

USING FLOW-CAST TESTING FOR FOOD-ADDITIVE HYPERSENSITIVITY AT A PRIVATE LABORATORY IN SOUTH AFRICA

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ABSTRACT

Introduction: Preservatives are often implicated in allergic diseases such as chronic idiopathic urticaria, angioedema, asthma and anaphylaxis.¹ The current literature reports that reactions to food additives are rare, with proven cases being 0.23% in a large population-based study.² The exception is sulphites, which have been shown to exacerbate asthma in a small group of asthmatic patients.³ An in-vitro tool, the flow cytometric basophil-activation test, is used to assess basophil-activation marker expression following antigen stimulation.⁴ It is a very useful test as it can be used in allergic disease where the mechanism is unclear. This is because it detects both IgE-mediated responses and non-IgE mediated hypersensitivity.⁴ In this article we examine the frequency with which Flow-CASTS (basophil-activation tests) are ordered by doctors at one of the main private laboratories in South Africa, and how often these tests yield positive results.

Methods: During the period 1 June 2017 to 31 May 2019, a retrospective analysis using a purposive sampling method was carried out at a private laboratory.

Results: The results showed that of all the tests requested 75% were negative, 16% were positive and 9% were indeterminate.

Conclusion: Ear, nose and throat specialists and general practitioners ordered the most Flow-CAST tests, but the tests ordered by general practitioners and paediatricians were more likely to yield a positive result.

Keywords: Flow-CAST testing, food additive hypersensitivity, private laboratory, South Africa

INTRODUCTION

Food preservatives are substances that are used in food preparation to prevent or delay food spoilage due to the action of micro-organisms, enzymes or physical agents.⁵ Preservatives inhibit the growth of mould, yeast and bacteria.⁶ The large demand for preservative use by the food industry is due to the growing necessity for food to be both chemically stable and safe and long-lasting.⁷ Preservatives may occur naturally or they may be synthetic, with this form being very effective in preserving foods for a long period of time.⁸ The common preservatives that are tested for at the laboratory are sodium benzoate, sulphites, monosodium glutamate (MSG), salicylates and nitrites.

Sodium benzoate is a sodium salt found in a variety of products such as margarine, sauces, marmalades, gelatine, liqueurs, beers, fruit juices and soft drinks.⁹ It also occurs naturally in tea, berries and cinnamon.⁹ Sodium benzoate is found in certain cosmetic and pharmaceutical products.^{10,11} Although the Food and Drug Administration (FDA) in the United States considers sodium benzoate to be harmless when used at levels that do not exceed good manufacturers' practice,¹² benzoates have been associated with various allergic conditions such as chronic urticaria, atopic dermatitis (AD), rhinitis and anaphylactic reactions.^{2,11}

Sulphites are ubiquitous chemicals found in the food, cosmetic and pharmaceutical industries.¹³ They inhibit microbial growth and are commonly found in wine, dried fruit, fresh shrimp, mincemeat and jams.¹³ Sulphites have previously been added to fresh fruits or vegetables in supermarkets and restaurants to prevent food discolouration; however, this practice is now illegal in many countries.¹³ They are also used in the manufacture of bleaching products, hair colourants and perfumes.¹¹ Sulphites are also found in various medications such as adrenaline, local anaesthetics and steroid creams.¹⁴ Population exposure to sulphites may be:

- via a dietary route through consuming food or drinks containing them;
- through using cosmetic products containing them, or
- due to sulphite exposure in an occupational setting.^{11,14}

Contact dermatitis has been observed in people who work in the food industry and are in frequent contact with sulphites.¹⁵ Other symptoms of sulphite hypersensitivity include dyspnoea, urticaria, diarrhoea and abdominal pain.¹⁴ Adverse reactions may differ in severity owing to various factors, such as the amount of sulphite exposure and the degree of individual sensitivity.¹³ For example, bronchoconstriction may occur in asthmatics when they are exposed to sulphites either via oral ingestion or through the inhalation of aerosolised sulphites in cold drinks.¹⁴ This is perhaps due either to low levels of sulphite-oxidase or to the parasympathetic system being stimulated by sulphites, resulting in bronchospasm in asthmatic patients.¹⁶ These patients typically have a severe form of asthma that is steroid-dependent.¹⁷

