Assessing risk factors associated with a positive graded oral challenge (GOC) in children with suspected amoxicillin allergy

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Abstract

The diagnosis of amoxicillin hypersensitivity is challenging in children given that the sensitivity of commercially available skin tests is low and oral challenges are often required to establish the diagnosis. We aimed to assess the diagnostic properties of a graded oral challenge (GOC) among children presenting with a rash during amoxicillin therapy and determine risk factors associated with positives challenges. A cohort study was conducted between March 2013, and March 2020, at the allergy clinic of the Montreal Children's Hospital and the Meadowood Medical Centre in Winnipeg. All children referred with suspected allergy to amoxicillin were recruited as part of the LAACTAM study (β-LActam and other Antibiotics allergy in Children: Tests, Assessment and Management) and a two-step GOC was conducted. Assessment of tolerance to a cephalosporin (cephalexin) was done when the GOC was positive. Eligible children were followed up for assessment of reaction to subsequent use of amoxicillin. Data were collected on demographics clinical characteristics of reaction and comorbidities. We found that an index reaction occurring within 5 minutes [aOR = 0.92; 95%CI, 0.88-0.96] and symptoms lasting more than 7 days [aOR = 1.02; 95%CI, 1.02-1.09] were associated with immediate and nonimmediate adverse reaction to the amoxicillin GOC respectively. The negative predictive value of the GOC was 85.3% which was found as a result of finding the false negative patients in our follow-up. Cross-reactivity between cephalexin and amoxicillin was evaluated at 11.5%.

GOCs provide an accurate and safe confirmatory test for adverse reactions to amoxicillin without prior skin tests. Based on the findings of this study, new strategies should be developed to appropriately diagnose amoxicillin allergy in children.

Résumé

Le diagnostic d'hypersensibilité à l'amoxicilline est difficile chez les enfants étant donné que la sensibilité des tests cutanés disponibles dans le commerce est faible et que les tests de provocation orales (TPO) sont souvent nécessaires pour établir le diagnostic. Nous avons cherché à évaluer les propriétés diagnostiques d'un test de provocation oral gradué en deux étapes chez les enfants présentant des symptômes dû à un traitement à l'amoxicilline et à déterminer les facteurs de risque associés aux tests positifs. Une étude de cohorte a été menée entre mars 2013 et mars 2020 à la clinique d'allergies de l'Hôpital de Montréal pour enfants, La Clinique pour Enfants et le Meadowood Medical Centre. Tous les enfants référés pour une allergie suspectée à l'amoxicilline ont été recrutés dans le cadre de l'étude LAACTAM (β-LActam et autres allergies aux antibiotiques chez les enfants : tests, évaluation et prise en charge) et un TPO gradué a été réalisé. L'évaluation de la tolérance à une céphalosporine (céphalexine) a été effectuée lorsque le TPO était positif. Les enfants éligibles ont été suivis pour évaluer les réactions à une utilisation ultérieure d'amoxicilline. Les données ont été recueillies sur les caractéristiques cliniques et démographiques des patients et de leurs réactions et de leurs comorbidités. Nous avons constaté que les réactions se produisant dans les 5 minutes suivant l'ingestion [aOR = 0,92; IC95%, 0,88-0,96] et des symptômes durant plus de 7 jours [aOR = 1,02; IC95%, 1,02-1,09] ont respectivement été associés à une réaction immédiate et non immédiate au TPO. La valeur prédictive négative du TPO était de 85,3%, d'après le nombre de faux négatifs dans notre suivi. La réactivité croisée entre la céphalexine et l'amoxicilline a été évaluée à 11,5%. Les TPOs fournissent un test de confirmation précis et sûr en ce qui a trait à l'allergie à l'amoxicilline sans tests cutanés préalables. Sur la base des résultats de cette étude, de

nouvelles stratégies devraient être développées pour correctement diagnostiquer l'allergie à l'amoxicilline chez les enfants.

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Contribution of researchers

Dr. Moshe Ben-Shoshan developed the idea for the thesis. The study design and protocol for B-LACTAAM were developed by Dr. Ben-Shoshan. Data collection, which involved recruiting patients in the allergy clinics and contacting patients annually for follow-up, was conducted by Rutherford Exius, Sofianne Gabrielli and Gregory Gooding at the Montreal Children's Hospital, and Dr. Elissa Abrams in Winnipeg. The literature review was done and written by Rutherford Exius. The data was entered in the database REDCap by Rutherford Exius, Sofianne Gabrielli, and Gregory Gooding. The data was extracted from the database by Greg Shand (lab manager). The data was statistically analyzed by Rutherford Exius. Every sections of the thesis were written by Rutherford Exius and revised by Dr. Ben-Shoshan.

Abbreviations

DHR – Drug Hypersensitivity Reaction
ADR – Adverse Drug Reaction
GOC – Graded Oral Challenge
SPT – Skin Prick Test
IDT – Intradermal Skin Test
PT – Patch Test

IgE – Immunoglobulin E
HLA – Human Leukocyte Antigen
TCR – T-Cell Receptor
APC – Antigen Presenting Cells
SJS – Stevens–Johnson syndrome
TEN – Toxic Epidermal Necrolysis
AGEP – Acute generalized Exanthematous Pustulosis
DRESS – Drug Rash with Eosinophilia and Systemic Symptoms
MPE – Malignant Pleural Effusion

NPV – Negative Predictive Value
CI – Confidence Interval
aOR – Adjusted Odds Ratio
IQT – Interquartile Range

MCH – Montreal Children's Hospital
TCC – The Children's Clinic

*The term "participants" is used to refer to the child patient and their parent/legal guardian who agreed to be part of the study by signing the consent form.

Introduction

Penicillin derivatives, mainly amoxicillin and cephalosporins are the major class of antibiotics used to treat common pediatric bacterial infections[1-3]. Up to 10% of children in North America and Europe are labelled as "allergic to antibiotics" upon developing rashes while on or directly after cessation of antibiotic treatment without being appropriately investigated by allergists[2, 4, 5]. There are no clear and accessible diagnostic algorithms available to accurately evaluate these children in the clinical setting. Therefore, most patients continue to avoid the suspect antibiotic, and often others from the same family or similar properties, throughout life. This practice can compromise the use of the most effective antibiotic in common childhood conditions and increase the rate of antibiotic resistance[6].

The diagnosis of immune-mediated reactions is challenging, as available skin tests have a limited role in the diagnosis of immediate reactions (any of the following symptoms within 1 hour of a graded oral challenge (GOC)[7]: urticaria, angioedema, wheezing, rhinitis, severe and repetitive vomiting, diarrhea, protracted abdominal pain, or shock) and no role in the diagnosis of non-immediate reactions (cutaneous symptoms which occur more than 1 hour after the challenge)[8]. Although a drug-specific oral provocation testing is considered the gold standard for diagnosis, it is rarely used in practice, owing to lack of data regarding its safety and accuracy in children[9]. Moreover, the approach to the diagnosis and management of patients with suspected allergy is complicated by concerns of cross-reactivity between penicillin derivatives and cephalosporins, which is reported to be as high as 30%[10, 11]. At this point it is also unclear if similar molecular structures (i.e. cross reactivity) or a general increased risk of antibiotic reaction (i.e. a co-allergy) account for these increased rates of reactions to both families of beta lactam

antibiotics. Given that skin tests are reported to have low predictive value[12] for identifying cross-reactivity most patients avoid both antibiotic classes without further investigation. Such avoidance increases the use of less effective and more toxic alternatives[13].

The overall goal of our research is to develop and evaluate effective and safe diagnostic strategies for antibiotic allergies in a pediatric population by GOC as well as evaluating the risk of allergy to a similarly structured cephalosporin among children with established amoxicillin allergy.

We hypothesize that a two-step GOC will provide a safe and accurate strategy to assess the percentage of immediate and non-immediate antibiotic allergic reactions in children presenting with suspected allergy to penicillin derivatives and confirm the safety of cephalosporin usage among children with established amoxicillin allergy.

Literature Review

Classification and pathophysiology of drug hypersensitivity

Drug hypersensitivity is a broader term which includes drug allergy, pseudo-allergy and the pharmacological interaction with immune receptor concept[14].

