Organizational challenges in setting up a breast cancer screening program in Valais and controlling for quality performance in specific IgE measurements

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Abstract

Contributing to organizational challenges through breast cancer prevention programs by mammography in Valais and establishing external quality schemes for specific IgE determinations for the Swiss Society of Allergology and Clinical Immunology has given me experience in bringing together health professionals and insurance agencies at times when practical actions could be taken. The two programs were set up by consensus, knowing that they should be affordable, progressively implementable and professionally managed. Future perspectives and options for development can be drawn from these involvements and could help to support upcoming decisions permitting the programs to evolve toward more equity and professional structures. In Switzerland, the perspective of organized national prevention programs could be built up from local cantonal programs, at least for breast cancer screening program with mammography. The Valais program, started in 1999 was organized as a continuously evaluated program, free of charge for women between 50 and 70 years old. Private and institutional radiologists were involved, six of them were accredited [...]
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Thesis submitted to the Medical School of the University of Geneva

for the degree of Privat-Docent

by

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- Geneva -

- 2013 -
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**Summary:**

Contributing to organizational challenges through breast cancer prevention programs by mammography in Valais and establishing external quality schemes for specific IgE determinations for the Swiss Society of Allergology and Clinical Immunology has given me experience in bringing together health professionals and insurance agencies at times when practical actions could be taken. The two programs were set up by consensus, knowing that they should be affordable, progressively implementable and professionally managed. Future perspectives and options for development can be drawn from these involvements and could help to support upcoming decisions permitting the programs to evolve toward more equity and professional structures.

In Switzerland, the perspective of organized national prevention programs could be built up from local cantonal programs, at least for breast cancer screening program with mammography. The Valais program, started in 1999 was organized as a continuously evaluated program, free of charge for women between 50 and 70 years old. Private and institutional radiologists were involved, six of them were accredited as second readers to include all the regions of the Valais. Indeed, we achieved a participation rate of 70% in five years, we were able to keep the participation free of charge and to have an external evaluation of the program performed every two years. We also participated in building up a national federation and contributed to the implementation of digital mammography, RIS-PACS transmission, multilingual computer programs and quality management in our program.
The external quality assessment program for specific IgE in Switzerland has a ten-year history. It shows that a definite scheme set up to ensure minimal requirements for all participants, in order to be reimbursed by insurances, cannot fulfill the needs for quality control of the larger laboratories and the challenge of new technologies, such as microarray.

The external quality assessment for specific IgE measurements was set up together with the “Centre Suisse de Contrôle de Qualité”, Geneva from 2003-2006 as an optional trial phase. A selection of three allergens (birch pollen, peanut and cat dander) was proposed at each distribution to be detected by the different methods used for specific IgE measurements. In the compulsory phase we were able to show that most participants succeeded either in a POC setting or in large laboratories. This program fulfilled the requirements established by the Qualab for other laboratory analyses.

The practical environment for allergen-specific IgE is rapidly changing to take into account the great increase of knowledge on the different molecular allergens. We have worked recently on the variation of specific IgE to molecular allergens in the course of immunotherapy. So far, we provided preliminary results that need to be validated to overcome many unresolved issues with these technologies, either molecular allergens or microarray in reproducibility. The clinical adequacy for each approach has not yet been settled. Moreover, it is not yet clear how to ensure a proper quality control for these specific technologies.

Innovative solutions can be found to adapt to our specific environment. National cancer prevention programs beyond early breast cancer detection by mammography is now becoming possible.
In a similar trend, the professional societies of laboratory medicine have done their best to set up external quality assessment programs in Switzerland in order for all practitioners to be able to fulfill the same requirements for reimbursement of equivalent medical procedures. The future will show if such efforts can evolve and cope with the challenge of new technologies and divergent needs in the application of medicine.
1. Organizational Challenge to setting up a prevention program: The breast cancer screening program by mammography in the Valais

1.1. Initial situation and interest of the medical community and concerned women in the Valais

The initial situation was very different in each canton in the French-speaking part of Switzerland in the mid-1990s, as far as the screening for breast cancer by mammography was concerned:

In Geneva, the individual opportunistic breast cancer screening by mammography was already well established for about 1/3 of the female population between 50 and 70 years old.

Three pilot sites were conducting a pilot phase program in Vaud, and one site, Aigle, was close to the Valais. At that time, there were less individual screenings by mammography being performed by gynaecologists in the Valais than in Geneva. There was a population-based focused interest for breast cancer screening by mammography by its inclusion in a federal law as a reimbursed procedure (1996).

The interest of radiologists participating in the pilot site program in Vaud (Aigle) was the leading motivation of the medical community, as well as the interest of women in the area
of Monthey wishing to benefit from the same opportunities as their counterparts in Aigle, less than 10 kilometers away.

1.2. Feasibility study and general principles that guided the setting up of the program

1.2.1 General principles for the constitution of the program in the Valais

In setting up the program, we decided to apply the European guidelines and the following points:

1. Strict concordance with the newly-edited federal law and application rules.
2. Creation of a large consensus to insure an ideal coverage of the targeted population of women in the Valais between 50 and 70 years old (fully paid coverage for all women, full translation in German of the program and opinion amplifiers in all regions of the Valais).
3. Have the agreement for the participation of the medical partners (radiologists public and private, gynaecologists and local practitioners) as well as paramedicals, health associations, the cantonal health department and insurance companies.
4. Make sure that the full coverage of the mammography could be financed, including the personal 10% participation, to maximize participation in the program.

1.2.2 Feasibility study

A feasibility study in the Valais permitted all the partners to be involved in advance to prepare the project: how to set up practically the program in the Valais? (Feasability Study, Elisabeth Marty, 1997, internal report)
1.3. Genesis of the screening program covering the entire canton

The program was set up initially in the entire canton of Valais with 11 radiographic institutions (Jean-Blaise Seppey, IUMSP, Lausanne 2002, internal report).

The mammography reading was done onsite and interpreted by the first radiologist, the radiographs were then taken to the Screening Center to be read by a second accredited radiologist. Initially they were six “second readers.” A third reading was done in case of divergent interpretations.

The participation was initially high, as compared with the participation ratios in other cantons and increased further after a few years.

1.4. Parallel evolution in other cantonal programs

At the same time other programs have been set up in the Vaud and Geneva cantons. We have been exchanging experiences and expertise especially with a view to certification of the equipment and personal.

A charter was elaborated between the cantons of Vaud, Geneva and Valais to define common principles and objectives of local breast screening programs. At the time, there was no other formal coordination between the cantons.

Under the authority of the different health departments, formal coordination was set up and pursued for more than 10 years.

In 2008, a Swiss Breast Cancer Screening Federation was created to allow the integration of other cantons who wanted to initiate programs of their own. The harmonization of the
programs became a necessity and added a common advantage, especially in sharing computer programs and transfer of digitalized mammograms to a central location. Finally, in 2012, the Federation changed its name to Swiss Cancer Screening to enable the use of expertise and know-how to set up new programs, as envisioned for colon cancer screening in the Vaud.

