

Interaction of mediators and effector cells in cashew nut-induced anaphylaxis



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ABSTRACT

Background: The underlying mechanisms of an immediate food-induced allergic reaction involve mast cell degranulation and recruitment of other effector cells, such as lymphocytes, eosinophils, and basophils. How the interaction of various mediators and cells results in anaphylaxis is not fully understood.

Objective: To evaluate changes in platelet-activating factor (PAF), platelet-activating factor acetylhydrolase (PAF-AH), tryptase, eosinophils, basophils, and eosinophil cationic protein (ECP) in cashew nut-induced anaphylaxis.

Methods: Open cashew nut challenges were performed on 106 children (aged 1–16 years), sensitized to cashew nut, with earlier allergic reaction to cashew nut or no known exposure. PAF, PAF-AH, tryptase, ECP, eosinophils, and basophils were measured at 4 time points.

Results: Of 72 challenges with positive results, 34 were defined as anaphylactic. Eosinophil count decreased progressively during an anaphylactic reaction at all 4 time points ($P < .005^*$) compared with baseline. Although significant PAF elevation was observed 1 hour from moderate-to-severe reaction ($P = .04^*$), PAF seemed to peak especially in anaphylaxis but did not achieve statistical significance. PAF peak ratio (peak PAF/baseline PAF) was significantly greater in anaphylactic reactions compared with the no-anaphylaxis group ($P = .008^*$). Maximal percentage change in eosinophils revealed negative correlation to severity score and PAF peak ratio (Spearman's rho -0.424 and -0.516 , respectively). Basophils decreased significantly in moderate-to-severe reactions and in anaphylaxis ($P < .05^*$) compared with baseline. Delta-tryptase (peak tryptase minus baseline) did not differ significantly between anaphylaxis and the no-anaphylaxis subgroups ($P = .05$).

Conclusion: PAF is a specific anaphylaxis biomarker. Marked decline of eosinophils during anaphylaxis may be related to robust secretion of PAF reflecting migration of eosinophils to target tissues.

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Introduction

Anaphylaxis is a severe, potentially lethal allergic reaction characterized by multiorgan involvement and rapid onset.¹ Although many biomarkers, such as tryptase, histamine, C-C chemokine ligand 2 (CCL2), and platelet-activating factor (PAF), have been identified,² the multifactorial mechanisms of human anaphylaxis are still partially unknown, and the usefulness of these biomarkers in clinical practice among children is largely undefined. Consumption of³ and anaphylactic reactions to cashew nut are increasing.⁴

A Canadian study reported post-reaction tryptase level elevation especially in children with severe anaphylaxis compared with those

with a mild-to-moderate anaphylaxis of various origin.⁵ In allergic food reactions, a 30% to 50% elevation in serum tryptase level has varied sensitivity and specificity in confirming anaphylaxis.^{6,7} Elevated baseline serum tryptase (BST) level has earlier been associated with allergic reaction severity.^{8–10} BST level was higher among children with an anaphylactic reaction to food compared with those without anaphylaxis.^{11,12}

PAF is a proinflammatory phospholipid produced and secreted by platelets, mast cells, monocytes, macrophages, basophils, neutrophils, eosinophils, and endothelial cells.¹³ PAF has a short half-life of 3 to 13 minutes.¹⁴ The platelet-activating factor-acetylhydrolase (PAF-AH) enzyme controls the PAF levels by degrading PAF.¹⁵ Among persons aged 15 to 74 years, PAF correlated directly and PAF-AH inversely to anaphylaxis severity of various etiologies.¹⁴ Changes in PAF correlated better to anaphylaxis severity than elevation in histamine and tryptase levels.¹⁶ In a pediatric population, baseline PAF-AH level was lower in children with severe anaphylaxis compared with those with milder anaphylactic reactions.¹⁷

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Eosinophils produce PAF but are likewise chemotactically attracted to the site of allergic reaction by PAF.¹⁸ Furthermore, PAF promotes eosinophil adhesion¹⁹ and degranulation.²⁰ Eosinophil cationic protein (ECP) is an intragranular protein produced mainly by eosinophils,²¹ and its release is triggered by various stimuli, such as IgE and IgG.²²

In an earlier study, we compared the accuracy of skin prick tests (SPTs), sIgE to cashew nut and Ana o 3, and basophil activation test (BAT) in predicting challenge outcome and reaction severity during open cashew nut challenge.³ The aim of this study was to evaluate why some patients develop anaphylaxis compared with other children with milder reactions to cashew nut. Our approach to use the data from repeated measurements during an allergic reaction offers novel insights to better understand the timing of appearance and interaction of various biomarkers and effector cells in the context of pediatric food-induced anaphylaxis.

Methods

Study Population

A total of 110 children were recruited between 2017 and 2020 from Helsinki University Skin and Allergy Hospital for open cashew nut challenges. The children were aged 1 to 16 (median 5.04) years. The inclusion criteria were previously proven sensitization to cashew nut or its storage protein (SPT ≥ 3 mm or IgE to cashew nut or Ana o 3 > 0.35 kU/L), an earlier allergic reaction to cashew nut, or no known exposure to cashew nut. The baseline characteristics of the patients are presented in eTable 1. An open cashew nut challenge was performed on 106 eligible children (4 children did not meet the inclusion criteria). Each patient received 5, 25, 50, 100, and 500 mg cashew nut protein at 30-minute intervals if any significant symptoms were not observed. The challenges were stopped and interpreted as positive according to PRACTALL guidelines.²³ We defined age-specific cutoffs for 4 diagnostic tests (ie, SPT, IgE to cashew nut and Ana o 3, and BAT) in predicting the challenge outcome and reaction severity. There were 72 children (68%) who experienced an allergic reaction. The modified Hourihane severity scoring by Kukkonen et al²⁴ was used to grade the reaction severity (eTable 2). There were 14 reactions (19%) graded as mild and 58 (81%) as moderate to severe. In addition, 34 reactions (47%) were anaphylactic,³ as defined by EAACI 2014 criteria.²⁵ The EAACI 2014 criteria for anaphylaxis are described in the eSupplement. Median age (interquartile range) in the anaphylaxis group was 5.48 (3.22–8.37) years; 17 children (50%) with anaphylaxis were male. In addition, 7 children (20.6%) in the anaphylaxis group were below 3 years old. The anaphylaxis and no-anaphylaxis subgroups were compared with each other, and only children with a positive challenge result were included in the no-anaphylaxis subgroup.

Procedures

We measured complete blood cell count (CBC) and serum tryptase level before each challenge. Both parameters were reassessed 1, 2, and 4 hours after initiation of an allergic reaction. In challenges with a negative result, tryptase level and CBC were reanalyzed once before discharging the patient. Delta-tryptase (highest post-reaction tryptase minus baseline tryptase) and tryptase ratio (highest post-reaction tryptase divided by baseline tryptase) were calculated. PAF, quantitative PAF-AH, and ECP were measured at the aforementioned time points. PAF peak ratio (highest post-reaction PAF divided by baseline value) and PAF/PAF-AH ratio (PAF divided by PAF-AH) were analyzed. A fresh blood sample for BAT was drawn just before the challenge, and the result was presented as percentage of activated CD63+ basophils (BAT%). The methodology of BAT analysis is presented in detail in our previous article.³

Blood was collected into VACUETTE CAT Serum Sep Clot Activator tubes (Greiner Bio-One, Austria) and kept at room temperature for 30 to 60 minutes. Serum was separated by centrifugation at 1984g for 10 minutes at room temperature. Serum samples were stored at –80°C. ECP concentrations in serum at different time points were measured with automated ImmunoCAP technology and the Phadia instrument system (Thermo Fisher Scientific, Uppsala, Sweden). PAF concentration was determined with competitive ELISA kit (Biovision, Milpitas, California) for the quantitative measurement of PAF in the serum, and PAF-AH was measured with a quantitative Quantikine ELISA kit (R&D Systems, Bio-Techne, Abingdon, United Kingdom).

Statistical Analyses

We investigated the changes in CBC, ECP, PAF, quantitative PAF-AH, and tryptase levels at various time points by reaction severity, and especially between the no-anaphylaxis and anaphylaxis subgroups. We used the Mann-Whitney *U* and Kruskal-Wallis tests to compare non-normally distributed data and Fisher's exact test for categorical data. We performed pairwise comparison of eosinophil, basophil, ECP, PAF, PAF-AH, and tryptase levels at different time points by using Wilcoxon signed rank test. Correlations were evaluated by Spearman's correlation test. All results were Bonferroni corrected. We used SPSS Inc. (Chicago, Illinois) version 25 software for our analyses. We used exploratory post-HOC analyses to evaluate the changes in anaphylaxis biomarkers and effector cells.

Ethics

The study followed the principles of the Declaration of Helsinki. The ethics committee at the Helsinki University Hospital of Children and Adolescents approved the study protocol, according to which one of the parents and patients aged above or equal to 6 years provided written informed consent.

Results

Symptom Combinations and Diagnostic Tests in Anaphylactic Reactions

The most common symptom combinations among those with anaphylaxis were skin, gastrointestinal, and cardiovascular (20%), followed by skin, gastrointestinal, and respiratory (18%), and gastrointestinal and cardiovascular (18%) symptoms. The symptom combinations in the anaphylactic reactions are found in Table 1. Epinephrine and antihistamine were administered in all 34 cases (100%) with an anaphylactic reaction and cortisone treatment was used in 32 of 34 (94%) children with anaphylaxis according to good clinical practice to prevent biphasic anaphylaxis. In moderate-to-severe reactions, 46 of 58 children received cortisone and 12 of 58

Table 1
Symptom Combinations in Anaphylactic Reactions

Skin and gastrointestinal, n (%)	4 (12)
Skin and respiratory, n (%)	0 (0)
Gastrointestinal and respiratory, n (%)	3 (9)
Skin and cardiovascular, n (%)	2 (6)
Gastrointestinal and cardiovascular, n (%)	6 (18)
Skin, gastrointestinal, and respiratory, n (%)	6 (18)
Skin, gastrointestinal, and cardiovascular, n (%)	7 (20)
Skin, gastrointestinal, respiratory, and cardiovascular, n (%)	4 (12)
Gastrointestinal, respiratory, and cardiovascular, n (%)	1 (3)
Skin, respiratory, and cardiovascular, n (%)	1 (3)
Decrease in blood pressure > 20% combined with other symptoms, n (%)	3 (9)
Decrease in blood pressure > 30%, n (%)	0 (0)

NOTE. Data are presented in number of patients and the percentage.

