Immunoassay Testing of Alpha-Gal Specific Immunoglobulin-E: Data from a National Reference Laboratory

Article:
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Guests: Drs. Abdul Saadalla and Patricia Slev from the University of Utah School of Medicine and ARUP Laboratories.

Randye Kaye: Hello, and welcome to this edition of *JALM* Talk from *The Journal of Applied Laboratory Medicine*, a publication of the Association for Diagnostics & Laboratory Medicine. I'm your host, Randye Kaye. Alpha-gal syndrome is a serious, potentially life threatening, acquired allergic condition that occurs after people eat red meat or are exposed to other products containin the carbohydrate known as "alpha-gal." A CDC report showed that between 2010 and 2022, more than 110,000 suspected cases of alpha-gal syndrome were identified in the US. However, because public health reporting is not required for this condition, it's likely to be an underestimation.

In the clinical laboratory, serology testing for alpha-gal specific IgE, which has high clinical sensitivity and specificity, is essential for establishing and confirming the diagnosis. The March 2024 issue of *JALM* features an article that presents alpha-gal specific IgE results from over 15,000 samples tested in the National Reference Laboratory. The authors correlated alpha-gal positivity to patient characteristics, time of the year, and results of other co-tested allergens.

Today, we're joined by two of the article's authors. Dr. Abdul Saadalla is an assistant professor in the Department of Pathology at the University of Utah School of Medicine, and Dr. Patricia Slev is a professor in the same department. Both Dr. Saadalla and Dr. Slev are also medical directors in the Immunology section at ARUP laboratories. Welcome, Drs. Saadalla and Slev.

Patricia Slev: Thank you so much for having us today and allowing us to share our exciting findings with the *JALM* audience.

Randye Kaye: First, can you give us some more background information about alpha-gal syndrome? Who can get it, and what are the symptoms?

Patricia Slev: So, a little bit about the background. Alpha-gal is actually an oligosaccharide, which is present on proteins and lipids in red meat and red meat derived products. It's actually present in all mammals except humans and old-world monkeys, which

are baboons and macaques for example. It's actually been estimated that humans lost the ability to produce the alpha-gal during primate evolution about 28 million years ago, and this is due to a loss of function mutation in the gene encoding the enzyme that catalyzes the generation of the alpha-gal. And why did this happen? It's believed that this loss is thought to enhance human immunity to bacterial infection and specifically bacterial induced sepsis. Another interesting thing about alpha-gal is about 1% of the entire IgG immunoglobulin repertoire is anti-alpha-gal specific in humans, and it's actually known that this is a major hindrance to xenotransplantation.

Now, who can develop alpha-gal allergy and what are the symptoms? When IgE antibodies to alpha-gal are present, this can lead to development of the alpha-gal syndrome, which, as you already mentioned, is generically known as an allergy to red meat. So this includes products like beef, lamb, and pork. This allergy is unusual in that the allergy symptoms typically occur two to six hours after meat ingestions, sometimes a little bit longer, depending on the timing of digestion of the glycoproteins and lipids which are found in alpha-gal. The symptoms can range from urticaria and general GI manifestations all the way to anaphylaxis. And what is unique is that patients are typically adults who had previously had no issues with eating any red meat. So this is actually becoming an increasingly recognized syndrome and quite a hot topic.

There is also strong epidemiologic and experimental evidence linking tick bites to adults subsequently developing allergies to red meat. The tick bite, specifically due to bite by the lone star tick, is what is believed to be involved in the US. It has been shown that the tick saliva contains alpha-gal glycans, which possibly induce inflammations when a human is bit, which leads to the alpha-gal syndrome in the human, and the allergy.

Now, what's also interesting about this is how it was originally discovered, this alpha-gal syndrome, red meat allergy. There was a drug that was developed primarily for colorectal and head and neck cancers called cetuximab. And it was discovered that a large proportion of individuals from certain geographic areas in the country developed this anaphylaxis and allergic syndrome when they were treated with cetuximab, and it was subsequently discovered that the IgE that was present in these patients, and it was present prior to initiating the therapy, was actually specific IgE against alpha-gal.

So that's an interesting discovery about this allergy, and also a bit of a hint about the geographical distribution of this allergy.

Randye Kaye: All right, thank you. And we're going to hear more about some of those things in just a moment. I have to tell you. I feel somewhat vindicated because I haven't eaten red meat in decades. And before it was fashionable to not eat red meat, I used to tell waiters I was allergic to red meat. So now I just feel totally validated. Can you tell us a little bit more about the role of serum IgE testing in the diagnosis of alpha-gal syndrome?

Abdul Saadalla: Yes, so it's detection of specific IgE to alpha-gal can be actually a very helpful test to clinicians to diagnose and establish alpha-gal syndrome diagnosis. So, our lab uses the ImmunoCAP assay, which is widely used by clinical laboratories. This assay has excellent performance. We actually offer the test ,meaning for alpha-gal specific IgE as a standalone, or in a panel that also includes specific IgE tests to beef, lamb, and pork, red meat, whole allergen extracts. Interesting finding from our studies that we often see that specific IgE to alpha-gal is often higher than the specific IgE to the co-tested whole red meat allergens, like, again, beef, lamb, or pork. And we think that knowing or seeing this pattern could be helpful to clinicians to diagnose alpha-gal syndrome.

