

## POLLEN-FOOD ALLERGY SYNDROME AMONG CHILDREN WITH SENSITIZED TO SPRING TREES

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### Abstract

It is known that among 40–70 % of patients pollinosis can run in conjunction with pollen-food allergy syndrome (PFS), while development of PFS is associated with the consumption of fresh fruits, vegetables, nuts and spices. Clinical course and severity of the disease depend on the sensitization profile, which can be represented by proteins-panallergens (PR-10, profilins, nsLTGs). However, there is little information about the sensitization profiles of patients with pollinosis caused by pollen of spring trees in Ukraine.

**Aim.** To study the profiles of sensitization of children with spring pollinosis.

**Methods.** We examined 61 children (aged 4–17 years) with spring seasonal allergic rhinitis/rhinoconjunctivitis (SAR). To establish the diagnosis, all children were given questionnaires, skin prick tests (SPTs) with commercial pollen extracts, and prick to prick tests with fresh fruits, vegetables and nuts. Component resolved diagnosis (CRD) were detected using an ImmunoCAP system. (Phadia, 100).

**Results.** It has been found that in 43 children (70.5 %) had pollinosis in combination with PFS, the main clinical manifestation of which was an oral allergic syndrome in 43 children (100 %). Among the causal food allergens that caused the manifestations of PFS were more apples, peaches, carrots and hazelnuts (consumption of which led to 11.6 % of children before the development of anaphylaxis). All of the examined children (100 %) had a positive IgE response to rBet v 1 at significant concentrations. In 9.3 % of children, panallergens were found at once from several botanical groups. Such panallergens: rBet v 2, rBet v 4, rPhl p 7, rPhl p 12, rArt v 3 are generally not defined in the control group children.

**Conclusions.** Birch related PFS are common in Ukrainian pollen-allergic children with nuts and fruits predominantly implicated. Sensitization profile of children with Birch-pollen syndrome is complex and associated with sensitization to panallergens. Clinicians should be worried of PFS in patients with a high degree of sensitization to birch pollen and even young children if they have birch sensitization.

**Keywords:** children, oral allergy syndrome, pollen food syndrome, seasonal allergic rhinitis, panallergens.

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## 1. Introduction

Pollinosis is allergic disease that caused by pollen of plants, characterized by acute allergic inflammatory changes in the mucous membranes, primarily respiratory tract and eyes [1, 2]. It has a distinct seasonality, which coincides with the period of flowering of certain plants. In recent years, pollinosis is increasingly combined with pollen-food allergy syndrome (PFS) [1, 2].

Pollen-food syndrome is an immunoglobulin E (IgE)-mediated reaction that occurs among patients with pollen sensitization, the basis of its formation is the cross-reactivity between homologous pollen molecules and plant food allergens [3, 4]. PFS is distinct from simple food allergies [5]. PFS is heterogeneous in relation to triggers, severity, medical history, concomitant diseases and response to treatment [6, 7].

The diagnosis of PFS should be detailed by clinical history but some patients have a mild discomfort and do not report this to the doctor, especially children who can't verbally describe their condition [8, 9]. Symptoms of PFS range from local manifestations in the oral cavity to the development of serious systemic reactions or even lead to life-threatening anaphylactic shock [10, 11]. The symptoms may sometimes develop into urticaria, conjunctivitis, nausea, vomiting, asthma [12]. The development of PFS significantly affects the quality of life associated with health, especially in patients who have food allergies to several foods at the same time [1, 8].

The highly cross-reacting molecules causing PFS are usually thermolabile, degraded by heat and digestive enzymes and can induce allergic reactions only in already-sensitized patients [13].

The most important panallergens include three protein clusters: pathogenesis-related class 10 proteins (PR-10), nonspecific lipid transfer proteins (nsLTP), profilins [14, 15]. Furthermore, component-resolved IgE testing has also improved our knowledge regarding the progression of IgE sensitization and development of symptoms and selection of immunotherapy [16, 17]. Panallergens are proteins that take part in key processes of organisms and are therefore ubiquitously distributed with highly conserved sequences and structures [18, 19]. Panallergens that have been convincingly demonstrated to be clinically relevant in ragweed, timothy grass and birch pollinosis-associated food allergies [20].

Unfortunately, in Ukraine there is no data on prevalence, peculiarities of formation, the profile of sensitization in children with pollinosis with PFS, which is caused by pollen spring trees [21].

