Final Report
Medium-term EAACI Research Fellowship
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“Clinical validation of an innovative multi-parametric test in allergic rhinitis”

This report aims to describe my main activities and achievements over the period of my fellowship (1st May 2017 – 31st October 2017) at the University of Charité (Berlin), dealing with a project targeted to validate novel diagnostic methods and approaches for chronic seasonal allergic rhinitis.

§1. Background
The etiologic diagnosis of pollen-seasonal allergic rhinitis (SAR), essential for the correct prescription of allergen specific immunotherapy (AIT), is traditionally based on a brief medical history (interview doctor-patient) and skin testing (SPT), performed almost exclusively by allergists. The clinical diary greatly improves the accuracy of the clinical history but it is rarely used because its analysis requires excessive time. The electronic clinical diary (eDiary) can overcome this difficulty at no cost. In the Mediterranean areas, at high aerobiological complexity, the traditional etiological diagnosis is relatively inaccurate in patients sensitive to highly cross-reactive pollens. The molecular diagnostics increases the accuracy of the etiologic diagnosis. However, molecular diagnostics (CRD) is poorly performed, as it is complex and difficult to be interpreted. Considering this combination of factors (and many others), only a small minority (2-6%) of eligible patients receive AIT, whose prescriptive precision is moreover often insufficient.

This study aims to validate novel diagnostic methods for chronic seasonal allergic rhinitis, including the determination of allergen-specific IgE antibodies in nasal secretions and the use of allergy-app and mobile health technologies for disease monitoring. Further, it targets to check if the combined use in an innovative algorithm (clinical decision support system, CDSS) of a multiplex molecular testing and a clinical electronic diary, for the interpretation of their results, may: improve the etiological diagnostics of SAR in a safe, effective and economical manner; favour a more accurate prescription of AIT; and enhance the diagnostic ability of the allergy specialist (AS) and general practitioner (GP).

§2. Study design and population
This project is an observational longitudinal bi-centric clinical study coordinated by Charité Medical University (Berlin, Germany). It investigates 99 adults (Pordenone) and 101 pediatric patients (Rome, both Italy) with Seasonal Allergic Rhinitis. The object of the study has been only in part the patient and primarily the doctor (i.e. the allergy specialist and general practitioner) who makes etiologic diagnosis in these patients.

Patients underwent a complete allergy diagnostic work-up, including not only a detailed retrospective clinical history collection and SPT, but also blood drawing and nasal secretion collection for IgE detections against allergen molecules, a prospective collection of clinical data with mobile-Health technology and allergen-specific nasal provocation test (NAPT) with the extract of the AIT-candidate pollen(s). The impact of the customized allergen-assay and the electronic clinical diary on AIT prescription of ASs and GPs have been also evaluated and their combined use in an innovative clinical decision support system algorithm (1st step, clinical history + SPT; 2nd step, clinical history + SPT + CRD; 3rd step, clinical history + SPT + CRD + eDiary). To this aim,
a workshop (WS) has been organized in each clinical center with 46 physicians collaborating with each centre on a regular base: 21 (ASs, n = 11; GPs, n = 10) attending the AIT-WS in Rome and 25 (ASs, n = 7; GPs, n = 18) the AIT-WS in Pordenone (both Italy). Doctors have expressed their prescriptive decision on the basis only of the clinical history and the results of SPT (traditional diagnostic approach) and then based on the previous information supplemented by the results from the customized assay and the electronic clinical diary.

§3. Activities

I have contributed personally to all the following phases of the study:

1) Staff training and standardization of procedures;
2) Recruitment of patients and clinical visits: informed consent, questionnaires, NAPT, SPT, basic diagnosis, collection of blood and nasal secretion samples;
3) Clinical-computer-aerobiological monitoring;
4) Workshop with allergy specialists and general practitioners;
5) Laboratory assays;
6) Data management and statistical analysis;
7) Preparation of scientific papers in English.

The first three phases were overall preliminary and preceding the fellowship period. During the latter, I focused on the other phases as better explained below.