The types of hypersensitivity that fall under the classical "drug allergy" term are the pathophysiological explanations for hypersensitivity brought up by immunologists Gell and Coombs[15]. This first form of drug hypersensitivity relies on drug-protein covalent binding forming new antigens (sensitization) *Figure 1*. Upon subsequent exposure, humoral and/or

cellular immune response may occur thus causing a reaction. Type I hypersensitivity reaction is an immediate reaction that occurs within 1 hour after exposure to the drug and are considered

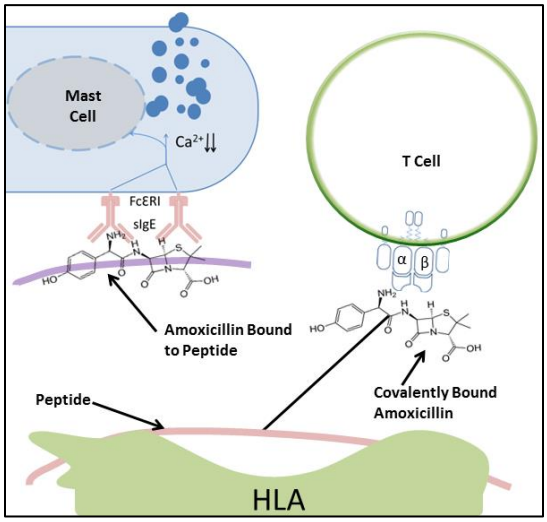


Figure 1. Formation of new antigens from a drug-protein covalent binding of the allergic-immune mechanism

Image by Greg Shand from I-DARE, permission of use by principal investigator Dr. Ben-Shoshan

IgE-mediated[16, 17]. They are characterized by symptoms such urticaria, angioedema, conjunctivitis, rhinitis, bronchospasm, gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain), and anaphylaxis or anaphylactic shock, the latter necessarily involve hypoxia [16, 18]. This reaction is a result of IgE production by antigen-specific B lymphocytes after sensitization which is induced by conjugation of the beta-lactam to carrier molecules[19]. Indeed, the drug antigen is thought to be a hapten-protein complex and a multivalent binding site is created for it when IgE antibodies bind to the affinity Fc R1 receptors on the surface of basophils and mast cells[18]. Upon following exposure to the drug, the antigen cross-links bound IgE, causing the release of preformed mediators, like histamine and tryptase, and the production of new mediators, (e.g., leukotrienes, prostaglandins, kinins, and other cytokines)[18].

Type II hypersensitivity reaction is a IgG and IgM-mediated cytotoxic reaction that is non-immediate[15, 20]. The symptoms include fever, hemolytic anemia, granulocytopenia, and thrombocytopenia[20]. Cell damage can occur through the direct action of macrophages, neutrophils and eosinophils that are usually linked to blood cells coated with immunoglobulins[14]. Another mechanism for the antibody-dependent cellular cytotoxicity is the activation of the

complement classical pathway. This results in cell lysis either because of binding of C3b or C3d to the target cells or to the binding of the C5b-C9membrane complex attack[14].

Type III hypersensitivity is a non-immediate immune complex reaction also involving IgM and IgG[20]. These reactions occur when antigens precipitate with antibodies (mainly IgM) in tissue spaces[14, 15]. Those microprecipitates are formed in and around small vessels and cause damage to cells. An excess of antigen is the source for the formation of soluble immune complexes and is the promoter of deposition in the endothelial lining of blood vessel walls. On a bigger scale, the depositions are often located in the lungs, joints, kidneys, and skin. Tissue damage begins with the local inflammation that involves the activation of complement. Moreover, cells like macrophages and neutrophils are attracted to the deposition site and contribute to the tissue damage.[15] With penicillin intake, the symptom is serum sickness which is clinically represented by rash, fever and arthralgia[20]. It is associated with the administration of low molecular weight molecules (<1,000 Da) like amoxicillin (365.4 Da). However, it is preferred to use the term "serum sickness-like disease", because evidences of circulating or deposited immune complex are rarely found.[15]

Type IV hypersensitivity is the most common nonimmediate reaction that doesn't involve antibodies and usually develops 6 hours to 10 days after drug exposure[21]. Patients who suffer from this type of reaction have not necessarily been sensitized. The reaction is T-cell mediated and usually manifests itself as a delayed cutaneous reaction when antigen-presenting cells present the drug allergen to T cells[14]. This results in the release of cytokines and lymphocyte stimulation.[15] Organs other than the skin can also be involved such as the liver, the lungs, kidney, and pancreas[21]. Mechanistically, beta-lactams and amino beta-lactams like amoxicillin can induce all four type of hypersensitivity reactions, but type I and IV reactions are thought to be

the most common. However , given that almost half of cases report reactions upon first exposure, immune-mediated reactions based on prior sensitization are unlikely to be the sole explanation underlying amoxicillin hypersensitivity [12, 22].

The second form of drug hypersensitivity, pseudo-allergy (drug intolerance or nonallergic hypersensitivity[14]), is represented by interactions with receptors of inflammatory cells *Figure 2*. Symptoms include urticaria and anaphylaxis.[14, 23] The reaction involves non-specific IgE cross-sensitization or the presence of a non-IgE-dependent mechanism[23]. The proposed mechanism is based on degranulation of mast cells through Mas-Related G-Protein-Coupled-Receptor -X2 (MRGPRX2)[23]. MRGPRX2 is found predominantly in the skin[24]. However, for beta lactam antibiotics this mechanism is unlikely given that these reactions usually involve drugs containing tertiary and quaternary ammonium structures (present in quinolones but not in amoxicillin).

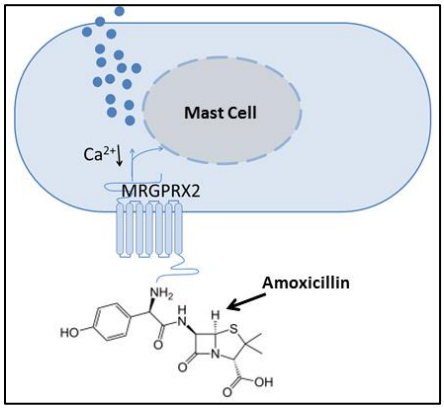


Figure 2. Drug interaction with receptor (MRGPRX2) of inflammatory cells (mast cell)

Image by Greg Shand from I-DARE, permission of use by principal investigator Dr. Ben-Shoshan

The third form of drug hypersensitivity is the pharmacological interaction with immune receptor concept (p-i concept) *Figure 3*. This concept was generated from the observation of non-covalent off-target effect of drug on immune receptors proteins leading to unorthodox,

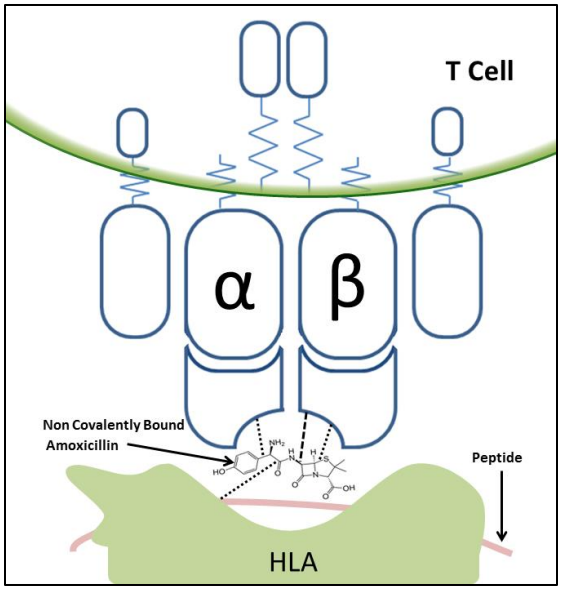


Figure 3. Pharmacological interaction with immune receptor concept

Image by Greg Shand from I-DARE, permission of use by principal investigator Dr. Ben-Shoshan

alloimmune-like stimulations of T-cells (immediate or nonimmediate). This concept can be split in two sub-categories: p-i HLA and p-i TCR. The clinical implications are similar to the type I to IV, but also include severe symptoms such as MPE, DRESS, SJS/TEN, AGEP.[14] Based on studies on abacavir, drugs can bind to the F pocket of HLA in the endoplasmic reticulum making it smaller and preventing other peptide, normally present, from binding.[25-27] This process has been shown to take hours and to be of high affinity. It has also been shown that the drug can bind the HLA on the cell surface. This type of binding has been shown to cause calcium ions influx in less than 19 minutes, thus it is associated with immediate reactions. Based on studies on sulphamethoxazole (SMX), drugs bind directly in a large loop in the CDR3 region of the TCRVα, which usually interacts with the peptide-HLA complex[14, 28]. Consequently, this binding leads to TCR-triggered cytokine secretion, proliferation upon interaction with HLA peptides presented by the antigen-presenting cell (APC)[14]. When drugs bind to the TCR-Vß, the altered TCR shows a 7-fold more affine interaction with the HLA-peptide complex, thus linking p-i HLA and p-i TCR. It has been hypothesized in the literature that viral infection lowers the activation threshold of drug-reactive T-cells[6, 22]. Recent studies suggest that amoxicillin related hypersensitivity reactions are mainly related to this third form of drug hypersensitivity reactions (DHR) [29].