## 2. Aim of the research

Therefore, the aim of our research was to study the profile of sensitization of children with pollinosis caused by birch pollen.

## 3. Material and methods of the research

### 3.1. Study population

The study was conducted in the allergy center and childrens clinics of the "Institute Pediatrics, Obstetrics and Gynecology after named academician O. Lukyanova of NAMS of Ukraine". Children were included from September 2015 to February 2016. We examined 61 children with birch pollinosis – 43 patients pollinosis sufferers with pollen-food syndrome (PFS+) and 18 children sufferers without pollen-food syndrome were included as a control group (PFS-). Criteria for eligibility were: age 4–17 years; clinical history of pollen-induced allergic rhinitis/rhinoconjunctivitis and/or asthma in one of the last two spring pollen seasons; SPTs for the relevant pollen extracts.

All patients were free of medication and specific immunotherapy. All investigations were performed out of the pollen season.

Parents of all participants provided informed written consent to clinical investigations. The study design and the procedures were approved by ethical committee.

### 3.2. Questionnaire

Demographic data, history of atopic disease, presence of PFS, implicated foods were recorded, other food allergies, which are not related to PFS.

### 3. 3. Skin prick tests

SPTs were performed with a panel of commercial extracts («Diater», Spain) such as birch, timothy grass, mugwort, ragweed. Skin prick to prick tests were performed with raw apple, peach, carrot, nuts (hazelnuts, nuts). Histamine 0.1 mg/ml were positive and negative controls. Readings were taken at 15 min and wheal  $\geq 3$  mm regarded as positive.

### 3. 4. IgE assays

Component resolved diagnosis (CRD) were performed to determine total IgE antibodies and specific IgE antibodies to PR-10 proteins, profilines, nsLTPs by ImmunoCAP (Phadia, 100). Results equal to or exceeding 0.35 kUa/l were considered positive.

### 3. 5. Statistical analysis

P value  $< 0.05$  was considered statistically significant. Variables were as mean  $\pm$  standard error of the mean (M  $\pm$  SEM), median (Me) and interquartile range (the difference between the third and first quartiles – the 75<sup>th</sup> (Q3) and 25<sup>th</sup> (Q1) quartiles) and/or as frequency and percentage. The Student t-test, the Mann-Whitney test (U) used to evaluate the differences among means and median. The dependence between pairs of parameters was evaluated as a simple linear correlation with the Spearman test ( $r_s$ ). The probability of the difference in frequency distribution was determined by Fisher's criterion  $\chi^2$ .

## 4. Results

### 4. 1. Study population and clinical parameters

In total, 61 patients with birch pollinosis (38 male and 23 female) were included for this study. The diagnosis of OAS was based on a compelling history of repetitive pruritus and/or angioedema of the lips, tongue, throat and/or palate due consumption of raw fruits and nuts.

The clinical characteristics of both of groups are shown in **Table 1**.

**Table 1**

Clinical and demographic data birch-pollen allergic patients

Variables	PFS+ (n=43)	PFS- (n=18)	P-value
Age (yr)	11.9 $\pm$ 0.3	8.3 $\pm$ 0.45	p=0.001
Age of onset (yr)	5.84 $\pm$ 0.28	4.05 $\pm$ 0.42	p=0.001
Pollinosis duration (yr)	5.9 $\pm$ 0.22	4.2 $\pm$ 0.31	p=0.001
Sex (male), %	26 (60.5)	12 (66.7)	p=0.65
Food allergies, (n, %)	7 (16.3)	3 (16.7)	p=0.96
Atopic dermatitis, (n, %)	14 (32.6)	6 (33.3)	p=0.95
Asthma, (n, %)	10 (23.3)	3 (16.7)	p=0.57
Atopic dermatitis with asthma, (n, %)	7 (16.3)	3 (16.7)	p=0.96
Oral allergy syndrome, (n, %)	43 (100)	–	p<0.05
Urticaria (n, %)	14 (32.6)	4 (22.2)	p=0.42
Angioedema (n, %)	6 (14.0)	1 (5.6)	p=0.35
Anaphylaxis (n, %)	5 (11.6)	–	p<0.05

According to the results of the obtained data, males in both groups were identical (p>0.05). The gender distribution boys was 1.5 times higher than girls in both groups (PFS+  $\chi^2=3.8$ , p=0.05; PFS-  $\chi^2=4.1$ ; p=0.04).