- **Workshop (WS)**
  - **Method setting and preliminary organization:** Setting and timing have been established. Clinical cases to be evaluated by physicians have been selected. Slides and the other informative materials have been prepared.
  - **WS phases:** a) educational training; b) clinical cases; and c) “feedback” survey. During the first part of the WS, the target, nature and methodology of the diagnostic tools proposed (questionnaires, SPT, CRD, eDiary) have been presented in comprehensive lectures. During the second part of WS, doctors were asked to express their therapeutic opinion concerning the 10 clinical index cases selected among the study population (n = 200). Each physician reported his/her own AIT decisions on the base of the primary data progressively added (clinical history+SPT; clinical history+SPT+CRD; clinical history+SPT+CRD+eDiary) concerning each individual patient. Finally, the doctors filled a questionnaire on the impact of CDSS and its perceived benefits as well as on the role of each diagnostic tool or step on the doctor’s “virtual” AIT prescription. In addition, participants were asked to express their satisfaction level on the entire AIT-WS (tutorial, clinical cases and feedback survey) in terms of content and general organization.

- **Laboratory assays**
  - **Method setting and standardization:** The methodology to test IgE in nasal secretions, recently established by Prof Matricardi (Berlin), Prof. Gavaert (Belgium), myself and others, have been adapted and validated in Berlin to the targets of the present project.
  - **Testing antibodies serum and in nasal secretions:** The biobank of serum and nasal secretions collected in 200 patients with SAR by the Italian clinical centers have been tested in Berlin with both a customized allergen-microarray and ImmunoCAP (gold standard) to determine IgE against a pre-determined panel of allergenic molecules. The molecules tested include: pollens (nOle e1, rPar j2, rBet v2, rBet v4, rPhl p1, rPhl p5, rPhl p7, rPhl p12, rCyn d1, rArt v1, rAmb a1, rPla a1, nCup a1, rCor a1, nSal k1); natural mold (Alt a 1); cat (rFel d 1, rFel d 2, rFel d 4); dog dander (rCan f 1, rCan f 2, rCan f 3, rCan f 4, rCan f 5, rCan f 6); mites (rDer p1, rDer p 2, rDer p 10, rDer f 1, rDer f2/
nDer p 1, rDer p 2, rDer p 4, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18, rDer p 21, rDer p 23, clone 16, nDer f 1 and rDer f 2).

- **Data management and statistical analysis**
  The databank (including data on clinical history, SPT, and response to NAPT, “virtual” AIT-decisions by ASs and GPs) together with lab data have been organized and regularly updated during their generation and, when completed, the data started to be analyzed. Descriptive and inferential statistical analyses have been performed and some are still ongoing. I have collaborated with the statistician in the more sophisticated statistical analyses.

- **Scientific reporting**
  I have presented the results so far obtained to the group and started to utilize them to write the scientific papers in English.

## §4. Results

During the research fellowship period, we managed to carry out all research activities proposed in the fellowship application. However, as reagents arrived later than expected, lab testing has been postponed compared to the scheduled plan. Therefore, some results still have to be calculated and analyzed and, thus, these cannot be included in this report. The main current results are summarized below.

- **Spectrum of clinically relevant pollen(s) and “virtual” AIT prescription results** - During the workshop, the spectrum of clinically relevant pollen(s) identified according to the proposed CDSS algorithm (integrating in three steps: a) clinical history + SPT; a) clinical history + SPT + CRD; a) clinical history + SPT + CRD + eDiary), has been compared with the “virtual” AIT prescription results from physicians. In patients with only one relevant allergen source according to the CDSS, the AIT agent most frequently prescribed by ASs coincided with the pollen identified as clinically relevant by the innovative CDSS (n = 8). When the CDSS identified two allergens (n = 6), one or both of them have been also prescribed by the allergy specialists. In the case of no (n = 2) or ≥ 4 (n = 4) clinically relevant allergens identified by CDSS, no AIT has been prescribed.

- **Spectrum of clinical relevant pollen(s) versus NAPT** – Eighteen patients (90%) underwent nasal provocation testing with one or more pollen(s) among the clinically relevant ones selected by the algorithm. The NAPT results were all positive, confirming the CDSS diagnosis (CDSS specificity, 100%).

- **Trend and concordance between ASs and GPs in “virtual” AIT prescription** – In both groups (ASs and GPs), the “virtual” prescription of AIT changed significantly through the three diagnostic steps proposed in our CDSS model (p < .01). Through this evolution, the AIT decisions harmonized within each medical category (GPs and ASs) (p <.01) [Figure 1].

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**FIGURE 1.** Changes (%) of the “virtual” prescription of allergen immunotherapy from the most prevalent final decision among allergy specialists for each medical category (allergy specialists and general practitioners) at each of the three diagnostic steps proposed in our algorithm in Rome (A) and Pordenone (B). CRD, component resolved diagnostics; eDiary, electronic clinical diary; Hx, clinical history; SPT, skin prick test. Chi squared test, when
CRD and eDiary impact on AIT prescription by participating doctors – Overall, the number of “virtual” AIT prescriptions increased when, in addition to clinical history and SPT, physicians considered also the CRD, and CRD and eDiary results.

