doses	250 (4.66)
Received 3 or more epinephrine doses	52 (0.97)
Received 4 or more epinephrine doses	8 (0.15)
Admission due to anaphylaxis	
ICU	47 (0.88)
Hospital ward	239 (4.46)

Note: H1 - antihistamines refers specifically to H-1 receptor antagonists.
*Reaction severity was determined based on symptoms experienced in the prehospital setting.
†Combined epinephrine IM, epinephrine IV, epinephrine SC, and epinephrine nebulizer.
‡Combination of prehospital treatment and in hospital treatment.

Table 2: Factors Associated with Receiving Two or More Doses of Epinephrine in the ED		
	Univariate	Multivariate
Characteristics	OR (95%CI)	OR (95%CI)
Age	1.0006 (1.0002, 1.0009)*	1.0005 (1.0001, 1.0008)*
Sex (Male)	1.0249 (1.0134, 1.0366)*	1.0263 (1.0147, 1.0381)*
Allergen – Reaction Trigger		
Milk	1.0095 (0.9845, 1.0351)	1.0201 (0.9945, 1.0465)
Egg	0.9921 (0.9683, 1.0166)	1.0028 (0.9780, 1.0282)
Peanut	1.0181 (1.0023, 1.0342)*	1.0220 (1.0054, 1.0390)*
Tree Nut	0.9950 (0.9769, 1.0134)	1.0041 (0.9853, 1.0232)
Sesame	0.9762 (0.9410, 1.0126)	0.9855 (0.9501, 1.0223)
Fish	0.9721 (0.9335, 1.0123)	0.9805 (0.9418, 1.0209)
Shellfish	0.9860 (0.9530, 1.0201)	0.9845 (0.9517, 1.0184)
Reaction Severity		
Mild	0.9871 (0.9725, 1.0020)	..
Moderate	0.9744 (0.9618, 0.9871)*	..
Severe	1.1012 (1.0783, 1.1246)*	1.0974 (1.0742, 1.1210)*
Prehospital epinephrine	0.9621 (0.9510, 0.9733)*	0.9552 (0.9434, 0.9670)*
Prehospital H1 - antihistamines	0.9748 (0.9638, 0.9859)*	0.9786 (0.9675, 0.9898)*
Prehospital corticosteroids	1.0008 (0.9689, 1.0336)	1.0002 (0.9678, 1.0336)
Prehospital β-agonist	0.9940 (0.9720, 1.0165)	1.0013 (0.9787, 1.0244)
Reported history of physician diagnosed food allergy	0.9916 (0.9805, 1.0029)	1.0089 (0.9964, 1.0215)
Reported history of physician diagnosed asthma	1.0091 (0.9932, 1.0253)	1.0081 (0.9919, 1.0245)
Reaction at home	0.9944 (0.9832, 1.0057)	0.9947 (0.9835, 1.0061)
* Indicates statistical significance (P<0.05)		

Table 3: Factors Associated with No Epinephrine Administration Prior to Arrival at ED		
	Univariate	Multivariate
Characteristics	OR(95%CI)	OR(95%CI)
Age	1.0018 (1.0010, 1.0027)*	1.0000 (0.9991, 1.0008)
Sex (Male)	0.9840 (0.9587, 1.0101)	1.0088 (0.9840, 1.0342)
Allergen – Reaction Trigger		
Milk	0.8516 (0.8040, 0.9020)*	0.9212 (0.8628, 0.9644)*
Egg	1.0354 (0.9790, 1.0950)	1.0226 (0.9681, 1.0801)
Peanut	0.9135 (0.8813, 0.9469)*	0.9858 (0.9510, 1.0219)
Tree Nut	0.9866 (0.9459, 1.0291)	1.0116 (0.9705, 1.0543)
Sesame	0.9945 (0.9139, 1.0821)	1.0635 (0.9815, 1.1523)
Fish	1.0412 (0.9484, 1.1430)	1.0300 (0.9426, 1.1248)
Shellfish	1.1268 (1.0420, 1.2185)*	1.0924 (1.0143, 1.1765)*
Reaction Severity		
Mild	1.1222 (1.0843, 1.1613)*	..
Moderate	0.9393 (0.9117, 0.9678)*	..
Severe	0.9307 (0.8864, 0.9771)*	0.8958 (0.8551, 0.9384)*
Reported history of physician diagnosed food allergy	0.7192 (0.7019 0.7371)*	0.7282 (0.7097, 0.7471)*
Reported history of physician diagnosed asthma	0.9175 (0.8846, 0.9517)*	0.9743 (0.9411, 1.0087)
Reaction at home	1.0551 (1.0280, 1.0828)*	1.0376 (1.0119, 1.0639)*
Israel	1.3247 (1.2250, 1.4323)*	1.2251 (1.1342, 1.3233)*
* Indicates statistical significant (P<0.05)		