Classification of antibiotics and Clinical Presentation

Beta (β)-lactams are antibiotics widely used in adults and children. Their effectiveness at eradicating common bacterial infections and low cost make them a prime choice to treat skin, ear, sinus and upper respiratory tract infections[30]. All β-lactams share the same core structure which is a four-membered cyclic amide β-lactam ring structure[31]. Differentiation among different beta-lactams stems from their nucleus [32]. The nucleus of penicillins consists of the β-lactam ring attached to thiazolidine ring[31]. Penicillin was discovered in 1928 by Alexander Fleming, it was derived from common mold, known as penicillin mold, and this group includes penicillin G and penicillin V, also called the natural penicillins, penicillinase resistant (methicillin, oxacillin, nafcillin, and cloxacillin), aminopenicillins (amoxicillin, ampicillin), aminopenicillins combined with beta-lactamase inhibitors (co-amoxiclav or amoxicillin/clavulanic acid and ampicillin/sulbactamz or ampicillin/flucloxacillin), mecillinams (pivmecillinam) [33, 34]. The extended-spectrum penicillins (carbenicilllin, mezlocillin, piperacillin, ticarcillin, and piperacillin/tazobactam), carbapenems (doripenem, ertapenem, meropenem Imipenem and cilastatin), and monobactams (aztreonam) are other types of beta-lactams[34]. As part of the beta-lactam family of antibiotics, we can also find the class of cephalosporins which is divided in five generations based mostly on their resistance to β-lactamases and bacterial susceptibility patterns[31]. The nucleus of a cephalosporin consists of a six-membered dihydrothiazine ring fused to the β-lactam ring core[32]. Differences among cephalosporins stems from the R1 and R2 chains attached to the nucleus[32]. Some of the first generation cephalosporins are cephalexin, cefadroxil, and cefazolin and they are considered to have good gram-positive coverage and poor gram-negative coverage[32]. The second generation of cephalosporin includes cefaclor, cefuroxime and they have the inverse coverage of the first generation.[32] Just like the second generation, the third

generation has a fairly good coverage of gram-positive cocci and an even more extensive coverage of the gram-negative ones. The third generation of cephalosporins includes cefixime and ceftriaxione[32]. The fourth and fifth generations are composed of cefepime and ceftaroline respectively and they both have good coverage of gram-positive cocci and even better coverage of the negative ones[32]. As for many other drugs, those antibiotics exhibits side effects and adverse effects. Cross reactivity among beta-lactams is also an issue especially between penicillins and first and second generation cephalosporins [31, 32]. Retrospective studies from the 1980's have demonstrated upwards of 10% cross reactivity between penicillins and first and second generation cephalosporins and 2-3% between penicillins and third generation cephalosporins[31, 32, 35]. This data has been the basis of cross-reactivity knowledge, but it was based of clinical history and exposure to first and second-generation cephalosporins known to have trace amount of penicillin[32]. Recent studies approximate cross-reactivity between penicillin and cephalosporins based on positive skin tests at ~2%[36-39]. The R1 side chain has been found to be the determining factor in immunologic cross-reactivity[32]. Aminopenicillins and cephalosporins with the same R1 side chain have been shown to have a higher rate of cross reactivity based on skin prick tests[32]. About 5% of children and 10% of adult report a β-lactam allergy, but very few are truly allergic[40]. The two clinical pictures that arise from this type of allergy are acute or immediate reactions and sub-acute or non-immediate reactions.

Prevalence

The proportion of individuals allergic to penicillin is reported to be 10% of those treated. However, it has been shown that over 90% of those patients are able to tolerate the drug after assessment[12]. The prevalence of beta-lactam allergy is likely decreasing in the general population according to recent studies[41]. The same trend is observed in the pediatric population[42]. This

reduction is probably due to the changing in the way antibiotics are prescribed. Indeed, the use of penicillin intravenously has decreased while in oral amoxicillin prescription increased[12]. This shift in prescription has led to the increased in the false diagnosis where viral rashes, more common in *per os* amoxicillin treatment[43], were inappropriately defined as allergies to amoxicillin[43]. In addition, patients who have a true IgE allergy to a beta-lactam tend to lose their sensitivity over time and studies have shown that.[12] Real representative data on the prevalence of true allergy is difficult to obtain because some side effects can be considered hypersensitivity reactions to the drug, coincidental events during treatment course can also lead to mislabeling, virus-antibiotic interactions are also a factor generating true allergy claims, and children tend to outgrow their beta-lactam allergy[42]. Moreover, diagnosis algorithms are not clear and optimal for the proper labeling especially in the pediatric population.

Diagnosis

Evaluation of clinical history

Based on International CONsensus (ICON) on drug allergy made by the International Collaboration in Asthma, Allergy and Immunology (iCAALL), formed by the European Academy of Allergy and Clinical Immunology (EAACI), the American Academy of Allergy, Asthma and Immunology (AAAAI), the American College of Allergy, Asthma and Immunology (ACAAI), and the World Allergy Organization (WAO),. history of the index reaction is the first crucial step required to confirm the diagnosis of drug allergy diagnosis[18]. It is important to query patients on the chronology of the symptoms (previous exposure to same or different antibiotic, time from last dose to onset of reaction), other medication taken at the time of the reaction and other drugs of the same class taken since, and the medical background of the patient (previous food, drug or environment/seasonal allergy). Clinical history is not a standalone diagnostic tool.

However, studies suggest that history often does not predict the presence of true allergy [44, 45]. Numerous factors may account for this observation including the presence of concomitant viral infection that can account for similar symptoms, the risk of recall bias[46] and the use of different drugs taken simultaneously during the treatment [18]. Pharmacovigilance algorithms are causality assessment methods carried out by pharmacologists that are designed to classify adverse drug reactions (ADR) based the likelihood of a causal relationship between the drug and the reaction[47]. This diagnostic tool is mainly based on the clinical history. Those algorithms are rarely specific for DHRs. Firm diagnosis of DHR can hardly be produced by the algorithms and allergy testing is often necessary.[48, 49] Stopping the use of a drug is not always synonym with stoppage of symptoms since rebounds of urticaria after drug withdrawal is possible for a few hours. Moreover, information and recall biases are important hurdles that makes drug causality assessment hard to conclude [18].

Skin tests

Most guidelines rely on the use of skin tests (mainly intra dermal) to diagnose drug allergy despite the lack of data confirming their sensitivity and specificity [12]. The European Academy of Allergy and Clinical Immunology recommends conducting a prick testing first for the culprit drug, followed by an intra dermal tests if the skin prick test is negative. According to the literature, the skin tests are recommended for beta-lactam allergy testing because of their simplicity, rapidity, low cost and the high specificity of the procedure. For T-cell mediated hypersensitivity to beta-lactams, late-reading intradermal tests (IDT) are often recommended[18]. However, more recently studies by our group and others[12, 22, 50] revealed that skin tests have low sensitivity and high rate of false positive tests. Mill et al reported a sensitivity of 5.9% when compared to the gold standard of drug challenge[22]. Ibanez et al. found that IDT has a high rate of

false-positive (80%). Patch test is suggested by some studies as a diagnostic tool for T-cell dependent DHRs. According to the protocol established by the European Society of Contact Dermatitis (ESCD), extemporaneous patch tests (PT) performed by an healthcare professional such as a nurse with the commercial drug used by the patient can be useful and reliable for identifying the culprit drug in a cutaneous ADR with a specificity of 28%[51] and a sensitivity of 9% [34]. Unlike many of the current diagnostic tools, PTs are thought to likely be safe and useful in testing patients with severe cutaneous reactions like SJS/TEN, DRESS and AGEP. Given that IDTs and PTs are considered to have low sensitivity, a drug challenge is often required to confirm the presence of true allergy [34].