Average age of children in the PFS+ group was significantly higher than in the PFS- (p=0.001). PFS+ was observed in children already in preschool-age and its frequency increased progressively

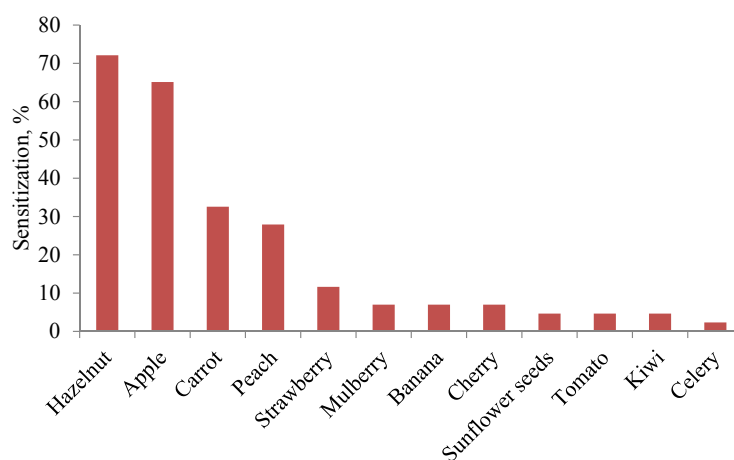
with age. The manifestation of the disease was previously reported in the PFS– ( $p=0.001$ ). Children with PFS+ showed a significantly longer SAR duration than patients without PFS ( $p=0.001$ ).

Among all the examined children 16 % had other food allergies (FA) not related to PFS (egg, cow's milk, soy, fish). 10 patients (16.4 %) were diagnosed as having asthma and felt their symptoms worsened in the spring among all patients. 32.8 % suffered from atopic dermatitis (AD) and 21.3 % suffered from asthma. 16.4 % had atopic dermatitis and bronchial asthma at one time.

No significant difference between groups was found related to allergic comorbidities: atopic dermatitis, asthma, urticaria, angioedema ( $p>0.05$ ).

Patients with PFS+ were more frequently affected by allergic comorbidities and statistically higher with manifesting as OAS and anaphylaxis.

According to questionnaire among the products which caused local allergic reactions, there were fresh fruits – apple, raspberry, strawberry, banana, peach, kiwi, mulberry, melon, vegetables – tomato, carrot, celery, hazelnuts, peanuts, walnuts, also mustard, sunflower seeds shown in **Fig. 1**.



**Fig. 1.** Food sensitization in children with birch pollen-related food allergy

Hazelnuts accounted for the greatest number of reported reactions ( $n=31$ , 72 %), followed by apples ( $n=28$ , 65 %), carrots ( $n=14$ , 32 %), peaches ( $n=11$ , 25 %).

Twelve patients (27.9 %) from 43 with PFS+ reported about reactivity to only one product. The most children (72.1 %) had problems with 2 or more products.

The most common allergenic nut was hazelnut in children with spring pollinosis. Five children reported about several cases clinical reactions and were very severe – anaphylaxis (11.6 %) after ingestion hazelnut.

The results of skin prick tests in both groups shown in **Table 2**.

**Table 2**

Positive skin prick test results and diameter of wheal results in PFS+ and PFS– groups

Allergen	Number of positive SPT (n, %)		Diameter of SPT wheal (mm)		P Value
	PFS+	PFS–	PFS+	PFS–	
birch	43 (100.0)	18 (100.0)	7.2±0.73	3.4±0.19	$p<0.05$
timothy grass	13 (30.2)	4 (22.2)	5.6±0.27	3.2±0.1	$p<0.05$
mugwort	10 (23.3)	2 (11.1)	4.8±0.22	3.4±0.0	$p<0.05$
ragweed	12 (27.9)	2 (11.1)	5.2±0.25	3.4±0.0	$p<0.05$

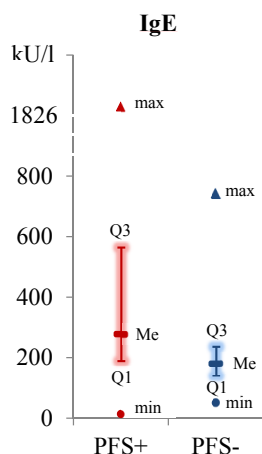
The children of both groups had positive tests with birch allergen in 100 %. In PFS+ group were monosensitized by SPTs to birch ( $n=25$ , 58 %) and other children were sensitized to two or

more species of pollen from different botanical groups. In PFS- group were monosensitized to birch (n=13, 72 %) and other were sensitized to several species of pollen.