Table 4: Factors Associated IV Fluid administration in ED		
	Univariate	Multivariate
Characteristics	OR(95%CI)	OR(95%CI)
Age	1.0060 (1.0055, 1.0065)*	1.0052 (1.0046, 1.0057)*
Sex (Male)	0.9754 (0.9600, 0.9914)*	1.0064 (0.9910, 1.0220)
Allergen – Reaction Trigger		
Milk	0.9545 (0.9207, 0.9896)*	0.9962 (0.9624, 1.0311)
Egg	0.9248 (0.8930, 0.9578)*	0.9834 (0.9507, 1.0173)
Peanut	0.9790 (0.9568, 1.0010)	1.0073 (0.9851, 1.0300)
Tree Nut	0.9488 (0.9241, 0.9741)*	0.9805 (0.9557, 1.0060)
Sesame	0.9213 (0.8738, 0.9713)*	0.9610 (0.9145, 1.0099)
Fish	0.9722 (0.9170, 1.0308)	0.9726 (0.9209, 1.0272)
Shellfish	1.0509 (1.0006, 1.1037)*	1.0086 (0.9634, 1.0560)
Reaction Severity		
Mild	0.9355 (0.9156, 0.9558)*	..
Moderate	0.9451 (0.9275, 0.9629)*	..
Severe	1.3324 (1.2937, 1.3724)*	1.2531 (1.2174, 1.2899)*
Prehospital epinephrine	0.9744 (0.9582, 0.9909)*	0.9761 (0.9599, 0.9925)*
Prehospital H1 - antihistamines	0.9790 (0.9631, 0.9952)*	0.9863 (0.9712, 1.0017)
Prehospital corticosteroids	1.1763 (1.1230, 1.2322)*	1.0594 (1.0133, 1.1077)*
Prehospital β-agonist	1.0126 (0.9804, 1.0458)	1.0236 (0.9924, 1.0557)
Reported history of physician diagnosed food allergy	0.9566 (0.9411, 0.9722)*	1.0020 (0.9853, 1.0190)
Reported history of physician diagnosed asthma	0.9977 (0.9751, 1.0209)	0.9853 (0.9640, 1.0072)
Reaction at home	0.9781 (0.9623, 0.9941)*	1.0031 (0.9877, 1.0186)
* Indicates statistical significance (P<0.05)		

Table 5: Factors Associated Hospital Admission (ICU and Hospital Ward)		
	Univariate	Multivariate
Characteristics	OR(95%CI)	OR(95%CI)
Age	1.0050 (1.0046, 1.0055)*	1.0040 (1.0035, 1.0045)*
Sex (Male)	0.9626 (0.9479, 0.9774)*	0.9872 (0.9727, 1.0018)
Allergen – Reaction Trigger		
Milk	0.9542 (0.9224, 0.9871)*	0.9906 (0.9585, 1.0238)
Egg	0.9448 (0.9142, 0.9764)*	0.9917 (0.9602, 1.0244)
Peanut	0.9748 (0.9544, 0.9956)*	1.0006 (0.9796, 1.0222)
Tree Nut	0.9318 (0.9090, 0.9551)*	0.9607 (0.9374, 0.9845)*
Sesame	0.9641 (0.9174, 1.0132)	0.9936 (0.9476, 1.0419)
Fish	0.9577 (0.9066, 1.0118)	0.9517 (0.9033, 1.0027)
Shellfish	0.9892 (0.9447, 1.0358)	0.9447 (0.9041, 0.9870)*
Reaction Severity		
Mild	0.9485 (0.9295, 0.9678)*	..
Moderate	0.9859 (0.9687, 1.0034)	..
Severe	1.1373 (1.1054, 1.1701)*	1.0724 (1.0432, 1.1025)*
Prehospital epinephrine	0.9596 (0.9447, 0.9748)*	0.9645 (0.9492, 0.9800)*
Prehospital H1 - antihistamines	0.9610 (0.9463, 0.9758)*	0.9634 (0.9493, 0.9777)*
Prehospital corticosteroids	1.3074 (1.2522, 1.3651)*	1.2328 (1.1814, 1.2864)*
Prehospital β-agonist	0.9891 (0.9595 1.0195)	0.9880 (0.9592, 1.0176)
Reported history of physician diagnosed food allergy	0.9426 (0.9284, 0.9570)*	0.9846 (0.9689, 1.0006)
Reported history of physician diagnosed asthma	1.0033 (0.9819, 1.0251)	1.0013 (0.9805, 1.0225)
Reaction at home	0.9346 (0.9205, 0.9489)*	0.9519 (0.9379, 0.9660)*
* Indicates statistical significance (P<0.05)		

