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Immunoglobulin E (IgE) Assay for the Diagnosis and Prevention of Pollinosis in the District of Abidjan

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Abstract: Allergology in Africa is booming. In addition to clinical studies, many studies in biology focus on molecular aspects. This work aims to list the species of the Ivorian flora incriminated in pollinosis and to indicate the pollens that may have molecular similarities with those from Europe. Biological tests using food and mixed allergens were used to search for IgE in 20 patients (14 men and 6 women). The people selected were those who had lived for more than a year close to the target plants and had given their consent for the study. A grid representing cross-allergies "Food-Pollens" was used to search for any possible reactions. The plant species of Côte d'Ivoire suspected to be the causes of allergies were *Cocos nucifera* (Arecaceae), *Elaeis guineensis* (Arecaceae) and *Petersianthus macrocarpus* (Lecythidaceae). The same patient may have one or more symptoms. Most of the patients showed ENT (80%), skin (40%), lung (35%), and eye (30%) symptoms. Allergic rhinitis was representative of ENT signs, but allergic asthma has reached 10% of people suffering from lung diseases. During the flowering of *Petersianthus macrocarpus*, 87% of the respondents present ENT and respiratory symptoms. Biological analyses revealed 70% of patients were sensitized to trophallergens and 60% to pneumallergens. The two patients with no clinical signs were the only negative patients in the biological tests. The most common cross-reactions were between the pollens of *Secale cereal* "Rye" and the fruits of *Corylus avellana* "Hazelnut". The study shows that, the European Panels are useful to start allergological investigations but insufficient to develop a precise diagnosis in Côte d'Ivoire. Therefore, a specific panel composed of Ivorian pollens is being established.

Keywords: Pollen, *Petersianthus macrocarpus*, IgE, Rye, Hazelnut, Allergies, Côte d'Ivoire

1. Introduction

Allergology is a rapidly expanding field of medicine [1]. Nowadays, allergy affects all age groups, especially the youngest, with an increasing prevalence of respiratory allergies. However, pollens are in pole position among pneumallergens [2]. Pollinosis are allergic conditions developed by atopic people when exposed to pollens. Recently, it has been observed that people with food allergy suffer from pollinosis. The cross-reactivity observed between a pollen and a food can explain positive reactions without clinical signs or an allergy to a wide range of pollens and foods [3]. Allergens are gathered into pneumallergens and trophallergens. These families allow the diagnosis and therapy [4]. The list of incriminated pollens is not complete, because several plant species are not described yet. Nevertheless, a best knowledge of the botanical families to which pollen allergens belong would provide a way of research.

Thus, a floristic and allergological exploration of four plant species namely, *Cocos nucifera* L. (Arecacea), *Ceiba*

pentandra (L). Gaertn (Malvaceae), *Elaeis guineensis* Jacq. (Arecaceae) and *Petersianthus macrocarpus* (P. Beauv.) Liben (Lecythidaceae) was conducted in 2015 in the District of Abidjan. This study revealed that all four species have potentially allergenic pollen. However, *Petersianthus macrocarpus* was the most incriminated species by about 16% of respondents [5]. This plant is present over a large part of the Ivorian territory, from the coast to the center of the country [6]. In addition, climatic factors favour the exposure of populations to *Petersianthus macrocarpus* pollens [7]. *Elaeis guineensis* and *Cocos nucifera* are present throughout Côte d'Ivoire and are predominant along the coast [8, 9].

In Côte d'Ivoire, pollen allergies are still very badly known to people and especially to at scientific level. Complete allergenicity studies have never been carried out on pollen. Allergic diseases such as asthma and rhinitis reported in Côte d'Ivoire have a prevalence of about 30% [10]. Among these allergies, the prevalence of pollinosis remains unknown. However, a severe exacerbation of respiratory diseases such as asthma has been noticed in recent years, with about 34% of allergic manifestations associated with asthma [11]. Pollens are the most important pneumallergen to consider in respiratory diseases.

However, it is difficult today to diagnose pollen-related allergies in Côte d'Ivoire, due to lack of diagnostic and monitoring tools related to ignorance of allergenic pollens in the atmosphere. What warning method could be developed for a best control of pollinosis in Côte d'Ivoire?

The objective of this study was to identify the pollens responsible for allergies in Côte d'Ivoire. Specifically, this work concentrates on plant species from the Ivorian flora whose pollens are incriminated in allergic signs and on similarities of these pollen allergens with European panels.

2. Method

2.1. Study Site

The district of Abidjan has 10 communes and five sub-prefectures. The choice of this site was based on the presence of plants with allergenic pollens around the house of the patients agreeing to participate in the study.

2.2. Study Population

Respondents were selected among 129 people suspected as being allergic during ethnoallergological surveys carried out in the District of Abidjan in 2015 [5, 12]. These people were called and visited at home. The study was carried out according the authorisation from the National Ethics Committee under the number N/Ref: 164-18/MSHP/CNESV-km on the 20th December 2018.

Of the 129 people suspected of having allergies, 43 persons have answered the call. However, on 23 persons were still living in the District of Abidjan. Among these respondents, 9 gave their consent for blood sampling. Thirteen (13) new persons regularly in contact with pollens of the target species also gave their consent. Finally, 20 persons were included in the study.

2.3. Surveys

The semi-structured interview was conducted from August 2018 to May 2019 at Nangui Abrogoua University. The information sought was the residence, age, sex, occupation, and the various symptoms and allergic manifestations felt by the respondent during the flowering of *Cocos nucifera, Elaeis guineensis* and *Petersianthus macrocarpus.*

2.4. Blood Tests (Specific IgE) with European Pollens panels

Serological tests were carried out at the Biology of Immunity Pole of the Pasteur Institute of Côte d'Ivoire.