Randye Kaye: All right, thank you. Now, you mentioned before that the study correlated the alpha-gal test results with several factors, but including geographic regions across the US. So, what exactly did you observe about the geographic distribution of antibody positivity?

Patricia Slev: So, first, I want to say that one of the surprising findings of our study was the prevalence of samples that were positive for specific IgE to alpha-gal, which was 37%. The positivity, or detectable antibody titers, were also highest in samples submitted to our lab from midwestern and southern states, which matches the known geographic distribution of the lone star tick. The lone star tick is known to be an aggressive biter type of tick, and the lone star tick range is expanding into various geographic territories. So originally, it was mostly found in the upper midwest, but now it's actually expanding into western states, like Nebraska and Oklahoma. Now, it's not necessarily clear why this is so. It could be due to changes in climate, forestation, or increase in deer populations. But of note, the fact that the range of this tick and the distribution of the tick is expanding could lead to an increased prevalence of this allergy in other parts of the country. So that is something to keep an eye on.

Randye Kaye: All right, thank you. So the lone star tick is also implicated in the transmission of Rocky Mountain spotted fever. So, you've already said quite a bit about this, but anything more to say

about finding any correlation between Rocky Mountain spotted fever serology titers and alpha-gal specific IgE levels?

Patricia Slev: Yeah, so that was one of the questions that we wanted to look at. Since it's believed that the lone star tick is actually involved in both of these, transmission of Rocky Mountain spotted fever and the alpha-gal syndrome, we wanted to look at how this correlates the syndrome and Rocky Mountain spotted fever in terms of lone star tick. So what we did is we identified a subset of serum samples that were co-tested for both Rocky Mountain spotted fever serology, both IgG and IgM, as well as alpha-gal specific IgE. And interestingly, we found that there were higher concentrations of alpha-gal specific IgE in samples that were also positive for Rocky Mountain spotted fever.

In addition, high antibody titers to Rocky Mountain spotted fever also showed a similar pattern for alpha-gal serology. What do I mean by that? I mean that there was actually a temporal correlation or a seasonality to this. So, it was highest in spring and summer for both RMSF, or Rocky Mountain spotted fever, as well as alpha-gal syndrome, which of course is supportive evidence of the role of lone star tick in AGS, as that's when people are out and about and exposed to lone star tick bites. Now, the Rocky Mountain spotted fever ELISA assay is not specific for the rickettsia, which is believed to be the causative agent of RMSF, and so there could be some cross reactivity. But that's one of the parts of testing that needs to be developed more, particularly with additional serology and molecular assays that could then differentiate between co-transmitted rickettsia species, whether it's pathogenic and how it's related to alpha-gal syndrome specifically.

Randye Kaye: All right, thank you. Well, what about patient age, patient sex? Any association between age and sex with the alpha-gal specific IgE titers?

Abdul Saadalla: Yes, so this was surprising and interesting finding of our study. We found that the titers of a specific IgE to alpha-gal were actually higher in older aged patients. We do not know why is that. It could be just because of longer times or more frequent exposures in older patients. Or, it could be related to, for example, age-related compromise in skin barrier function or integrity, or changes in immune system response over time with age that could make it more allergic, or what we call that T-helper 2 phenotypes. So this is not clear to us, but I think it's an area of more research.

Randye Kaye: All right, thank you. One final question. Let's kind of sum it up. Overall, what do you think are the most important findings from the study, and then are there any questions

that remain to be answered in the future for alpha-gal syndrome?

Abdul Saadalla: I think we think the most striking finding from our study is really the high prevalence. So, like, out of 15,000 samples to find that almost 37% have detectable specific IgE to alpha-gal, this is striking to us. But for research, I think future studies focused on understanding how local skin and systemic immune responses could affect or impact alpha-gal pathogenesis. This is certainly an area of further investigation and whether there is any pathogenic role for co-transmitted bacteria that are present and detect saliva, this would also be very interesting. So hopefully a lot more to come, and we definitely need a lot more work.

Patricia Slev: We just need to do more studies on the mechanism and why some people do develop and whether everybody develops this when they are bit by a lone star tick. I don't think that's known yet.

Abdul Saadalla: Yes, great.

Randye Kaye: A lot of unknowns there. All right, well, thank you so much. Very interesting, and thank you for joining us today, doctors.

Patricia Slev: Thank you.

Abdul Saadalla: Thank you for having us.

Randye Kaye: That was Drs. Abdul Saadalla and Patricia Slev from the University of Utah and ARUP Laboratories, discussing the *JALM* article "Immunoassay Testing of Alpha-Gal Specific Immunoglobulin-E: Data from a National Reference Laboratory." Thanks for tuning in to this episode of *JALM* Talk. See you next time and don't forget to submit something for us to talk about.