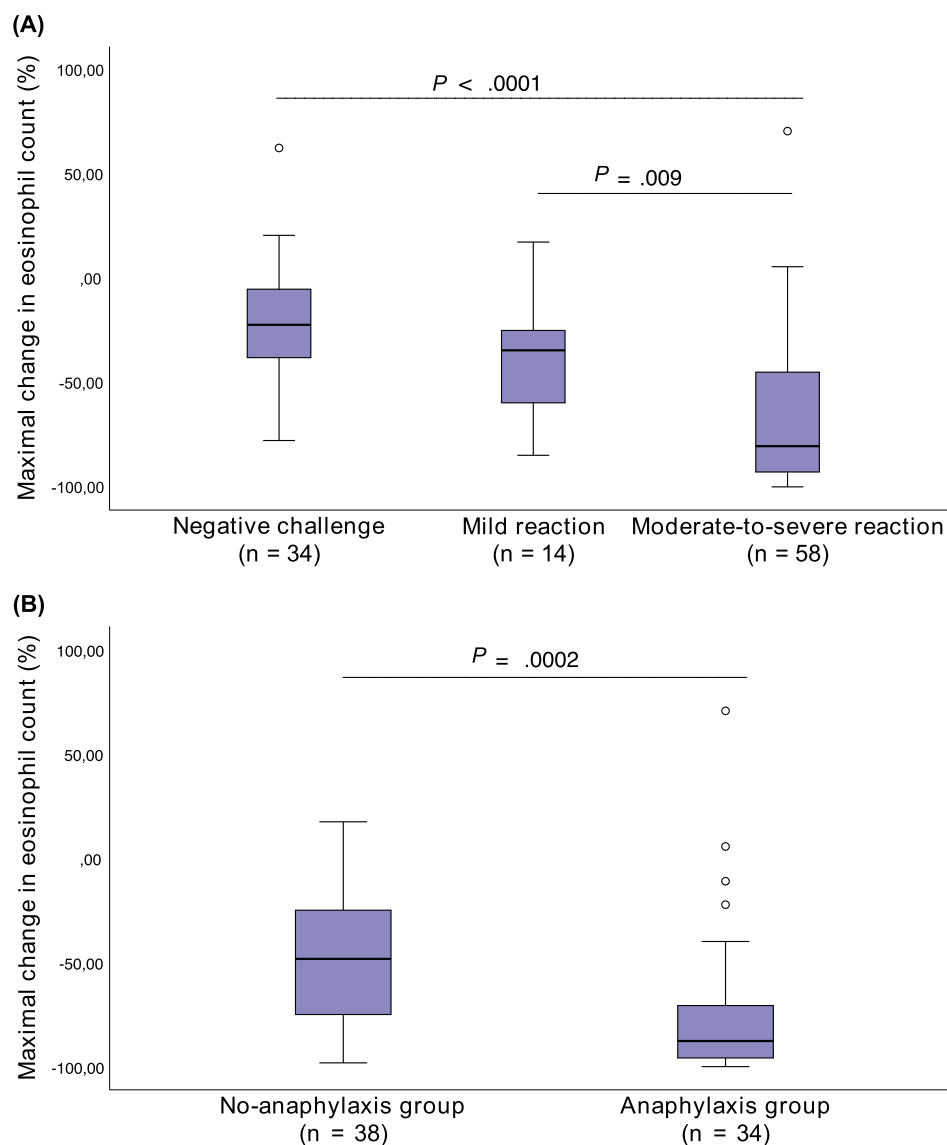


Figure 1. (A) Maximal percent change in absolute eosinophil count by reaction severity and (B) in the no-anaphylaxis and anaphylaxis subgroups among those who reacted.

did not. Furthermore, IgE to Ana o 3 or wheal size in SPT to cashew nut did not differ between the no-anaphylaxis and anaphylaxis groups. However, cashew nut IgE and BAT% were significantly greater in the anaphylaxis group compared with the no-anaphylaxis group ($P = .02^*$ and $P = .01^*$, respectively) (eFig 1A–D). Median (interquartile range) for cashew nut IgE and BAT% was 0.60 (0.17–2.94) kU/L and 4.4% (2.50–23.60) in the no-anaphylaxis group and 5.06 kU/L (0.82–15.03) and 36.20% (19.35–59.70) in the anaphylaxis group.

Leucocytes, Eosinophils, Basophils, and Eosinophil Cationic Protein

Maximal percentage change in absolute leucocyte count was significantly greater in the anaphylaxis group than in the no-anaphylaxis group ($P < .001^*$). The increase in leucocyte levels was progressive and statistically significant between baseline and 1- 2-, and 4-hour samples both in the subgroup with moderate-to-severe reactions and that with anaphylaxis (eFig 2A–C). Among the patients with anaphylaxis, the neutrophil subset level elevated significantly between the baseline and 2- and 4-hour time points ($P < .001$).

The maximal percentage change in absolute eosinophil count differed significantly between children with a negative challenge result and moderate-to-severe reaction ($P < .001^*$) and between those with

mild reaction and moderate-to-severe reaction ($P = .009^*$). The percent decrease in absolute eosinophil count was significantly more profound in children with anaphylaxis compared with those with no anaphylaxis (median decrease -88% vs -48% , $P = .001^*$) (Fig 1A and B). Eosinophil count decreased significantly between all time points and baseline in the moderate-to-severe reaction group ($P < .001^*$) and between baseline and 1- and 2-hour samples in the mild reaction group ($P = .007^*$ and $P = .009^*$, respectively). The decrease of eosinophil count continued until the last 4-hour measurement point both in the anaphylaxis and in the moderate-to-severe reaction groups (Fig 2A and B). The absolute eosinophil count was statistically similar at baseline between those with a negative challenge result or mild or moderate-to-severe reaction ($P > .05$). A significant decrease in eosinophil count between baseline and 1- and 4-hour time points ($P = .02$ and $P = .048$, respectively) was also found in moderate-to-severe reaction subgroup without any glucocorticoid administration.

Baseline basophil count did not differ significantly by reaction severity or between the no-anaphylaxis and anaphylaxis subgroups ($P < .05$) among children who had a reaction. In those with moderate-to-severe reactions, the basophil level decreased significantly at the 1-, 2-, and 4-hour time points compared with baseline ($P = .007^*$, $P < .001^*$, and $P < .001^*$, respectively). In the anaphylaxis subgroup, a significant

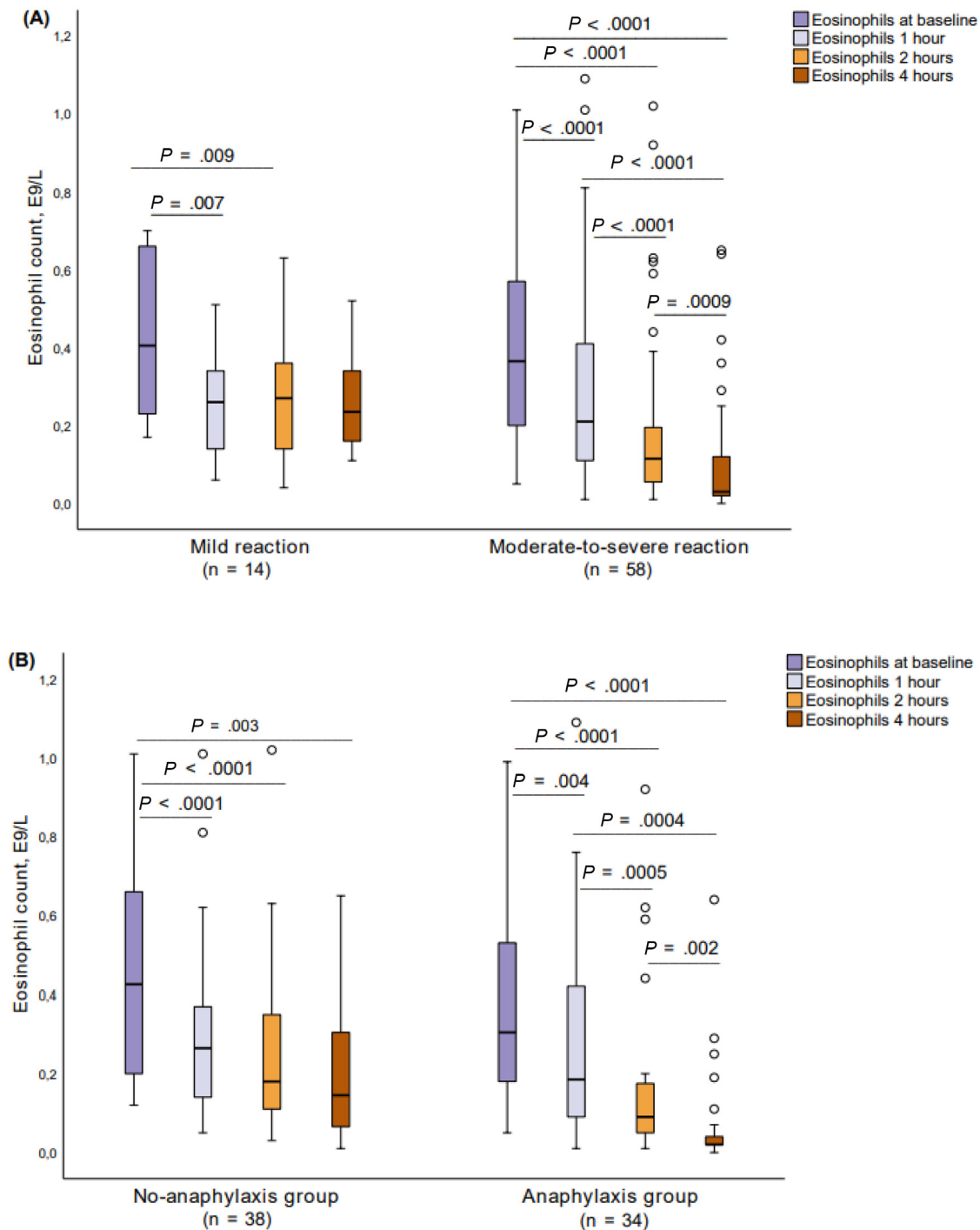


Figure 2. (A) Absolute eosinophil count by reaction severity and (B) in the no-anaphylaxis and anaphylaxis subgroups and (C) absolute basophil count by reaction severity and (D) in the no-anaphylaxis and anaphylaxis subgroups at baseline, 1 hour, 2 hours, and 4 hours after an allergic reaction. Only patients with a positive challenge result are included.

decline in basophils was observed between the baseline value and the 2- and 4-hour samples ($P = .001^*$ and $P = .001^*$, respectively). The basophil levels by reaction severity at 4 measurement points in children with a positive challenge result and between the no-anaphylaxis and anaphylaxis subgroups are presented in Figure 2C and D.

ECP levels at baseline were similar between the groups with negative challenge result, mild reaction, and moderate-to-severe reaction ($P > .05$). Furthermore, in those with moderate-to-severe reactions, ECP levels decreased significantly during the reaction compared with baseline. Significant changes were not observed in the subgroups with mild reactions, with no anaphylaxis, or with anaphylaxis, among those who had a reaction (Fig 3A-C).

Platelet-Activating Factor and Platelet-Activating Factor Acetylhydrolase

In the moderate-to-severe reaction group, the PAF level at baseline was significantly lower than in that with a negative challenge result ($P = .001^*$) (eFig 3). PAF peak ratio was significantly greater in the group with moderate-to-severe reactions than in those with a negative challenge result or a mild reaction ($P < .001^*$ and $P = .003^*$, respectively) and significantly greater in children with anaphylaxis compared with those with no anaphylaxis ($P = .008^*$). In the moderate-to-severe reaction group, PAF peaked significantly 1 hour after initiation of an allergic reaction ($P = .04^*$) (Fig 4A-D).

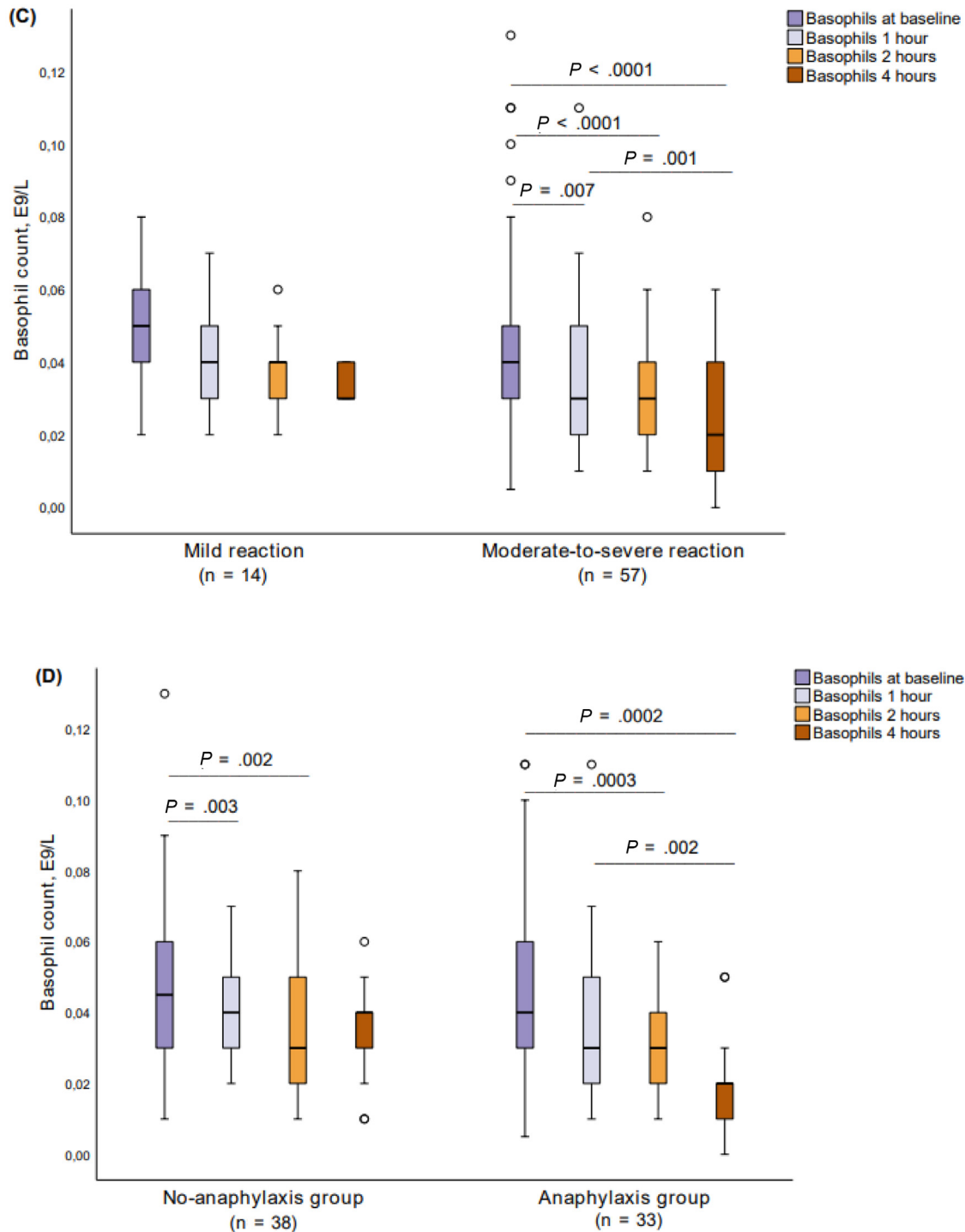


Figure 2 Continued.