In vitro tests

There are two main *in vitro* tests used to detect specific IgE antibodies to β-lactam. The first consists of detection of antibodies in serum by immunoassays. The second is based on basophil activation upon contact with hapten (PPL, MDM and AX), by quantification of the release of mediators (histamine or leukotrienes) or basophil activation marker expression[19]. These methods were reported to have low sensitivity for the diagnosis of β-lactam allergy in patients with a history of anaphylactic shock and negative skin tests[19, 52]. Moreover, like many of diagnostic tools, there is a lack of information regarding sensitivity, specificity, positive and negative value in the pediatric population.

Cellular tests are biological tests that also leverage the capacity of basophils to be activated and release different mediators for diagnosis[19]. These tests consist of cellular stimulation with allergens for the quantification of sulphidoleukotrienes (LTC4 and its metabolites LTD4 and LTE4). The sulphidoleukotrienes are produced and released when blood

leukocytes, usually basophils, are stimulated by a drug in vitro [53]. Due to its low sensitivity and low specificity, this test is not recommended as a routine laboratory method [19].

Another cellular test is the (basophil activation test) BAT. It is based on the flow-cytometric evaluation of CD63 on the surface of blood basophils, a marker that appears in activated basophils following incubation with drugs or other allergens *in vitro*[19]. The sensitivity of the BAT has been reported to be 48.6% for beta-lactams and even higher for detection of culprit cephalosporins with a sensitivity of 77% [52].

Oral challenge

The consensus regarding oral challenge (OC) is that it is the gold standard for drug allergy diagnosis [18]. It is referred to as graded oral challenge, drug provocation test, test dosing or drug challenge. In all guidelines and algorithms regarding potential drug allergy, oral challenges are the last confirmatory step. This is due to their inherent risks according to many practitioners[18]. Even though recent studies have shown the safety of amoxicillin challenges in children presenting with non-life threatening cutaneous reactions[22]. The goal of conducting OC may differ. The US Practice Parameters recommend this test to demonstrate tolerance to a drug already less likely to be the culprit of the DHR[54]. The BSACI recommend the usage of this test to exclude hypersensitivity reaction induced by the culprit drug. The EEACI-DAIG/ENDA holds similar views to the BSACI regarding GOC, but also preconizes the use of alternatives in some clinical practice situations[55]. Albeit, the EEACI-DAIG/ENDA does mention the altruistic and scientific value of the oral challenge[55]. In clinical practice challenge is often not used due to several reasons. Some practitioners argue that challenges should not be conducted for a drug that is infrequently used and for which many alternatives exist[18]. In addition, patients may be reluctant to re-exposure to a drug they deem harmful[18]. Severe reactions are not amenable to

challenges[18]. Finally, the challenge requires resources including a well-trained medical team and a setting that can manage allergic reactions. However, recent large studies in children and adults have found that negative predictive value for challenges is 94-98% for beta-lactam allergy testing and the test has been shown to be quite safe since most of the reactions reported by the patients were mild and nonimmediate[56, 57].

Health risks and socioeconomic downsides of misdiagnosis

Misdiagnosis of beta-lactam allergy is a major health problem associated with direct and indirect economic/health costs. It is commonly known that mislabeling of β-lactam allergy leads to the prescription of broader spectrum antibiotics or less effective agents, thus leading to the increased rates of antibiotic resistance[6]. In fact, according to the CDC's 2019 report *Antibiotic Resistance Threats in the United States*, more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result[58]. Further alternative medications are reported to be associated with higher risk of side effects. For example, fluoroquinolones are contraindicated in children because of chances of cartilage damage, based on animal studies after high dose administration [59]. Clindamycin is associated with important side effects such as vomiting, diarrhea, and nephrotoxicity[60]. An increased in hospitalization is associated with the label of a beta-lactam allergy. A cohort study has shown the patients with this label spend 9.9% more days (0.59 days: 95% CI, 0.47-0.71) in hospital than controls[61]. Furthermore , it was reported that among patients testing negative, 85 (38%) subsequently received beta-lactams, preventing 504 inpatient days and 648 outpatient days on alternative agents[62]. Another downside of misdiagnosis of beta-lactams regarding the use of alternative drugs is the lower efficacy of those drugs compared to the ones mainly prescribed when children are not labeled as allergic to penicillin. Indeed, β-lactam antibiotics, mainly amoxicillin and

penicillin, are the most effective medications for streptococcal pharyngitis[63], otitis media[64], and infectious endocarditis[65]. Lastly, cephalosporins often are the first-line treatment in penicillin-allergic patients[66]. However, it is common practice for allergists to suggest avoidance of cephalosporins with the same side chains because of the enhanced risk of cross reactivity[6]. In the case of mislabeled patients, this is another reason to take an alternative drug that is less effective and that may have more severe side effects. A 30-year-old study targeting the pediatric population in the USA, found that patients labeled as allergic penicillin had significantly more medical visits, more antibiotic prescription, and higher average wholesale drug cost for those antibiotics when compared with a random sample of controls in the population [67].

In a previous study conducted by our group on 818 Canadian children, it was demonstrated that there is direct health economic benefit of assessing true amoxicillin allergy by oral challenge [22]. Among the seventeen immediate reactors in the sample, only one had a positive skin test. It was estimated that the direct health care cost of a unique skin test is CAD $170 (US $126, including the cost of physician and nurse services as well as the PRE-PEN ampule). According to common allergy diagnostic algorithms, the other sixteen patients with negative skin test were eligible for an oral challenge. If all participants had solely an oral challenge, they approximated the cost at around $3740 (US $2782). However, the cost of a skin test and a challenge for those 17 participants is approximately CaD$6420 (US $4776; including 1 patient with a skin test only and 16 with skin tests and challenge). A study published in the New England Journal of Medicine in 2019 also echoes these findings [68]. Based on their review, several studies from North America and Europe have documented higher costs of inpatient and outpatient care for patients with penicillin allergy[68]. Furthermore, it is estimated that penicillin-

allergy testing and delabeling lead to cost savings, with the largest study showing a reduction in total health care expenses of $1,915 (in U.S. dollars) per patient per year[68].

Treatment

In the case of suspected reaction, symptoms associated with beta-lactam hypersensitivity reaction are promptly treated and alternative medications like cephalosporins are considered [66] . For the relief of symptoms, health practitioners recommend stopping the use of the culprit drug. In the case of a suspected IgE-mediated reaction, prompt epinephrine administration is required [18]. Antihistamines are then prescribed due to the usual nature of those reactions. Corticosteroids can also be prescribed for the reduction of inflammation or itching[18]. If no appropriate substitution is available, desensitization is considered mainly for IgE-mediated reactions[69].The principle of desensitization is simple. It was first used by O'Donovan during the Second World War. He added increasing amount of oral penicillin to milk until the target dose was reached without side effects in the soldiers he was treating and had had anaphylactic shock when using intramuscular penicillin[68]. Protocols are in place for oral and intravenous desensitization with success rates of 100%[68]. Desensitization, which is a form of immunotherapy, is not a cure nor does it answer the question whether a patient is truly allergic to penicillin or its derivates[68].

Study Objectives

Overall objectives

There are no clear-cut diagnosis algorithms regarding beta-lactam allergy in children due to the paucity of data regarding safety and accuracy of oral challenges to amoxicillin. Hence, the overall goal of our research is to develop and evaluate effective and safe diagnostic strategies for antibiotic allergies in a pediatric population based on the use of GOC.

Primary objective

To determine the accuracy and safety of GOC in children.

Secondary objectives

1. To determine the risk factors associated with immediate and nonimmediate reactions to the GOC.
2. To evaluate risk factors associated with a positive GOC or subsequent reaction to amoxicillin.
3. To evaluate the potential of cross-reactivity between amoxicillin and first-generation cephalosporin with a similar R1 chain (cephalexin).

Study Methodology

Study Design

All children referred to the allergy clinic of the Montreal Children's Hospital (MCH) and The Children Clinics (TCC) in Montreal, and the Meadowood Medical Centre in Winnipeg for the assessment of a suspected penicillin allergy were approached for the study. We excluded children with history of drug reaction with eosinophilia and systemic symptoms, Stevens-

Johnson syndrome or toxic epidermal necrolysis (TEN)/anaphylaxis. In order to participate to the study, participants were given a consent form to sign which explained the study. The parents/legal guardian signed the consent form on the behalf of children (<7 years-old), older children and adolescents co-signed with their parents/legal guardian.