Biological tests using food allergens "Panel 3" and mixed allergens "Panel 1" [13] were used to test the 20 patients for IgE. Five (5) ml of blood was collected from each person in EDTA tubes. After centrifugation at 3000 rpm for 5 min, 2 ml of plasma was collected in cryotubes and stored at - 20°C for IgE testing. Before testing, the membrane, reagents and patient plasma were brought to room temperature (20-25°C). Also, a wash buffer was prepared in a graduated cylinder by adding 120 ml of distilled water to 5 ml of Wash 25x. All incubation steps were performed at room temperature on the orbital shaker. After each incubation, the membrane was emptied and tapped on an absorbent support.

At the beginning, each membrane was initially moistened by covering it with 500 µl of Wash Buffer, followed by incubation for 1 min. Then the membrane was filled with 400 µl of patient plasma and incubated for 30 min. The membrane was then washed with 400 µl of Wash Solution followed by incubation for 1 min. This washing is repeated three times. The membrane is then filled with 400 µl of antibody and incubated for 45 min followed by the three washes as above. Then, the membrane was filled with 400 µl of "conjugate" and incubated for 20 min. Subsequently, it was rinsed over a sink with 1000 µl of wash buffer. This rinsing operation is performed three times. The membrane was again filled with Wash Buffer (400 µl) and incubated for 1 min. This operation was carried out twice. After this, the membrane was filled with 400 µl of Substrate and incubated for 15 min in the darkness.

The membrane is filled again with 400 µl of Wash Buffer and incubated for 1 min. It is then emptied as in all steps, but without tapping. This membrane is finally filled with 400 µl of distilled water and incubated for 1 min. Finally, the membrane is dried for at least 30 min in the open air or in cold air. The results are exploited when the film of liquid on the membrane has completely evaporated, showing a membrane that shows the bands corresponding to the allergens that are present in the plasma and the five reference (standard) bands that validate the test. The membrane reading is taken when the test is valid. The reading is then performed using a 3D color flatbed scanner validated by R-Biopharm in combination with the RIDA qLine® Soft software. The values obtained were expressed in IU/ml or RAST (Radio Allergo Sorbent Test). A positive result is obtained for values greater or equal to 0.35 IU/ml (or ≥1RAST). Positive

patients to only one category of allergens are mono-sensitized [14]. Those who are two-positive are bi-sensitized and those who are three-positive are tri-sensitized [15].

2.5. Systematic Classification of Pollen Allergens

Plant species corresponding to the pollen allergens of Panel 1 and their botanical families were classified according APG IV [16]. Their ranges were determined by the African Plants Database [17]

2.6. Data Processing

The membranes were read using a R-Biopharm-validated 3D color flatbed scanner in combination with the RIDA qLine® Soft software. The data was processed in Excel.

3. Results

3.1. Socio-demographic Data

All 20 respondents lived in the District of Abidjan, nine in the commune of Abobo, five in the commune of Yopougon, three in Cocody, two in Port-Bouët and one in the sub-prefecture of Anyama (Figure 1). They are divided into 14 men and 6 women, with age ranging from 18 to 47 years old. They were one female trader, three teachers and 16 students (Table 1).

Table 1. *Socio-demographic data.*

COMMUNES		Abobo	Anyama	Cocody	Port-Bouët	Yopougon	Total
Sex	Men	6	1	3	1	3	14
	Women	3	0	0	1	2	6
Ages	Total	9	1	3	2	5	20
	[18-27]	0	0	0	1	1	3
	[28-37]	7	1	2	0	3	12
	[38-47]	2	0	1	1	1	5
	Total	9	1	3	2	5	20
Professions	Shopkeeper	0	0	0	1	0	1
	University students	9	1	3	0	3	16
	Teachers	0	0	0	1	2	3
	TOTAL	9	1	3	2	5	20

Table 2. *Persons allergic to pollens of the three plant species.*

Plant species indexed for clinical signs	Patients	Number of patients
Petersianthus macrocarpus	Pat2, Pat3, Pat4, Pat7, Pat8, Pat10, Pat11, Pat12, Pat13, Pat14, Pat15, Pat16, Pat17, Pat18, Pat20	15
Cocos nucifera	Pat 1, Pat 19	2
Elaeis guineensis	Pat 9	1
No species	Pat5, Pat6	2