1.5. Evaluation of the program’s quality and monitoring

Professional evaluation by an external institution has been an permanent preoccupation of the initiators of the program and the reference group. It was carried out on a regular basis by the IUMSP in Lausanne by Jean-Luc Bulliard. The evaluation periodically addressed different issues such as the organization, the follow up of the intermediate results and compared them with European guidelines and with the performance of the other Swiss programs. The evaluation was carried out by invitation cycles or “tours.” Two general reports have been published (*Evaluation à 6 ans et à 10 ans*, IUMSP, Jean-Luc Bulliard, Lausanne).

| Tableau 4.1: Indicateurs de qualité du Programme valaisan de dépistage du cancer du sein et comparaisons avec les normes européennes, 1999-2009 |
|---|---|---|---|
| Indicateurs de qualité (%) | Tour prévalent (n=32′771) | Tour incident (n=54′794) | Normes européennes prévalent | Normes européennes incident |
| **Qualité des lectures** | | | ≤70 | ≤7 |
| Taux de 3ème lecture* | 14,3 | 6,7 | | |
| Taux de reconvocation | 7,2 | 3,1 | <5-7 | <3-5 |
| **Qualité des investigations** | | | ≤0,5 | ≤0,25 |
| Rendement biopsique** | 0,73 | 0,26 | | |
| Taux d’imagerie additionnelle | 6,5 | 2,7 | <1-5 | |
| Cytoponction avec matériel insuffisant | 9,7 | 10,2 | <15-25 | |
| Cancer avec cytoponction ininterprétable | 3,1 | 8,8 | <5-10 | |
| Diagnostic non-opératoire de malignité** | 73,8 | | >70-90 | |

*Cet indicateur peut varier selon les critères utilisés dans la grille de lecture radiologique. La norme proposée est indicative et correspond aux objectifs recommandés par les évaluateurs, de concert avec la direction du Programme.*

**Voir le glossaire pour la définition.*
A third report focused on the progress of the radiologist’s performance and analysis of interval cancers in the program has been released in spring 2013 (Performances radiologiques: Evolution et déterminants 2002-2012, IUMSP-UEC, Jean-Luc Bulliard, Raisons santé 214, Lausanne 2013).

As president of the reference group of the Valais breast cancer screening programme, I was involved in the feasibility study, the strategy planification, the choice of the direction of the programme, the evaluation mandates, the corrective actions and the coordination of partners.

1.6. Pertinence of breast cancer screening programs and comparison with international standards

The pertinence of organized breast cancer screening programs has been evaluated for Switzerland by including our program in the academic evaluation of the IUMSP in Lausanne. International comparisons with others national or regional programs have confirmed the adequacy of our programs and their compliance with European guidelines\(^{(2,3)}\).

However, we had difficulty complying with the minimal participation requirements because of the two competing strategies—opportunistic and organized—for breast cancer screening, as proposed by the federal application rules for screening, are reimbursed. The trend of lesser participation was most manifest in the city cantons (Geneva), were individual screening was regularly proposed and reimbursed without complying with the guidelines of organized screening programs.
1.7. Cost-efficiency of organized program versus opportunistic mammographies in Switzerland

The cost-efficiency of the organized screening program was evaluated in parallel to the opportunistic individual mammographies in the Valais. There were a few features of the Valais program that allowed a direct comparison of the two approaches in term of cost-efficiency. One of these feature was the fact that most of the breast biopsies were processed in the same pathology institute, allowing parallel follow up of all mammographies. In summary, it was established that for a cost ratio inferior, the organized program accomplished a similar efficacy to that of the opportunistic individual mammography screening.\(^{(4)}\) Moreover, the organized program allowed an overall analysis of the performances and their monitoring.

1.8. Controversies in the evaluation of the benefits of mammography screening programs

In the last few years, conflicting controversies over the benefits and pitfalls of breast cancer screening programs have been reported by the press. The statistical methods used to evaluate efficacy have been challenged, as well as the actual role attributable to the breast cancer screening procedure, the balance between the benefits, overdiagnosis and the psychological difficulties induced by the procedure.\(^{(5)}\)

Without minimizing the methodological problems, the actual limits of the applied strategy dictated by feasible opportunities in population-scale screening and the consequences of many positive screenings not being followed by a definite cancer, we need to consider the
major step forward that the implementation of a cantonal program has allowed, especially in the Valais.

We have increased the awareness of the targeted population to the first cause of death in women of that age. We have contributed to increasing the knowledge of the practitioners and gynaecologists to a useful strategy leading to early detection.

We have assessed, accredited and increased the capabilities of the radiologists in performing optimal mammography with adequately controlled, regularly verified equipment. Their own individual performance has been confronted to their peers performance and to standardized criteria. The results were reported to them individually in comparison with the overall performance, taking care of the proper protection of their privacy (they were the only ones who knew their results and they could compare them with the performance of the group). This procedure allowed us to better target an effective common goal in a public health vision, without having to perform too many additional procedures.

1.9. Comparing screening strategies in different cantonal programs

In their own history, the first initiating cantons (Vaud, Valais and Geneva) have elaborated programs that differ significantly in their settings, especially in the number of second readers, as well as population coverage. This permitted analyses of the variations in performance, while taking into account the particularities of each canton.
Figure 1: Comparative analysis of 3 french speaking cantonal programmes a) detection rate versus false-positive rate  b) Positive predictive value versus reference rate.

Clearly, strategies competing with the organized program are deleterious. This tendency is increasing as time goes on, because the informed women wish to start proper breast screening at an early age, which cannot be done in the organized program starting at the age of 50 and being conducted every two years till the age of 70. We observed this development especially in larger towns in the Valais in our recent monitoring statistics (annual internal report 2011).

Recently, we also published a comparative analysis of the performance of the three initial French-speaking programs and showed that decreasing the number of second readers of
mammograms is a critical point in ensuring the best performance of the programs. This was specifically the case in the Valais and recommendations to modify the strategy have been proposed in a recent evaluation report.\textsuperscript{(6)}

In the first place, we decided in 2006 to intervene in order to specifically train all the radiologists with a known dataset of selected difficult cases comparing radiohistological correlations of false positive radiological cases. Radiologists had to sign an agreement to comply to public health requirements and from then on their individual performance was reported to them on a yearly basis. After an initial improvement in the homogeneity of the first and second reader’s group, we recently observed the recurrence of major differences between radiologists. In the canton of Vaud, the performance increased significantly, most likely correlated to a low number of specifically trained second readers.\textsuperscript{(7)}

New opportunities are offered by the digitalization of mammograms and their transfer to a common Swiss database in Bern, allowing the distant second reading to be integrated into the strategy of local programs (e.g. the Valais).

We are now at a strategic crossroad where new opportunities should be taken to increase the efficacy of local programs. Digitalization and transfer of the mammograms to a common Swiss database will permit multiple professional interactions, especially for the second reading of the mammograms. This will enable local programs to favor more professional second readers and to be able to meet the known standards of the European guidelines.

At the same time we should not loose the ingredient of the initial enthusiasm of the local radiologists, practioners who have permitted our local program to be population-based until now.
1.10. Future and perspectives

Many determinant factors have been identified for the successful implementation of a screening program. The development of the Swiss Federation of Breast Cancer Screening Programs is a major step from a public health point of view. The Breast Cancer screening Federation originated from the French-speaking coordination of the cantonal programs and allowed the new interested cantons to take advantage of the available expertise and to share the expenses on a population scale for computer programs, information and organizational expertise.

