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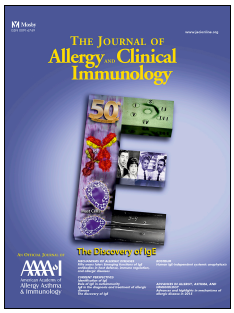

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Basophils, high-affinity IgE receptors and CCL2 in human anaphylaxis

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ABSTRACT

Background: The role of basophils in anaphylaxis is unclear.

Objective: To investigate whether basophils have an important role in human anaphylaxis.

Methods: In an emergency department study, we recruited 31 patients with acute anaphylaxis, predominantly to *hymenoptera* venom. We measured expression of basophil activation markers (CD63, CD203c), the absolute number of circulating basophils, whole-blood *FcεRI*, *CPA3* and *HDC* gene expression, and serum markers (CCL2, CCL5, CCL11, IL-3, TSLP) at three time points (during the anaphylactic episode, and in convalescent samples 7 and 30 days later). We recruited 134 *hymenoptera*-allergic and 76 healthy controls for comparison. We then investigated whether the changes observed during venom-related anaphylaxis also occur during allergic reactions to food in 22 peanut-allergic individuals undergoing double-blind placebo-controlled food challenge to peanut (DBPCFC).

Results: The number of circulating basophils was significantly lower during anaphylaxis (median 3.5 cells/µl) than 7 and 30 days later (17.5 and 24.7 cells/µl, $P<0.0001$), and compared to venom-allergic and healthy controls (21 and 23.4 cells/µl, $P<0.0001$). *FcεRI* expression during anaphylaxis was also significantly lower than in convalescent samples ($P\leq0.002$) and venom-allergic controls ($P<0.0001$). CCL2 (but not other serum markers) was significantly higher during anaphylaxis (median 658 pg/ml) than in convalescent samples (314 and 311 pg/ml, 7 and 30 days, $P<0.001$). Peanut-induced allergic reactions resulted in a significant decrease in circulating basophils compared to pre-challenge samples ($P=0.016$), a decrease in *FcεRI* expression ($P=0.007$), and an increase in CCL2 ($P=0.003$).

Conclusions: Our findings imply an important and specific role for basophils in the pathophysiology of human anaphylaxis.

Keywords: anaphylaxis, basophils, CD63 activation, *FcεRI* expression, CCL2, serum tryptase

Key Messages

- Human anaphylaxis involves a substantial reduction in circulating basophils, which inversely correlates with serum CCL2, a major basophil chemotactic factor
- This decrease was confirmed by reduced whole blood *FcεRI*, *CPA3* and *HDC* gene expression
- These data imply an important and specific role for basophils in the pathophysiology of human anaphylaxis

Capsule summary

We demonstrate a substantial reduction in circulating basophils and whole blood FcεRI gene expression during acute anaphylaxis. There was an increase in the major basophil chemotactic factor CCL2, which correlated with a decrease in basophils.

Abbreviations:

CCL2: chemokine ligand 2

CCL5: chemokine ligand 5

CCL11: chemokine ligand 11

CCR2: chemokine receptor 2

CCR3: chemokine receptor 3

CPA3: carboxypeptidase A3

DBPCFC: Double-blind, placebo-controlled food challenge

ED: Emergency department

FcεRI: high-affinity IgE receptor

HDC: L-histidine decarboxylase

TSLP: thymic stromal lymphopoietin

INTRODUCTION

Anaphylaxis is a potentially life-threatening, rapidly-progressing systemic allergic reaction that may lead to death due to airway obstruction or vascular collapse following exposure to allergens (including insect venom, foods and medication).[1] Mast cell activation is postulated to have a pivotal role in anaphylaxis,[2] and an increase in serum mast cell tryptase can confirm the diagnosis.[1] However, in individuals experiencing anaphylaxis, it is not unusual to find normal serum tryptase in the context of increased plasma histamine,[3–5] suggesting that anaphylaxis may also involve basophil activation. However, there is little published data demonstrating a direct contribution of basophils to IgE-mediated anaphylaxis in humans.