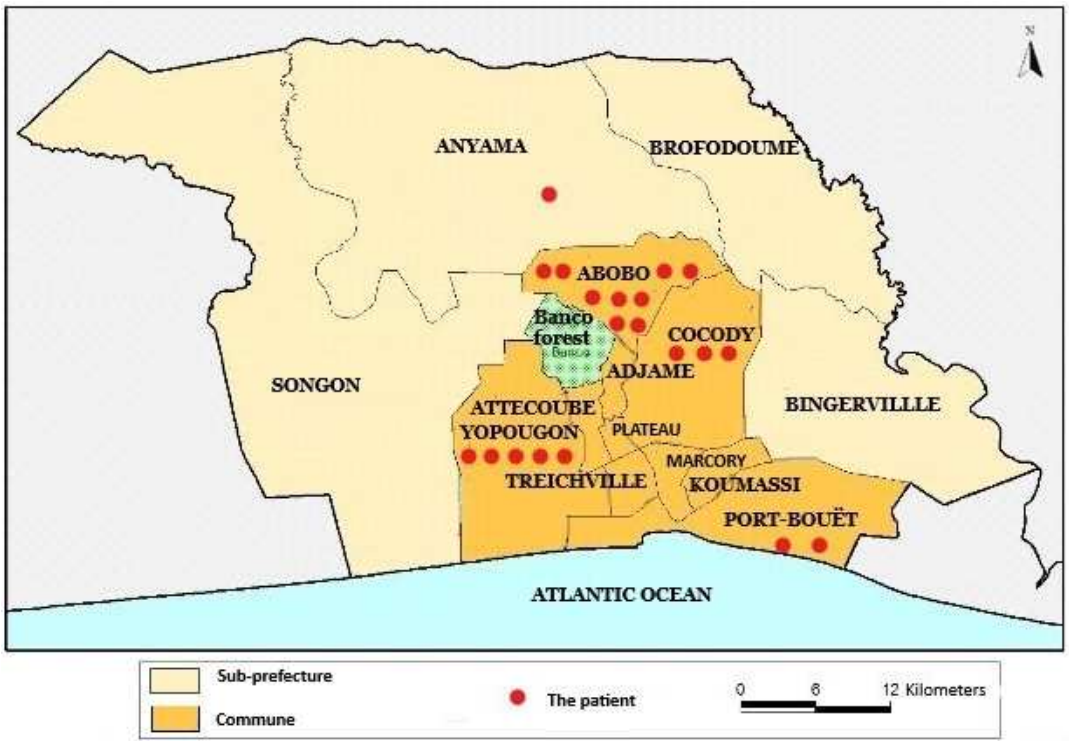

Figure 1. *Location of patients in the district of Abidjan. (Source: INS, 2015 modified by Yomanfo, 2020).*

3.2. Ethnoallergological Surveys

The clinical signs in studied people during the flowering periods of *Cocos nucifera, Elaeis guineensis* and *Petersianthus macrocarpus* were identified after the survey.

In contact with the pollens of these plant species; especially during their flowering periods, 15 persons showed allergic manifestations in the presence of *Petersianthus macrocarpus*, two for *Cocos nucifera* and one person (Pat 9) for *Elaeis guineensis* (Table 2).

Overall, of 20 people surveyed, 16 had ENT signs, 6 had eye signs, 8 skin signs, and 7 showed cardiovascular signs. A person may have one or more allergic manifestations the same time after the contact with airborne pollens.

3.3. Prevalence of Clinical Signs

Among the ENT signs, the prevalence of rhinitis was the highest with 80% of patients. In case of skin signs, itching was the most common (40%). Among the cardiovascular signs, the highest value was obtained for cough observed in 35% of the people surveyed and the lowest was asthma (10%). Ocular manifestations occured in 30% of the studied people (Figure 2). Among the persons with allergic reactions in contact with pollens of *Petersianthus macrocarpus,* 87% suffered from rhinitis (Figure 3).

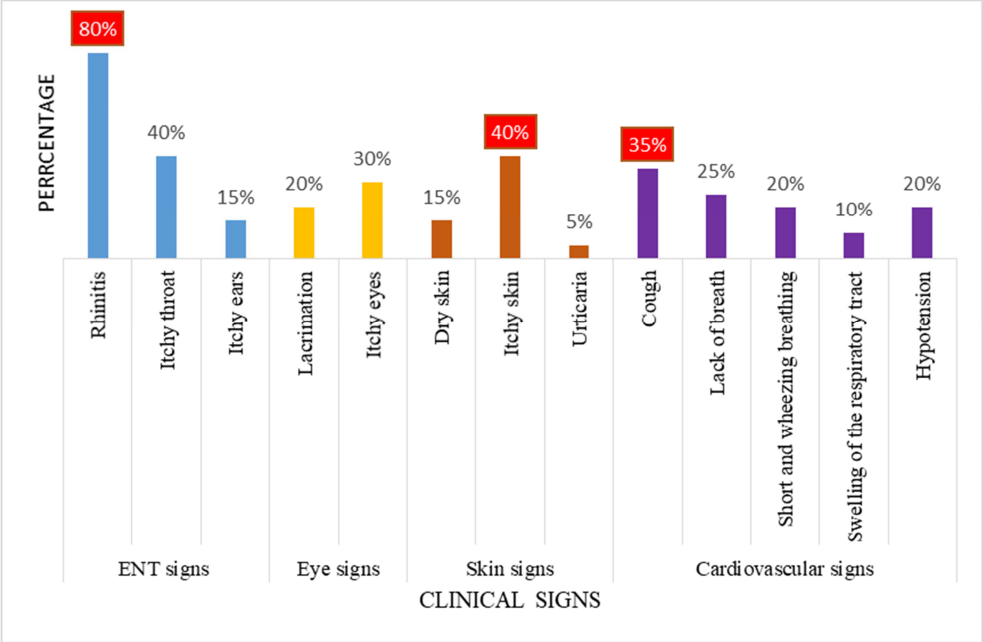

Figure 2. *Percentage of Clinical Signs in Allergic Manifestations.*

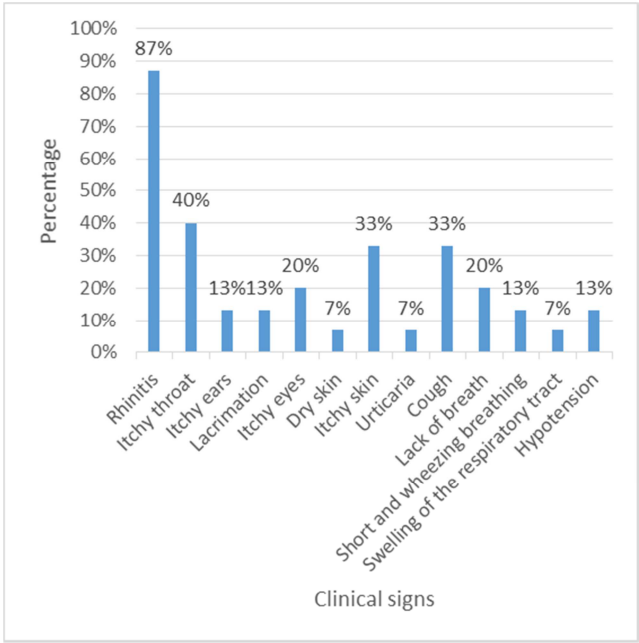

Figure 3. *Percentage of clinical signs to Pollens of P. macrocarpus.*

3.4. Reactions to Panels

3.4.1. Panel 1

Reading pat9 patient membrane

The results obtained after reading the Pat9 patient membrane was an illustration of the possible results of Panel 1. The green color curve indicates a negative result. However, other color indicated a positive result that was proportional to the coloration (Figure 4).