A special focus can now be set on implementing standardized quality procedures and optimal qualification for second reader radiologists. New participants can directly integrate the new guidelines into their programs and rapidly make up for the lost time in starting their local programs.

This development enabled us to envision a real national prevention program in Switzerland, with all the necessary tools already at work in the different languages. However, one should not underestimate the complexity in setting up a national public health vision in a private-based medical environment. In that sense, a major decision will be the modification of federal application rules for the reimbursement by the insurance companies of breast cancer screening mammography. At this point, individualized/opportunistic mammography can no longer be accepted as a procedure fulfilling the proper conditions of achievement, in terms of quality standards as well as monitoring requirements for a global evaluation.

The recent acceptance of the transformation of the federation into Swiss Cancer Screening is another major development in being able to set up other prevention screening programs,
as envisioned for the Vaud pilot program of Colon Cancer Screening program. Practical modifications of the computer software have already been made to accommodate these specific needs.

Finally, in Switzerland, the practical factors of success in treating early breast cancers have been studied specifically. Many factors in the chain of care that go far beyond screening programs are crucial and should be integrated into a population-based objective of care. These clinical settings taking care of breast cancers are heterogeneous in Switzerland and have been shown to be linked to the academic expertise of the surgeon and of the local breast center.(8)

From the point of view of a canton like the Valais, the development of this global project was appealing because:

- It offered the opportunity to set up early an overall population-based program. Within a few years, this program equipped a peripheral canton (Valais) with comprehensive coverage equivalent to that of an urban canton (Geneva).
- It permitted breast cancer screening by mammography to all women between 50 and 70 years of age, free of charge in order to increase the overall participation.
- It set as mandatory requirement, a repetitive external evaluation by a specialized institution, which contributed to the credibility of the local program, the adjustment of the initial objectives and the monitoring of performance. The same external institution has recently revealed the results of the first national monitoring of the breast cancer screening programs by mammography, and they confirmed the excellent quality of the Valais program (Bulliard, Dec. 2012, internal report).
- It allowed local public health programs to participate in the effort of coordination of, starting with a charter of objectives between three programs, and going on to the
coordination by the health department of each canton of the French-speaking part of Switzerland, finally becoming a Swiss Federation of Breast Cancer Screening programs. The latter recently developed into the Swiss Cancer Screening Foundation, in order to be able to propose other programs.

- It helped us realize that the advantages of an organized monitored regional program, initially representative of a large part of the population, is progressively losing the participation of the initially screened women (50-55 years old in the more urban areas)\(^9\). These women have already been screened on an individual basis by the time they should enter the program. The advantages and quality of the regional program will be difficult to maintain without a clear federal decision in favor of a monitored national program.
2. Organizational challenge: Program to control quality performance of allergy diagnosis, in particular specific IgE measurements

2.1. Introduction

Allergy diagnosis is a complex medical procedure that integrates personal history and exposure to paramedical procedures. New tools are available for in vitro diagnosis. The knowledge of the molecular allergens is fast growing and will profoundly change the practice of allergology. The requirements for a better control of performance and definition of the settings in which they should be realized will have to be clarified before becoming common allergological practice.

There has been a growing interest among healthcare partners to be able to demonstrate the quality of the algorithms and the medical procedures leading to diagnosis and treatment of allergic diseases. From the laboratory point of view, since the beginning of the 1990 in Switzerland, there have been individual initiatives to set up reproducible laboratory procedures that could be performed according to standards and guidelines and compared between different laboratories. These initiatives have gained recognition through the voluntary accreditation of medical laboratories since 1995 in main areas of laboratory medicine such as clinical chemistry, hematology and microbiology.
In parallel, with the support of the Swiss National Office for Public Health (OFSP), the different professional partners elaborated the first basis of the requirements for performing laboratory analysis that would be reimbursed by health insurance companies. A preliminary convention was set adopted by the “Commission Suisse pour l’assurance de qualité dans le laboratoire medical” (QUALAB) on 7/1/1994 (http://www.qualab.ch).

The “Loi fédérale sur l’assurance maladie” (LAMal), which is still in force today, formulated the integration of the QUALAB concept and the programs of external quality control in a law (art.58 LAMal) and its application decree (art. 77 OAMal). A national fee schedule ("Liste des Analyses") was introduced at the same time, describing the various analyses to be performed by each type of partner, as well as the minimal quality requirements (CFLAM). The QUALAB has been responsible for keeping the “Qualab concept” up to date, organizing and coordinating the development of the specific requirements for each domain through the Swiss Centers for Quality Control (CSCQ, Geneva and MQ, Zurich).

As a representative of the Swiss Society for Allergology and Immunology, I have been involved since 2000 in setting up the minimal requirements for the reimbursement of medical analyses in our field, along the lines of the previous work. We first established a convention with the Qualab for the delegation of performance control in the area of Immunology and Allergology diagnosis. We set up an external quality control program for specific IgE measurements. This assessment program was designed for the medical laboratories, headed by a Immunology specialist FAMH and also practicing allergologists, with the title of Allergology and Clinical Immunology FMH, who performed specific IgE
determinations at their own responsibility and wanted to have these specific analyses reimbursed by health insurances.

The panel of analyses being controlled in the field of Immunology has recently been extended to immunassays, protein determination and autoantibody testing. Due to the low number of participants from the Swiss laboratories, the latter panel is conducted abroad by the UK NEQAS.\textsuperscript{(10)}

### 2.2. External quality control program for measurement of serum specific IgE in Switzerland

The program was set up in two phases:

The first phase program from 2003-2006, was offered to voluntary participants. It was targeted at the feasibility of a design that could be performed by specialists in Allergology FMH, usually equipped with near patient setting devices (POC), as well as by medical laboratories headed by an specialist in Immunology FAMH, usually equipped with large automated systems performing quantitative measurements. First, the various methods available were described.\textsuperscript{(11)} Then a panel of three allergens (birch pollen, cat dander and peanut) was chosen to be performed in each distribution of the program. In order to be able to compare the results in the different assays available, we chose an ordinale scale of RAST classes (0, 1, 2, 3, 4 and above; 0 being negative). The tolerance set by the Qualab was one class around the target class and more than 75\% of the results had to be correct (minimum three out of four participations per year).

The compulsory phase for all performers of serum specific IgE was started on 1/1/2006, on the basis of this initial trial, with a few adjustments. A detailed analysis of the results of
a three-year trial has been published.\textsuperscript{(12)} We were able to show that the results of most participants were in the target (more than 95% overall) defined by the QUALAB tolerance. Such an external quality control (CQE) program was designed to improve the allergy diagnosis in practice, as it was reported in other European assessment schemes.\textsuperscript{(10)}

A few conclusions could be drawn from the detailed analysis. First, we observed a significant improvement over the first six years of the semi-quantitative methods. In the first phase, either the supplier was able to provide good results for the CQE program with his equipment or they stopped supplying the test in Switzerland themselves. In the second phase, however we observed more misses with semi-quantitative methods, especially with cat dander in the two first years. The misses diminished significantly in 2008.

We also observed that it took time for the participants to perform and transmit the results in the set delay with the proper format. The reporting, after a phase of trial and error, clearly improved, and this reinforced the necessity for a feasibility phase before implementing a compulsory trial.