Baseline quantitative PAH-AH was similar between the 3 reaction groups and between the no-anaphylaxis and anaphylaxis subgroups. PAF/PAF-AH ratio was elevated between baseline and 1-hour value both in moderate-to-severe reaction group and more distinctly in the anaphylaxis subgroup, although statistical significance was not reached (Fig 5A-D).

Tryptase

Baseline tryptase level did not differ significantly by reaction severity or between the no-anaphylaxis and anaphylaxis subgroups

($P > .05$) (Fig 6A). Tryptase level was elevated in the anaphylaxis subgroup 1 hour, 2 hours, and 4 hours after onset of allergic symptoms compared with the baseline value ($P = .001^*$, $P = .001^*$, and $P = .009^*$, respectively). Among children with a positive challenge result, delta-tryptase level was higher in the anaphylaxis group than in the no-anaphylaxis group (median 1.35 and 0.90 $\mu\text{g/L}$, respectively) with borderline significance ($P = .05$). Peak tryptase ratio was statistically similar between the no-anaphylaxis and anaphylaxis subgroups ($P = .07$). Baseline tryptase, tryptase levels at 4 time points, delta-tryptase, and tryptase ratio in the no-anaphylaxis and anaphylaxis subgroups are found in Figure 6B to D.

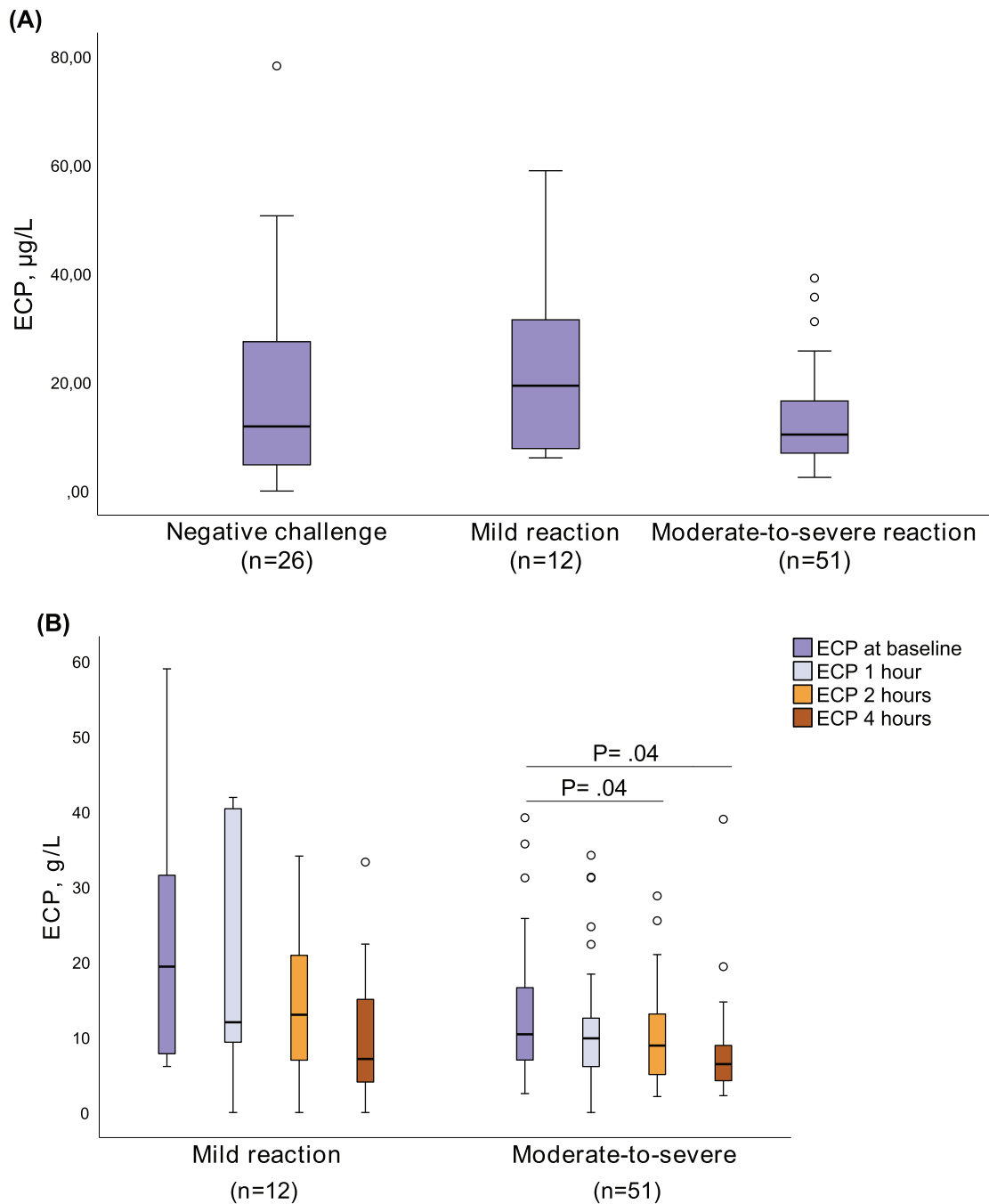


Figure 3. ECP (A) at baseline, (B) by reaction severity, (C) and in the no-anaphylaxis and anaphylaxis subgroups at 4 time points among those who reacted. ECP, eosinophil cationic protein.

Correlations

The correlations between biomarkers, severity score, and changes in effector cells are found in [eTable 3](#).

The median values and range for PAF, quantitative PAF-AH, tryptase, BAT%, and ECP at baseline, and in PAF peak ratio, delta tryptase, and maximal percent change in absolute eosinophil and basophil count between the no-anaphylaxis and anaphylaxis subgroups are presented in [eTable 4](#).

Discussion

Children with confirmed anaphylaxis seem to have several immunologic characteristics that differentiate them from those with milder reactions to cashew nut. The decrease of eosinophils among patients with an anaphylactic reaction was strikingly profound when compared with those with milder reactions or a negative challenge result. PAF peaked substantially 1 hour from initiation of anaphylaxis, and PAF peak ratio was significantly higher among those experiencing anaphylaxis.

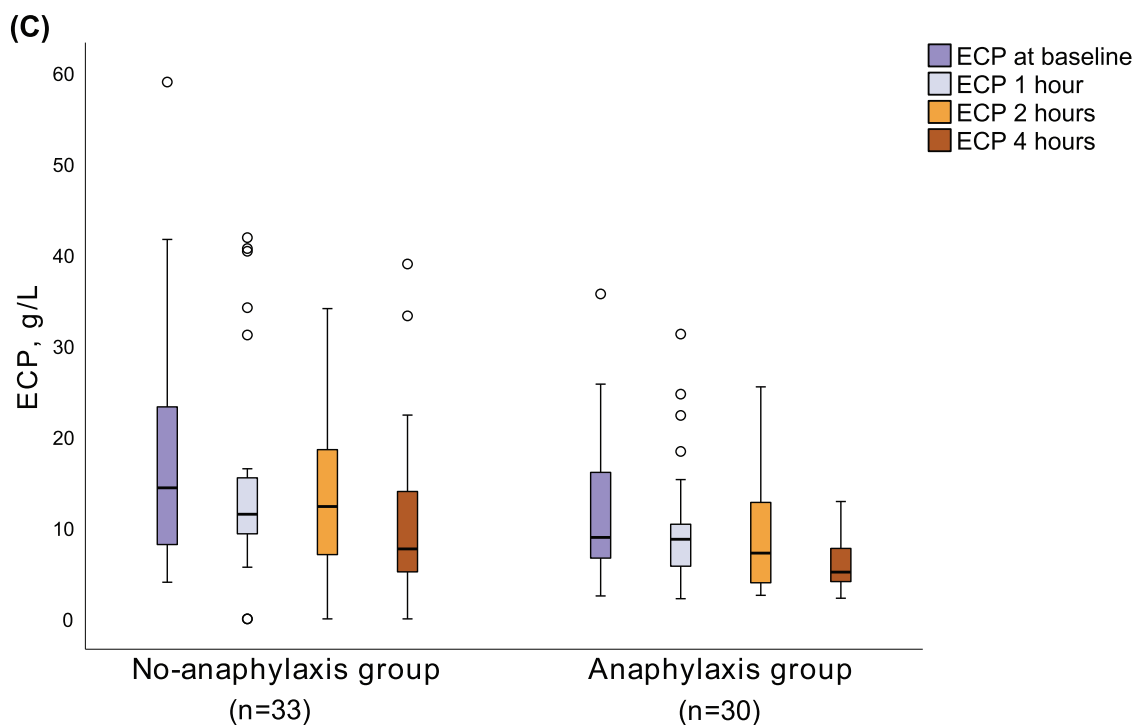


Figure 3 Continued.

Reaction severity was associated with the degree of eosinophil reduction. We observed a significant percent decrease in eosinophil count between children with a negative challenge result and those with moderate-to-severe reactions and between the mild and moderate-to-severe reaction groups. Similarly, children with anaphylaxis had a significantly more prominent decrease in eosinophil count compared with the no-anaphylaxis group. The eosinophil count dropped significantly between all 4 time points, and it also decreased progressively until the last 4-hour measurement point in both the group with moderate-to-severe reactions and children with an anaphylactic reaction. The same phenomenon was found to a lesser extent in the groups with mild reactions and with no anaphylaxis. We suggest that eosinophil decline may reflect eosinophil migration from the peripheral blood to the target tissues during an allergic reaction, which seems to be further boosted by reaction severity.

Niggeman et al.²⁶ reported a significant eosinophil decline after a positive food challenge result and increase in ECP 8 hours after the clinical reaction. Another study revealed that leucocyte levels decreased during a double-blind, placebo-controlled (DBPC) food challenge independent of challenge outcome and without simultaneous change in eosinophil count.²⁷ Earlier studies revealed a significant decrease in basophil count during anaphylaxis,^{28,29} but no significant decline in other blood cells was reported. CCL2 seemed to chemotactically induce basophil migration. CCL2 and tryptase levels were found to have a positive correlation with anaphylaxis, and CCL2 level is elevated significantly during anaphylaxis.²⁸

Similarly, ECP level decreased significantly during a moderate-to-severe reaction, reflecting the concomitant fall in eosinophil count. This phenomenon is probably linked to transition of blood eosinophils to tissues induced by an ongoing allergic reaction, and thus the level of free ECP is reduced. A possible increase in serum ECP levels after degranulation of eosinophils in the target tissues may have been observed if extra blood samples were drawn at a later stage after reaction. An earlier German study reported ECP level elevation 8 hours after the clinical reaction.²⁶

Leucocyte levels, and especially neutrophil subset, increased significantly during moderate-to-severe reactions. This was most pronounced during anaphylaxis which may be due to intravenous glucocorticoid treatment. An increase in leucocyte count, however, may reflect overall leucocyte activation during the immunologic process initiated by an allergic reaction. Conversely, an earlier study by Beyer et al.²⁷ reported a decrease in leucocyte count in DBPC food challenges regardless of the challenge outcome.

