EAACI Research Fellowship 2020: Final Report

Project title: In Vitro Diagnostic Tests for Perioperative Hypersensitivity and Immediate Hypersensitivity reactions to antibiotics.

Research Fellow: Gustavo Jorge Molina Molina

Type of Fellowship: Short term Research Fellowship

Host supervisor: Prof. Didier Ebo

Location: Immunology, Rheumatology and Allergy Department of the Antwerp University

Duration: 3 months (January 2020 – March 2020)

I have been awarded with the EAACI Research Fellowship grant 2020 to perform short term training in another European country. I spent three months in the Immunology, Rheumatology and Allergy department of the Antwerp University in Belgium.

The questions addressed:

The aim of my fellowship was to broad my knowledge in the use of *in vitro* diagnostic tools in allergy, with focus in perioperative hypersensitivity reactions (POH) and hypersensitivity reactions to antibiotics.

POH are rare but can be life threatening. Unfortunately, correct diagnosis of suspected immediate perioperative allergic reactions can pose significant challenge. The standard reference test for accurate diagnosis of suspected immediate perioperative allergic reactions is a controlled drug provocation test. However, because of obvious ethical and practical limitations, the drug provocation test is currently mainly advocated in difficult cases who demonstrate equivocal or negative results. Therefore, in clinical practice, diagnostic approach, of suspected immediate perioperative allergic reactions, generally starts with judicious skin testing and quantification of drug-reactive specific IgE antibodies (sIgE). sIgE determinations has no risk for recurrence of immediate reactions and also point to the potential pathomechanistic process of a POH. The basophil activation test is another technique that could help in the diagnostic approach of POH.

Unfortunately, the reported performance of serologic testing, that is, measurement of serum sIgE shows great variation, especially for β-lactam antibiotics and NMBAs. Correct serologic diagnosis of rocuronium allergy is mainly hindered by clinically irrelevant positive sIgE results because of nonspecific binding to the solid phase in cases of elevated serum total IgE (tIgE). Therefore, it has been proposed that calculation of a drug-specific could help to clarify the interference of tIgE on sIgE results and depict the clinical relevance of a positive sIgE result. Moreover, in the context of drug allergy, it has been shown this strategy to be helpful in optimizing serologic diagnosis of allergy to β-lactam antibiotics such as aminopenicillins and cefazolin. However, no data regarding the application of the sIgE/tIgE ration in other drug allergies are available.

The main question was if this strategy could be useful to improve in vitro diagnosis of rocuronium allergy, a major cause of perioperative allergy and anaphylaxis.

Nature of the Research:

Dr. Ebo and his team have a wide experience in the diagnostic approach and treatment of perioperative hypersensitivity reactions. My research fellowship was divided in two main tasks.

On one hand, I reviewed and included new patients into a database of POH. During the review of the database, we selected patients diagnosed as allergic to rocuronium (ROC+).
Positive diagnosis of rocuronium allergy was confirmed by skin test (ST) and basophil activation test's (BAT) positivity, requiring in addition a measurement of total IgE (tIgE) and specific IgE (sIgE) independently of the values. Evaluation of sensitization to morphine and pholcodine in ROC+ patients was made with the determination of sIgE as it has been described previously that they could be used as marker of sensitization to tertiary and quaternary ammonium determinants.
Negative controls for groups and subgroups were also analysed. All of control patients were assessed for allergy to their respective suspicious drugs after an initial exposition using ST, BAT and were declared negative after the finding of another trigger that explained the initial reaction, requiring as in the same case as allergic patients, the serum determination of tIgE and sIgE against the drugs of each group.
The measurement of tIgE and sIgE to rocuronium, morphine and pholcodine were carried out using an ImmunoCAP System FEIA (Thermo-Fisher, Uppsala, Sweden) according to the manufacturer's instructions.

On the other hand, I performed basophil activation test on the new patients with suspicion of rocuronium allergy that underwent the diagnostic approach mentioned above. After the result of the tests, those patients were included in the database as ROC+ or controls. This technique was performed in the Immunology laboratory of the Antwerp University.

After all the inclusion of patients, we carried out the statistical analysis.

Our primary endpoint was to explore the clinical utility of the sIgE/tIgE ratio for rocuronium, compared to the quantification of sIgE rocuronium, in the diagnosis of rocuronium allergy. Secondarily, to study the value of similar ratio's for morphine and pholcodine in the diagnosis of rocuronium allergy.

What was the result?

Regarding the database analysis, I have improved in my skills in the use of software used to process data as well as statistical analysis. During my lab training I also learned the protocols of basophil activation test as well as the use of flow cytometer and its software to analyse the results.