Furthermore, we had trouble convincing the large laboratories performing quantitative measures of specific IgE that the reporting format in a semi-quantitative scale was sufficient for qualification by the QUALAB. In fact, the program was designed to compare all the methods together and provided the same requirements for all. This did not fit with the perception of the larger laboratories who wanted to evaluate quantitative methods of specific IgE in kU/L. Such quantification was shown moreover, in the past years to have a predictive value for clinical symptoms in specific situations. However, being also involved in a large medical laboratory, we still believe that this procedure is correct and necessary to qualify all the performers in an identical specific IgE scheme. The expertise of
a large laboratory to detect specific IgE, can further be evaluated on a voluntary basis for accreditation purposes, for example in the UK NEQAS. This qualification goes beyond the needs of quality for reimbursement and defines a further step of expertise for special allergens and quantitation.

We are also convinced that the external quality control for specific IgE has increased the awareness of potential problems among all the partners in the determination of these parameters. This is a continuous quality assessment that will contribute to the ongoing training through yearly re-certification by the Qualab.

Finally, entering the tenth year of the program, we believe that the time has come to really validate and support those who participated for years in external quality control by applying an exclusion rule that would disqualify those who still do not participate in the CQE. The positive list project of the QUALAB, reimbursing only those partners who can provide proof that they have accomplished the proper quality control for a specific parameter, is in preparation. We think it should be put into action as soon as possible.

### 2.3. Practical environments for allergen specific IgE testing

#### 2.3.1 The use of specific multidisc IgE.

A mixture of allergens can be used in a single well to detect confined sensitization to multiple allergens. There are useful studies that have confirmed the negative predictive value of Phadiatop in excluding an allergic sensitization in a low pretest probability context. This can further be improved for pediatric ages by the inclusion of common food allergens (Fx5).
We contributed in 1995 to an interesting study in the special setting of an school for army recruits. 289 subjects performing a standardized exercice were analysed for bronchospasm and pulmonary functions and gave blood samples. We were able to show that, outside of the pollen season, the non-atopic subjects with exercice induced bronchospasm (EIB) were more frequently sensitized to pollen (Rx1; OR: 7.4) and perennial allergens (Rx2; OR: 8.8). (13) We concluded that the “not yet recognized” low level IgE sensitization to allergens is a predicting factor for exercise induced bronchospasm.

2.3.2 The conflicting measurements of specific IgG or IgG4 to food.

The speciality commission of the SSAI has taken position on the absence of significance and indication of the extensive determinations of IgG directed to food in allergological practice. (14) I contributed to the position statement of the SSAI published in the Bulletin des médecins suisses (2005) by Brunello Wuthrich.
3. New developments in allergy diagnosis related to specific serum IgE measurements

3.1. Additional comprehension of the molecular families of allergens

The complexity of the allergens in most plant extracts is better recognized (for example the birch pollen extracts contain more than 20 different cloned molecular allergens). The tools to help us understand the real profile of sensitization have greatly improved in the last 10 years. The cloning of the various proteins and the analysis of the glycosylation pattern have increased the understanding of the various pattern of allergenic reactivities.

3.1.1 Study of the profile of allergenicity to the specific plant components.

The major allergenicity profile has been defined for most type of extracts. The reactivity to specific molecules could be related to major or minor allergens reactivity in groups of allergic patients. The new clinical evaluation based on the reactivity to known molecular allergens was termed “Component Resolved Diagnostics (CRD).” Different clinical profiles were related to profiles of specific IgE to molecular allergens.
3.1.2 Cross-reactivities between different molecular allergens were related for most protein allergens to a structure homology.

The structure homology from the different plant protein families varied from one family to the other, usually being higher in the most phylogenic conserved proteins (i.e. profilin). The crossreactive allergenicity was associated with different biological and clinical parameters. However, at least for the peptidic epitopes, they were most often associated with the percentage of sequence homology. Crossreactivity was less observed if the homology between two proteins was low. Conformational epitopes and glucidic epitopes were not as dependent on the structure homology. For example, the structure homology of rBet v1 with the PR-10 family proteins (called Bet v1 homologue) is more that 80%. Similarly, the structure homology between most of the Lipid Transfer Proteins, LTP) is above 50%, which leads to a more diverse pattern of reactivity between the various LTPs.\(^{16}\)

The cloned proteins from a given species had similarities with other plant proteins with identical function. The allergens of a major plant family crossreacting with other plants were defined as “panallergens.” On the contrary, the proteins contained in a defined allergenic source and only those taxonomically closely related were defined as “genuine allergens” or initial sensitizing allergens.
3.1.3 The molecular nomenclature of allergens and the principal allergen families among plants and animals.

The allergen nomenclature was officially defined by the International Union of Immunological Societies Allergen Nomenclature Subcommittee and currently updated and displayed on their official web site: http://www.allergen.org

The official spelling of molecular allergen is constructed from the first three letters of the organism genus name, followed by a single letter (sometimes two or a letter for variants) from the species name and a number indicating the order of identification of the allergen.

For example, the major allergen for birch pollen is called Bet v 1: Bet (genus: betula), v (species: verrucosa), 1 (order of identification, occasionally with homologous groups).

Isoforms and variants can play a critical role in current diagnosis and eventually in therapy.

The purification origin of the molecular allergen is usually indicated at the beginning as a single letter: n for native or r for recombinant.

Allergen subsets can also be defined on the basis of their clinical phenotype. They can be distinguished between major allergens, usually “genuine,” and minor allergens, or “panallergens.” Examples of the most relevant allergens follow:
1. **Major or “genuine” allergens.**

They are normally the primary sensitizer for a given species and most often responsible for triggering clinical symptoms. They define a closely-related taxonomic family. For example, **nOle e1**, the major allergen of olive pollen, is able to crossreact with other group 1 allergens from the *Oleaceae* family with more than 80% homology, like **Fra e1** from *fraxinus*. Such a tool can significantly help to define primary sensitization to this specific tree or tree family, among the sensitizations to the others close-related trees, often found positive in skin prick tests on the basis of reactions to a mixture of crossreactive allergens. Other important markers of specific primary sensitization are **Phl p1** and **Phl p5**, as markers of grass sensitization; **Cup a1**, as markers of the *Cupressaceae* group 1 (cedar,
cypress, juniper). Der p1 and Der p2, Der f1 and Der f2 are called genuine allergens of the dust house mite.

2. Minor allergens, mostly crossreacting, called “panallergens”

These are most often a co-sensitization phenomenon appearing with molecular allergens from other species that are not necessarily taxonomically related. They are responsible for the triggering clinical symptoms, sometimes to a lesser degree with unrelated species. The “panallergens have similarities (sequence homologies), but are not identical between botanical or zoological families. Their recognition by specific IgE is variable from patient to patient.

They can, however, strongly affect the skin tests and the specific IgE determinations. In particular, such minor or panallergens in pollen are profilins and calcium binding proteins, called "polcalcins" for plants. For example, in birch and grass pollen, Bet v2, Phl p12 and Bet v4, Phl p7 are representative of the profiling and the polcalcin family respectively.