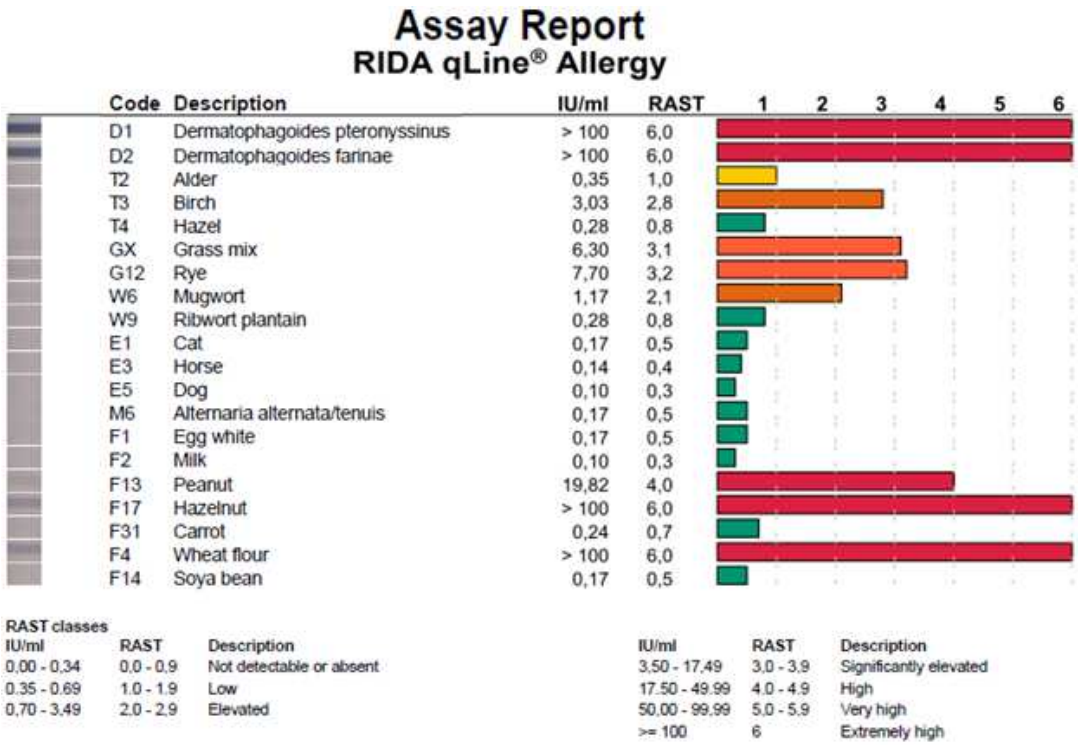

Figure 4. *Patient Pat9 Membrane Reading.*

Botanical observations

The seven pollen allergens: Mugwort, Alder, Birch, Hazelnut, Plantain lanceolate, Rye and the herbal mixture come from plant species from Europe. These species are missing in flora of Côte d'Ivoire. Whereas Betulaceae and Plantaginaceae are only native from temperate zones. The Poaceae and Asteraceae are also present in tropical Africa, including Côte d'Ivoire (Table 3).

Table 3. *Location of Panel 1 pollen allergens in Côte d'Ivoire.*

Scientific names and botanical families	Vernacular Names	Distribution of plant species	Distribution of families
Artemisia vulgaris L. (Asteraceae)	Mugwort	Absent	Present
Alnus glutinosa (L.) Gaertn. (Betulaceae)	Alder	Absent	Absent
Betula pendula Roth (Betulaceae)	Birch	Absent	Absent
Corylus avellana L. (Betulaceae)	Hazelnut tree	Absent	Absent
Plantago lanceolata L. (Plantaginaceae)	Launched plantain	Absent	Absent
Secale cereale L. (Poaceae)	Rye	Absent	Present

Overview Prevalence of Panel 1 allergens

Pneumallergens represent 45% of the allergens in panel 1, with 10% of mites all causing positive responses and 35% of pollen, 30% of which cause positive responses.

Trophallergens represent 50% of the allergens in panel 1, with 25% of animals all giving negative results and 25% of plants of which 15% cause positive results. Mould represents 5% of the allergens in panel 1, but it does not cause any positive results. In summary, 55% of the allergens in Panel 1 gave positive results in the study population**.**

Sensitizations to the allergens in Panel 1

In Panel 1, no patient was sensitized to animal-derived trophallergens, three patients (Pat5, Pat 6 and Pat15) were negative to the tests. Four patients (Pat1, Pat4, Pat9, Pat17) were sensitized to dust mites, pollen and food allergens. These patients were therefore polysensitized. The sensitivity of patients Pat1 and Pat9 to food allergens was highly accentuated. Five patients were di-sensitized, three (Pat2, Pat11, Pat19) to dust mites and food and two (P14, P16) to pollens and food. None of the patients was sensitized to pollens and mites.

Therefore, all di-sensitized patients were positive for food allergens. Eight patients were monosensitized, five (Pat8, Pat12, Pat13, Pat18, Pat20) to food allergens, two (Pat3, Pat7) to mites and only one (Pat10) to pollen. All nine mite sensitized patients were positive for *Dermatophagoides farinae*, but two were negative for *Dermatophagoides pteronyssinus* (Figure 5).

