*Clinical Chemistry* Trainee Council
Pearls of Laboratory Medicine
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**TITLE:** **Allergy**

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**Slide 1:**
Title slide

**Slide 2:**
Before discussing the specifics of allergic disease, it is important to understand the various types of hypersensitivity reactions. There are four classes of hypersensitivity reactions, each associated with specific mechanisms and mediators. The type I reaction, also known as immediate hypersensitivity, is dependent on IgE antibodies and mast cells. The type II and type III reactions are also antibody-mediated reactions, although they are dependent on IgM and IgG antibodies. The difference between these two reactions is that the type II hypersensitivity is dependent on soluble antibodies while the type III hypersensitivity is dependent on immune complex formation between the IgG or IgM antibody and an antigen. Lastly, the type IV hypersensitivity reaction, also known as the delayed-type hypersensitivity reaction, is independent of antibody production and is caused by activation of CD4+ and CD8+ T cells. In the context of allergic disease, we will focus on the type I or immediate hypersensitivity reaction.

**Slide 3:**
As stated previously, IgE and mast cells are the primary mediators of a type I reaction. IgE is the least abundant circulating immunoglobulin isotype, generally on the order of 10 to 20 ng/mL. It also has the shortest half-life of any immunoglobulin – around 2 days. It is generally not present free in circulation, but rather is bound to mast cells through a high-affinity Fc receptor. Mast cells are a type of granulated leukocyte. The granules within the mast cells hold a variety of pre-formed inflammatory mediators that are released when the mast cell becomes activated. Mast cells reside in most tissues, but they are found in relatively high density within the skin and gastrointestinal tract.

 

**Slide 4:**
There are 3 phases of a type I hypersensitivity reaction. During the sensitization phase, an individual is exposed to a specific antigen, or allergen, and produces an IgE antibody specific for that antigen. In this phase, the allergen-specific IgE is produced but no clinical symptoms manifest. However, if the patient is re-exposed to that allergen, mast cells are activated through cross-linking of the high-affinity IgE receptors. This is followed by the effector phase, in which the inflammatory mediators are released from the mast cells, leading to the clinical presentation we view as an allergic response.

**Slide 5:**
In making a diagnosis of allergic disease, it is critical to obtain a thorough clinical history and establish a correlation between exposure to the candidate allergen and the onset of clinical symptoms. The next step is to identify the allergen responsible for the clinical symptoms, which can be done indirectly by determining whether the patient has an IgE antibody that specifically recognizes a given antigen. This can be assessed either by *in vivo* skin testing or *in vitro* allergen-specific IgE testing. Lastly, it may be necessary, in a minority of patients, to perform an allergen challenge and demonstrate that exposure to the allergen results in clinical disease. This is generally not necessary to establish a diagnosis of allergic disease, and the decision to perform this challenge should only be made by a clinician.

**Slide 6:**
The laboratory evaluation for allergic disease includes testing for total IgE, allergen-specific IgE, and inflammatory mediators. Identification of the allergen-specific IgE, either by skin testing or by *in vitro* immunoassay, is useful in establishing a diagnosis of allergy. In contrast, evaluation for inflammatory mediators, specifically histamine and tryptase, are not useful in diagnosing an allergic response but rather to evaluate a suspected anaphylactic episode.

**Slide 7:**
For total IgE testing, the most common methodology used in the clinical lab is a solid-phase sandwich immunoassay using 2 anti-IgE antibodies. Most clinical assays are well-standardized, and are calibrated against the WHO 75/502 human reference serum. Total IgE concentrations may be reported as IU/mL or ng/mL, with the conversion being 1 IU/mL is equal to 2.44 ng/mL.

**Slide 8:**
There are a variety of clinical uses for total IgE testing. It is one of the diagnostic criteria for allergenic bronchopulmonary aspergillosis, with concentrations greater than 1000 ng/mL being consistent with that diagnosis. It is also useful for evaluating patients with suspected hyper IgE syndrome. This is a rare heritable primary immunodeficiency that is characterized by increased concentrations of IgE, hypereosinophilia, and recurrent staphylococcal infections. Lastly, total IgE also plays a role in the evaluation of suspected allergic disease. Although increased concentrations of total IgE are consistent with an allergic response, it is important to note that significant allergy can occur in patients with normal total IgE concentrations. In addition, total IgE concentrations are useful in patients who are already diagnosed with allergic disease to identify candidates for anti-IgE therapy and to establish the proper dosing. Generally, patients would be considered for this therapy if the total IgE concentrations are between 70 and 800 IU/mL.

 

**Slide 9:**
Next, we will move on to the allergen-specific IgE testing. This type of testing measures only the IgE antibodies that will recognize and bind to a specific allergen molecule. Skin testing is an *in vivo* bioassay in which a small amount of allergen is injected into the skin. If there are IgE antibodies that recognize the injected allergen, the allergen will bind to the IgE and induce cross-linking of the Fc receptors, resulting in activation of the mast cells present within the skin. The subsequent reaction, known as the "wheal and flare response," is caused by the mediators that are released from the mast cell granules.