Several panallergens families have been described:

Bet v1-like proteins (Fagales-related proteins) are widely distributed in plants acting as a defence proteins. They belong to the "pathogenesis-related protein 10" or PR-10 family, are present in tissue devoted to reproduction (pollen, seed or fruit) and are not resistant to heat and digestion. Symptoms caused by ingestion of PR-10 proteins contained in food are usually mild (class 2 food allergens). The oral allergy syndrome (OAS) is the classical example, though more severe reactions have been reported for soya.

The PR-10 proteins, in addition to the birch pollen, are present in most Fagales family trees (i.e. beech, oak, chestnut, hazel) and have been recognized for their very confusing sensitization, especially for Fraxinus in our area.
Furthermore, patients allergic to birch pollen often suffer from OAS to various types of fruit and vegetables sharing homologous, crossreactive allergens in the PR-10 protein family. The homologous proteins are present in *Rosaceae* (apple, pear, peach and some nuts), *Apiaceae* (celery, carrot), *Fabaceae* (soy beans, peanut, etc.)

**Profilins** are proteins present in all eucaryotic cells. Those belonging to the plants have a high sequence homology (more than 75%). Ten to twenty percent of the allergic patients (grass, birch, olive, pellitory) are sensitized, with specific IgE to profilins. They cause a highly crossreactive syndrome often involving pollens, vegetables and even latex. They cause similar manifestations by inhalation, ingestion or contact, but are usually mild (class 2 food allergens).

**Polcalcins** are proteins from the ubiquitous Calcium Binding Protein family (CBP). They have been called polcalcin to distinguish them from mammalian CBP, with which they share no crossreactivity. They are highly crossreactive between plants, are present in trees (i.e *Bet v4*), grasses (i.e *Phl p7*), and seeds (i.e *Bra n4, Par j4*).

**Lipid transfer protein (LTP)** are true panallergens with a variable degree of crossreactivity. They are plant proteins involved in the defense against microbial invasion. The highest expression of LTP has been found in peripheral epithelial cells in cuticles of epidermal tissues. Due to their resistance to pepsin and heat, LTP are considered as food allergens that might cause severe reactions. (class 1 food allergens). After ingestion, but occasionally also by inhalation or contact, the clinical symptoms are diverse, ranging from OAS, often associated with violent abdominal cramps, to anaphylaxis. LTP allergy occurs at any age and exhibits crossreactivity between unrelated species.
Seed storage proteins are the required nutrients available to plant seeds during sprouting. They include groups of proteins such as the 11S globulins, 2S albumins and the 7S vicilins, which is largely present in most allergenic seeds: mustard, walnuts, sesame, castor beans, cashews, pistachios, peanuts. They are resistant to cooking and digestion (class 1 food allergens). Seed storage proteins are first among the food allergens to be responsible for severe anaphylactic reactions in adults. In children, they are ranked third after milk and egg allergies.

Tropomyosins are proteins that regulate muscle contraction in invertebrates. They are widespread animal proteins to which there is a daily exposition corresponding to the quantity of arthropods that infest homes (mites, spiders, silverfish, cockroaches) and that are regularly found in house dust. They have been identified as inhalant allergens (mites and cockroaches) and as food allergens (crustaceans, molluscs, and the fish parasite Anisakis simplex). Tropomyosins retain their IgE binding activity after prolonged heating or proteolysis. They are highly crossreactive between the different allergenic sources and have long confused the diagnosis of house dust mite allergy. All allergen extracts did not have the capacity to separate the crossreactive tropomyosin sensitized patient (i.e. Der p10) from the genuine sensitization to dust mites (i.e. Der p1, Der p2). More than 100 tropomyosin homologues have been described, some of them representing IgE protective response to parasites, mainly nematodes.

Parvalbumins are proteins present in fish and frogs and are involved in muscle contraction and controlling calcium flux in the sarcoplasm. They are the major allergen among fish allergic patients. Resistance to boiling and proteolysis expose patients to severe allergic reactions (class 1 allergens).
Chitinase and glucanase. The most obvious relevance of molecular diagnosis is exemplified by the understanding of the latex allergic patient profile\(^{(17)}\). Among more than 10 characterized allergens, the major allergens in latex sensitized patients, have been identified as **Hev b1** (rubber elongation factor), **Hev b3** (small rubber particle protein), **Hev b4**, **Hev b5** and **Hev b6**.

- **Hev b2** (1,3-Beta glucanase) are enzymes widely distributed in plants including PR-2 type proteins. They have been associated with hypersensitivity to foods, especially avocado, banana, chestnut, fig, kiwi and potato.\(^{(18)}\)

- **Hev b6** (hevein and prohevein) are homologues of some cereal lectins and chitin binding proteins. Chitinase are defense proteins against mold and insects. They contain an N-terminal domain with chitin binding properties; the allergenicity is inactivated by heat and enhanced by artificial fruit ripening. Chestnuts, avocados and bananas belong to this group and elicit major allergic reactions (class 1 food allergens). Latex being the sensitizer and fruit chitinase the elicitors of allergic reaction.
The most important allergenic families and their characteristics are shown below (adapted from Caducéus 2011, E. Dayer, at [www.hopitalvs.ch](http://www.hopitalvs.ch)).

**Tableau 1: Most important allergenic families and their characteristics**

<table>
<thead>
<tr>
<th>Famille</th>
<th>Chaleur et protéases</th>
<th>Exemples d'allergènes végétaux d'aliments</th>
<th>Clinique</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-10 Bet v1 homologue)</td>
<td>sensible</td>
<td>Bétulacée, rosacée, apiacée et fabacée</td>
<td>Syndr. Allergie oral (SAO)</td>
</tr>
<tr>
<td>Protéines de transfert des lipides</td>
<td>résistant</td>
<td>Bétulacée, rosacée Mais, arachide, raisin, chou</td>
<td>Réaction systémique Sud-Nord</td>
</tr>
<tr>
<td>Protéines de stockage (albumine 2s.globulines5,7,11s)</td>
<td>stable</td>
<td>Amandes, noix, graines Ex: Arachide, soja, fruits à coques</td>
<td>Réactions systémiques courantes</td>
</tr>
<tr>
<td>Profilines (Bet v2 homologue)</td>
<td>sensible</td>
<td>Répandus chez végétaux Ex: agrume, melon, banane tomate</td>
<td>Pertinence clinique moindre</td>
</tr>
<tr>
<td>CCD (cross reactive carbohydrate Determinant)</td>
<td>sensible</td>
<td>Répandus chez végétaux Allergénicité pour cèleri tomate, courgette</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*PR-10: noisette, celeri, arachide, soja*
3.2. Implications of recombinant allergens for allergy diagnosis

As reported by Pr G. Pauli, Strasbourg\textsuperscript{(19)}, we can no longer ignore the development of molecular allergens for the day-to-day practice of allergology. It has changed our perception of the tools that we work with. The increase in complexity has to be countered by more precise studies of the molecular allergens place and algorithms for allergological practice. As such, it will increase the specific knowledge of the field and favor referral to specialists.

Laboratory suppliers have already integrated the addition of recombinant allergens to their extracts in order to obtain a higher sensitivity; for example, the spiking of f17 (cherry) with increased amounts of Pru av1. Similar spiking has been reported for latex, kiwi, litchi, fruit (apricot, peach, cherry), nuts, and hazel. Many other plants or food allergens, however, are spiked with major recombinant allergens.