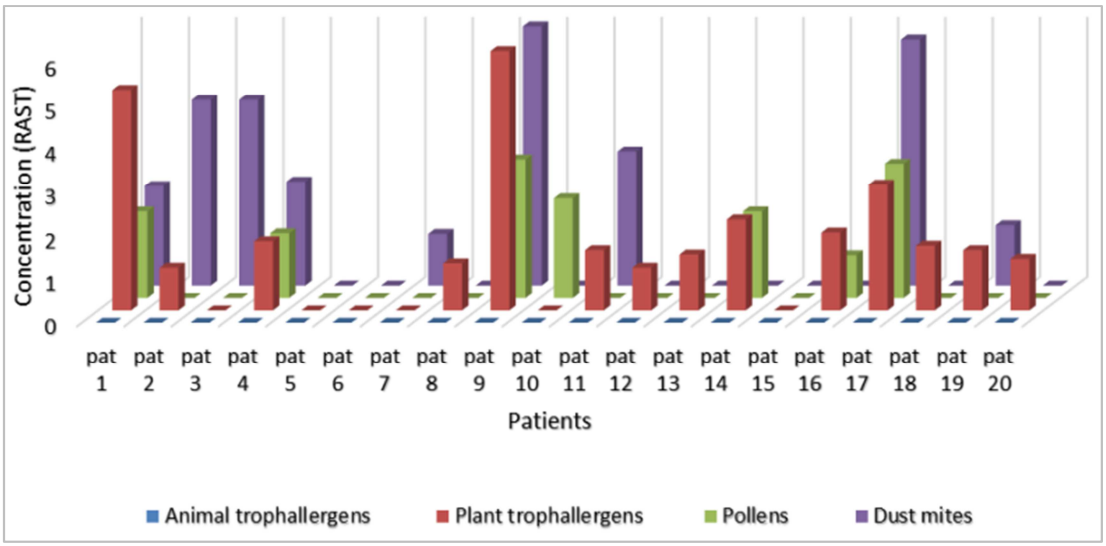

Figure 5. *Overview of panel 1.*

Prevalence of the mono and polysensitized

Among patients, 85% were sensitised to panel 1 allergens, with 20% tri-sensitized, 25% bi-sensitized and 40% mono-sensitized. Monosensitization was 5% to pollens, 10% to house dust mites and 25% to plant trophallergens (Figure 6). The same person could be sensitized to all categories of allergens. Therefore, 70% were sensitized to trophallergen and 60% to pneumallergen.

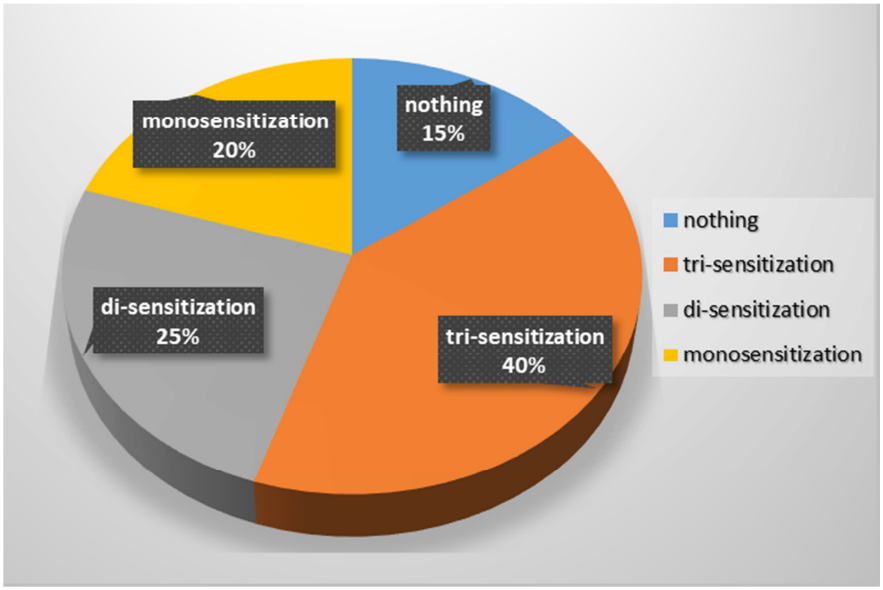

Figure 6. *Prevalence of sensitization to Panel 1 allergens.*

Pollinosis

Patients Pat1, Pat4, Pat9, Pat14, Pat10, P16 and Pat17 were sensitized to Mugwort, Alder, Birch, Hazelnut, Rye and Herb Mix pollens. These seven patients representing 35% of the study population were therefore susceptible to pollinosis. In patient Pat 9, Rye, Mixed herbs and Birch agglutinated the most IgE at 7.7 IU/ml, 6.3 IU/ml and 3.03 IU/ml respectively. With other patients, the amount of agglutinated allergen was less than 2 IU/ml. All seven pollinosis-predisposed patients were positive for Rye. Only Patient Pat 9 was positive for Mugwort and Alder and finally Patient Pat 14 alone was positive for Hazelnut (Figure 7). Poaceae pollens were at the top of the list with 83% of patients whose IgE has agglutinated allergens. Allergens from mixtures herbal accounted for 67% of the patients. However, Asteraceae and Betulaceae had an identical percentage of 17% (Figure 8).

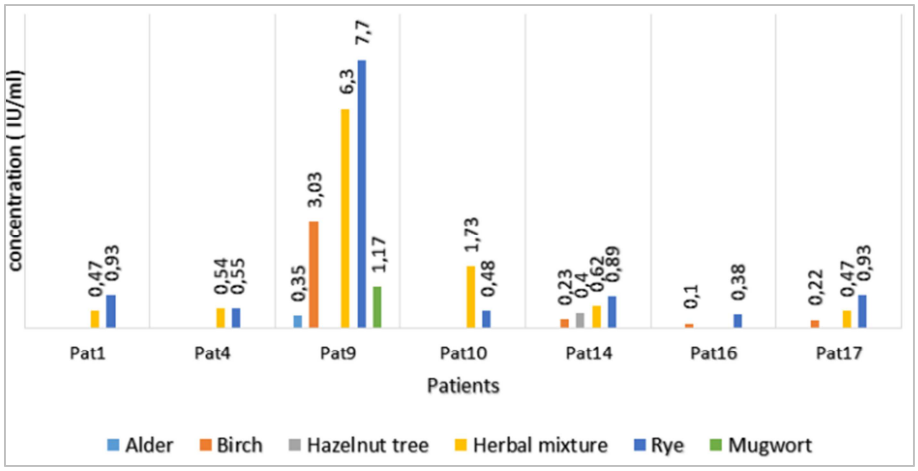

Figure 7. *Concentration of pollens involved in the sensitization of each patient.*