In an earlier study by Vadas et al.,¹⁴ PAF level was higher in patients with anaphylaxis compared with the control group and PAF correlated with anaphylaxis severity. The anaphylaxis etiology varied and was not restricted to pure food anaphylaxis, and therefore, non-IgE-mediated mechanisms may also have been involved. The patients in the anaphylaxis group were significantly older (15–74 years)¹⁴ than our purely pediatric population. In a recent pediatric study, tryptase and PAF levels elevated during anaphylaxis, but the triggers of anaphylaxis varied and children with only urticaria and angioedema were included to the anaphylaxis group in the analysis.¹⁷ In those with moderate-to-severe reactions, PAF level was elevated statistically significantly 1 hour after the reaction and PAF levels seemed to peak even more substantially 1 hour after initiation of an anaphylactic reaction. The lack of statistical significance for PAF level elevation in the anaphylaxis group is probably because of a large distribution of PAF values at the 1-hour measurement time point and limitations in the anaphylaxis group sample size. However, PAF peak ratio was statistically significantly higher in the anaphylaxis group compared with the no-anaphylaxis group, and the similar significant difference was also observed between those with moderate-to-severe reactions compared with those with mild reactions or a negative challenge result. To our knowledge, we present the first human study that reveals a significant elevation in PAF levels by using repeated measurements during a moderate-to-severe allergic reaction, and not just in anaphylaxis. Larger studies on the role and timing of PAF in pediatric food-induced anaphylaxis are needed.

In the study by Upton et al.,¹⁷ PAF-AH activity seemed to be lower in those with severe anaphylaxis compared with those with mild-to-

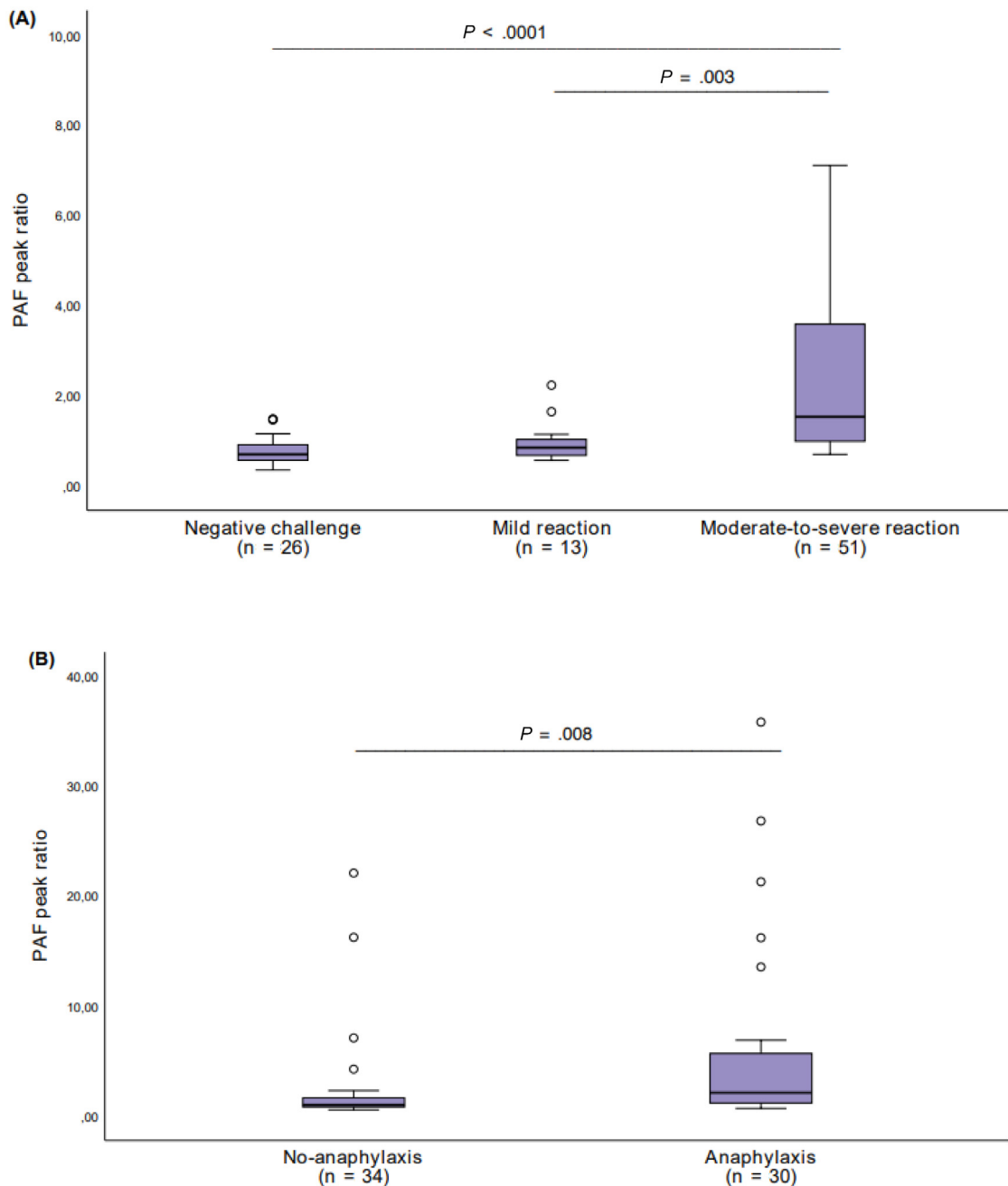


Figure 4. PAF peak ratio (A) by reaction severity and (B) in the no-anaphylaxis and anaphylaxis subgroups. PAF at 4 time points (C) by reaction severity and (D) in the no-anaphylaxis and anaphylaxis subgroups. Only patients with a positive challenge result are included. PAF, platelet-activating factor. In panel C, in moderate-to-severe reactions, 5 outliers at the 1-hour time point and 1 outlier at 2-hour time point were excluded. In panel D, 1 outlier at the 2-hour time point in the no-anaphylaxis group and 5 outliers in the anaphylaxis group at the 1-hour time point were excluded.

moderate anaphylaxis. However, the number of patients in the severe anaphylaxis group was low ($n = 12$), and patients with only skin symptoms (ie, urticaria and angioedema) were included in the mild anaphylaxis group.¹⁷ We did not observe significant difference in quantitative baseline PAF-AH between the no-anaphylaxis and anaphylaxis subgroups. Upton et al¹⁷ measured biological activity of PAF-AH in their study compared with our quantitative PAF-AH assay, which may explain their results regarding PAF-AH activity in predicting risk for anaphylaxis. However, in comparison to accidental exposures to varied allergens, our study was performed in the hospital ward under highly controlled circumstances regarding the up dosing process with a specified provocative food allergen and possibility for repeated biomarker measurements during the anaphylaxis. The

potential cofactors (ie, infections, aeroallergen exposure, NSAID use, exercise) were also controlled as potential anaphylaxis confounders. In our study, the PAF/PAF-AH ratio in the anaphylaxis group clearly seemed to peak 1 hour after initiation of the reaction, and even more strikingly than the PAF levels at the same time point. This may indicate the inadequate neutralizing effect of PAF-AH among patients experiencing anaphylaxis. The wide distribution in the PAF/PAF-AH ratio probably accounts for this robust elevation not being statistically significant in the anaphylaxis group.

Baseline serum tryptase level in this study population did not differ between the anaphylaxis and the no-anaphylaxis subgroups in contrast to some earlier studies.^{11,12} BST did not predict the reaction severity. In some previous studies, BST is associated with anaphylaxis

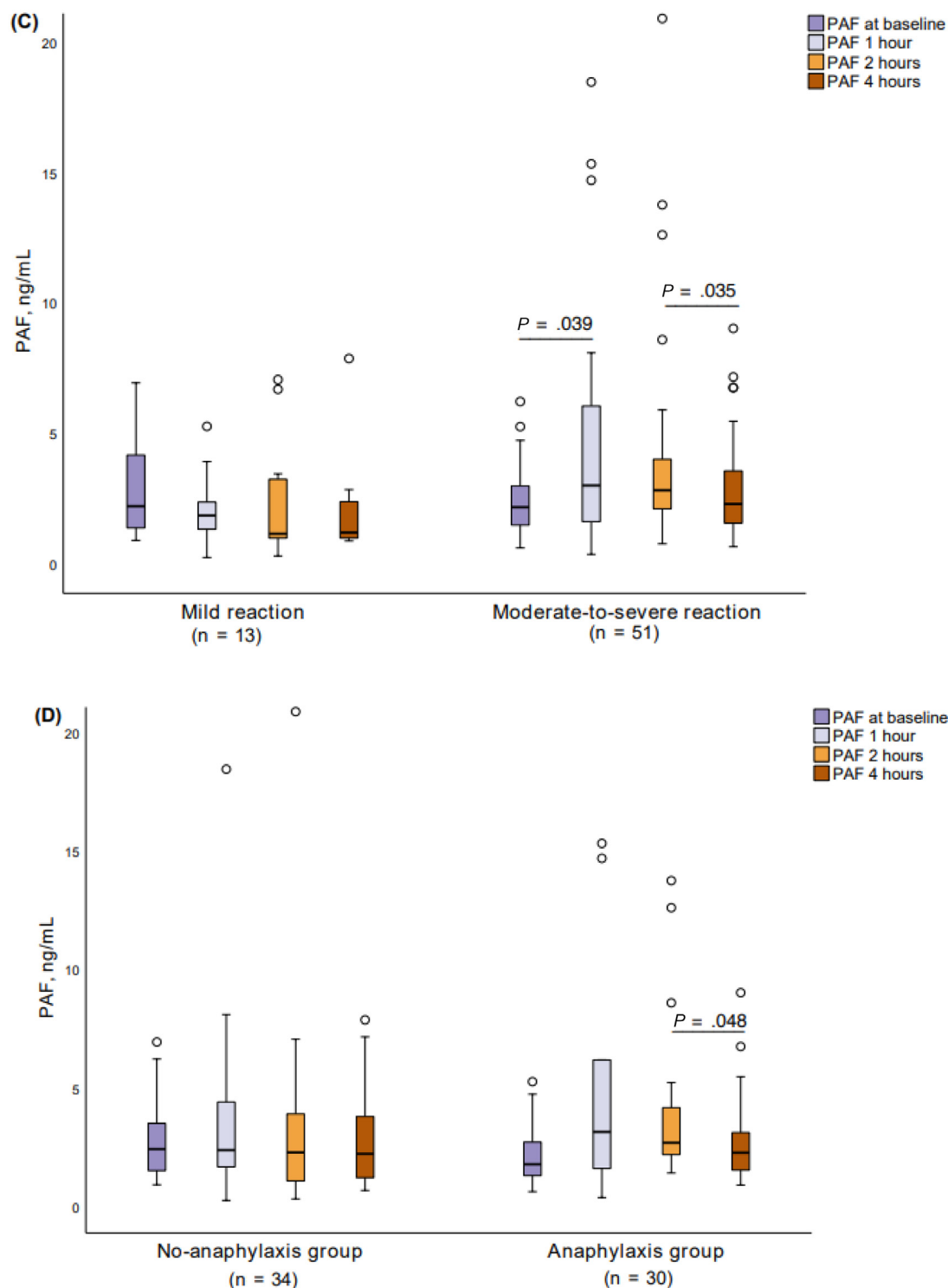


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severity.^{9,10} In our study population, delta-tryptase seemed to be higher in the anaphylaxis group compared with the no-anaphylaxis group, although this did not reach statistical significance.

Regarding the timing of significant changes in eosinophil and basophil counts, ECP, and anaphylaxis markers (PAF, tryptase) in children with anaphylaxis, PAF level peaked most profoundly 1 hour after the initiation of an allergic reaction and subsequently decreased rapidly. This may be due to the short half-time of 3 to 13 minutes.¹⁴ Furthermore, we found that PAF peak ratio is a specific marker of anaphylaxis. Tryptase level also peaked rather early in the

anaphylaxis group (1 to 2 hours after onset of a clinical reaction), albeit significantly elevated tryptase levels were evident even 4 hours after initiation of an anaphylactic reaction. Among children experiencing anaphylaxis, the decrease of eosinophils was progressively more apparent at each measurement point; ECP followed the decreasing trend accordingly.