Skin prick test extracts are not suited yet for the addition of some recombinant allergens; standardization of the extracts is a regulatory issue in progress. Some extracts are said to contain almost pure LTP (Peach extract, ALK; not available commercially), similar to a date palm profilin extract containing almost pure profilin.\textsuperscript{(20)} However, no extracts are commercially available for practical recombinant allergen skin tests.

Testing for individual recombinant allergens by ImmunoCAP is available for the large number of allergens. This can be included in a strategy of testing either in sequence or alone the different allergens or mixtures of allergens (all and/or recombinant combinations).
3.3. Implications of individual or combined recombinant allergens specific IgE testing for clinical practice

It is thought that, with approximately twenty recombinant molecular allergens, we could detect and investigate most of the common allergy syndromes. As reported below these recombinant allergens have been documented and are already used in practice (adapted from Caduceus 2011, www.hopitalvs.ch).

**Tableau 2: Suggested combination of recombinant allergens with specific symptoms**

<table>
<thead>
<tr>
<th>Allergie</th>
<th>Source d’allergène</th>
<th>Composant</th>
<th>Combinaison d’IgE sp. utile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printemps</td>
<td>Bouleau/frêne</td>
<td>Bet v1/ Ole e1</td>
<td>Bet v1 et Ole e1</td>
</tr>
<tr>
<td>Juin</td>
<td>Graminées</td>
<td>Phl p1/p5</td>
<td>Phl p1/p5 vs p7/12</td>
</tr>
<tr>
<td>Août</td>
<td>Armoise</td>
<td>Art v1</td>
<td>Art v1</td>
</tr>
<tr>
<td>Bouleau et SOA</td>
<td></td>
<td>PR-10 protéine</td>
<td>Bet v1 ev. PR-10 spécifique</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profiline, polcalcine</td>
<td>Bet v2/v4, CCD</td>
</tr>
<tr>
<td>Oeuf</td>
<td></td>
<td>Œuf et ovomucoide</td>
<td>Œuf, Gal d1</td>
</tr>
<tr>
<td>Anaphylaxie</td>
<td>Pêche</td>
<td>Pru p3</td>
<td>Pêche, Pru p3</td>
</tr>
<tr>
<td></td>
<td>Noisette</td>
<td>Cor a8</td>
<td>Noisette, Cor a8</td>
</tr>
<tr>
<td>Arachide</td>
<td>Ara h2, h1/h3</td>
<td>Arachide, Ara h2</td>
<td></td>
</tr>
<tr>
<td>Abeille+,guêpe+</td>
<td>Crevette</td>
<td>Tropomyosine</td>
<td>Crevette, Pen a1</td>
</tr>
<tr>
<td></td>
<td>Venin hyménop.</td>
<td>Api m1, Ves v5, Pol d5</td>
<td>Dénistage venin, Api m1, Ves v5</td>
</tr>
</tbody>
</table>

However, some of the major controversies are not yet solved and we need to be cautious in applying simplified algorithms that have not yet been fully approved by consensus.

The most important question for each recombinant plant family is: is there an ideal candidate that represents the sensitization to this given allergen in the majority of patients? For example, the sensitization to nOle e1 is representative of most Oleaceae...
sensitization. In this case, the answer to this question is negative: intensive investigation of patients sensitization to the variants of this molecular allergen in the different plant families should be considered, including by multiplexing or ISAC.

1. Individual and coupled recombinant molecular allergens as diagnostic or prognostic tools for inhalant allergy.

Birch and grass allergies are the most prevalent inhalant allergies. Immunotherapy has been shown to reduce the symptom scores and decrease the extension of allergen sensitization and progression to asthma for rhinitis patients. Prognostic factors, such as mono-sensitization have been known for a long time, however, it has only recently been related to a biological process. Sensitization to the major allergens was associated with a better outcome, although not very persuasively.

To cite an example, P. Schmid-Grendelmeier reported his own retrospective analysis in an academic setting.\(^{(21)}\) in which he showed that more than 50% of his day-to-day patients were sensitized to minor allergens (\textbf{Bet v2, Bet v4, Phl p7, Phl p12}). Sixty percent of these patients had a good response to standard practice subcutaneous immunotherapy. The good response rate was lower than in patients with sensitization only to the major allergens (\textbf{Bet v1 or Phl p1/ Phl p5}).
Tableau 3: Response rate in percent according to the sensitization to major, minor allergens combination

<table>
<thead>
<tr>
<th>Allerg. majeurs</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allerg. mineurs</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bonne réponse</td>
<td>284</td>
<td>87%</td>
<td>197</td>
<td>60%</td>
</tr>
<tr>
<td>Mauvaise réponse</td>
<td>41</td>
<td>13%</td>
<td>133</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
<td>325</td>
<td>43%</td>
<td>330</td>
<td>44%</td>
</tr>
</tbody>
</table>

Cat dander sensitization identified by recombinant \textbf{Fel d1} is at least as sensitive as the all cat extract in detecting cat allergy, the levels of specific IgE are a risk factor for developing cat allergic asthma.\textsuperscript{(22)} Moreover, in a large follow up cohort (BAMSE birth cohort), cat sensitization identified by recombinant \textbf{Fel D1} specific IgE was predictive of the future development of asthma.\textsuperscript{(23)}

2. Individual and coupled recombinant molecular allergens as diagnostic or prognostic tools in food allergy and effort associated allergy

In egg allergy, \textbf{Gal d1} can predict the tolerability to boiled egg as well as the increased risk.\textsuperscript{(24)} Relationships with the levels of corresponding IgG4 have been studied; so far, only the ratio IgE/ IgG4 for Gal d1 has been related to a prognosis.\textsuperscript{(25)} In milk allergy, similar ratios of exposition vs. sensitization have been shown to parallel clinical symptoms, but the clinical usefulness of this is not clear.\textsuperscript{(26)}
In effort-associated wheat allergy, the absence of sensitization to the omega-5 gliadin (Tri a19) has a high negative predictive value for the implication of wheat hypersensitivity in the anaphylaxis associated with exercise.(27)

3. Individual and coupled recombinant molecular allergens as diagnostic or prognostic tools in food allergy crossreactive to plants

Severe reactions (class 1 allergen reaction) have been reported after LTP ingestion.(28) Pru p3 has been recognized as a frequent marker of sensitization to LTP, as well as Api g2.(29)
However, with structure homology between LTP down to 50%, the reactivity was found to be highly variable between different patients and may be associated with exercise, at least in Italy.(30)

Based on the interest in predicting the severe reaction, algorithms have been proposed for foods. In storage proteins sensitization, severe reactions to Ara h1, h2, h3 are usually found with the most severe reactions in peanut allergy.
Another example of a definite use of recombinant allergen is tropomyosin, as the major allergen in shrimp allergy. Pen a1 has a high negative predictive value of 91%.

3.4. Needs for multiplex testing in allergy diagnosis

Polysensitized patients represent the most difficult cases in the rational work up of an allergy diagnosis. Understanding their individual pattern of sensitization may provide clues permitting us to offer them more rational food eviction, exposition to inhalants or immunotherapy.
Individual recombinant allergen determination can be justified to answer a simple clinical question: for example, in the presence of a negative CAP result of specific IgE for Pen a1, was the food allergic reaction following a meal with shrimp likely to be related to shrimp sensitization? Knowing the high negative predictive value (91%) of this analysis in this context the answer is most likely no.