This led us to find that in rocuronium allergic patients, the median for sIgE rocuronium, morphine, and pholcodine was significantly higher than in controls. The median of the sIgE/tIgE ratios for morphine and pholcodine were significantly higher in ROC+ as compared to controls. This was not the case for sIgE/tIgE rocuronium. We analyzed sensitivity and specificity of the different diagnostic methods using Receiver Operator Curves (ROC). Our analyses showed that sIgE values to rocuronium performed significantly better than the sIgE/tIgE ratio for differentiating between patients and controls.

This research activity has produced one manuscript, already submitted in a journal but not accepted yet where the EAACI fellowship was properly acknowledged.

How will the findings impact future research?

Correct diagnosis of suspected immediate perioperative allergy presents a significant clinical challenge with serious consequences of diagnostic error. Accurate diagnosis is critical for patient management and prevention of subsequent reactions, whereas overdiagnosis can lead to unnecessary avoidance measures and, more importantly, failure to identify the real culprit(s). In this regard, it is dissuaded to rely upon a positive sIgE result in isolation to confirm their clinical suspicion, because of the risk of clinically irrelevant results due to nonspecific binding. As this nonspecific binding is mainly observed in patients with elevated titters of tIgE, calculation of a sIgE/tIgE ratio could be used as a simple and safe approach to improve diagnostic performance of serology. From our data emerged that calculation of such a drug - specific sIgE/tIgEratio does not benefit serologic diagnosis of rocuronium allergy.

Unlike sensitization to benzylisoquinoline NMBAs, sensitization to rocuronium can also be explored indirectly by measuring IgE reactivity to tertiary and quaternary substituted ammonium structures that are considered to be the major epitopes of NMBAs by using morphine -based and/or pholcodine assays. Unfortunately, sIgE to these opiates are prevalent in the general population. As for the rocuronium sIgE/tIgE ratio, we observed a sIgE/tIgE ratio for both opiates not to benefit diagnosis of rocuronium allergy.

In conclusion, the application of specific/total IgE ratios for rocuronium, morphine and pholcodine does not benefit the positive likelihood and overall diagnostic performance of serologic testing for rocuronium allergy.

Adaptation of the research from the original plan

First we wanted to analyse not just in vitro techniques for perioperative hypersensitivity diagnosis, but also for antibiotics. After considering the amount of data available regarding the NMBAs and the relevance of the use of basophil activation test, as it was mainly used in the correct diagnostic approach of POH than in antibiotic allergy, we finally decided to limit our research to rocuronium suspicion of allergy, carrying out the project described above.

Personal reflection

This fellowship was an important step in my training to develop my researcher skills, focused in drug hypersensitivity. The experiments performed during the stay helped me to improve my laboratory skills, not only regarding the performance of basophil activation test, but also it made me see the dynamics of a research lab. Moreover, I had the opportunity to spend three months in a top-level university and also to attend clinical staff meetings where clinical cases were discussed, letting me to participate taking into consideration my opinions as a clinician. In addition, professor Ebo's team asked me to make a presentation about LTP food allergy in Spain with the aim of sharing our experiences as the pattern in Belgium and Spain is different. For me this was a clear reflection of the collaborative spirit that clinicians and researchers from different geographical place have to show in medicine.
Furthermore, in the research staff meeting, all the researchers of the lab shared every week the progress in their respective projects. When I advanced enough on my own project, I felt part of the team so we helped each other sharing ideas or possible solutions to the different difficulties that appeared in an open-minded atmosphere.

Finally, living in Antwerp during three months allowed me not just to know better Belgium, its weather and the most important cities. It made me contact with other behaviours in their people, different from Spanish but friendly and open-minded too. At the end I saw Belgium as a second home where I also leave new colleagues and friends.

Acknowledgements

First, I would like to thank to Dr. Didier Ebo for providing the valuable fellowship chance in Antwerp. It was my honour to join the Immunology, Rheumathology and Allergy Department at the Antwerp University. I enjoyed the staff meeting and discussions of clinical cases and research. I would also like to warmly thank all the members of the research group, who made me feel part of the team from the first day. I wanted to thanks Christel Mertens for spending a lot of her time introducing me into the procedure of basophil activation test as well as the use of the software to interpret the results. I really appreciate her good mood and all the effort she put on answering all my questions. Also, special thanks to Jessy Elst for her support during my acquisition of laboratory skills, always ready to give me counsel when I had doubts.

This EAACI Fellowship has helped me to develop my research skills by learning a new laboratory technique in an international scientific research environment. Furthermore, I am sure this will be the beginning of future collaborations with the team of Dr. Ebo.

Finally, I would like to thank to the EACCI Headquarters for this opportunity. This short research fellowship has fulfilled my expectations and I would encourage other researchers to apply for the next openings.