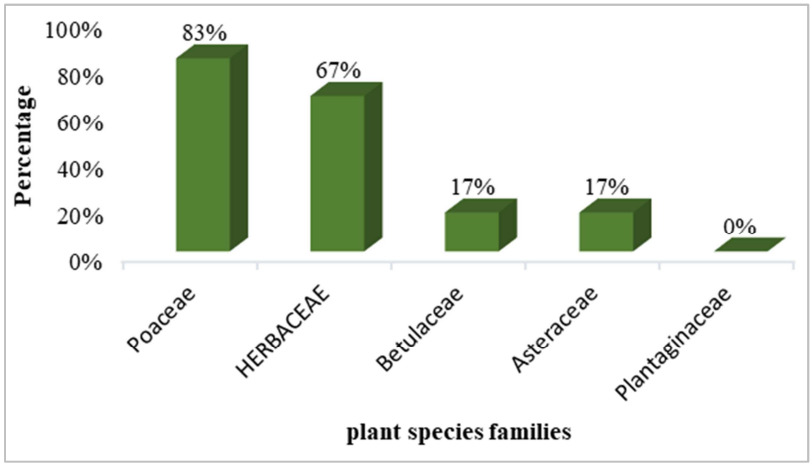

Figure 8. *Percentage of botanical families incriminated in pollen sensitization.*

Sensitization to plant trophallergens

Fourteen (14) patients were sensitized to plant trophallergens. All these patients were tested positive for Hazelnut. Four (4) were positive for wheat flour, three (3) for peanut and two (2) for carrot. The four plant allergens (peanut, hazelnut, carrot and wheat flour) significantly agglutinated the IgE antibodies of patients Pat 1, Pat 9 and Pat 17. But the highest peak (6 RAST) was reached in patient 9 with hazelnut and wheat flour (Figure 9).

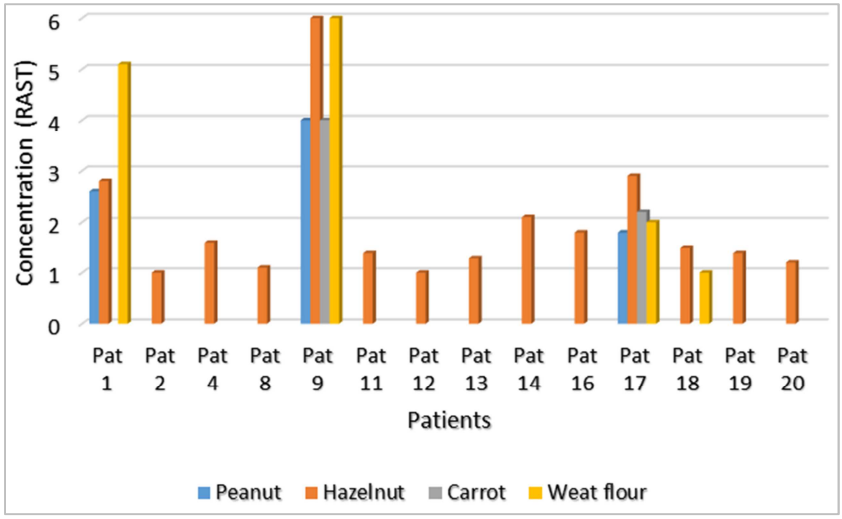

Figure 9. *Patients sensitization of trophallergens plant to Panel 1.*

3.4.2. Panel 3

In Panel 3, eight patients (Pat 2, Pat 3, Pat 8, Pat 10, Pat 11, Pat 15, Pat 19) out of 20 reacted positively to allergens. Also nine allergens (Hazelnut, Peanut, Almond, Celery, Crab, Orange, Wheat flour, Rye flour, Sesame) out of 20 were incriminated. Patient 8 was sensitised to 1/3 of the allergens with the peak (5.1 RAST) reached with almond (Figure 10). Only patients Pat 3, Pat 8, Pat 10 were sensitized to peanut, orange and celery respectively. Patients Pat 2, Pat 8 and Pat 11 were sensitized to crab. Patients Pat 3, Pat 8, Pat 10 and Pat 15 were test positive to rye flour, but only Patients Pat 8 and Pat 10 were positive to wheat flour. Finally, patients Pat 3, Pat 8, Pat 19 and Pat 20 were tested positive for hazelnut (Figure 11).