However, the daily exposition of allergologist to polysensitized patients forces them to evaluate more complex individual clinical histories. Many diagnostic questions arise to determine the extent of sensitization to multiple allergens. Among them some will effectively modify their treatment approach and justify further investigation. For example: Could the spring or summer co-sensitization to specific trees or weeds require additional immunotherapy? Could the identification of the cross-sensitization to food components at risk better define the prevention of severe reactions and limit the eviction diets?

3.5. Implications of multiplex recombinant allergens testing for clinical practice

3.5.1. The proposed indications

There is a break-even point at which, from the point of view of cost-effiency (somewhere between testing for five to eight individual allergens and molecular allergens), it is more rational to invest in specific IgE multiplexing detection to obtain a complete picture of the reactivity to the various allergens families. The indication for such determination should include the proper a priori motivation for at least three clinical questions that would benefit from specific IgE multiplex testing. These questions have been addressed in the
literature\textsuperscript{(31,32)} and can be summarized in three of the most common types for clinical practice, not considering research:

- **Unforeseen allergens and diagnostic specificity**

In idiopathic anaphylaxis, 50\% of patients will exhibit reactivity in the ISAC test (Phadia); it is not yet clear how many of the challenged patients effectively reacted to the putative allergens. Similar unforeseen allergens are revealed in the literature in heretofore idiopathic or allergy associated conditions such as eosinophilic esophagitis, steroid dependent asthma, acute urticaria and atopic dermatitis.

Diagnostic specificity of individual recombinant allergens can favor the use of specific IgE multiplex testing when at least three relevant possibilities could be addressed by such a test.

- **Planning immunotherapy (IT) in polysensitized allergic patient**

Apart from the pauci-sensitized allergic patients, allergological practice has to take care of a majority of referred polysensitized patients for whom there is a clear need for specific IT after an insufficient response to symptomatic drugs. Selecting the appropriate targeted therapy is the foremost goal and predictive factor for treatment success. Moreover, this treatment has been shown to be efficient in two-thirds of the patients, and recently also after oral pill IT (Grazax)

- **Challenge test avoidance**

Peanut, latex, and hazelnut allergy are classical situations in which there is a need to test for many (more than six molecular allergens) to have a clear picture of the risk of future reaction. Typically at least three storage proteins (\textit{Ara h1}, \textit{Ara h2}, \textit{Arah3}) confer a risk of
severe reaction to peanuts. On the contrary, sensitization to Ara h8 (a PR10 family protein) usually leads to a local oral allergy syndrome. An LTP (Ara h9) found to be particularly relevant in an Italian population may explain the geographical differences found in the sensitization associated with severe reactions in Europe. In children, the prediction of severe reactions based on the profile was not so clear, but Ara h2 was associated with the prediction of clinical allergy to peanuts.\(^{(33)}\)

Latex is the typical example in which specific IgE multiplex testing has added value, especially in discriminating between fruit crossreactivity (Hev b2, Hev b6, Hev b11) with serious outcomes and those of the PR-10 and profilin families respectively (Hev b7, Hev b8) with usually only less severe reactions.

In hazelnut allergy, in vitro molecular allergen analysis revealed substantial differences across Europe in terms of IgE profiles between hazelnut allergic and tolerant patients.\(^{(32)}\) The Mediterranean area had more cases of severe reactions than Northern Europe and these were related to sensitization to LTP (Cor a8) in Spanish patients. In Denmark and Switzerland, no evidence of LTP sensitization causing severe reactions could be confirmed. Other proteins, like storage proteins (i.e. Cor a9) could also be associated with severe reactions. Linked to the excellent clinical sensitivity and the number of CAPs to be performed, clearly specific IgE multiplex testing would be more efficient, as long as performance is comparable.

3.5.2. Technical differences between UniCAP and ISAC

Immune Solid-phase Allergen Chip test (ISAC, Thermo Fisher Scientific) is a multiplex slide microchip coated with more than 100 spots of recombinant or highly purified allergens. Individual binding of specific IgE from patient serum to allergens in triplicates
can be measured using a Laser Scan Confocal Microarray Reader (LuxScan 10k/A CapitalBio, Beijing, China). Results are analysed automatically through a Microarray Image Analyzer and reported for individual allergen in arbitrary units (ISU). An expert system of interpretation is also provided, as well as a reporting by allergen families.

Figure 3: Immune Solid-phase Allergen Chip (ISAC) assay principle: Slide microarray spotted with allergens and binding of specific IgE from serum detected by fluorescent reader

Assay conception and characteristics on different supports detecting the same recombinant allergen, from the same provider can have different consequences on the interpretation of the results of specific IgE measurements. Initial characteristics of the microarray for screening of specific IgE were described\(^{34}\), but since the technology has evolved and been
better standardized.\(^{(35)}\) The comparison between the UniCAP and ImmunoCAP ISAC, CDR 103 testing a cohort of 321 allergic patients and 92 controls has been published.\(^{(36)}\) The results showed excellent correlations with Unicap detected specific IgE values, negative percent agreement and variable positive percent agreement (PPA) according to the specific recombinant allergens. The PPA was inferior to 75% for ambrosia, aspergillus and dog allergens when comparing values above 1 kUA/L and 1 ISU/L. In line with other published studies, discrepant values were more frequent at lower levels and more marked for the LTPs that need improvement\(^{(37)}\). Cross-sectional surveys of 23,077 subjects using microarray techniques confirmed the accuracy and prevalence for the various recombinant allergens.\(^{(38)}\) A recent study of the variability of the ImmunoCAP ISAC CDR 103\(^{(39)}\) showed that the intra-class correlation coefficients were excellent overall and that most recombinant allergens performed well individually, with an intra-assay variation inferior to 15% and with an inter-assay variation (CV) for most allergens between 20-40%. In this study, the recombinant allergens with the higher inter-assay CV were Api g1, Gal d3 and Phl p6 and left room for improvement.

A new ImmunoCAP ISAC CDR 112 was recently launched, together with an interpretative software program, Xplain. The characteristics of this new version have been improved in many ways. First, the technical features have been updated. It is still a semi-quantitative determination, based on fluorescence intensity; results are reported within a measuring range of 0.3-100 ISU-E (ISAC standardized units calibrated on the ImmunoCAP specific IgE units). Representative examples of comparison with ImmunoCAP have been reported for Alt a1, Canf 1, Phl p1, Ara h2, Der p2, Prup3. Precision was reported for intra-assay and inter-assay variation coefficients per component below 25 % above 1.0 ISU-E; overall, the mean coefficient of variation was below 10%, in the order of other immunoassay
systems. New recombinant or native allergens have been included in the food allergens: i.e. shrimp (nPen m2, nPen m4), walnut (nJug r1, nJug r2, nJug r3), peanut (nAra h6, rAra h9), wheat (rTri a14), as well as aeroallergens; i.e. olive (nOle e7, nOle e9), plane tree (rPla a3), weeds (rChe a1, rPla l1), dog (rCan f5), horse (rEqu c1), mite (rBom t5, rLep d2)), and particular allergens such as hymenopter (rPol d5, rVes v5) and CCD marker (nMUXF3). Confirmation of these characteristics and the extension of these improvements will have to be validated in clinical applications.