Mast cells enter tissues as immature progenitors, where they undergo the final stages of their development and remain resident *in-situ* for weeks/months. In contrast, basophils typically mature in hematopoietic tissues and subsequently circulate in the blood, with a half-life of less than one week.[6] Local allergen challenge studies in humans have demonstrated an influx of basophils to inflammatory sites within several hours of allergen exposure, demonstrating the existence of mechanisms for basophil recruitment from the circulation to the site of allergen exposure.[7–9] Both mast cells and basophils may rapidly secrete histamine and similar (but not necessarily identical) mediators and cytokines following IgE cross-linking.[2] In murine studies, basophils contribute to IgG-mediated anaphylaxis.[10] In contrast, human basophils cannot be activated through IgG receptors, and their function is inhibited by IgG-mediated triggering *via* FcγRIIb receptors; moreover, they lack protease-activated receptors and antigen-presenting functions.[11,12]

We hypothesized that basophils play an important role in human anaphylaxis, and specifically that: (1) basophils are activated during human anaphylaxis; (2) there is a basophil migration during anaphylaxis; and (3) basophil-related biomarkers may be useful to confirm anaphylaxis. We addressed our hypotheses in a series of inter-linked studies. First, in an

106 emergency department (ED) study we investigated the up-regulation of CD63 expression (the
107 most commonly used basophil activation marker[13]) during and after anaphylaxis
108 (predominantly caused by *hymenoptera* venom allergy). We monitored the absolute numbers
109 of circulating basophils, the corresponding whole blood gene expression of *FcεRI*,
110 carboxypeptidase A3 (*CPA3*) and L-histidine decarboxylase (*HDC*), and serum levels of the
111 major basophil chemotactic factors, including the CCR2 ligand CCL2, and the CCR3 ligands
112 CCL11 and CCL5.[14,15] We also measured T cell-derived IL-3 (an important basophil priming
113 and growth factor), and epithelial cell-derived thymic stromal lymphopoietin (TSLP) which
114 promotes IL-3-independent basophil development and activation.[6,16,17] We then proceeded to
115 assess whether the changes seen during venom-related anaphylaxis also occur during allergic
116 reactions to food under the controlled setting of a double-blind placebo-controlled oral food
117 challenge (DBPCFC) in peanut-allergic individuals.

118

METHODS

Study participants

Emergency Department (ED) study: We prospectively recruited 31 patients (13 female, age 18-79 years) presenting with an acute episode of anaphylaxis to the ED of the University Hospital Golnik, Slovenia (June-August 2011; July-November 2013). Severity of reactions was graded according to Mueller criteria.[18] We collected blood samples during the reaction (at presentation to the ED) and in convalescent samples seven and/or 30 days after the anaphylactic episode (Table E1).

Hymenoptera venom allergic controls and healthy subjects: We recruited two groups of control participants for comparisons: (1) 134 patients (49 females, age 23-67 years) with confirmed venom anaphylaxis from whom blood samples were obtained at least two months after the last sting reaction, and prior to initiation of venom immunotherapy; and (2) 76 healthy controls (47 females, age 17-79 years).

To assess for possible confounding by treatment with corticosteroids and its effect on basophil activation, absolute cell count, *FcεRI* expression and soluble markers, 17 healthy subjects received a single dose of 64 mg of oral methylprednisolone and were monitored for up to 24 hours after the treatment (Table E2).

Peanut allergy study: We recruited 22 peanut-allergic individuals (Table E3) in whom peanut allergy was confirmed by the DBPCFC (details in the Online supplement). Blood samples were collected prior to challenge, at cessation of challenge due to the onset of objective symptoms[19] (but prior to administration of any treatment), and 2-4 hours post-challenge.

Ethical approval was obtained from the Slovenian National Medical Ethics Committee (ED study and control participants), and the London Central Research Ethics Committee (peanut allergy study). All subjects provided written informed consent.