Monosodium glutamate (MSG) is a preservative that is often added to savoury foods. It also occurs naturally in some foods such as tomatoes and is used to cure certain meats, such as ham.¹⁹ MSG has been associated with a variety of medical conditions such as headaches, asthma, urticaria and angioedema, and allergic rhinitis (AR).⁹ 'Chinese restaurant syndrome' is a term that describes pain and numbness spreading in the neck and upper arms with associated flushing, headache, abdominal pain and heart palpitations. It follows the ingestion of foods with high doses of MSG. Symptoms occur within 30 minutes of the meal and may last for up to two hours. The condition resolves spontaneously.²⁰ The mechanism proposed is an exaggerated sensitivity to MSG.²² Following ingestion, MSG is metabolised to glutamate, which is an excitatory amino-acid neurotransmitter.²⁰ The reaction occurs only when high doses of MSG are added to food, therefore MSG is generally considered to be safe.¹⁹

Salicylates occur naturally and are widely distributed in plant foods.²¹ Salicylate intolerance has been defined as 'a non-specific antigen-induced, pseudo-allergic hypersensitivity reaction to salicylic acid, its derivatives or other related organic or inorganic acids of similar chemical structure' as a result of naturally occurring salicylates.¹⁶ A variety of conditions, such as nasal polyposis, sinusitis and rhinoconjunctivitis, are associated with salicylate intolerance.¹⁶ Other presentations such as hyperactivity and diseases of the gastrointestinal tract (GIT) have been implicated in salicylate intolerance; however, limited evidence supports this.²² Aspirin, or acetyl salicylate, is a

synthetic salicylate that is associated with aspirin-exacerbated respiratory disease (AERD), an inflammatory disease that comprises three features:

- sensitivity to aspirin;
- asthma; and
- sinus disease with recurrent nasal polyps.

Nitrites are used commonly to preserve foods, enhance flavours and colour foods. They are found in processed meats and fish. Symptoms of nitrite allergy are rare but include headaches and chronic urticaria.¹⁸

AERDs affect up to 20% of asthmatic subjects as adults and 40% of patients with nasal polyps.²³ The basophil-activation test for aspirin hypersensitivity is the lys-aspirin, which differs from the sodium salicylate test that is performed for food additive hypersensitivity.²² Double-blind studies have not shown salicylates found in food to cause adverse reactions in aspirin-sensitive patients.²² Aspirin desensitisation is an effective treatment for managing the symptoms of AERDs.¹⁶ This procedure should be performed by a trained allergologist in a hospital setting.²⁴

The current literature reports that reactions to food additives are uncommon, with proven cases being 0.23% in a large population-based study.²⁵ The possibility of food additives and chemicals being the cause of adverse symptoms is very difficult to show consistently because a combination of factors may result in these events.²³ These include the dose of the individual additive ingested,^{9,14,25} potential co-factor effects⁹ and the form of preservative ingested. For example, naturally occurring preservatives may cause more adverse events when compared to synthetic preservatives.¹⁶ Furthermore, the mechanisms of hypersensitivity to a preservative are still poorly understood.^{9,26} Because of these various factors, the results of similar studies have been found to differ considerably.^{20,27}

Preservative testing includes both CAST ELISA and flow cytometric basophil-activation testing. Our data come from Lancet Laboratories, which uses the flow cytometric basophil-activation test (BAT). This test is useful in allergic disease where the mechanism is unclear as IgE-mediated responses and non-IgE-mediated hypersensitivity can both be detected using this test method.⁴

The exclusionary diets for preservative hypersensitivity are controversial. The risk–benefit ratio should be considered when recommending elimination diets because many of these foods are ubiquitous and confer health benefits.²³ In addition, the lack of these foods may cause nutritional deficiencies.¹⁹ The removal of high histamine and sulphite foods has been shown to provide symptom relief;²⁵ however, limited evidence is available for the efficacy of low-salicylate²⁵ and low-sodium benzoate diets.²⁷

Allergy, in general, is a common condition that is managed by a variety of practitioners, including ear, nose and throat (ENT) specialists, general practitioners (GPs), paediatricians and physicians. A common cause of allergy is thought to be preservatives, namely, sulphites, salicylates, sodium benzoates, glutamates and nitrites. Although it has been established that

these preservatives are associated with allergic disease, testing for them is particularly difficult, with the chances of a positive result being poor.² Despite this, experience has shown that testing for these preservatives is widespread. This study investigated the ordering patterns of different groups of practitioner who attend to allergy patients in an attempt to see if there is any difference in the ordering patterns between the groups. This study is novel in that data from South African cohorts on Flow-CAST testing and preservatives are scarce, with little published on this topic.

STUDY DESIGN

We conducted a retrospective study. Permission was obtained from Lancet Laboratories.