The wheal diameter in the PFS+ group was larger than those in PFS-. Increasing diameter of the SPT wheal was found to be related to rising age in patients with PFS.

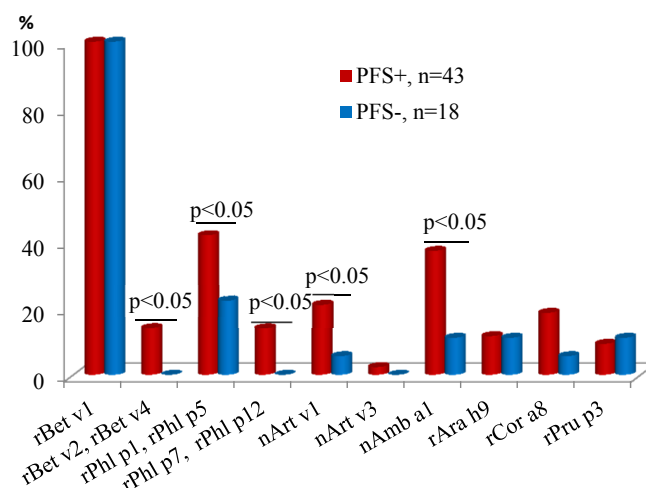
#### 4. 2. Sensitization profile

We compared the levels of total IgE and this indicator in children with PFS+ was higher than in group PFS- (median, 278 kU/l; range, 192–564 kU/l and median 180 kU/l; range, 140–236 kU/l, respectively,  $U=214,5$ ;  $p<0,05$ ) and shown in Fig. 2.



**Fig. 2.** Levels in total IgE in examined children: IgE – immunoglobulin E; PFS+ – with pollen-food syndrome; PFS- – without pollen-food syndrome

All patients showed a positive IgE response to rBet v 1 (100 %) shown in Fig. 3 was detected at significant concentrations (ME-45.4 [17.2-100], range 1.25-100 in the PFS+ group vs ME-12.5 [10.8-26.2], range 4.2-100 in the control group,  $U=232$ ,  $p<0.05$ ). Most patients in PFS- group had monosensitization to rBet v 1 (72.2 %) compared with the PFS+ group, where only one third of the children were monosensitized (32.6 %) ( $\chi^2=7.93$ ,  $p<0.005$ ).



**Fig. 3.** Sensitization rates of component allergens in PFS+ and PFS- groups

When assessing the sensitization patterns of the examined patients with major allergen-positive sensitization to rBet v 1, it was found that children sensitized to birch panallergens – rBet v 2, rBet v 4 (14.0 %) were only in the PFS+ group. We found that panallergens by grasses rPhl p 7,