PAF plays a key role in human anaphylaxis.^{13,14} Eosinophils and PAF are strongly linked to each other. Eosinophils secrete PAF, and PAF interacts with eosinophils by recruiting eosinophils to the site of an allergic reaction.¹³ On this basis and our recent findings, we

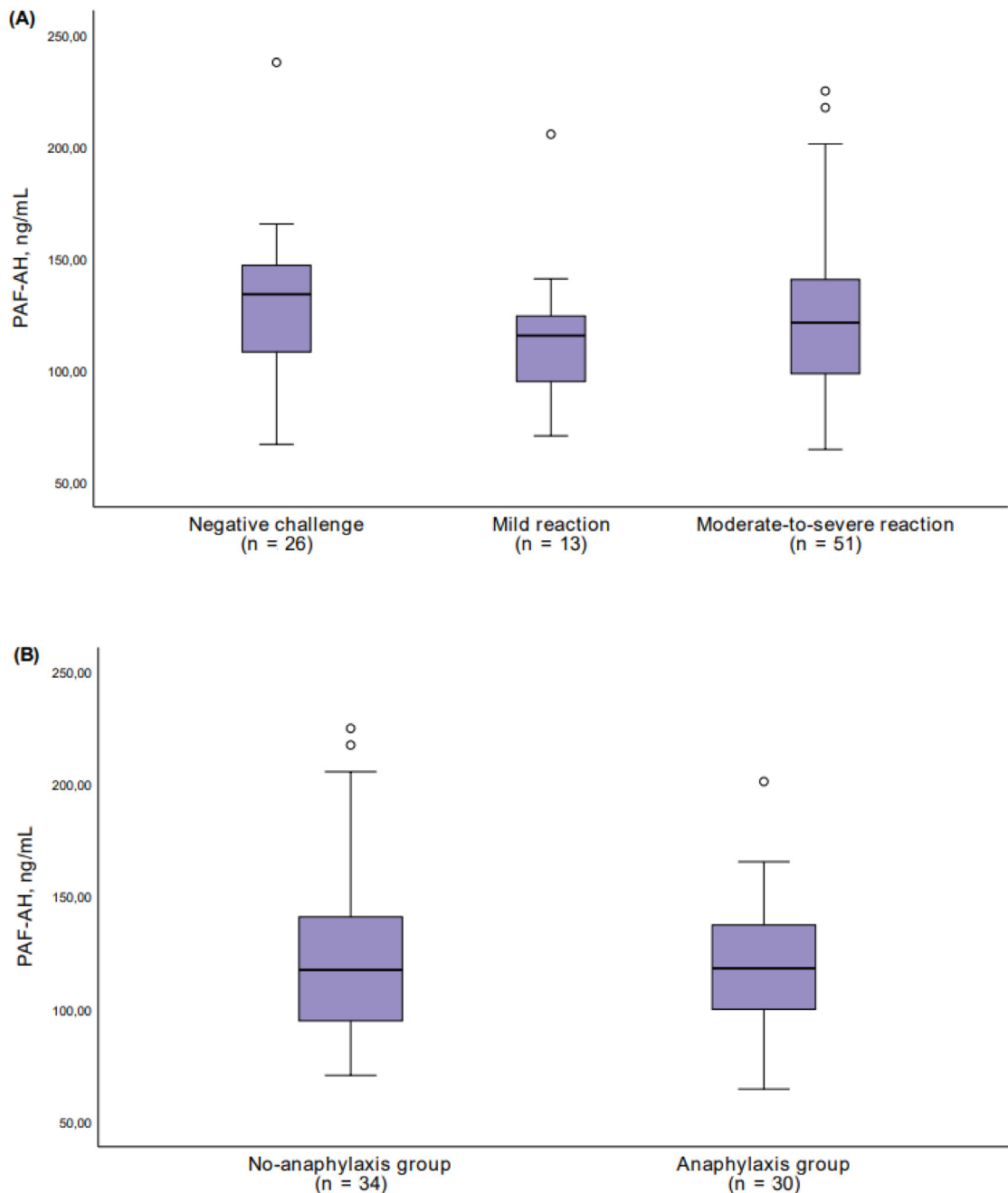


Figure 5. Quantitative PAF-AH at baseline (A) by reaction severity and (B) in the no-anaphylaxis and anaphylaxis subgroups and PAF/PAF-AH ratio at 4 time points (C) by reaction severity and (D) in the no-anaphylaxis and anaphylaxis subgroups. Only patients with a positive challenge result are included. PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetylhydrolase. In panel C, in moderate-to-severe reactions, 5 outliers at the 1-hour time point and 3 outliers at the 2-hour time point were excluded. In panel D, in the anaphylaxis group, 5 outliers at the 1-hour time point and 1 outlier at the 2-hour time point were excluded.

suggest that the profound decline of eosinophils in children with a positive challenge result (and strikingly pronounced in anaphylaxis) may be strongly related to the role of PAF as a significant chemotactic protein in anaphylaxis. The moderate negative correlation between maximal percent change in eosinophils and PAF peak ratio supports the strong linkage between PAF and eosinophils in cashew nut-induced anaphylaxis. Furthermore, PAF has the ability to promote eosinophil adhesion¹⁹ and degranulation,²⁰ and by this mechanism, it may drive more than understood the clinical manifestations of anaphylaxis induced by various intracellular compounds derived from eosinophils. Moreover, the potency of migrating eosinophils to secrete PAF may further promote the direct effects of PAF in the target tissues (ie, skin, cardiorespiratory system, gastrointestinal tract,

and central nervous system). This complex interaction of eosinophils and PAF at the site of an allergic reaction should be investigated further in novel studies, which may reveal new insights into identifying patients more susceptible to anaphylaxis. Moreover, the assessment of ECP and other intracellular compounds of eosinophils in the later phase after a clinical reaction is a relevant topic for future studies.

In earlier studies, the BAT was found to be promising in predicting reaction severity in peanut and cashew nut allergies.^{3,30} Vantur et al²⁸ reported a significant decrease in basophil count measured at 1 time point during anaphylaxis compared with a baseline level assessed later. However, no significant change in eosinophil levels was observed, which contrasts with our results. CCL2 seems to have a role in human anaphylaxis and affects basophil migration.²⁸ The

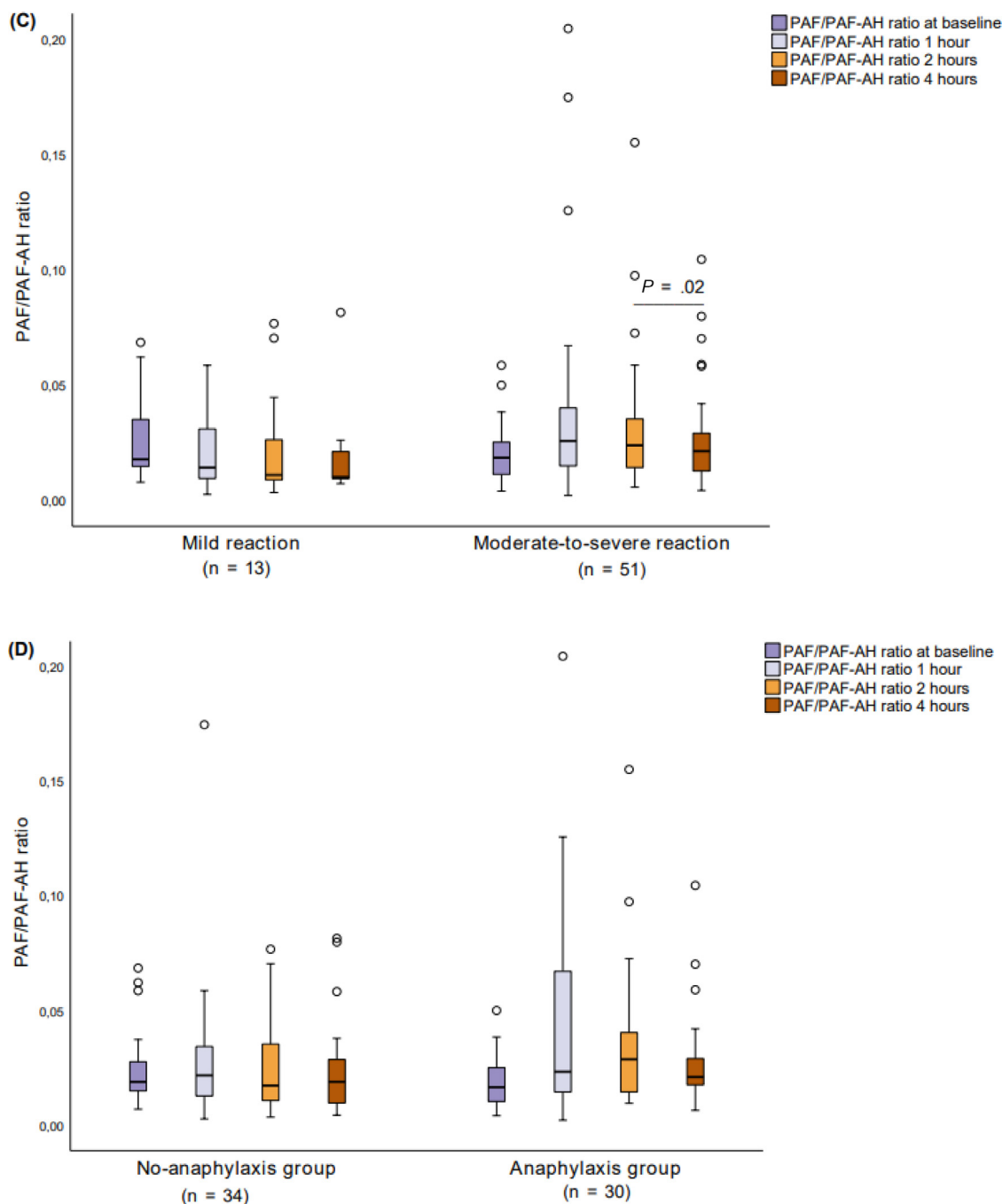


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decline of basophils during anaphylaxis in our study population may be explained by a CCL2-induced influx of basophils to target tissues during an allergic reaction. As proposed in earlier studies, mast cells³¹ or eosinophils³² may be potential sources of CCL2 production. Nevertheless, the evident negative correlation between maximal percent change of absolute basophil count and PAF peak ratio emphasizes the potential interaction between PAF and basophils during an anaphylactic reaction. The positive correlation between BAT% and delta-tryptase suggests a potential IgE-mediated activation of both basophils and mast cells. Moreover, the observed significant decrease in both eosinophils and basophils underlines the relevance of both these effector cells either individually or simultaneously in affecting a large group of mediators in food-induced anaphylaxis. The significantly higher BAT% in the anaphylaxis group than in the no-anaphylaxis group supports the essential role of basophils in the clinical

manifestations of anaphylaxis. Delta-tryptase exhibited a weak negative correlation to maximal change in eosinophils, whereas the negative correlation between PAF peak ratio and maximal percent change in eosinophils was clearly more evident. This may provide further evidence of PAF as a major contributor in chemotactically attracting eosinophils to target tissues during cashew nut-induced anaphylaxis. Mast cell-, eosinophil-, and basophil-driven reactions most likely coexist in human food-induced anaphylaxis.