SUPPLEMENTS

eTable 1: Comparison of characteristics between prospective and retrospective patients.			
Variable	**Prospective (n=2098)**	**Retrospective (n=3266)**	**P-Value**
Age, median (IQR)	8.05 (2.50, 16.4)	9.2 (3.3, 17.3)	0.1838
Proportion Test: Prospective vs Retrospective (P-R)			
Variable			**Difference (95%CI)**
Sex (Male)			-2.39 (-5.15, 0.37)
Severe reaction classified according to Muraro et al. (2007)			0.67 (-0.0082, 0.0216)
Reported history of physician diagnosed food allergy			3.60 (0.68, 6.24)
Reported history of physician diagnosed asthma			2.06 (0.12, 4.02)

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Discussion

Data concerning factors that can predict oral food challenge outcomes are scarce. In order to address this gap, I undertook an extensive study in Canada, the largest and first of its kind to utilize log-transformed sIgE component levels, to assess various predictive models for positive OFCs specifically in children diagnosed with severe CM allergy. My results reveal that two factors

in particular: log-transformed BLG-sIgE levels and prior use of epinephrine are valid predictors of challenge outcome in children with milk allergy. I developed a model that has high specificity (0.875) and sensitivity (0.913) in accurately predicting the presence of true CM allergy in children with severe CM allergy. Furthermore, I identified that a log-transformed BLG value of 1.9 serves as a threshold, which, when surpassed, displays a specificity of 1.00 and a sensitivity of 0.738 in effectively anticipating positive outcomes in OFCs. The use of log-transformed components offers greater accuracy in diagnosing true CM allergy when compared to conventional diagnostic tests.

In addition to addressing the scarcity of data on predictors for OFC outcomes, I also conducted the largest and most recent study to assess the outcomes of prehospital treatments of anaphylaxis. The study revealed crucial insight into the relationship between prehospital medications and the resulting outcomes of anaphylaxis. Not only did the findings emphasize the importance of prompt epinephrine administration and potential protective effect of H1 - antihistamines, but also revealed potential harmful effects of prehospital corticosteroid administration. Both prehospital epinephrine and prehospital H1 - antihistamines were associated with a lower likelihood of requiring two or more doses of epinephrine in the ED and a lower likelihood of hospitalization. Patients treated with prehospital epinephrine were also less likely to need intravenous (IV) fluids in the ED. Conversely, the use of prehospital corticosteroids was associated with an increased likelihood of needing IV fluids in the ED and an increased likelihood of hospitalization.

Current diagnosis of IgE-mediated food allergy is based on clinical history, skin prick test (SPT) measurements and specific IgE (sIgE) levels. SPTs are relatively easy to perform, have low cost, are sensitive, but have low specificity. The SPT is reported to have a sensitivity and specificity of approximately 90% and 50% respectively.[1-4] Furthermore, SPT results may be

difficult to interpret when values are below the positive predictive value.[1, 32] A previous study assessing the implications of early peanut introduction for the prevention of peanut allergy found that up to 20% of high-risk patients (patients with either egg allergy or onset of moderate to severe eczema by 6 months old) with SPT wheal measure >4mm could tolerate peanut.[31] This study also found that 28% of patients with negative SPT had detectable peanut-sIgE.[31] Additionally, SPT cut off values vary from different age groups and food allergen being tested, further convoluting their interpretations. [1-3, 31] While SPTs have a high negative predictive value, a positive SPT is indicative of sensitization to a food but does not necessarily correlate with allergy and is not diagnostic on its own.[1, 3]