Basophil activation, absolute cell count, gene expression and serum markers

Detailed methodology is described in the Online supplement. Briefly, expression of CD63 and CD203c (markers of basophil activation), and the enumeration of basophils (CD123+HLA-DR- cells), lymphocytes and polymorphonuclear leukocytes (PMNs) were determined by flow cytometry as previously described.[20–22] In samples from peanut-allergic patients, we determined the absolute basophil count using a similar methodology, with basophils identified as CRTh2+CD303-CD123+ cells.[23]

FcεRI (*FCER1A*), *CPA3* and *HDC* gene expression was analyzed in whole blood samples (PAXgene, PreAnalytiX, Hombrechtikon, Switzerland) as previously described.[22]

We measured serum concentrations of CCL2, CCL5, CCL11, IL-3 and TSLP using ELISA according to the manufacturers' instructions (Quantikine R&D Systems, Minneapolis, MN, USA and Abcam, Cambridge, UK). For IL-3 measurements, we also performed spiking experiments (Online Supplement). We measured serum total tryptase (α+β) using ImmunoCAP 100 (ThermoFisher, Uppsala, Sweden); tryptase concentrations that exceeded 11.4 µg/L were considered increased.

Statistical analysis

The distribution of data was assessed using the D'Agostino and Pearson test. We used appropriate non-parametic and parametric tests for comparisons between the groups, including Wilcoxon's signed-rank test, Mann-Whitney *U*-test, *t*-test with a Welch correction and Pearson correlation. Data are expressed as the median unless otherwise stated. We compared the performance of basophil-related biomarkers in discriminating between patients with anaphylactic reactions and those without using receiver operating characteristic (ROC) curve analysis. Analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

RESULTS

Study participants

ED study and controls: Table E1 and Figure E1show detailed information on demographic characteristics, clinical and emergency treatment and sampling data of 31 ED patients. The reaction was caused by an insect sting in 28 patients. The median time from the onset of symptoms to sample collection was 105 minutes (range 20 minutes to 5 hours, Figure E1). Convalescent samples were collected from 28 patients seven days after the anaphylactic episode, and from 23 patients after 30 days (Table E1); two patients provided samples 24 hours after the acute episode.

We measured basophil activation and counts in all ED patients and controls, and serum tryptase in all ED patients and venom-allergic controls (Table E4). We ascertained gene expression in 15, chemokines and IL-3 in 17, and TSLP in 14 ED patients, and analyzed *FcεRI* expression in 37 venom-allergic controls, and CCL2 in 71 healthy controls (Table E4).

Peanut allergy study: Basophil counts were determined in 22 peanut allergic patients prior to, and during both active and placebo arms of the DBPCFC. CCL2 levels (n=22) and *FcεRI* expression (n=12) were ascertained during the active arm of the DBPCFC.

Basophil markers in ED study and controls

Basophil activation: The percentage of CD63-activated basophils in ED patients during anaphylactic episodes was low (median 3.8%). These values were marginally higher compared to seven (median 2.9%; P=0.01) and 30 days later (median 2.9%, Fig. 1A; P=0.05). Only four patients had >5% activated basophils, and only one exhibited an activation of >10%. This was mirrored by a small, but significantly higher percentage of CD63-activated basophils during anaphylaxis compared to venom-allergic controls (median 3.1%, P=0.01), or

191 healthy controls (median 2.4%, P=0.001, Fig. 2A). Expression of the activation marker
192 CD203c correlated highly with that of CD63 (Fig. E2).

193 *Circulating basophils:* The absolute number of circulating basophils was significantly lower
194 during reactions (median 3.5 cells/µl) compared with seven and 30 days later (17.5 and 24.7
195 cells/µl respectively, P<0.0001, Fig 1B). This marked decrease (median 83%, range 53%-
196 99%) was evident in 30/31 patients. Basophil numbers in ED patients during the acute
197 reaction were significantly lower compared to venom-allergic controls and healthy subjects
198 (median 21 and 23.4 cells/µl respectively, P<0.0001, Fig. 2B).