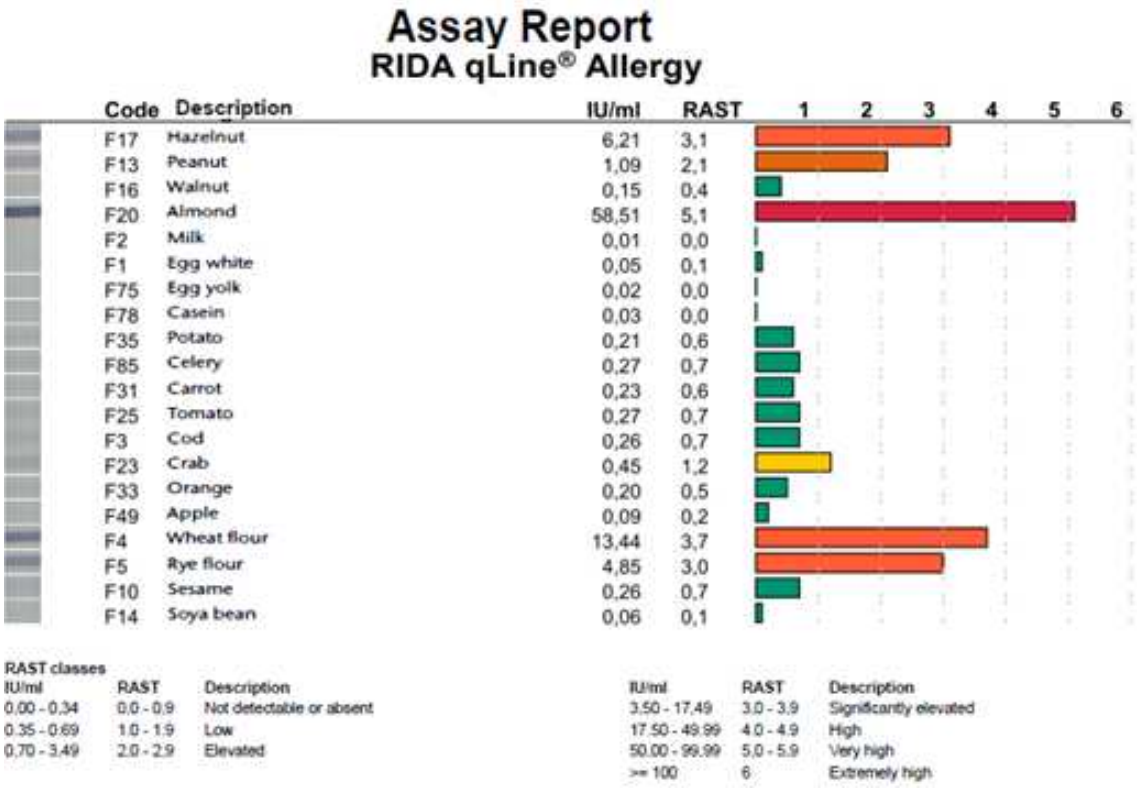

Figure 10. *Reading the patient Pat8 membrane on the panel 3.*

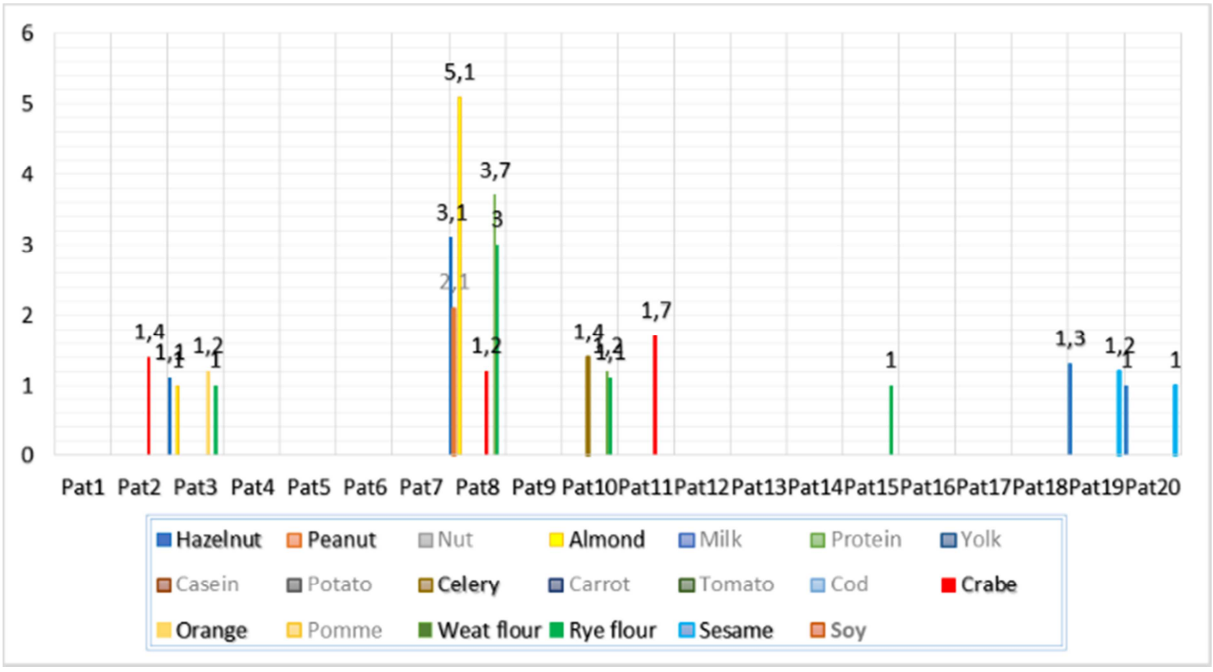

Figure 11. *Allergens inducing positive results in Panel 3.*

3.4.3. Combination of Panel 1 and Panel 3

Two of the patients were not sensitized to Panel 1 and Panel 3 allergens. The remaining 18 were sensitized differently. Of these, seven were sensitized to the allergens of Panel 1 and Panel 3 (Table 4)

Table 4. *Patient reactions to IgE tests.*

Patients (Pat) / Allergens	Pat2, Pat3, Pat8, Pat10, Pat11, Pat19, Pat 20	Pat1, Pat4, Pat7, Pat9, Pat12, Pat13, Pat14, Pat16, Pat17, Pat18	Pat 15	Pat5, Pat6,	Total
PANEL 1	+	+	-	-	
PANEL 3	+	-	+	-	
Amount	7	10	1	2	20

-: Absent +: Present Pat: Patient

3.4.4. Identification of Cross-Allergies

The pollens contained in the herbal mixtures and rye have crossed allergens from hazelnuts. The patients concerned were Pat1, Pat4, Pat9, Pat11, Pat14, Pat16 and Pat17. Thus, seven patients were predisposed to cross-allergy (Table 5).