Similarly to SPTs, sIgE level interpretation is nuanced. Previous studies have found that sIgE level testing has a positive predictive value of approximately 90% to 95%, depending on the allergen and patient age, in patients with high sIgE level and is a useful tool in determining whether an OFC is necessary or not.[2, 5, 65, 66] More specifically, one study established the sensitivity of sIgE testing for egg, CM, soy, and wheat to be 97%, 83%, 69%, and 79% respectively, while the specificity was 51%, 53%, 50%, and 38% respectively.[6] Specific IgE cut offs for determining the necessity of an OFC have not been well defined for patients with low sIgE levels.[5, 32, 65] Additionally, 10% to 25% of patients with undetectable sIgE levels may still have clinical reactions upon challenge.[5] Therefore, the use of sIgE levels is limited as most cases that are difficult to interpret when levels are below the 95% positive predictive value. Like SPTs, high sIgE values are indicative of sensitization to an allergen but do not correlate with clinical allergy reaction severity, and thus on its own is not diagnostic of clinical allergy.[3] In fact, high false negative rates for sIgE were exemplified in a population based study in the US. The study reported that more than 90% of the those who have high levels of sIgE in the general population tolerate milk, egg, or peanut.[67]

As such, the OFC is considered the gold standard for the diagnosis of IgE-mediated food allergies. However, this test is also not without its drawbacks. Patients undergoing an OFC are at elevated risk of developing a severe reaction to the allergen being tested.[4, 5, 7, 32, 68] Oral food challenges are also time consuming and necessitate constant supervision of the patient, making them resource intensive.[4, 5, 7, 32, 68] Therefore, improved strategies to diagnose an IgE-mediated food allergy are required.

Recent efforts have explored the use of sIgE component testing for the diagnosis of other food allergies. Specific IgE component testing differs from conventional sIgE testing in that sIgE antibodies to individual proteins of an allergen are measured, as compared to conventional testing which measures the presence of sIgE antibodies to the whole allergen protein.[7] Component testing of the Ara h2 component has been shown to have higher sensitivity (60%) as compared to whole peanut protein sIgE (sensitivity: 26% while using 95% positive predictive value) in the identification of true peanut allergy.[8] This study has also found that using Ara h2 testing if a patient has prior SPT between 3mm and 8mm would result in a 2.5-4 times reduction in the number of OFC to establish an accurate peanut allergy diagnosis.[8] A study investigating the role of cashew protein component, Ana o 3, in cashew allergy diagnosis found that this component is more sensitive than cashew sIgE in detecting true cashew allergy with an AUC of 0.94 as compared to an AUC of 0.78.[9] Sensitivity and specificity were found to be 0.91 and 0.94 respectively when using 0.4 kUA/l as an Ana o 3 cut-off.[9] A small retrospective study investigating the use of component testing in the diagnosis of CM allergy found the casein component to have the greatest AUC (0.98), followed by the BLG component (AUC=0.923).[10]

While these studies have shown the utility of component test, none have incorporated aspects of medical history, such as previous epinephrine use within their predictive models.

Additionally, none have utilized log-transformed data to account for potential skews in data. Including this additional component to my model offers a more holistic approach to predicting OFC outcome. My group developed a comparable model with a mean AUC of 0.92, specificity of 0.875, and sensitivity of 0.913, using log-transformed BLG-sIgE values and previous epinephrine use for a CM triggered reaction. I also found that ALA, and casein are significant predictors of a positive OFC in a univariate logistic regression. These findings are in line with the previous mentioned findings. While my model does provide insight on the utility of component testing in CM allergy, future larger scale studies are needed to further develop an accessible predictive model to detect true CM allergy without the need for OFC. Doing so would decrease the need for OFC to diagnose true CM allergy, reducing the burden that OFC have on both patients and the health care system. This would also allow patients with limited access to an allergist to be accurately diagnosed and provide tailored education on the risk of anaphylaxis when coming into contact with their allergen.

Whether it is during an OFC or from accidental exposure to an allergen due to cross-contamination, anaphylaxis is a significant risk for patients diagnosed with IgE-mediated food allergy. The risk of fatality due to anaphylaxis is low, yet unpredictable.[11, 12, 69] Therefore, it is important to properly manage the reaction.[11, 12, 69] The first lime treatment for anaphylaxis is prompt epinephrine.[11, 14, 70] However, studies have shown the underutilization of epinephrine, which is often replaced with H1 - antihistamines and corticosteroids.[11, 13] There are many contributing factors to the underuse of epinephrine, such as, the increasing cost of epinephrine autoinjectors, lack of availability in schools, camps, and among patients, and incorrect technique.[11] My findings are consistent with previous reports outlining the protective effects of epinephrine and the importance of prompt administration.[14, 64, 70, 71] While some individuals with cardiovascular

conditions may be more cautious with epinephrine use, a recent review found that adverse cardiovascular events are rare and most of the time associated with incorrect dosage/administration.[11]