199 *Gene expression:* We observed significantly lower expression of *FcεRI*, *CPA3* and *HDC*
200 during the acute reaction compared with the expression seven and 30 days later (P≤0.002, Fig
201 1C-E); median decrease [range]: 89% [54%-100%], 80% [29%-98%] and 86% [57%-98%],
202 *FcεRI*, *CPA3* and *HDC* expression respectively). *FcεRI* expression in ED patients during
203 reactions was significantly lower compared to venom-allergic controls (P<0.0001, Fig. 2C).
204 Gene expression correlated highly with the absolute number of circulating basophils (r=0.75,
205 r=0.64 and r=0.62, P<0.0001; *FcεRI*, *CPA3* and *HDC* respectively, Fig. 3A-C). Of note, we
206 observed lower basophil counts and *FcεRI* expression in ED patients across different reaction
207 severities (Mueller grade I-II and III-IV, Fig. E3A-B)

208 *Serum markers*: CCL2 concentrations in ED patients during reactions (median 658 pg/ml)
209 were significantly higher than that measured in convalescent samples taken seven and 30 days
210 later (median 314 and 311 pg/ml respectively, P=0.0002, Fig. 4A), and compared to 71
211 healthy controls (median 201 pg/ml, P<0.0001, Fig. 2D). CCL2 increased during the acute
212 reaction (median increase 113%, range 50%-477%) in all 17 patients (Mueller grade I-II and
213 III-IV, Fig. E3D). There was a significant negative correlation between serum CCL2 and the
214 absolute number of circulating basophils (r=-0.58, P<0.0001, Fig. 3D). There were no

differences between the three time points in CCL5 (46.9, 49.5 and 46.7 ng/ml), CCL11 (109, 108 and 96 pg/ml), IL-3 (23, 17 and 23 pg/ml) and TSLP (54, 60 and 58 pg/ml) (Fig. 4B-E).

The median concentration of serum tryptase in ED patients was significantly higher during the acute reaction (17.5 µg/L) than seven and 30 days later (5.2 and 5.6 µg/L respectively, P<0.0001, Fig. 1F), and compared to venom-allergic controls (3.8 µg/L, P<0.0001, Fig. 2E). Using a binary cut-off of 11.4 µg/L, tryptase was increased during the acute episode in 22/31 (71%) patients (4/7 with Mueller I-II, and 18/24 with Mueller grade III-IV reactions; Fig. E3C).

Other blood cells: There were no differences in the PMNs and lymphocyte absolute count during the acute reaction compared to seven and 30 days later (PMNs: median 3292, 2618 and 2738 cells/µl respectively, Fig. 1G; lymphocytes: 1431, 1724 and 1547 cells/µl, Fig. 1H). Of note, in some patients, an increase in PMN to >10,000 cells/µl, and a decrease in lymphocytes to <500 cells/µl were observed (Fig. 1G-H).

Inter-assay variability and potential confounding by treatment: Detailed results of these experiments are presented in the Online supplement (Figs. E4-E7). Briefly, there was a fast and substantial (>two-fold) increase in the absolute number of PMNs 2.5-3 hours after the administration of methylprednisolone, and a slower decrease in the absolute number of blood basophils and in *FcεRI* expressions (Fig. E4B-D). There were no changes in CD63 activation, CCL2, CCL5, CCL11 and IL-3 levels (Fig. E4A; E5A-D).

Changes in basophil markers during acute allergic reactions to peanut

Circulating basophils: There was a significant decrease in the absolute number of circulating basophils during the active arm of the DBPCFC compared to the matched pre-challenge sample (P=0.016); no such difference was observed during the placebo arm of the challenge (Fig. 5A). The decrease in circulating basophils was significantly greater in the active

compared to the placebo arm of the DBPCFC (median decrease [range], -23% [-57%-33%] *vs.* -4.5% [-36%-141%], active *vs.* placebo, P<0.05).