3.5.3. Contribution to the utility of microarray specific IgE testing (ISAC CDR 103) in the follow up of immunotherapy in polysensitized patients

We evaluated the clinical response of nine polysensitized patients with 31 courses of subcutaneous immunotherapy (SIT) of three years and analyzed their sera before and after IT treatment by microarray (ImmunoCAP ISAC CDR 103) for specific IgE and IgG4 (EAACI, London 2010, SSAI annual meeting, St Gallen, 2010). We were able to show a median improvement of symptoms score as assessed by physician of 72% at the end of the three-year course of SIT. The clinical improvement was correlated with the results of ISAC specific IgE after SIT, but not with the ImmunoCAP specific IgE after SIT. The clinical benefit was best correlated with the modification of the IgE/IgG4 ratio during SIT. The median modification of the ratio was of 2 log (100x).
Organizational challenges in prevention program and in controlling laboratory procedures

Figure 4: The physician evaluation of the immunotherapy success correlated with IgE by ISAC after IT and the variation of the IgE and IgG4 ratio during IT

These results, although generated from a small heterogeneous cohort, represented significant evidence for a parallel biomarker of the clinical response, especially considering the evolution of the IgE/IgG4 ratio to the major recombinant allergens included in the SIT preparation. Questions were raised by the study, but no clear answer could be foreseen/concluded without further clinical studies and in vitro inhibition studies. The use of the ratio of specific IgE/IgG4 to evaluate response to immunotherapy (IT) has been reported in past studies. There is a clear indication from the bee venom response to IT, that it can be a reliable indicator of response and even set the goal of a normal ratio seen in hyperimmune beekeeper protected from regular bee stings.\(^{41}\) This phenomenon has been confirmed in pollen IT\(^{42\text{-}43}\) and partially understood, showing that sublingual immunotherapy is associated with an increase in allergen specific IgG4, IgA and serum inhibitory activity for IgE facilitated allergen binding to B cells.\(^{44}\) Furthermore, for example in food allergy, the high IgE/IgG4 ratio to ovalbumin (Gal d2) and/or ovomucoid (Gal d1) measured by UniCAP has been reported to be reacting to baked egg, while the
low IgE/IgG4 ratio were tolerant to baked egg. With no defined cut-off, this information is not yet applicable to clinical practice\(^{(25)}\).

Multiplex allergens analysis for IgE and IgG4 in parallel in 2 detection signals could allow direct comparison, as long as the method is reproducible and the proper software program to interpret the results are available.

The other main issue of the particular results obtained in the study was the divergent results for specific IgE measured after SIT between the ImmunoCAP and the ISAC CDR 103 assays. It has been known for some time that no significant differences in specific IgE results could be measured by ImmunoCAP IgE in the course of SIT\(^{(45)}\), unlike the ImmunoCAP IgG4. We came to the same findings in our study with the ImmunoCAP IgE, but this was not true for ISAC CDR 103 IgE, which showed a significant decrease after the course of IT.

There might be methodological reasons to explain these discrepancies between the two techniques: ImmunoCAP is an end stage assay in large excess of antigen, minimizing affinity modification and interference by other Ig subtypes. The properties of ISAC CDR 103 IgE are different, binding less antigen and more influenced by specific IgG concentration. Little has been published on the silicium binding properties of recombinant allergens in the ISAC. Since fewer antigens are to be bound on the support, the assay might be more sensitive to modifications of interference and affinity. In an abstract, Hamilton has reported that IgG antibodies interfere with IgE binding in the ISAC, diminishing the accuracy of the chip-based allergen specific IgE assay by ISAC.\(^{(46)}\) This was not the case with the ImmunoCAP IgE. Such inhibition associated with the parallel increase in IgG4
found after IT could explain our results in part, but does not modify the pertinence of the observation.

More studies should be performed to define the properties of the different assays in relationship to various clinical treatments and to evaluate the potential of the IgE/IgG4 ratio to specific allergens as a predictor of clinical response to immunotherapy.

3.5.4. **Quality control of specific IgE by microarray is a challenge for the future**

At the moment, only the basic quality performances of specific IgE assays are measured in Switzerland. The CSCQ scheme controls the performance for three allergens (birch pollen, peanut and cat dander). This scheme is suitable for determination by simple near the patient devices and automated laboratory instruments. It represents the basic requirements for reimbursement of these analyses by the insurance companies in all settings.

This scheme was by no means intended to qualify specialized allergy laboratories detecting numerous allergens (usually more than hundreds of allergens) in quantitative assays. The laboratory commission of the Swiss Society of Allergology and Clinical Immunology recommended individual laboratories to apply on a voluntary basis for European external quality evaluations, such as UKNEQAS, to fulfill the requirements for accreditation in this specialized field. Such programs provide controls for a larger number of allergens, informations on the real variability of thesens assays and allow the comparison of more than 200 participants; however, it cannot be imposed on allergologists with a small laboratory.

A further degree of complexity will be to control for quality performance of specific IgE microarrays (ISAC), in which more than 100 different allergens are coated on the support. The performance of the analyses is above all a challenge for the supplier in order to provide assays with interlot and interassay performances below a 20% variation. As
published, the variation coefficients of individual allergens are not yet fully satisfactory.\(^{(37,39)}\) In the new version of the ISAC, the CVs should be improved, as new recombinant allergens have been included and the methodology improved.

Moreover, as suggested by the recent poster, the specific IgE detected microarray, such as ISAC, will be modified by the interference of other IgG subclasses, in particular IgG4 appearing during the course of immunotherapy or recurrent exposition as exemplified by the beekeeper hyperimmune status.

In conclusion, it will of critical importance to restrict the conduct of these analyses to specialized allergological settings, combining on one side an accredited allergological laboratory and, on the other side, the proper clinical interpretation of the patient status in terms of relevant expositions, past immunotherapies and specific medical history. According to the present European guidelines, confirmatory procedures such as proper provocation tests should be performed to ascertain relevant sensitization and the exact balance with the hyperimmune status for a specific allergen.
4. Conclusions

Contributing to organizational challenges in the breast cancer prevention program by mammography in the Valais and establishing external quality schemes for specific IgE determination for the Swiss Society of Allergology and Clinical Immunology has been a very gratifying experience for me.

The knowledge acquired from local cantonal programs is serving as preliminary experimentation for a national public health prevention program by mammography for breast cancer. The external quality assessment program for specific IgE in Switzerland has ten years of experience and has set up the minimal requirements for all specific IgE measurements expected by the providers for reimbursement. Such a scheme, however, will not fulfill all the needs for quality control in the larger laboratories and with newly available technologies.

Innovative solutions will have be set up to adapt the specific Swiss environment to technical developments. National cancer prevention programs beyond early breast cancer detection by mammography are now becoming a reality.

According to a similar trend, the professional medical societies involved in laboratory diagnosis have done their best to set up external quality assessment programs in Switzerland in order for all practitioners to be able to fulfill the same requirements for the reimbursement of equivalent medical procedures. The future will show if such efforts can evolve in the face of the challenges of technological developments and divergent needs in the application of medicine.
5. References


Organizational challenges in prevention program and in controlling laboratory procedures


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