Patients with suspected amoxicillin allergy were offered a two-step non-blinded GOC with 10% of the therapeutic dose, then 20 minutes later 90% of the therapeutic dose and were observed for 1 hour after the last dose. The dose ranged for the challenge in the case of amoxicillin is 550 mg to 1,500 mg of amoxicillin, depending on the child's weight. For cephalosporins, the dose range was determined according to the standard dose recommended in the Canadian Pediatric Society position paper[70].

This observational study features both retrospective and prospective arms. After obtaining parental consent, the suspected reactions to antibiotics were retrospectively characterized through a standardized questionnaire that captures the clinical characteristics, co-morbidities (including history of atopy and use of medications regularly and during the suspected reaction), suspected antibiotic exposure and management of the reaction. This data was coded and stored anonymously in a REDCap database at the Research Institute of the McGill University Health Centre for Innovative Medicine. GOC outcomes and future use of antibiotics were investigated prospectively. The latter was tracked via a five-year annual follow up which consists of contacting families annually by phone and by email to assess antibiotic use and development of future reactions starting one year after the GOC and included four follow up calls.

Ethics Approval

All appropriate ethics reviews and approvals were obtained before beginning this study. The study was approved by the McGill University Ethics Committee and the Research Ethics Board at the University of Manitoba.

Statistical Analysis

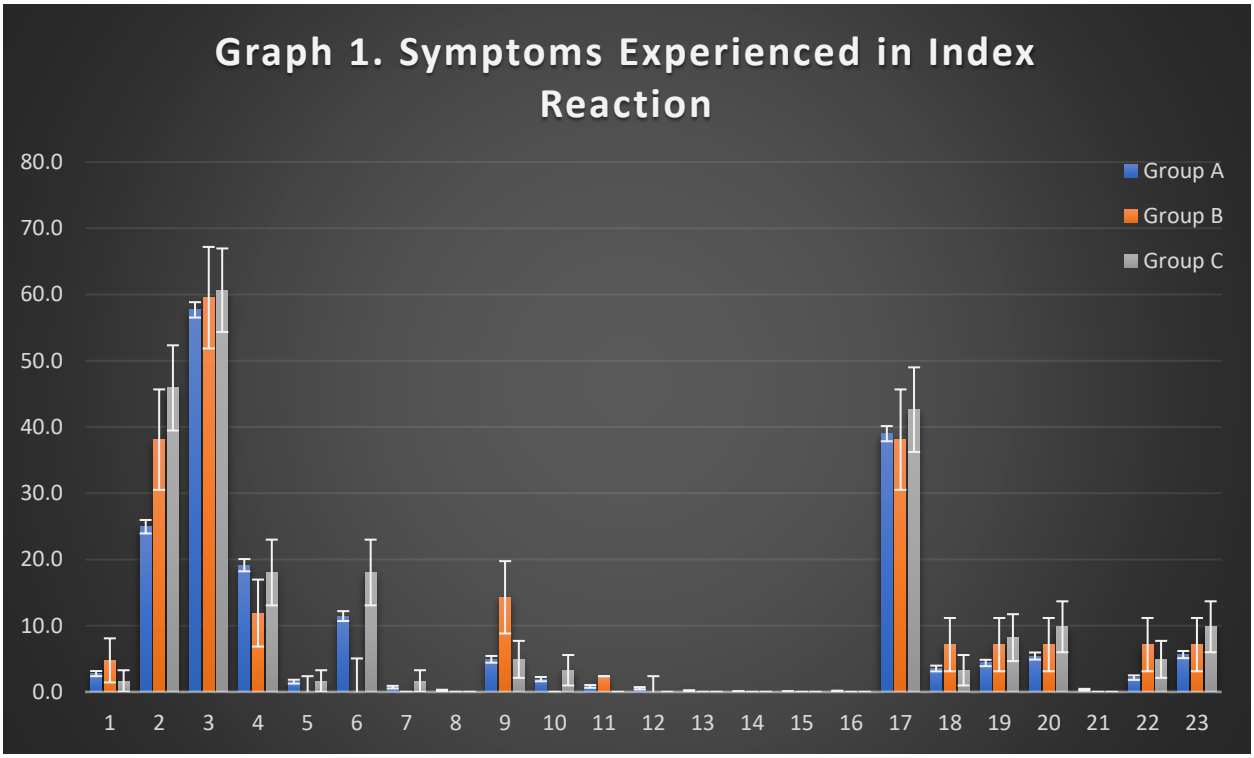
Descriptive statistics were used to summarize demographic and clinical characteristics of reactions through averages, medians, and interquartile ranges. Univariable and multivariable logistic regression analyses were done to assess potential confounders and evaluate the effects of sociodemographic predictors (including age and sex), presence of comorbidities (e.g., atopic diseases, use of other medications such as nonsteroidal anti-inflammatory drugs and acetaminophen during the reaction and on a daily basis), family history of drug allergy and atopy in biological parents, and clinical characteristics of the reaction (time between initial suspected reaction and challenge, whether this was the first exposure and clinical symptoms of the suspected reaction, i.e., type of rash, presence of arthritis or arthralgia or of symptoms involving the respiratory, digestive, or cardiovascular system) on immediate and nonimmediate reactions to the GOC. Model selection was done by including medically relevant variables, by following the general rule of 10 events per variable and based on the Akaike Information Criterion. All statistical analyses were conducted using the statistical software RStudio version 1.1.456 – © 2009-2018 RStudio, Inc.

Results

Demographics and Clinical Characteristics

From March 2013 to March 2020, 1844 children from Montreal (TCC and MCH) and 70 from Winnipeg with suspected amoxicillin allergy were recruited by trained members of each centers (involved in the study) The median age was 1.7 years [interquartile range, 1.0-3.6 years] and the majority [53.8%] were males. The median time from the patient's reaction to their GOC was 1.1 years [IQR,0.4-3.6] (*Table 1*). Patients were grouped into three groups, according to outcome of the GOC: tolerance to GOC (group A), immediate reaction (< 1 hour after GOC, group B) or nonimmediate reaction (>1 hour after GOC, group C) (*Table 1*). Group A included most of the participants with 1811 patients (94.6%) who tolerated the provocation test. Statistical description of the symptoms of the reaction to the GOC and the nature of those symptoms are at the basis of the safety criterion. Group B had 42 (2.2%) participants who developed mild immediate reactions including pruritus (localized or generalized), urticaria, flushing, throat tingling, gastrointestinal symptoms (vomiting once), maculopapular rash, arthritis and/or arthralgia, and mild mottling. Group C contained 61 (3.2%) individuals who developed nonimmediate reactions that included pruritus (localized and generalized), urticaria, flushing, angioedema, gastrointestinal symptoms (vomiting multiple times), breathing difficulties, maculopapular rash, arthritis and/or arthralgia, fever, serum-sickness-like reaction (SSLR),