Great majority of children experiencing anaphylaxis received cortisone treatment according to common good clinical practice to prevent biphasic anaphylaxis. However, 12 of 58 children in the no-anaphylaxis group with a moderate-to-severe reaction did not receive cortisone treatment, and significant decrease in eosinophils was still evident. This supports the significant role of an allergic reaction itself in the post-reaction eosinophil decline. At least partial

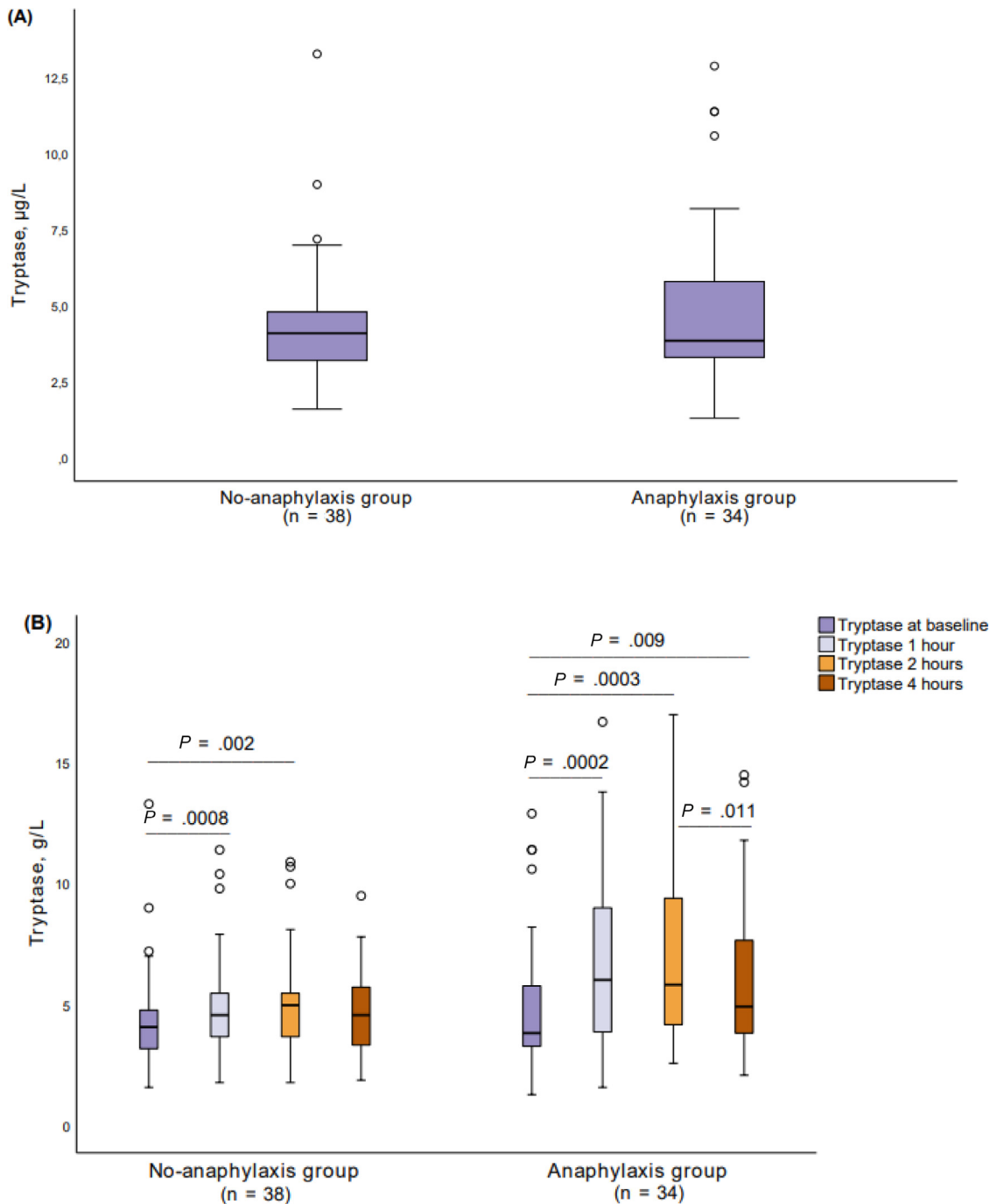


Figure 6. Tryptase (A) at baseline and at 4 time points (B) in the no-anaphylaxis and anaphylaxis subgroups. (C) Delta-tryptase and (D) peak tryptase ratio in the no-anaphylaxis and anaphylaxis subgroups. Only patients with a positive challenge result are included.

role of cortisone on the profound decrease of eosinophils in anaphylaxis is difficult to exclude because of this major confounder. It is still important to emphasize that the very same patients who received cortisone treatment experienced also the most severe, usually generalized symptoms. However, glucocorticoids do not necessarily have significant effect on eosinophil migration or chemotaxis.³³ Larger in vivo studies concerning effects of single-administered glucocorticoids to eosinophil count in anaphylaxis are still lacking.

The strengths of this study were a large sample size, a significant number of patients with anaphylaxis, multiple prospective measurements of various biomarkers during anaphylaxis, and the study population restricted to pediatric patients with an IgE-mediated food

allergy. The low median age of the children with anaphylaxis also provides a new perspective in evaluating the role of anaphylaxis biomarkers in younger age groups with food allergy. To our knowledge, similar studies restricted to a pediatric study population which have investigated changes in all the anaphylaxis biomarkers in question (ie, PAF, PAF-AH, tryptase, ECP, eosinophils, and basophils) by repeated measurements have not been published previously.

Our study has some shortcomings. Challenges were not performed in a DBPC fashion. Although there were a rather large number of patients in the anaphylaxis subgroup, the multivariate analysis of the data was not possible because of the sample size. The great majority of patients in the anaphylaxis group were treated with glucocorticoids, which hampers the analysis. The wide cross-correlation

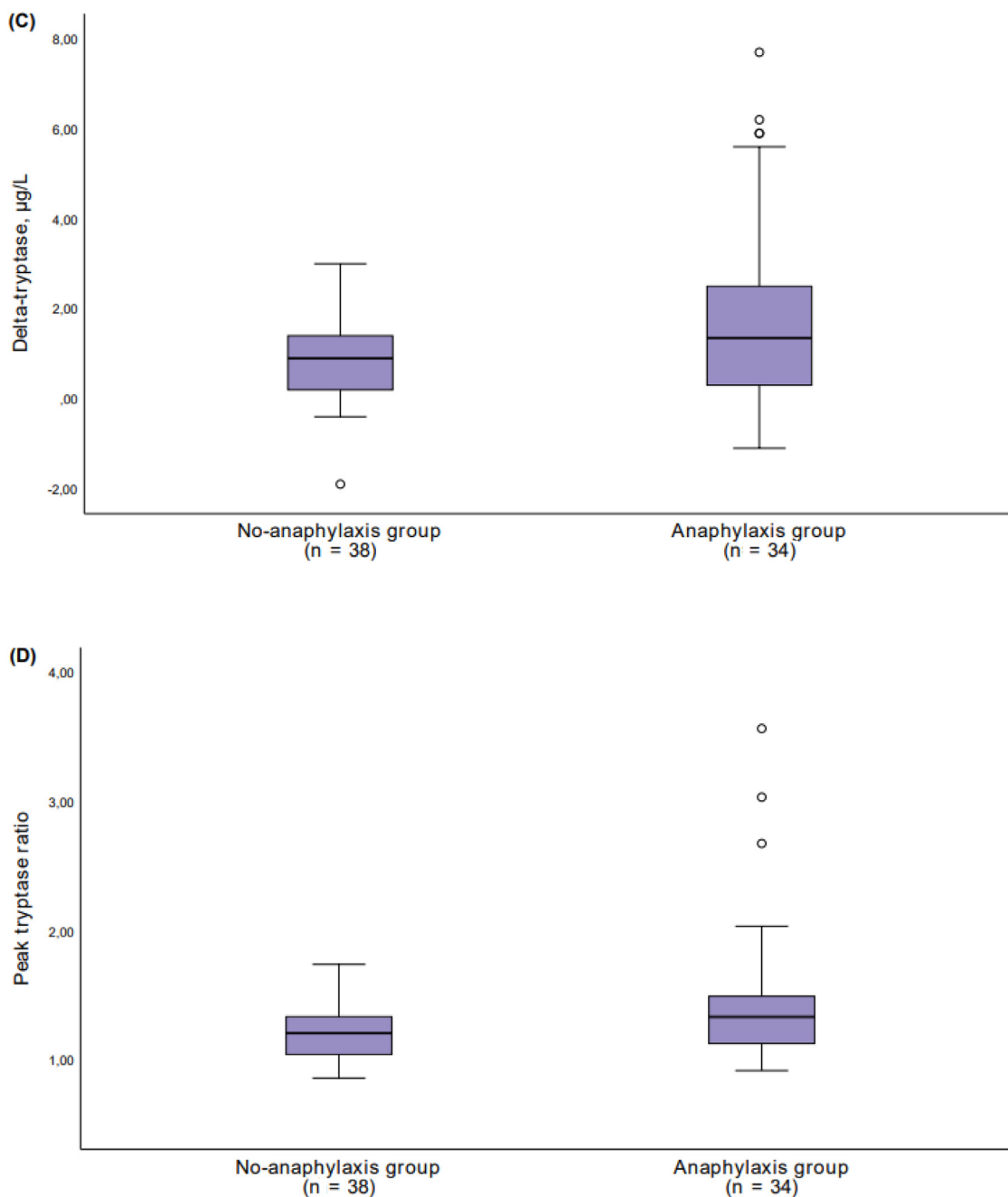


Figure 6 Continued.

between various biomarkers and changes in effector cells limits the conclusions based purely on correlations observed.

In conclusion, a profound decrease of eosinophils in cashew nut-induced anaphylaxis suggests that eosinophils are a more crucial player in food-induced anaphylaxis than previously understood. The simultaneous decrease in basophils may indicate a concurrent basophil-driven pathway during an anaphylactic reaction. PAF is also a specific anaphylaxis biomarker among children, and PAF increases robustly when assessed in the early phase of anaphylaxis. The complex interplay between PAF, eosinophils, basophils, and other anaphylaxis biomarkers should be studied in further detail to better understand the multifactorial pathogenesis of anaphylaxis. The findings of our study highlight the need for new perspectives to better understand the interactions between these multiple biomarkers and effector cells, and thus the various immunologic pathways and mechanisms in food anaphylaxis.

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Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.anai.2023.04.014>.

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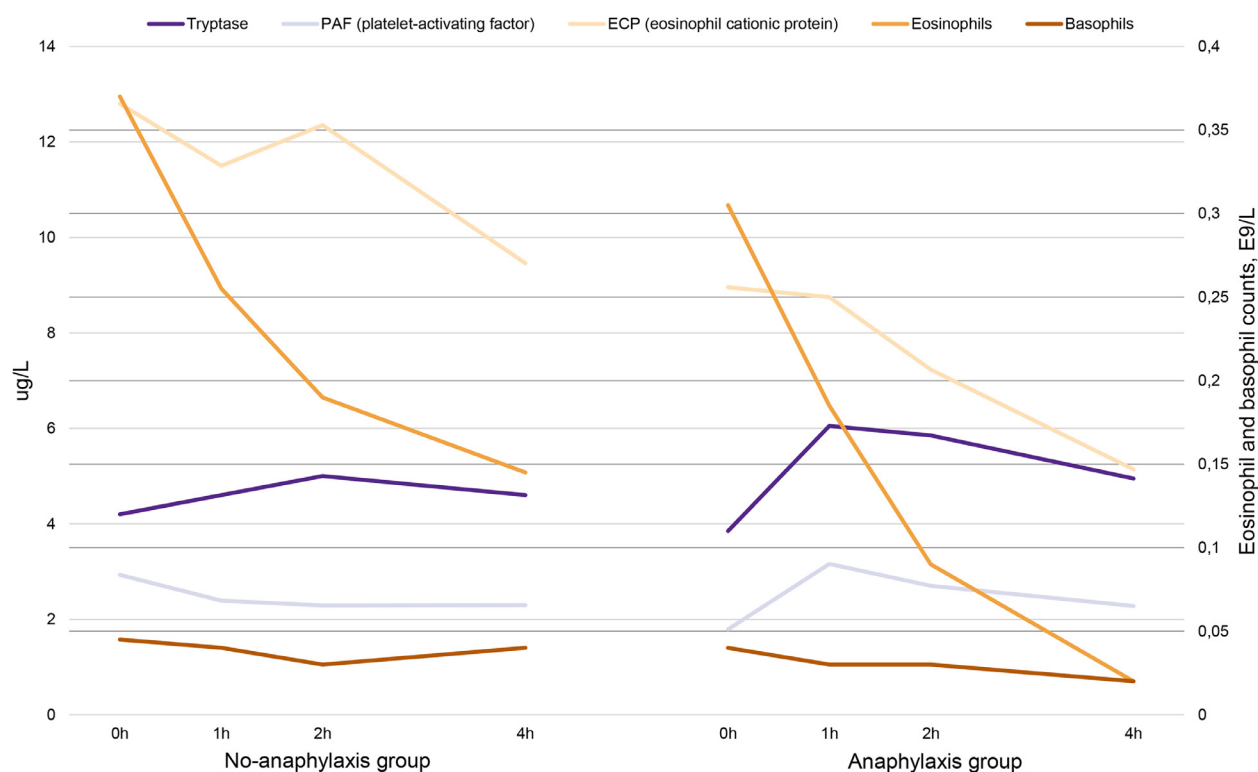
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Supplementary Data

eSupplemental Material

The European Academy of Allergy and Clinical Immunology 2014 anaphylaxis guideline: Anaphylaxis according to European Academy of Allergy and Clinical Immunology 2014 guidelines is characterized with a rapid onset of allergic symptoms from at least 2 organs (involvement of the skin, persistent gastrointestinal symptoms, respiratory compromise, reduced blood pressure or related symptoms), or low age-specific systolic blood pressure, or greater than or equal to 30 mm Hg decrease in systolic blood pressure.²⁵