Table 5. *Cross-allergy possibilities.*

Patients	Cross Allergens	
	Pollens	Foods allergens
Pat1	Herbal mixture, Rye, Birch,	Groundnut, hazelnut, froment
Pat4	Herbal mixture, Poaceae, Rye	hazelnut
Pat 9	Herbal mixture, Rye, Mugwort, Birch	Hazelnut, Groundnut, Rye flour
Pat11	Dust mites	Crab
Pat14	Herbal mixture, Hazelnut tree, Rye	Hazelnut
Pat16	Rye	Hazelnut
Pat17	Herbal mixture Mugwort, Rye	Hazelnut, Groundnut

3.4.5. Percentage of Risk of Cross-Allergy

The 18 allergen-sensitized patients from the two panels together correspond to 90% of the patients in the study, seven patients were predisposed to cross-allergy. Therefore, an estimate 90% of the studied population may develop an allergy and that a sensitized person had a 38% risk of developing a cross-allergy.

4. Discussion

This study was carried out in two phases. In first phase, clinical signs in the respondents were investigated and characterized. The second phase used IgE test to detect allergens to which patients are sensitized. The study population was adult, with ages ranging from 18 to 47 years.

During the interview, 15 people showed allergic manifestations in the presence of *Petersianthus macrocarpus*, two showed in the presence of *Cocos nucifera* and one person (Pat 9) in the presence of *Elaeis guineensis* (Arecaceae). This patient was sensitized to Poaceae pollen with a very high peak for *Secale cereale* (Rye) pollen according to biological tests. Poaceae and Arecaceae Monocotyledons. Therefore, it is possible a person sensitized to Rye pollens being allergic to *Elaeis guineensis* pollens.

Among the allergic manifestations experienced by respondents, rhinitis, itchy skin, cough, itchy eyes and asthma had prevalences of 80%, 40%, 35%, 30% and 10% respectively. The high prevalence of rhinitis is similar to that reported in a study on prevalence and risk factors of respiratory and skin genes [18]. Thus, allergic rhinitis could be a guide in the search for an allergic cause.

While Betulaceae are missing from the Ivorian flora, Poaceae and Asteraceae are present. Special attention should therefore be paid to the species of these two botanical families, especially in their flowering periods, using the usual precautions such as wearing glasses and avoiding physical activity near these species [19, 20].

All patients predisposed to pollinosis were all positive for rye pollen. However, rye pollen is one of the most aggressive allergens [21]. For this reason, affected patients should continue with their diagnosis and avoid consuming foodstuffs containing rye pollen.

With the exception of patient Pat9, all patients positive for rye pollen reported allergic manifestations during the flowering period of *Petersianthus macrocarpus*. Therefore, people who may be allergic to Rye pollen can also be allergic to *Petersianthus macrocarpus* pollen.

The IgE of patient Pat 9 was agglutinated at 7.7 IU/ml by rye pollens. This patient had allergic manifestations in the presence of *Elaeis guineensis* pollens. *Elaeis guineensis* and *Secale cereale* have identical amino acid sequences [22]. The allergens of these two species may therefore cross or cause identical symptoms and allergic manifestations. Therefore, individuals sensitized to *Secale cereale* pollens may be sensitized to *Elaeis guineensis* pollens.

In addition, depending on the possibility of cross-allergy, all patients who were positive for Rye or Birch pollen are likely to have a cross-allergy to hazelnut. A similar case, reveal that, pollens are the most frequent pneumallergens that cross with food allergens [23]. Therefore, a best knowledge of the pollens crossing these foods would be a great means of preventing populations from pollinosis.

Moreover, of the seven patients sensitized to pollens, only patient Pat10 was monosensitized. This confirms the results of Chiriac and Demoly [24], who state that pollen allergy is rarely isolated.

The 14 patients sensitized to plant trophallergens were tested positive for hazelnut (*Corylus avellana* L.). The seeds of *Coula edulis* Baill. (Olacaceae) (African hazelnut), are highly appreciated by local populations and its taste permit to remember those of the common hazelnut (Corylus avellana L.) [25]. Therefore, the consumption of *Coula edulis* by the African population needs to be regulated, as this plant is widely distributed in the forest area of West and Central Africa, from Sierra Leone to the Democratic Republic of Congo. *Coula edulis* belongs, to the phytochory of the Guinean-Congolese domain [26].

In panel 3, patient 8 reacted to 1/3 allergens with the peak (5.1 RAST) reached with the almond. However, the allergic characteristic of the almond in several food products has been proved [26]. Such a patient is thus warned on possible allergies to almond. This same patient was sensitized to animal allergen: the crab. However, the crab and many other crustaceans are widely consumed in Abidjan [28]. Therefore, there is a need to control this consumption in Côte d'Ivoire.

In addition, the seven patients who may contract a cross-allergy were tri-sensitized. This is in full agreement with the results of Demoly in 2017 [15], which states that polysensitized people are rare, but all suffer from cross-allergy.

Finally, an allergic person had 38% risk of cross-allergy. This is below the prevalence listed in molecular studies. In these studies, the prevalence of allergenic risk is 60% [23]. Therefore, the interest is to isolate and identify the pollen proteins from the flora of Côte d'Ivoire, responsible for allergies.

Regarded multiple sudden death that could be caused by anaphylactic shock [29], it is advisable to include IgE tests into health check-up in order to avoid such attacks as much as possible.

5. Conclusion

Petersianthus macrocarpus pollens were the most suspected cause of these allergic manifestations in the District of Abidjan. After biological tests, a cross-allergy between pollen and food was detected in great part of studied people. Some persons were polysensitized (pollen, food allergens, and dust mites).

This study revealed that European Panels may be useful to start an allergological investigation, but not sufficient to develop a precise diagnosis on human health in Côte d'Ivoire. Therefore, there is an urgent need to set up a specific panel composed of allergens from the flora of Côte d'Ivoire.

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