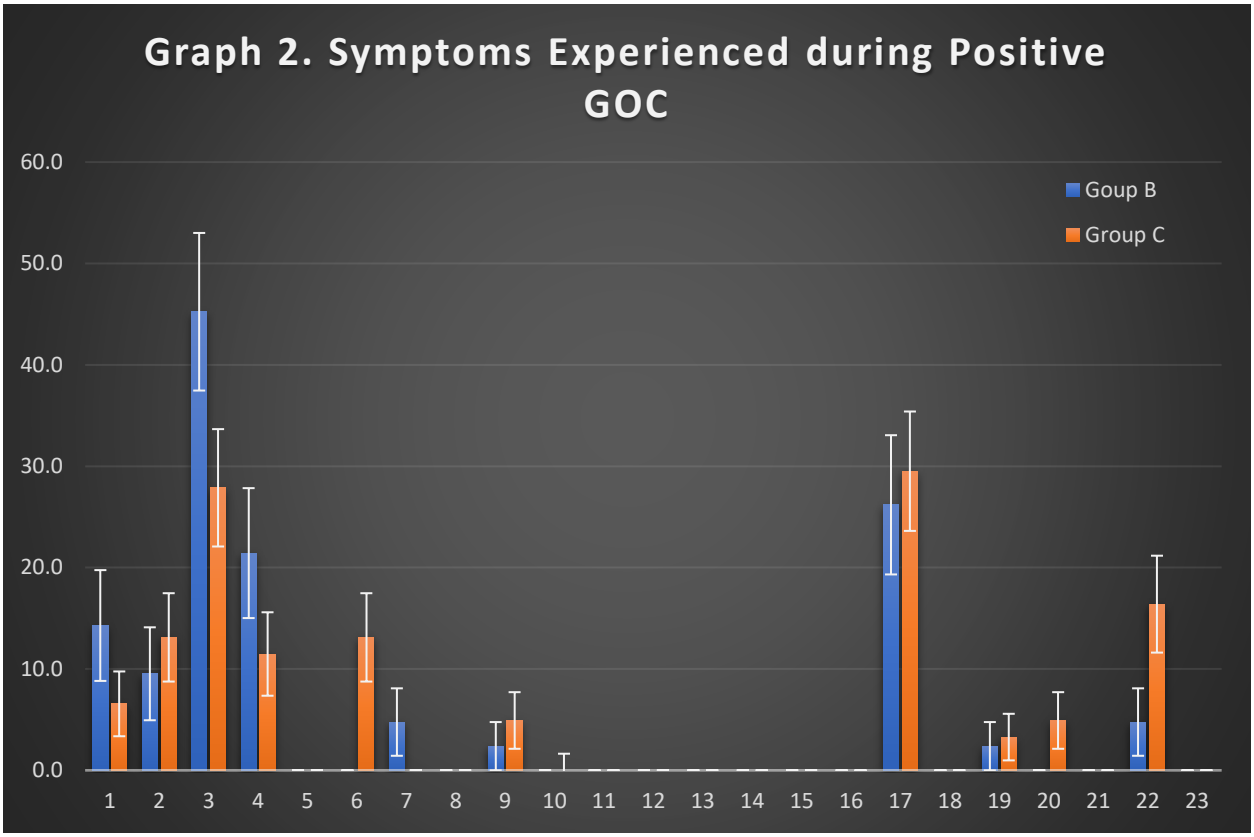
swelling and burning feeling of the skin of genitals, and dizziness (*Graph 1*).



Legend:
1 = Pruritus (localized) ;2 = Pruritus (generalized) ;3 = Urticaria ;4 = Flushing ;5 = Rhino conjunctivitis ;6 = Angioedema ;7 = Throat tingling ;8 = Stridor ;9 = Gastrointestinal ;10 = Breathing difficulties ;11 = Wheezing ;12 = Cyanosis ;13 = Circulatory collapse ;14 = Hypotension ;15 = Hypoxia ;16 = Incontinence ;17 = Macular/papular rash ;18 = Erythema multiforme ;19 = Arthritis/arthralgia ;20 = Fever ;21 = Involvement of mucosal membranes ;22 = Other ;23 = SSLR. Error bars indicate standard error

No participant who reacted to the challenge, whether immediate or nonimmediate, had anaphylaxis (*Graph 2*). Mild reactions were defined as symptoms limited to the oral mucosa or the skin; severe reactions included cardiovascular or respiratory symptoms or involvement of any 4 systems; and all other reactions were classified as moderate[22]. The immediate reactions were mild to moderate, no severe reactions occurred (*Graph 2*). Nonimmediate reactions were not observed on site, they were reported by the participants to our team. Most of the nonimmediate

reactions were also considered mild or moderate (*Graph 2*).



Legend:
1 = Pruritus (localized) ;2 = Pruritus (generalized) ;3 = Urticaria ;4 = Flushing ;5 = Rhino conjunctivitis ;6 = Angioedema ;7 = Throat tingling ;8 = Stridor ;9 = Gastrointestinal ;10 = Breathing difficulties ;11 = Wheezing ;12 = Cyanosis ;13 = Circulatory collapse ;14 = Hypotension ;15 = Hypoxia ;16 = Incontinence ;17 = Macular/papular rash ;18 = Erythema multiforme ;19 = Arthritis/arthralgia ;20 = Fever ;21 = Involvement of mucosal membranes ;22 = Other ;23 = SSLR

Other important results from our study show that 58.5% of the patients from group A were taking amoxicillin for the first time. The numbers are 54.8% for group B and 67.2 % for group C (*Table 1*).

Table 1. Sociodemographic and Clinical Characteristics of Patients Receiving a GOC for Amoxicillin

	Group A n = 1811	Group B n = 42	Group C n = 61
Participants	94.6	2.2	3.2
Male	53.8	54.8	52.5

Median age at initial suspected reaction (IQR), yrs.[a]	1.8 (1.0-3.6)	2.1 (1.2-6.1)	3.0 (1.0-2.9)
Median age at GOC (IQR), yrs.[b]	4.1 (2.1-7.7)	4.9 (1.7-9.6)	3.5 (1.8-8)
Time between initial suspected reaction and GOC, median (IQR), yrs.[c]	1.1 (0.4-3.7)	0.5 (0.3-3.2)	1.2 (0.3-2.7)
Antibiotic given for:			
1, Acute otitis media	66.3	61.9	68.9
2, Pneumonia	7.3	14.3	8.2
3, UTI	0.4	2.4	0.0
4, Streptococcal infection	5.5	7.1	4.9
5, Viral infection	1.0	0.0	0.0
6, Sore throat with unknown cause	7.3	11.9	8.2
First exposure	58.5	54.8	67.2
Route of exposure [d]			
1, Oral	100	100	100
2, Topical	0.0	0.0	0.0
3, Inhaled	0.0	0.0	0.0
4, Parenteral	0.0	0.0	0.0
Concurrent drug use			
1, NSAID [e]	21.0	11.9	24.6
2, Acetaminophen [f]	30.0	14.3	36.1
3, Antacids	0.2	0.0	0.0
4, Steroids [g]	0.9	0.0	0.0
5, Stimulants [h]	0.1	0.0	0.0
6, Antihistamines [i]	0.3	0.0	0.0
7, Beta-agonists [j]	0.9	0.0	0.0
8, Antileukotriene [k]	0.1	0.0	0.0
9, Opiates [l]	0.1	0.0	0.0
10, Antibiotics [m]	0.1	0.0	0.0
11, Antiviral [n]	0.1	0.0	0.0
12, Antifungal [o]	0.1	0.0	0.0
13, Unknown/other [p]	8.0	4.8	1.6
14, No treatment	48.2	69.0	41.0
Location			
1, Home [q]	90.3	95.1	88.1
2, Workplace [r]	0.2	0.0	0.0
3, School/day care [s]	4.9	2.4	8.2
4, Healthcare institution [t]	0.9	0.0	0.0
5, Vacation [u]	1.4	2.4	1.6
6, Third party's home/secondary home [v]	0.5	0.0	0.0

7, Public places/outdoors [w]	0.5	0.0	1.6
8, Unknown	1.5	0.0	0.0
Time to initial suspected reaction [x]			
<5min	2.3	12.5	1.6
5-60 min	11.7	7.5	3.3
1-8h	31.9	37.5	36.1
>8h	41.1	30.0	44.3
Unknown	13.0	12.5	14.8
Duration of symptoms in initial suspected reaction [y]			
1-3 days	63.5	70.0	54.1
4-7 days	25.3	15.0	26.2
More than 7 days	7.4	12.5	19.7
I do not know	3.9	2.5	0.0
Reaction occurred after how many days of treatment [z]			
1-3d	52.0	61.9	42.6
4-7d	25.4	21.4	31.1
>7d	16.4	7.1	21.3
1-3d post treatment	2.6	2.4	4.9
4-7d post treatment	0.6	0.0	0.0
>7d post treatment	0.4	4.8	0.0
Do not know	3.2	2.4	1.6
Treated outside of the health care facility with:			
1, Epinephrine IM (e.g. EpiPen, twinject)	0.1	0.0	0.0
2, Antihistamines (e.g. Benadryl, Atarax, Claritin, Reactin)	34.2	31.0	37.7
3, Anti-H2 (e.g. Zantac, Cimetidine, Tagamet, Pepcid, Famotidine)	0.2	0.0	1.6
4, Short acting inhaled beta agonists (e.g. Ventolin, Salbutamol, Bricanyl)	0.2	0.0	0.0
5, Corticosteroids (e.g. Cortisone, prednisone)	0.9	0.0	1.6
6, IV fluids	0.1	0.0	0.0