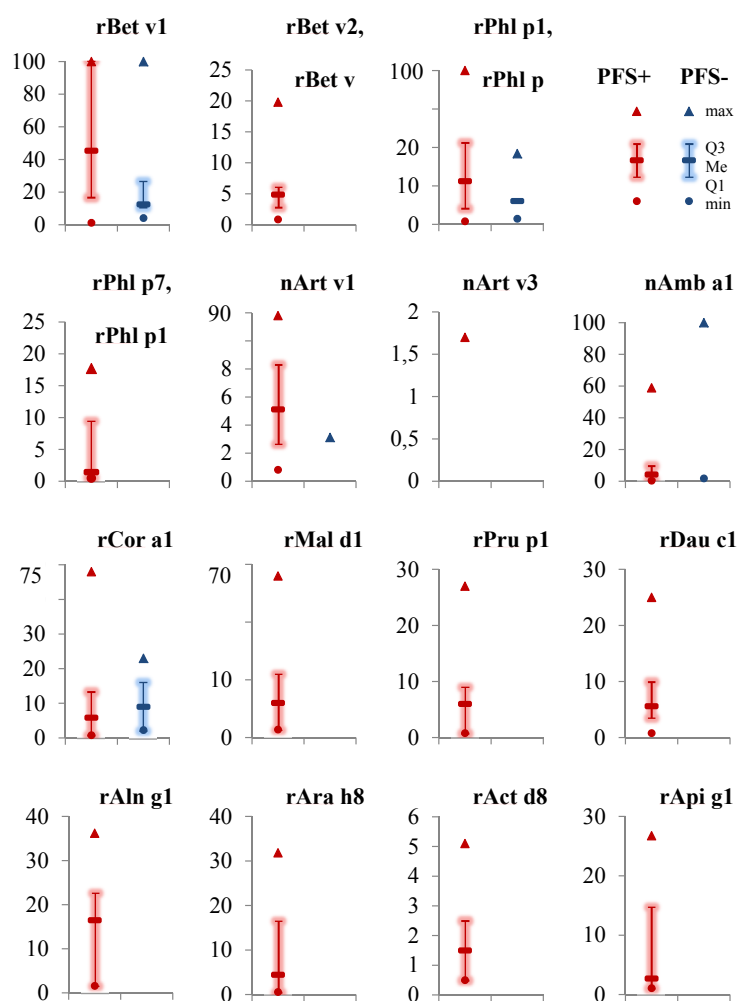
rPhl p 12 (14.0 %) was also found only in the PFS+ group ( $p < 0.05$ ) which simultaneously had a sensitization to rBet v 1.

In group PFS+ of 4 children (9.3 %) had a major sensitization to rBet v 1 and the same times panallergens from two botanical groups (rBet v 2, rBet v 4 and rPhl p 7, rPhl p 12).

Three patients demonstrated sensitization to lipid transfer protein from mugwort – nArt v 3 in PFS+ group. Also we found that one patient were sensitized to nsLTP (nArt v 3), panallergens (rPhl p 7, rPhl p 12) and PR-10 (rBet v 1) concurrently from group PFS+.

Such panallergens (rBet v 2, rBet v 4, rPhl p 7, rPhl p 12, nArt v 3) were not identified at all children in control group.

Sensitisation rates of component allergens in children with spring pollinosis in PFS+ and PFS- groups were shown in Fig. 4.

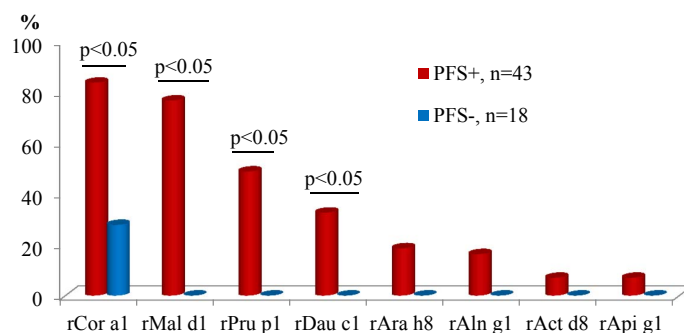


**Fig. 4.** Sensitisation rates of component allergens in children with spring pollinosis in PFS+ and PFS- groups

Higher positive rates of IgE responses to rPhl p 1, rPhl p 5 ( $n=18$ , 41,9 % vs.  $n=4$ , 22,2 %) (ME-11.3 [4.3–21.2], range 0.8–100 in the PFS+ group vs ME-6.1, range 1.5–18.4 in the control group), nArt v 1 ( $n=9$ , 20,9 % vs.  $n=1$ , 5,6 %), nAmb a 1 ( $n=16$ , 37,2 % vs.  $n=2$ , 11,1 %) were observed in group PFS+ than the PFS-, respectively ( $p < 0.05$ ).

Patients with a high rates major allergen-positive sensitization to proteins nArt v 1 (ME-5.1 [2.7–8.3], range 0.8–89.7) and nAmb a 1 (ME-4.3 [1.3–9.5], range 0.4–58.9) in group PFS+, which did not demonstrate the presence of symptoms of pollinosis in the flowering season of weeds and such results we evaluate as clinically insignificant.

**Fig. 5** summarizes the sensitization to different recombinant and purified rBet v 1 homologues. We found statistical significance is reached with all PR-10 (rBet v 1 homologues) allergens in group PFS+.