Feedback survey on doctors’ perception regarding diagnostic tools – Doctors filled a questionnaire on the role of each diagnostic tool or step on their own decisions expressed in the AIT-prescription survey. Additionally, their opinion on the algorithm proposed in our innovative CDSS has been assessed. All physicians considered the application of a CDSS useful and recognized its potential in ameliorating the traditional diagnostic procedures [Table 1]. There was agreement also concerning the role of molecular diagnostics in improving the accuracy of AIT prescription (100%). The reliability of clinical history was assessed lacking (70-100%) and optimizable by electronic clinical diary (82-100%). In addition, all respondents judged the latter as easier to be filled by patients and to be interpreted by physicians in comparison to a paper diary. Furthermore, the majority of doctors agreed on a potential role of an electronic diary in the diagnostics of other allergic diseases (e.g. asthma and food allergy). Significant discrepancies were registered among the two medical categories (ASs vs GPs) in one center (Pordenone) concerning the physicians’ opinion on eligibility to AIT of patients sensitized to more than four aeroallergens (p = .03). Participants were overall satisfied by the workshop (educational training, clinical cases and feedback survey) in terms of content and general organization [Table 1].

Table 1 - Answers to the "feedback survey" among allergy specialists and general practitioners for each clinical center in the context of the diagnostics of pollinosis

<table>
<thead>
<tr>
<th>Items</th>
<th>ROME</th>
<th></th>
<th>PORDENONE</th>
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<tr>
<td></td>
<td>Allergy Specialists (%)</td>
<td>General Practitioners (%)</td>
<td>Allergy Specialists (%)</td>
<td>General Practitioners (%)</td>
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<tr>
<td></td>
<td>n= 11</td>
<td>n= 10</td>
<td>n= 7</td>
<td>n= 18</td>
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<td></td>
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<td>Y y n N</td>
<td>Y y n N</td>
<td>Y y n N</td>
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<td>Clinical decision support system</td>
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<td>Reliability of the clinical history</td>
<td>55 36 0 9</td>
<td>60 40 0 0</td>
<td>1.00</td>
<td>86 14 0 0</td>
</tr>
<tr>
<td></td>
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<td>55 45 0 0</td>
<td>70 30 0 0</td>
<td>1.00</td>
</tr>
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<td>Reliability of the clinical history</td>
<td>0 18 55 27</td>
<td>0 30 60 10</td>
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<td>Component resolved diagnosis</td>
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<td>Increase in accuracy of the AIT prescription</td>
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<td>90 10 0 0</td>
<td>3.31</td>
<td>100 0 0 0</td>
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<td>Usefulness of a single assay only for pollinosis</td>
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<td>50 30 20 0</td>
<td>1.00</td>
<td>43 57 0 0</td>
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<td>Electronic diary</td>
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<td></td>
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<td>Contribution to optimal monitoring of pollinosis</td>
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<td>70 30 0 0</td>
<td>1.00</td>
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<td>Reliability of the data registered by the patient</td>
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<td>20 70 10 0</td>
<td>1.00</td>
<td>14 86 0 0</td>
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<td>Electronic compilation easier than on paper</td>
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<td>70 30 0 0</td>
<td>1.00</td>
<td>43 57 0 0</td>
</tr>
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<td>Interpretation easier in comparison to paper diary</td>
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<td>50 50 0 0</td>
<td>0.27</td>
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<td>Potential role in diagnostics of asthma and/or food allergy</td>
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<td>80 20 0 0</td>
<td>2.51</td>
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<td>Exhaustiveness and well-leading of the preliminary tutorial</td>
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<td>Pivotal methodological errors in the workshop (tutorial, clinical cases and survey)</td>
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<td>Eligibility of polisensitized patients to AIT§</td>
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</tr>
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</table>

p*: *Fisher test was used to evaluate the association of categorical data between independent groups.

Considering the preliminary results, I am confident that the innovative concepts proposed in this study will open new avenues to a multi-parametric diagnosis and management of the patient with allergic rhinitis and possibilities of precision medicine.
Acknowledgments

I would like to thank my host supervisor, PD Dr.med. Paolo Maria Matricardi, and all the people have been involved in the project. Finally, I do not want to lose the opportunity to thanks EAACI for providing such an opportunity to promote quality allergy research among young researchers.

Essential bibliography