The other approach to identify allergen-specific IgE antibodies is to use an *in vitro* immunoassay. This is similar to the total IgE measurement, except that an immobilized allergen is used to capture the IgE, which is then detected with a labeled anti-IgE antibody. Although it is said that this testing is used to "diagnose allergic disease," what both methods are really doing is identifying sensitized individuals, or individuals who happen to have an IgE antibody that is specific for a given allergen. The mere presence of a specific IgE does not determine whether that individual, when naturally exposed to that allergen, will actually mount a clinical allergic response. However, there does appear to be a relationship between the amount of specific IgE present and the probability that exposure to that allergen will ultimately lead to an allergic reaction.

**Slide 10:**
The *in vitro* immunoassays have evolved over time, and there have been many improvements with each subsequent generation of testing. The first generation tests used a solid phase that had limited allergen-binding capacity. In the subsequent generations of testing, the solid phase was improved and the binding capacity was increased significantly. This has resulted in improved analytical sensitivity for the allergen-specific IgEs and decreased time required to perform the testing.

Another significant change involved the detection antibody, with a move away from a radioactively-labeled anti-IgE antibody to an enzyme-labeled anti-IgE, generally used with a fluorescent substrate system. The general term "RAST" came from the first-generation assays, and stands for radioallergosorbent test. Although the radiolabeled detection antibody is mostly no longer used, the term RAST is still used generically to refer to all *in vitro* testing for allergen-specific IgE antibodies. Lastly, the quantitation of the specific IgEs has also improved, primarily due to the fact that the calibration curve for total IgE that is traceable to the WHO standard is used for all allergen-specific IgEs as well.

**Slide 11:**
The clinical utility of specific IgE testing is to provide evidence to support the diagnosis of allergic disease through the identification of a sensitized individual. Although the presence of an allergen-specific IgE does not absolutely establish that exposure to the allergen will result in an allergic response, it does define a certain probability that a response may occur. This is related, at least for a number of allergens, to the quantitation of the allergen-specific IgE antibodies. Lastly, by identifying the allergen-specific IgEs, the clinician may better optimize the treatment strategy for a given patient, by advising ways to avoid exposure to the allergen or perhaps to guide possible immunotherapy.

**Slide 12:**

Lastly, we come to the evaluation of histamine and tryptase in the context of an anaphylactic reaction. Anaphylaxis can be a manifestation of a severe allergic response. It is generally an acute response that is systemic in nature. The consequences of an anaphylactic reaction can be severe, and prompt medical attention is critical. To evaluate a suspected case of anaphylaxis, histamine and tryptase, which are two of the preformed mediators found with the granules of mast cells, can be measured.

Histamine is a bioactive nitrogen compound that acts as both a neurotransmitter and as a regulator of the immune response. It is rapidly metabolized to N-methylhistamine, which is subsequently released into the urine.

Tryptase is a neutral protein with enzymatic activity similar to trypsin. There are two forms of tryptase – immature and mature. Immature tryptase is released spontaneously from unstimulated mast cells while mature tryptase is released only from mast cells that have undergone activation and degranulation. Tryptase and histamine are useful in the evaluation of anaphylaxis as both are markers of mast cell activation. Tryptase is measured only in plasma, while histamine can be measured in either plasma or urine. In addition, tests for the N-methylhistamine metabolite in urine are also available.

The challenge with measuring histamine and tryptase relates to their relatively short half-lives. The half-life of histamine in plasma is approximately 10 to 15 minutes; the half-life of tryptase is around 2 hours. As a result, it is critical that samples for histamine and tryptase be collected as soon as possible after initiation of the suspected anaphylactic reaction. For histamine, it is recommended that plasma be collected within 30 minutes of the reaction, although this time can be extended for histamine and N-methylhistamine analysis in urine. For tryptase, blood samples should be collected within 1 to 2 hours of the reaction. This is clearly not always possible, which is a significant limitation to this type of testing.

**Slide 13:**

So, in thinking about evaluating a patient with suspected allergic disease, it is important to guide test selection based on the specific patient. A thorough history is critical, as it can help to identify potential causative allergens. It is important that exposure to the allergen be considered in the context of when the clinical symptoms developed. At that point, rational choices can be made regarding testing for allergen-specific IgE antibodies. One of two approaches can be taken. Testing can be done for a limited number of allergen-specific IgE antibodies for which there is a high degree of clinical suspicion. Or, multi-allergen panels can be used as a screening test. These are panels in which multiple allergens are included in a single reaction with the generation of a single result. If the result is negative, then the presence of an IgE antibody to any of those allergens has been ruled out. On the other hand, if the result is positive, then the interpretation is that the patient has an IgE antibody to at least one of the allergens and follow up testing is necessary to identify the specific antibodies.

The overall message is that testing for many individual allergen-specific IgE antibodies in a given individual should be avoided. Rather, the selection of appropriate testing should be based on known exposures for that individual that have a higher likelihood of being associated with the allergic response.