Graphical Abstract

eTable 1
Baseline Clinical Characteristics and Baseline Values by Challenge Outcome

Clinical characteristics	Positive n = 72 (68%)	Negative n = 34 (32%)	P value
Male (%)	38 (52.7)	12 (35.3)	.09
Age, median (range), y	5.5 (1.4–16.7)	4.5 (1.6–15.1)	.10
History of reaction to cashew nut (%)	47 (65.2)	19 (55.9)	.35
Latest reaction to cashew nut (%)			
<1 y	15 (20.8)	7 (20.6)	.98
1–3 y	16 (22.2)	4 (11.8)	.20
>3 y	16 (22.2)	8 (23.5)	.88
Convincing pistachio allergy (%) ^a	6 (8.3)	3 (8.8)	.93
History of IgE-mediated milk, wheat, egg, or other nut allergy, n (%) ^a	45 (62.5)	18 (52.9)	.35
Atopic eczema, ever (%)	57 (79.1)	24 (70.6)	.33
Asthma, ever (%)	28 (38.9)	7 (20.6)	.06
Allergic rhinitis, ever (%) ^a	37 (51.4)	11 (32.4)	.07
Total serum IgE, kU/L, median (range)	208 (18–6226)	175 (9–1598)	.35
Cashew nut IgE, kU/L, median (range)	2.85 (0.16–81.30)	0.20 (0.03–38.70)	<.001
Ana o 3 IgE, kU/L, median (range)	1.82 (0.11–96.90)	0.08 (0.00–31.20)	<.001
Cashew nut SPT, median (range), mm ^b	10 (0–18)	4 (0–14)	<.001
Pistachio nut SPT, median (range), mm	7 (0–15)	4 (0–8)	<.001
Sensitized to birch (%) ^a	47 (65.2)	14 (41.2)	.019
Baseline blood eosinophil count, median (range), E9/L	0.37 (0.02–1.01)	0.34 (0.03–1.46)	.66
Baseline serum tryptase on a challenge day, median (range), µg/L	4.0 (1.3–13.3)	4.35 (1.8–15.0)	.54
Cumulative reactive dose, median (range), mg ^c	80 (5–730)	680 (430–680)	<.001

Abbreviations: IgE, immunoglobulin E; SPT, skin prick test.

NOTE. P Value < .05 is considered as significant and significance is stated as bold.

^aSkin prick test ≥ 3 mm or specific IgE ≥ 0.35 kU/L.

^bn = 105.

^cPatients with a negative challenge median and range have cumulative dose protein. Significant P value < .05 calculated by Pearson χ^2 , Fisher's exact, or Mann-Whitney U test.

eTable 2
The Modified Severity Scoring of Allergic Reactions at a Food Challenge

Cumulative dose (mg) of protein at which the symptoms appear			
Symptoms	>100 mg	6-99 mg	≤5 mg
Localized urticaria < 5 lesions on the face or extremities or pruritus	1	2	3
Mild stomach pain < 30 min and resolves without medication	3	4	5
Generalized urticaria	6	7	8
Abdominal pain > 30 min, vomiting, diarrhea, or angioedema	9	11	13
Laryngeal edema or mild wheezing: hoarseness of voice, stridor, cough, wheezing, pain in the throat	14	15	17
Severe respiratory symptoms or a collapse: drop in the blood pressure, severe bronchial obstruction, oxygen saturation < 92%, pallor, collapse	21	22	23

NOTE. Modified from Hourihane by Kukkonen et al.²⁴

Severity of the reaction is the highest, not cumulative, score: 1 to 5 indicate mild, 6 to 23 moderate-to-severe allergic reactions.

eTable 3
Correlations by Spearman Rho and *P* Values Between PAF Peak Ratio (Highest PAF/Baseline PAF), Severity Score, Baseline Tryptase, Delta-Tryptase (Highest Tryptase Minus Baseline Tryptase), Maximal Change in Eosinophils in Percent, Baseline Eosinophil Cationic Protein, Maximal Change in Basophils in Percent, and BAT%

Parameters	PAF peak ratio	Severity score	Baseline tryptase	Delta-tryptase	Maximal change in eosinophils (%)	Baseline eosinophil cationic protein	Maximal change in basophils (%)	BAT%
PAF peak ratio		0.396 <i>P</i> = .001	−0.045 <i>P</i> = .67	0.344 <i>P</i> = .001	− 0.516 <i>P</i> < .001	−0.076 <i>P</i> = .48	− 0.410 <i>P</i> < .001	0.395 <i>P</i> = .001
Severity score	0.396 <i>P</i> = .001		0.178 <i>P</i> = .14	0.154 <i>P</i> = .20	− 0.424 <i>P</i> = .001	−0.117 <i>P</i> = .36	− 0.246 <i>P</i> = .045	0.448 <i>P</i> = .001
Baseline tryptase	−0.045 <i>P</i> = .67	0.178 <i>P</i> = .14		0.027 <i>P</i> = .08	0.089 <i>P</i> = .37	0.025 <i>P</i> = .82	0.112 <i>P</i> = .27	−0.037 <i>P</i> = .72
Delta-tryptase	0.344 <i>P</i> = .001	0.154 <i>P</i> = .20	0.027 <i>P</i> = .08		0.312 <i>P</i> = .001	−0.122 <i>P</i> = .25	− 0.282 <i>P</i> = .004	0.498 <i>P</i> < .001
Maximal percent change in eosinophils (%)	− 0.516 <i>P</i> < .001	− 0.424 <i>P</i> = .001	0.089 <i>P</i> = .37	− 0.312 <i>P</i> = .001		0.170 <i>P</i> = .11	0.593 <i>P</i> < .001	− 0.441 <i>P</i> < .001
Baseline eosinophil cationic protein	−0.076 <i>P</i> = .48	−0.117 <i>P</i> = .36	0.025 <i>P</i> = .82	−0.122 <i>P</i> = .25	0.170 <i>P</i> = .11		0.173 <i>P</i> = .11	−0.101 <i>P</i> = .35
Maximal percent change in basophils (%)	− 0.410 <i>P</i> < .001	− 0.246 <i>P</i> = .045	0.112 <i>P</i> = .27	− 0.340 <i>P</i> = .001	0.593 <i>P</i> < .001	0.173 <i>P</i> = .11		− 0.386 <i>P</i> < .001
BAT%	0.395 <i>P</i> = .002	0.448 <i>P</i> = .001	−0.037 <i>P</i> = .72	0.498 <i>P</i> < .001	− 0.441 <i>P</i> < .001	−0.101 <i>P</i> = .35	− 0.386 <i>P</i> < .001	

Abbreviations: BAT%, percentage of activated CD63+ basophils; ECP, eosinophil cationic protein; PAF, platelet-activating factor.

NOTE. Data are presented as Spearman's rho and *P* value. *P* value < .05 is considered as significant and bold indicates *P* < .05.

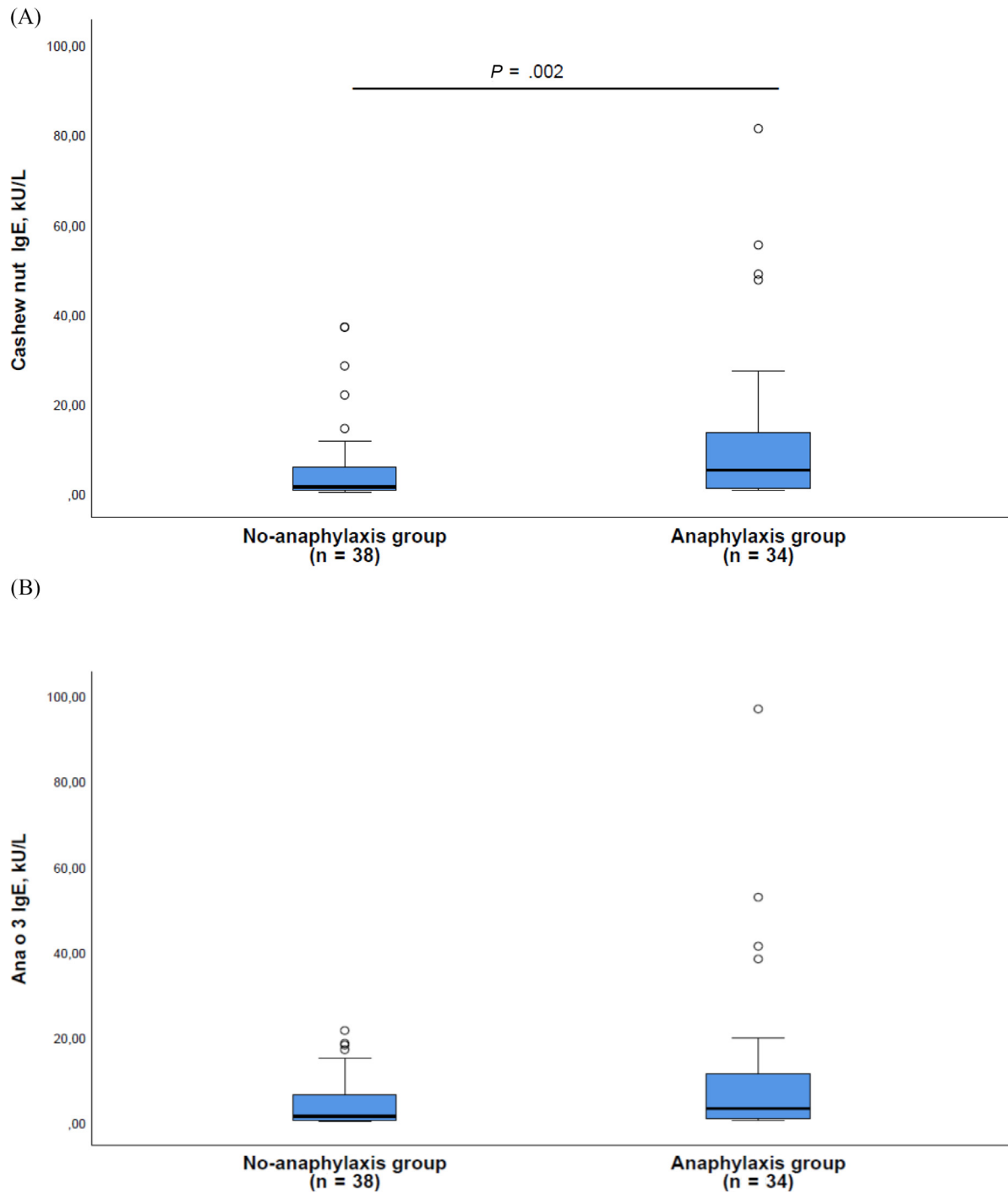
eTable 4
Median Values and Range Between the No-Anaphylaxis and Anaphylaxis Subgroups for PAF, Quantitative PAF-AH, Tryptase, BAT%, and ECP at Baseline and PAF Peak Ratio (Highest Post-Reaction PAF Divided by Baseline PAF), Delta-Tryptase (Highest Post-Reaction Tryptase Minus Baseline Tryptase), Maximal Percent Change in Absolute Eosinophil Count, and Maximal Percent Change in Absolute Basophil Count