**Fig. 5.** Sensitisation rates of component allergens to rBet v 1 homologous in PFS+ and PFS- group

The obtained data from the results of CRD regarding the profile of sensitization to the family PR-10 (rCor a 1, rMal d 1, rPru p 1) clinically coincided with the development of PFS in children after the use of these products: hazelnuts, apples, peaches, carrots. Children who had a panallergens in sensitization profile (rBet v 2, rBet v 4 and rPhl p 7, rPhl p 12) more often complained of PFS manifestations also after use of kiwi, celery, tomatoes and bananas.

It was found that among the family PR-10-Bet v 1 homologues in the control group significant concentrations were found only for hazelnut protein – rCor a 1 in 5 children (27.8 %), no other homologues were detected.

In 36 children, the hazelnut protein rCor a 1 in serum was determined at significant concentrations (ME-5.9 [2.2–13.3], range 0.8–75.2 in the PFS+ group, respectively, ME-9.0 [2.5–16.0], range 2.3–23 in PFS- group). 21 patients were sensitized to peach protein – rPru p 1 and 33 had high levels of sIgE to apple protein – rMal d 1. This pattern corresponded to the clinical history in almost 94 % and 12 % of children had asymptomatic sensitization to the hazelnuts and apples. We established a strong direct correlation between rCor a 1 and rMal d 1 ( $r_s=0.573$ ,  $p=0.001$ ), moderate between rCor a 1 and rPru p 1 ( $r_s=0.423$ ,  $p=0.005$ ), weak between rMal d 1 and rPru p 1 ( $r_s=0.328$ ,  $p=0.03$ ).

## 5. Discussion

In North European countries, birch pollen sensitization leads, in a considerable part of the affected patients to PFS, after contact with plant food [6, 22]. Conversely, polysensitization to variety of pollens associated with food allergy manifesting as OAS is typical of Southern European countries [6].

Polinosis is associated with pollen-food syndrome in 40–70 % of patients [11]. We also found that 70.5 % of this study population with birch pollinosis experienced food allergy.

Our data confirm previous observations in adults and emphasize that PFS in childhood is very complex with early onset in pre-school age.

Major sensitization acts as triggers in the development of clinical manifestations of allergic diseases in most cases. Minor allergens are considered as markers of multiple pollen sensitization [23]. In our study, we did not find monosensitization only to panallergens among all the examined children. All patients had positive values of specific IgE in major birch allergen in 100 % of cases. It has also been found that panallergens are found in the profile only in children of PFS+ group.

Patients with major proteins of mugwort and ragweed in the sensitization profile at sufficiently high concentrations did not demonstrate the clinical manifestations during the flowering season of weed.

Our results have important implications for future studies about PFS in our country.



## 6. Conclusion

1. Birch related PFS is common in Ukrainian pollen-allergic children with nuts and fruits predominantly implicated. Sensitization profile of children with Birch-pollen syndrome is complex and associated with sensitization to panallergens.

2. In our study prevalence of PFS in children with spring pollinosis was 70.5 % and with the beginning already in preschool age.

3. The most frequent causative food were hazelnuts followed by apples, carrots, peaches.

4. Clinicians should be worried of PFS in patients with a high degree of sensitization to birch pollen and even young children if they have birch sensitization.

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## EVALUATION OF CENTRIFUGING REGIMES FOR THE PURPOSE OF OPTIMIZING THE PLATELET RICH PLASMA HARVESTING PROTOCOL

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### Abstract

**Aim:** Based on the classical principles, to determine the optimal conditions for centrifugation, PRP harvesting (platelet-rich plasma). To conduct a quantitative assessment of the substrate obtained under different conditions of centrifugation.

**Materials and methods.** Based on the basic principles of obtaining platelet-rich plasma (PRP) by centrifuging in containers with an anticoagulant followed by phase separation to obtain the final substrate, the efficiency of the technique under the conditions of single and double centrifugation as well as under different conditions of acceleration and centrifugation was evaluated.

Blood for follow-up was collected from 20 healthy volunteers (11 men, 9 women) average 25.3±4.1 in syringes of LuerLock design with ACD-A anticoagulant solution, and centrifuged. Centrifugation was carried out under controlled conditions using a centrifuge with rotating bowls of the rotor. Centrifugation was performed at an acceleration of 100–400 g in time intervals up to 20 minutes. Activation of the substrate was performed with calcium chloride solution.

Quantitative evaluation of platelets of whole blood and the final substrate of PRP was carried out with a semi-automatic analyzer.