8, No, there was no treatment prior to the arrival at the health care facility [aa]	56.9	69.0	54.1
9, I do not know [bb]	5.6	2.4	0.0
10, acetaminophen [cc]	0.7	0.0	1.6
11, NSAID [dd]	0.7	0.0	0.0
12, Lotion [ee]	1.0	0.0	1.6
Seen in the ER for index reaction, the patient was treated with:			
1, Epinephrine IM (e.g. EpiPen, Twinject)	0.5	2.4	0.0
2, Antihistamines (e.g. Benadryl, Atarax, Claritin, Reactin)	14.7	21.4	16.4
3, Anti-H2 (e.g. Zantac, Cimetidine, Tagamet, Pepcid, Famotidine)	0.2	2.4	0.0
4, Short acting inhaled beta agonists (e.g. Ventolin, Salbutamol, Bricanyl)	0.4	0.0	0.0
5, Corticosteroids (e.g. Cortisone, prednisone)	2.6	2.4	3.3
6, IV fluids	0.8	0.0	0.0
8, I do not know	2.8	0.0	1.6
9, No treatment	13.2	54.8	63.3
10, NSAID	0.7	0.0	0.0
11, Acetaminophen	0.3	0.0	1.6
Seen at the clinic or by GP and treated with:			
1, Epinephrine IM (e.g. EpiPen, Twinject)	0.0	0.0	0.0
2, Antihistamines (e.g. Benadryl, Atarax, Claritin, Reactin)	7.0	7.1	4.9
3, Anti-H2 (e.g. Zantac, Cimetidine, Tagamet, Pepcid, Famotidine)	0.0	0.0	0.0
4, Short acting inhaled beta agonists (e.g. Ventolin, Salbutamol, Bricanyl)	0.1	0.0	0.0
5, Corticosteroids (e.g. Cortisone, prednisone)	0.8	2.4	1.6
6, IV fluids	0.1	0.0	0.0
7, Other	0.2	0.0	0.0
8, NSAID	0.1	0.0	0.0
9, Acetaminophen	0.1	0.0	0.0
10, No treatment	30.2	33.3	54.1

11, Don't know	1.6	0.0	1.6
Avoided amoxicillin after reaction	90.8	87.8	86.2
Use of other antibiotics post index reaction			
Yes	58.2	47.6	59.3
No	29.9	42.9	33.3
Don't know	11.9	9.5	6.7
Comorbidities			
Allergy	13.4	16.7	9.8
Asthma	14.0	19.0	13.1
Eczema	25.6	26.2	26.2
Chronic urticaria (CU)	1.1	9.5	1.6
None	49.0	38.1	59.0
Other	7.8	17.1	3.3
Don't know	4.2	2.4	0.0
Medication taken regularly			
NSAID	0.4	2.4	0.0
Antibiotics	0.6	0.0	0.0
Other	13.6	14.3	11.5
None	80.6	76.2	86.9
Don't know	4.5	7.1	0.0
Skin test			
Positive	0.0	2.4	0.0
Negative	0.8	19.5	0.0
Not Done	65.3	51.2	58.3
Patient entered before this question added	33.8	26.8	41.7
Parental drug allergy	26.7	31.0	54.1
Race/ethnic background [ff]			
White	48.5	52.4	52.5
East Asian	2.1	4.8	3.3
Black (African, African American/Canadian, Caribbean)	2.2	0.0	4.9
Arab	5.5	4.8	1.6
Latin American	4.4	2.4	8.2
South Asian (e.g. East Indian, Pakistani, Sri Lankan, etc.)	1.8	0.0	0.0
Southeast Asian (e.g. Cambodian, Indonesian, Filipino, Vietnamese, etc.)	1.7	4.8	0.0
West Asian (Afghan, Iranian, etc.)	0.8	0.0	0.0

Indigenous (First Nations, Inuit, Metis, etc.)	0.8	2.4	1.6

Table 1

N_{total} = 1914 participants. All frequencies are presented in percentage.

[a] missing data = 26 in group A. [b] missing data = 10 in group B. [c] missing data = 26 in group A. [d] missing data = 40 in group A, 1 in group C. [e] ibuprofen (Motrin, Advil). [f] paracetamol/acetaminophen (Tempra, Tylenol) [g] fluticasone (Avamys), fluticasone/salmeterol (Advair), ciprofloxacin/dexamethasone (Ciprodex), mometasone (Nasonex), fluticasone propionate (Flovent), dexamethasone, beclometasone (Qvar), prednisolone, ciclesonide (Alvesco). [h] Biphentin. [i] Loratadine (Claritin), diphenhydramine (Benadryl). [j] salbutamol (Ventolin), fluticasone/salmeterol (Advair). [k] montelukast (Singulair). [l] morphine. [m] bacitracin/polymyxin B (Polysporin), mupirocine (Bactroban). [n] oseltamivir (Tamiflu). [o] ketoconazole (Ketoderm). [p] ADHD medication (1), rectal anti-pyrectic (1), eye drops (2), natural products (1), and ear drop (1); acetaminophen or ibuprofen (5). [q] missing data = 40 in group A, 2 in group C; separated parents, Canada, Europe, and Mexico. [r] missing data =39 in group A. [s] missing data = 39 in group A, 1 in group B. [t] missing data = 39 in group A, 1 in group B; MCH, St Mary's, hospital in Haiti, Sainte-Justine, osteopath. [u] missing data = 39 in group A, 1 in group B; International: Cuba, Jamaica, Mexico, United States of America, Algeria, Columbia, Europe, Peru National: Lac Saint-Jean. [v] missing data = 39 in group A, 1 in group B; grandparent house, friend's house, chalet. [w] missing data = 39 in group A, 1 in group B; Jehovah Witness center, summer camp, public pool, camping, birthday party, shopping center, playhouse (Zig Zag Zoo), airplane, outdoors. [x] missing data = 62 in group A, 2 in group B. [y] missing data = 10 in group A, 2 in group B. [z] missing data = 8 in group A. [aa] includes stopping the medication. [bb] includes participants that don't remember the "other" treatment. [cc] Tylenol, Tempra. [dd] Motrin, Advil. [ee] anti-itch cream, calamine combined with bath. [ff] missing data = 729 in group A.

Predictors of positive reaction to GOC

One of the objectives of this study was to find predictors of positive reactions based on the index reaction which is the reaction for which the participant was referred to an allergist. This target relied on the memory of the participants and analysis of members of our teams when presented with information (e.g. classification of symptoms based on pictures). History of reaction happening less than 5 minutes after last dose was the reference category. The three other levels of this variable were: history of reaction happening between 5 minutes and 1 hour after last dose [aOR = 0.92; 95%CI, 0.87-0.96], history of reaction happening between1 hour and 8 hours after last dose [aOR = 0.93; 95%CI, 0.89-0.97], and history of reaction happening more than 8 hours after last dose [aOR = 0.92; 95%CI, 0.88-0.96]. Chronic urticaria [aOR = 1.14; 95%CI, 1.08-1.21] was associated with immediate reactions. These odds were obtained while controlling for age at reaction, sex, comorbidity, and time to initial reaction.

The odds of having a nonimmediate reaction to an amoxicillin GOC for a participant experiencing symptoms for more than 7 days were 1.06 [95%CI, 1.02-1.09] times higher than the odds of a participants whose symptoms last between 1 and 3 days. Parental drug allergy [aOR =

1.04; 95%CI, 1.02-1.06] was associated with nonimmediate reactions when controlling for duration of symptoms, age at reaction, sex, comorbidity, drug-naïve state, and parental drug allergy.

Follow-up

In order to detect false negative results of the GOC, at least one year after their visit to the clinic, patients with negative GOC were contacted to assess tolerance to amoxicillin during subsequent use. 265 participants had reused amoxicillin at least one year after their challenge and responded to the follow-up call. Among those patients, 226 participants (85.3%) reported tolerance, while 39 (14.7%) had mild reactions. Allergists agree that GOCs are the gold standard of allergy testing and it is possible that subsequent reactions are related to infection and not to the use of antibiotics [18]. However, when assuming that tolerance upon subsequent use is the ultimate reflection of the absence of allergy, the GOC had a negative predictive value of 85.3%. This measure could be as discussed, an upper bound while the true percentage of true allergy may be lower. Classification of symptoms were done by the participants with our research team member. Based on the index reaction for which the patient was refer to an allergist, patients whose symptoms lasted more than 7 days were more likely [aOR = 1.30; 95%CI, 1.13-1.49] to have a subsequent reaction compared to patients whose symptoms lasted 1 to 3 days and patients whose parents reported a drug allergy [aOR = 1.14; 95%CI, 1.04-1.25] were also more likely to have a reaction to a subsequent use of amoxicillin. Those factors were obtained while controlling for age at reaction, sex, race, parental drug allergy, and duration of symptoms.