Parameters	No-anaphylaxis (n = 38)	Anaphylaxis (n = 34)	<i>P</i> value not Bonferroni corrected	<i>P</i> value Bonferroni corrected
PAF at baseline, ng/mL	2.44 (0.92-6.96)	1.79 (0.63-5.28)	.09	.18
PAF-AH at baseline, ng/mL	117.37 (70.76-225.00)	118.08 (64.60-201.36)	1.00	1.00
PAF peak ratio	0.99 (0.56-22.07)	2.21 (0.69-77.08)	.008	.02
Tryptase at baseline, µg/L	4.10 (1.60-13.30)	3.85 (1.30-12.90)	.72	1.00
Delta-tryptase	0.90 (−1.90 to 3.00)	1.35 (−1.10 to 7.70)	.05	.100
Maximal percent change in eosinophil count (%)	−48.33 (−98.18 to 17.39)	−87.71 (−100.00 to 70.59)	.001	.001
Maximal percent change in basophil count (%)	−40.00 (−75.00 to 100.00)	−50.00 (−100.00 to 100.00)	.07	.13
BAT%	12.90 (2.30-72.10)	32.20 (2.30-82.00)	.005	.01
ECP at baseline, µg/L	14.40 (4.03-59.00)	8.96 (2.52-35.71)	.04	.07

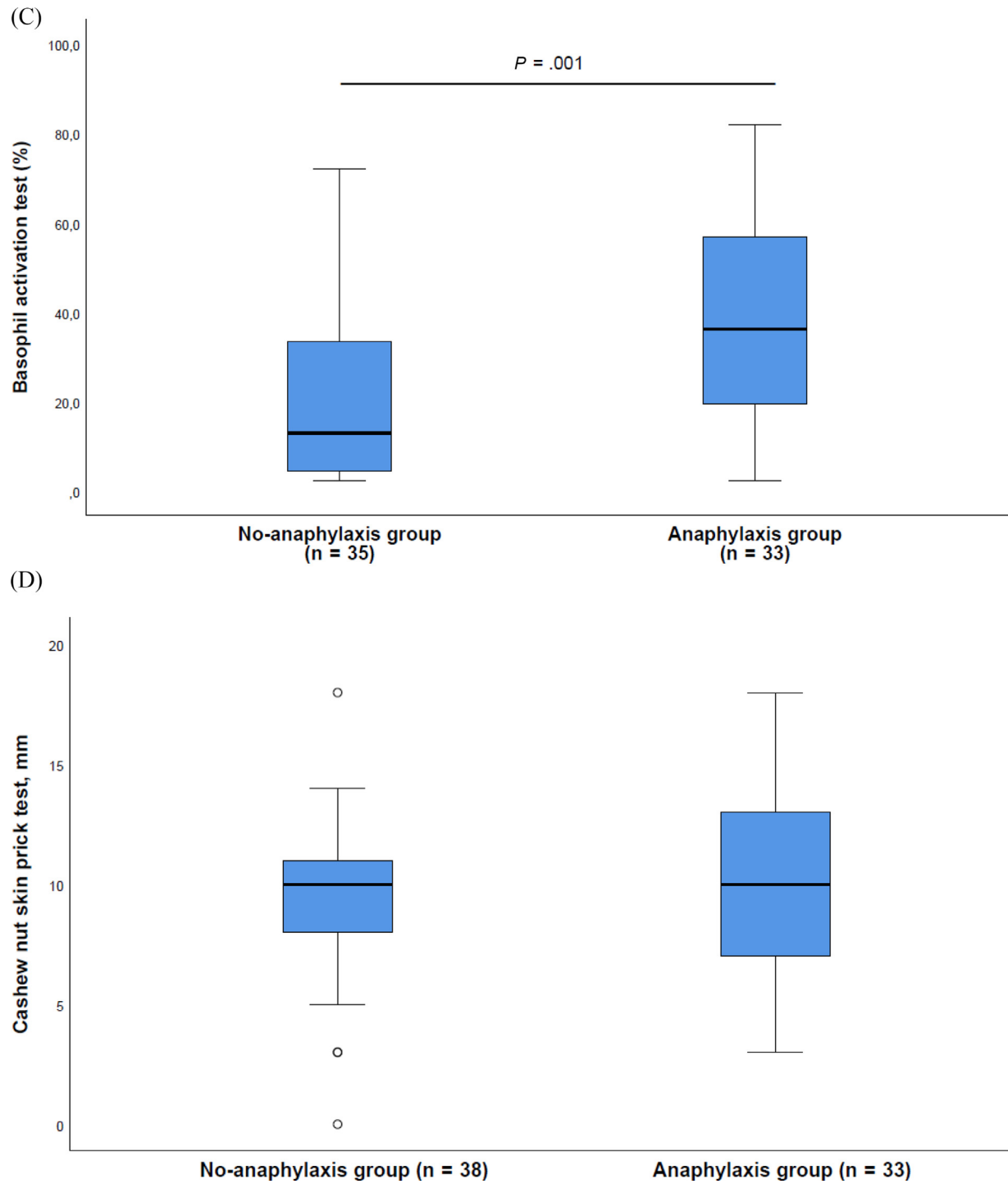
Abbreviations: BAT%, percentage of activated CD63+ basophils; ECP, eosinophil cationic protein; PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetylhydrolase.

NOTE. Only patients with a positive challenge result are included in the no-anaphylaxis subgroup.

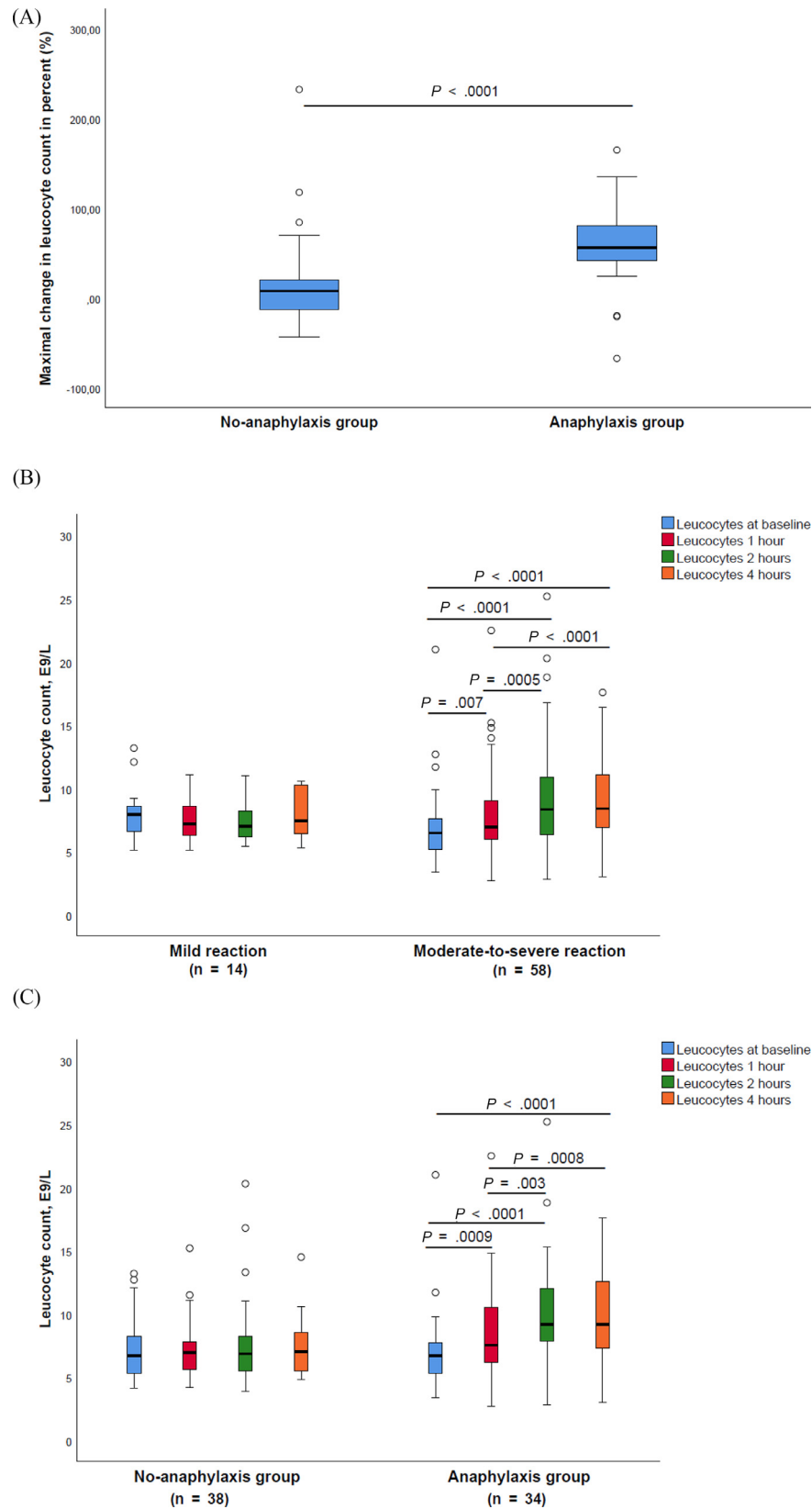
Data are presented as median (range). *P* < .05 is illustrated in bold.



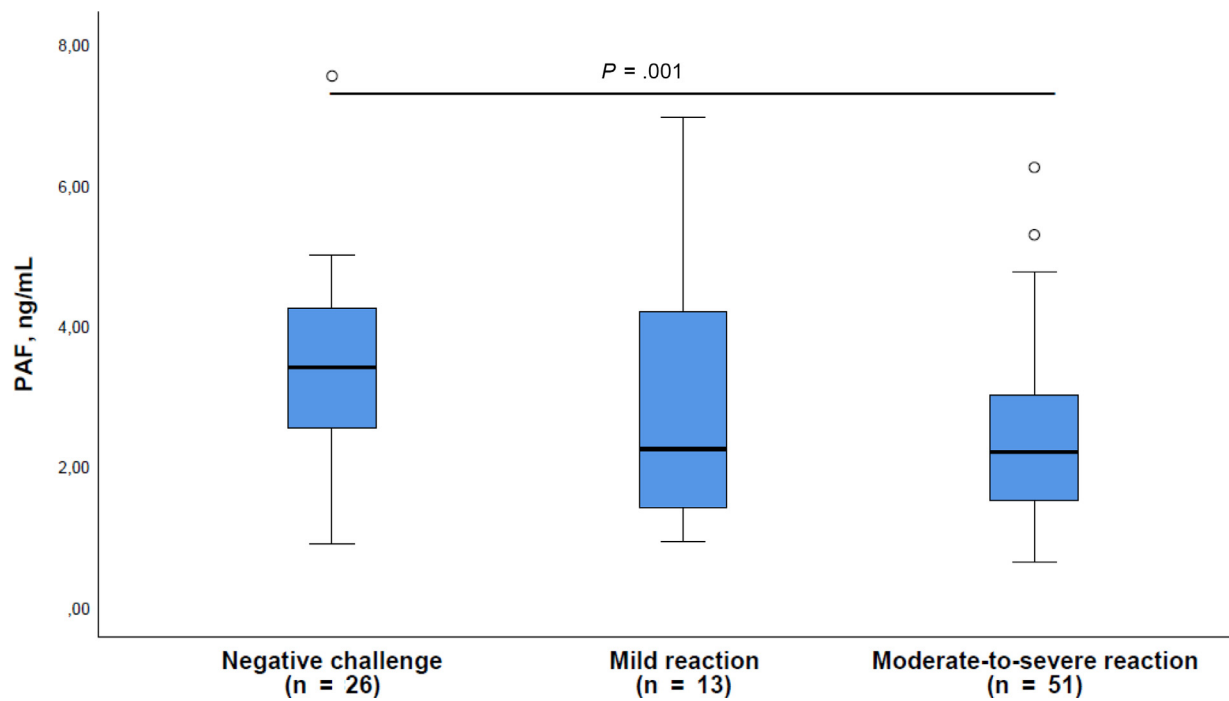
eFigure 1. Among the patients who reacted in the challenge between the no-anaphylaxis and anaphylaxis subgroups: (A) cashew nut IgE, (B) Ana o 3 IgE, (C) basophil activation test, and (D) skin prick test to cashew nut. IgE, immunoglobulin E.



eFigure 1 Continued.



eFigure 2. (A) Maximal percent change in absolute leucocyte count in the no-anaphylaxis and anaphylaxis subgroups among the patients who reacted in the challenge. (B) Absolute leucocyte count at 4 time points by reaction severity. (C) Absolute leucocyte count in the no-anaphylaxis and anaphylaxis subgroups among the patients who reacted in the challenge.



eFigure 3. PAF at baseline by reaction severity. PAF, platelet-activating factor.