STUDY SETTING

A purposive sampling method was used to select expert information sources from Lancet Laboratories. Our study examined requests for preservative Flow-CASTS from the period 1 June 2017 to 31 May 2019 at a private laboratory in South Africa. A total of 2 608 tests were requested during this period. The data were anonymous and unlinked to patient identifiers, and the only information obtained about the patients was their sex and age. The referral doctors' specialty, the Lancet Laboratory branch where the blood was drawn and which Flow-CAST test that was ordered, were included in the set of data.

INSTRUMENTS

EDTA samples are used for Flow-CAST testing. The test is performed within 48 hours of collection and the cell stimulation is either performed immediately or the samples are kept refrigerated. An increase in CD63 on the cellular surface of basophils using flow cytometry suggests basophil activation. Both a stimulation buffer and an allergen are added to whole blood. During basophil activation, intracellular compounds containing CD63 fuse to the cellular membrane. Controls are used in the form of one negative control and two positive controls. The erythrocytes are removed by a lysing reaction and after that they are centrifuged and suspended in a wash buffer.²⁸ The test results were described as either positive, negative or indeterminate. An indeterminate result implies a problem with one of the controls. If the negative control fails, for example, or the basophil count is too low, then this is reported as indeterminate.

CAST STATISTICAL METHODS

The data were captured and recorded on a Microsoft Excel spreadsheet and exported into the statistical program STATA 13.1 for analysis. After describing the range, the media and the interquartile ranges, the age variable was recoded into four main categories for ease of description (see Graph 1). Age was then further analysed using the Chi-square test (and the Fisher's Exact Test – where appropriate) along with the other categorical (nominal) variables. The Alpha level (the probability of rejecting the null hypothesis in error) was set at 5% (0.05).

CAST RESULTS

A total of 2 608 tests were requested during the period, of which only one had missing data and this was excluded from the analysis.

GENDER

Of the 2 607 tests analysed, 57.2% were for female patients and 42.8% were for male patients.

PATIENT AGE

The patients' ages ranged from less than one year of age up to 88 years of age, with the median age being 34 years (interquartile range 10–46 years) (see Graph 3). The largest age group was 36–55 years (35%), followed by <1–18 years (33%), 19–35 years (19%), and >55 years (13%)

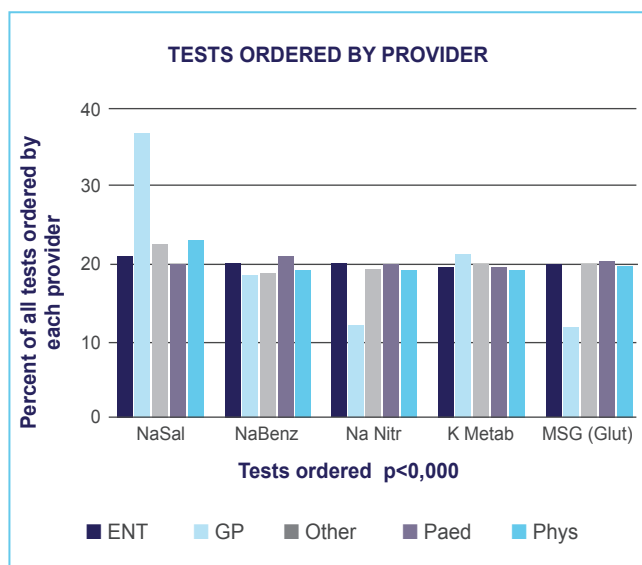
THE PROVIDERS REQUESTING THE TESTS

The largest provider groups who requested the tests were ENT surgeons (32%) followed by GPs (24%), adult physicians (19%) and paediatricians (18%). The remaining providers were grouped into a category termed 'Other', which was made up of gastroenterologists (1.7%), pathologists (1.2%), dermatologists (0.8%), gynaecologists (0.8%), allergologists (0.6%), surgeons (0.4%), five cases from the National Health laboratory Service (NHLS) and one from a homeopath.

THE TESTS REQUESTED

Five CAST tests were requested, namely:

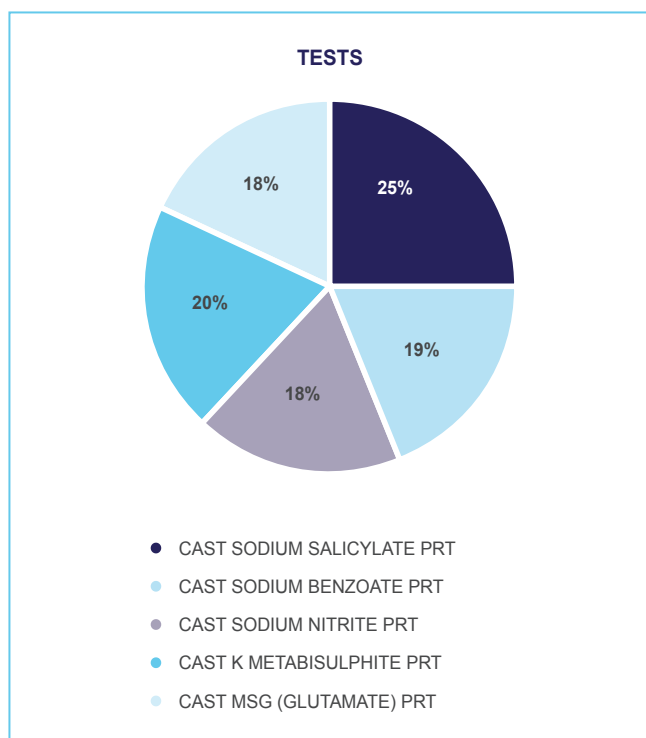
- CAST sodium salicylate PRT (25%)
- K metabisulphite PRT (20%)
- Sodium benzoate (19%)
- CAST MSG (glutamate) PRT (18%)
- Cast sodium nitrite PRT (18%) (see Graph 2).



Graph 1: Test request patterns of the different provider groups

Test results: Of all the tests requested, 75% were negative, 16% were positive and 9% were indeterminate (see Graph 3).

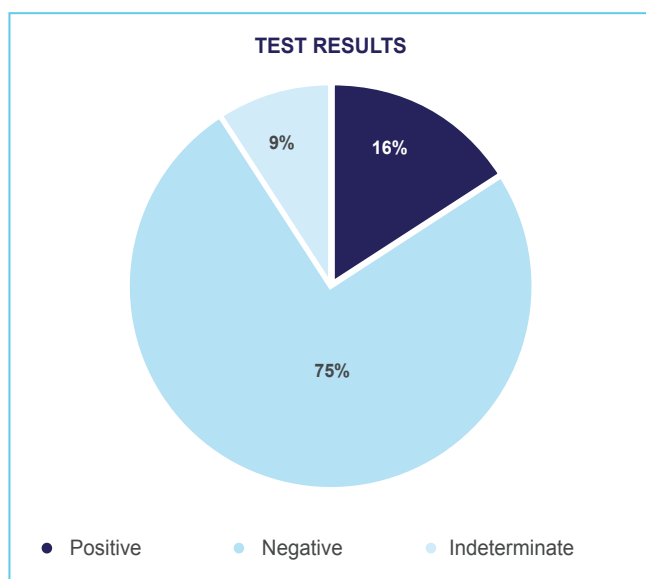
Test request pattern: There was significant association between the specific type of test requested and the provider requesting the tests ($p < 0.000$). The ENTs, paediatricians, physicians and the other group appear to be non-selective about the type of tests they were ordering. But the group of GPs appear to be more likely to request the CAST sodium salicylate and less likely to request the CAST sodium nitrite and the CAST MSG (glutamate) tests than the other providers (see Graph 1).



Graph 2: The CAST tests requested

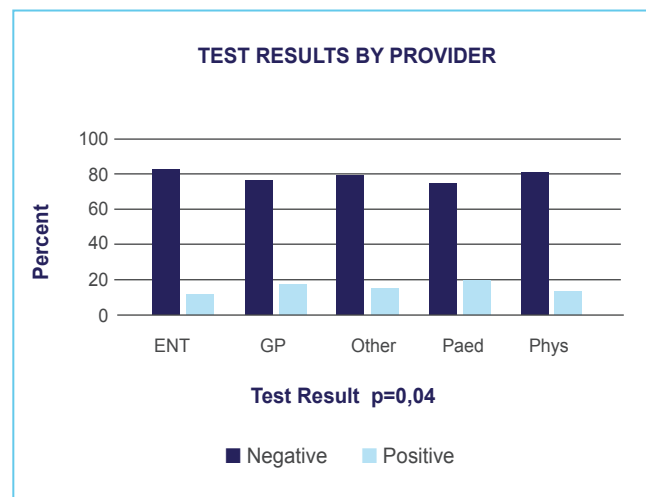
TEST OUTCOME PATTERN OF THE DIFFERENT PROVIDER GROUPS

In order to analyse the association between the test outcome and the different provider groups, the indeterminate results were excluded from this part of the analysis. The results showed a significant association between the test outcome result and the different provider groups ($p = 0.04$). It appears that the group of GPs and paediatricians were more likely to get a positive result on testing than the rest of the providers (see Graph 4). In addition, no association was found between the age of the patients and the test outcomes ($p = 0.252$).