Cross reactivity

In order to assess the potential of cross reactivity between amoxicillin and cephalexin (Keflex), participants who reacted to the amoxicillin GOC (immediate and nonimmediate) were given a GOC with cephalexin. In this branch of the study, 26 participants agreed to this oral

challenge. Among them, 14 had an immediate reaction to amoxicillin and 12 had a nonimmediate reaction. There were three reactions (3/26; 11.5%) to the cephalexin GOC. Two of them were immediate reactions (2/14; 14.3%). Those two participants also had immediate reactions to their GOC with amoxicillin. Their symptoms were mild, limited to their skin and included rash and flushing. The nonimmediate reaction (1/12; 8.3%) was reported as localized pruritus and flushing and this patient also had a nonimmediate reaction to their oral challenge.

Discussion

Our results show that the vast majority (94.6%) of children were tolerant to amoxicillin and only 5.4% of patients reacted to the GOC (2.2% immediate and 3.2 nonimmediate) with mild symptoms. Patients who tested positive were considered true positive because oral challenges are considered the gold standard in allergy testing[18]. These results are consistent with recently published reports by our group and others[12, 50] suggesting that fewer than 10% of reactions occurring while an individual is receiving β-lactam treatment are true allergic reactions[22, 71-74]. Disparity in prevalence between suspected and true reactions may be due to viral-induced delayed exanthems and viral urticaria which are often mislabelled as allergic reactions [75]. More than half of participants claimed that they reacted to amoxicillin on their first exposure. This prevalence can be explained by recall bias. Indeed, participants may not remember using the antibiotic. However, this is unlikely to be the best reasoning because participants bring their drug records to the clinic. There is also the possibility of previous exposure through the womb which has been evaluated at around 29% for commonly prescribed antibiotics [76]. In all likelihood, this can be better explained with the p-i concept as we know that reactions can happen in drug-naïve patients through this form of drug hypersensitivity[14].

One of the objectives of this study was to determine predictors of positive GOC. We found higher odds of immediate reaction to the GOC among patients with a history of a reaction occurring less than five minutes after exposure compared to more than 8 hours [aOR = 0.92; 95%CI, 0.88-0.96]. This observation is likely due to the fact that reactions occurring in such a short time interval are more likely due to type I hypersensitivity[68]. Chronic urticaria [aOR = 1.02; 95%CI, 1.01-1.03] was also associated with immediate reaction to the GOC. Chronic urticaria (CU) is a long-lasting skin disease characterized by widespread, transient wheals occurring daily or almost daily for at least 6 weeks[77]. In most cases, it is idiopathic and is related to mast cell activation (likely to auto-antigens)[68]. The prevalence in the pediatric population is thought to be lower than the one in the adult population (0.5-5%), but precise numbers are not yet available for children[68]. Sánchez-Borges et al have shown that penicillins were involved in triggering CU (frequency = 0.2%)[78]. However, CU patients of group B did not report symptoms lasting up to 6 weeks likely due to different mechanisms not involving mast cells that operate in non-immediate reactions.

Patients experiencing symptoms for more than 7 days [aOR = 1.02; 95%CI, 1.02-1.09] historically and parental drug allergy [aOR = 1.04; 95%CI, 1.03-1.06] were associated with nonimmediate reactions while adjusting for age at reaction, sex, race, parental drug allergy, and duration of symptoms. Most common viral rashes in children last up to 7 days [79], thus cutaneous symptoms with longer duration may reflect a true amoxicillin allergy. The association between nonimmediate reactions and reported family history of drug allergy is supported by previous reports on the association between specific genetic loci and other nonimmediate drug allergies, suggesting a familial effect [80, 81].

The annual follow-up was designed to confirm negative challenges and find false negatives within group A. Only 14.7% among those who have used amoxicillin at least a year after their visit to the clinic (n=265) experienced an ADR. This arm of the study allowed to find a negative predictive value of 85.3% which demonstrated the usefulness of the GOC for tolerant patients. The rate of false negative can be explained by coincidental events at the time of antibiotic use reported by parents that could lead to information bias. An interaction between a virus [82, 83] and antibiotic or the underlying infection itself can lead to cutaneous reactions [1, 3]. Moreover, exanthems are most likely secondary to underlying infection, especially in children less than 5 years of age [84, 85]. Therefore, it is safe to assume that the follow-up has likely captured amoxicillin-related hypersensitivity reactions that would have been missed even when conducting more prolonged challenges in a child that is not sick.

Our results reveal for the first time an upper bound for the risk of reactions with future treatment and predictors for a subsequent reaction based on index reaction history. Symptoms lasting more than 7 days [1.30; 95%CI, 1.13-1.49], and parental drug allergy [aOR = 1.14; 95%CI, 1.04-1.25] were factors associated with a subsequent reaction to amoxicillin. In this arm of the study, the duration of the symptoms may be reflecting the imperfection of the GOC, but most likely it is evidence for viral infection side effect, virus-antibiotic interaction, or non-allergic side effects of the antibiotic. It has been proposed to increase the time of the GOC to account for the possibility of concurrent viral infection during exposure to amoxicillin. However, Van Gasse et. al have reported that prolonged challenges have no advantage over a GOC [86]. Parental drug allergy, in this arm of the study, could have been subject to recall bias. Indeed, parents self-report those allergies and often they have not received an oral challenge to confirm their claim. It would be safe to say that the children from group C who turned out to be

subsequent reactors had parents who were not truly allergic. This is supported by a review study that shows that 90% of adults who self-report antibiotic allergy are not truly allergic[87]. Therefore, the subsequent reactions may be due to reasons other than the patient's genetic inheritance.

Previous results from our group have shown that cefixime is a safe alternative to amoxicillin whether patients had an immediate or nonimmediate reaction to their GOC with amoxicillin. Cross-reactivity among antibiotics is reported to be related to the resemblance of their R1 side chain group [80]. Amoxicillin and cefixime do not have similar R1 nor do they have a shared cross reactivity with their beta-lactam ring [88]. The R1 side chains of amoxicillin and cephalexin are similar but not identical [89]. Our findings suggest 14.3% and 8.3% cross reactivity in immediate and nonimmediate reactors, respectively. Cross-reactivity between cephalexin and amoxicillin is evaluated at less than 2% in the literature for cephalosporins and penicillins with different side chains [88]. This higher chance of cross reactivity could be caused by the R1 side chain of amoxicillin and cephalexin which are almost identical [88]. There is a potential sampling bias combined with a caveman effect with such a small sample and patients reacting to drugs with the same R1 chain. Indeed, we only had 3 reactions (2 immediate and 1 nonimmediate) and the groups had 14 and 12 patients, respectively. With a larger sample, we would probably see a decrease in cross-reactivity, but the rate might not necessarily be of 2% or lower. However, our findings are still reassuring given that all reactions were of mild severity and limited to the skin.

Conclusion

Our results establish the safety and accuracy of performing a GOC to diagnose true amoxicillin allergy without prior skin testing in children with suspected amoxicillin allergy. We were able to identify factors associated with immediate and nonimmediate positive two-step graded oral challenge (GOC) with amoxicillin; the most prominent ones being an index reaction

occurring within 5 minutes [aOR = 0.92; 95%CI, 0.88-0.96] for an immediate GOC and symptoms lasting more than 7 days [aOR = 1.02; 95%CI, 1.02-1.09] for a nonimmediate GOC. The negative predictive value of the GOC was 85.3% which was found as a result of finding the false negative patients in our follow-up. Cross-reactivity between cephalexin and amoxicillin was evaluated at 11.5%. Future large studies are required to establish the risk of cross reactivity between cephalosporins and amoxicillin,

New guidelines based on our findings should be developed in order to contribute to a safe and appropriate diagnostic algorithm of true amoxicillin allergy in children.

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