H1 - antihistamines have a role in treating allergic reactions, especially cutaneous reactions. However, their use in acute anaphylaxis management is scrutinized, seeing as H1 - antihistamines have no role in the treating respiratory and cardiovascular symptoms exhibited during anaphylaxis.[13, 64, 72] My study found that H1 - antihistamines decreased the odds of certain negative outcomes.[11, 14, 70] These findings complement previous works by our group and others shedding light on the potential supportive role of H1 - antihistamines.[14, 70] Guidelines recommend the use of second-generation H1 - antihistamines. First-generation H1 - antihistamines have been associated with sedation, respiratory depression, and may potentiate hypotension.[72-74] While H1 - antihistamines should not replace rapid epinephrine administration, the extended cutaneous relief they provided may contribute to a patient's overall wellbeing throughout anaphylaxis and they have a role as an adjunctive treatment.[14, 70] However, future, randomized controlled trials should be conducted to solidify their potentially supportive role in anaphylaxis management.

The potent anti-inflammatory effects of corticosteroids in the management of asthma, allergic rhinitis, and atomic dermatitis have been well documented.[75] However, multiple reviews have outlined the lack of convincing evidence surrounding their use in acute anaphylaxis management.[74, 76, 77] Despite corticosteroid peak plasma concentration levels being attained approximately one to two hours after administration, they continue to be utilized, often replacing epinephrine, in anaphylaxis management.[78] My findings show the increased odds of hospitalization and need for IV fluids in the ED following prehospital corticosteroid administration. A potential explanation for this association is that corticosteroids may have been used in preference to

epinephrine, inadequately treating the reaction. Given the rapid onset and need for immediate treatment to prevent severe or fatal anaphylaxis, it is unlikely that corticosteroids reduce the severity of anaphylaxis.[78, 79] Moreover, although corticosteroids were thought to decease the likelihood or prevent biphasic anaphylaxis, this is not supported by the evidence.[78, 80, 81] In fact, studies have pointed to potentially harmful effects of corticosteroids, such as negating the effect of epinephrine and paradoxically increasing short term inflammation and IgE production as a result of β lymphocytes being stimulated by IL-4.[14, 82-84] In line with my findings, corticosteroid use has also been associated with lengthier hospitalization, higher total hospitalization costs.[82] Seeing as there is a lack of supporting evidence for corticosteroid use in anaphylaxis management and potential negative effects, their use managing anaphylaxis should be further investigated through randomized-controlled trials.

My studies have potential limitations. While the predictive model did have high specificity and sensitivity for detecting true CM allergy, it was based on a sample of children with physician diagnosed CM allergy. As a result, this led to imbalanced outcomes, with most patients having positive challenges. Having a non-normally distributed, small sample may affect the generalizability of the findings. However, log transformation, different statistical measures, and cross validation was utilized to mitigate the potential effects of these limitations.[85] In addition to the specific sample, certain variables were incomplete and thus required data imputation for the development of a model. In order to decrease the potential statistical bias present in imputed data, only variables with less than 30% missing data from either positive or negative OFC subgroups were included in the analysis. My large cohort study on anaphylaxis management was also not without its limitations. Multiple definitions have been put forward by different research groups, all of which have their strengths and weaknesses.[24-27] The definition used in my study was

according to National Institute of Allergy and Infectious Disease (NIAID), as this definition has been associated with less bias.[27] However, having a more specific definition may result in over exclusion of patients and thus a smaller sample of patients with more specific anaphylaxis cases. As such, the true associations detected may be larger or smaller than what has been reported in my study. Additionally, some symptoms, such as hypotension, degrees of abdominal pain may introduce additional variability in the results given that they are challenging for patients to qualify.

Conclusion

Overall, my work contributes significantly to the understanding of predicting oral food challenge outcomes and optimizing prehospital treatment strategies for anaphylaxis. My studies showed the usefulness of CM sIgE component testing in the development of accurate OFC predictive models in CM allergy, and reinforced the importance of prompt epinephrine administration, potential protective effect of prehospital H1 - antihistamines administration, and potential negative effects of prehospital corticosteroid administration, in the management of anaphylaxis. These findings have the potential to inform medical practices, enhance patient care, and improve outcomes for individuals with severe CM allergy and anaphylaxis. Further research and exploration in these areas is required to better understand and establish management protocols to benefit those affected by food allergy and anaphylaxis.

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