Graph 3: The test results

The group of 'Other' providers was analysed separately in order to ascertain the validity of including them all in one group. In this analysis, no association was found between the types of test ordered ($p = 1.000$), the outcome of the test results ($p = 0.767$) and the different providers in this group. In addition, no association was found between the age of the patients and the test outcomes ($p = 0.864$) in this group either.



Graph 4: Test outcome pattern of the different provider groups

DISCUSSION

Preservatives are ubiquitous and have been implicated in a spectrum of allergic diseases. Flow-CAST testing is available to help to diagnose preservative hypersensitivity. In the laboratory test requests from one private laboratory using data from South Africa during the period 1 June 2017 to 31 May 2019, the following results were found:

- Overall, of the requested tests, 75% were negative, 16% were positive and 9% were indeterminate.
- Slightly more preservative CAST requests were requested for females than for males.
- The most common age groups testing was requested for were the 36–55 age bracket, followed by <18 age group.
- Doctors who requested the most tests were ENT surgeons followed by GPs. They were followed in turn by adult physicians and paediatricians.
- Five CAST tests were requested, with sodium salicylates being requested the most, followed by potassium metabisulphite; sodium benzoate, MSG and sodium nitrite tests were ordered with almost equal frequency.
- GPs and paediatricians were more likely to get positive results than the other groups of doctors. This suggests that they selected their tests more carefully. This is most likely due to these doctors having a special interest in allergies.

The data suggest that GPs were specific in ordering CAST tests, with differences noticed in the types of test requested. ENT surgeons, paediatricians and physicians seemed to be less discriminate in test ordering and seemed commonly to request the panel of the five available preservatives.

Although the reason for a referral was not available from the laboratory data, certain assumptions may be made. Requests were slightly higher in the female group of patients than in the male group. A possible reason for this may be that females seek medical advice more regularly than males;²⁹ however, the type of presenting allergic disease yielding preservative testing may be more common in females than males. An example of this would be chronic urticarial, which occurs twice as often in the female than in the male population.³⁰ Hormonal factors are cited as one possible explanation for these differences in the gender prevalence of chronic urticaria.²¹

In the preservative testing requests and the different age groups, one-third of the patients in this study ranged from 36–55 years (35%), which was the biggest age group that had CAST tests done. As ENTs were the main providers requesting the tests, it seems that certain conditions relating to the nose – such as chronic AR or nasal polyps, which commonly present in adulthood and have been associated with preservatives – may have been the reason these tests were performed.¹⁷ The next largest group were patients under the age of 18 years. The high rate of preservative test requests in this age group may be due to the high prevalence of food allergies,³⁰ asthma and eczema³¹ that are seen during childhood. These doctors may have felt their patients' clinical pictures were associated with or exacerbated by preservative ingestion or exposure.

GPs ordered these tests commonly, second only to ENTs. On closer inspection, the majority of this group of GPs have a special interest in allergies and have attained diplomas in allergology. This would explain both the high frequency of tests being ordered and the reason for this group being more selective when ordering these tests: they opted for specific preservatives as opposed to the entire preservative panel. This may also be a reason why GPs attained more positive results than many of the other groups of doctors who ordered these tests.

Salicylates were the most commonly requested preservative. As the data are limited, we do not know the clinical picture of the patients who were tested. Salicylate sensitivity may present with sinusitis, rhinoconjunctivitis or nasal polyps and, for this

reason, present more often to GPs and ENTs than to other groups of doctors. In this study, GPs and ENTs requested the most preservative tests; therefore, this may be a reason for the high proportion of salicylate test requests.

The ordering pattern for preservative Flow-CASTS seems to differ between the four different groups of practitioners who commonly request these tests: that is, ENTs (32%), GPs (24%), paediatricians (18%) and physicians (19%). Likewise, the chances of getting positive results were more likely for GPs and paediatricians than the other groups, which suggests that they may be selecting the patients for testing more carefully than the other groups are. It is impossible to say from the data what percentage of allergy patients seen by each group was referred for allergy testing, and the patients referred for testing may represent those cases that were more difficult to explain than the rest. Our findings suggest that this type of training is likely to direct allergy testing in a way that is tailored to the specific patient, but further studies will be needed to confirm this suspicion. We hope that this data will be useful in encouraging practitioners who deal with this group of difficult-to-manage patients to consider more directed training in the management of allergy.

LIMITATIONS

A significant limitation of this study was that we were unaware of the reason for the preservative CAST request.

CONCLUSION

This study analysed the ordering patterns of preservative Flow-CAST tests by a range of different practitioners who manage allergic disease. The most common specialties ordering these tests were ENTs, GPs, physicians and paediatricians. Those who seemed to order more directed tests and whose tests yielded more positive results were those doctors who had an interest in allergies and had studied this topic further.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

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