FcεRI expression: During the active arm of the DBPCFC, there was a significant decrease from baseline in *FcεRI* expression, both at the time of objective symptoms (but prior to any treatment being administered, P=0.007), and 2 to 4 hours post reaction (P=0.002), Fig. 5B.

Serum CCL2 levels: CCL2 increased significantly at the time of objective symptoms during the active arm of the DBPCFC compared to baseline levels (P=0.003, Figure 6A). CCL2 levels returned to baseline within two hours of the onset of symptoms (Fig. 6B); the rate of increase in CCL2 was significantly greater in the active compared to the placebo arm of the DBPCFC (P=0.008; Fig. 6B).

Predictors of anaphylaxis

As indicated by the estimated area under the ROC curve (AUROC), CCL2 and *FcεRI* expressions were the most accurate readouts in discriminating between patients with anaphylactic reactions from those without, followed by basophil counts and tryptase levels: AUROC (95% CI), CCL2 0.99 (0.98-1); *FcεRI* expression 0.98 (0.94-1); basophil count 0.93 (0.88-0.97); tryptase level 0.88 (0.81-0.95); and basophil activation 0.73 (0.63-0.83); Fig. E8 (for further details, see Online supplement). With a cut-off of >334 pg/μL, the estimated sensitivity and specificity of CCL measurements were 94% and 96% respectively, compared with 93% and 92% for *FcεRI* expression (cut-off<0.2) and 87% and 81% for basophil counts (cut-off>12 cells/μL).

DISCUSSION

Our study demonstrated a substantial (~80%) reduction in circulating basophils during anaphylactic reactions to *hymenoptera* venom. Decreased gene expression of *FcεRI*, *CPA3* and *HDC* confirmed the flow cytometry data. We also observed an increase in CCL2, which correlated with a decrease in circulating basophils. We replicated these findings in peanut-allergic individuals experiencing allergic reactions during DBPCFC to peanut. Compared to the reactions in the emergency department, which were generally more severe, we observed more modest (but nonetheless significant) changes at the time of objective symptoms during the peanut challenges. Taken together, these data suggest that anaphylaxis induces a rapid and considerable basophil migration. The mechanism of anaphylaxis-related basophil migration appears to be selective, because no significant changes were seen for lymphocytes, PMNs, or chemotactic factors which may affect other effector cells such as eosinophils (e.g. CCL5 and CCL11).

Limitations

The nature of the management of anaphylaxis (including administration of high-dose corticosteroids) makes it difficult to exclude the potential confounding by treatment and draw an unequivocal interpretation of the decrease in basophils in the ED setting. In our ED study, 94% of patients received methylprednisolone, and 42% epinephrine. Corticosteroids have a well-described effect on blood leucocytes, including an increase in circulating neutrophils and decrease in lymphocytes and basophils.[24,25] The kinetics of the response of various leukocytes to corticosteroid administration varies, with neutrophilia and lymphopenia preceding the onset of basopenia,[25] which was confirmed in our study. Compared to healthy controls who received oral corticosteroids, the reduction in blood basophils (but not lymphocytes or PMNs) was much greater and occurred at an earlier time in patients with acute anaphylaxis, suggesting that the changes in basophils were not related to treatment. Moreover, we replicated the

observed changes in basophil markers in the controlled setting of peanut-allergic individuals undergoing DBPCFC, where the study design allowed for blood sampling both prior to challenge and before any treatment. This avoids the issue of confounding by treatment (both with corticosteroids and epinephrine), and allows comparison with pre-reaction samples (something not possible in the ED setting). We acknowledge that two previous reports failed to detect a change in absolute basophil counts following food challenge.[26,27] However, these studies involved fewer patients experiencing only mild allergic symptoms, and used methods for basophil detection less sensitive and specific than that employed in our study.

Several cytokines and chemokines are involved in basophil migration, with the CCR2 ligand CCL2 and the CCR3 ligand CCL11 eliciting the most potent migratory responses.[15] However, there is a difference in the cellular specificity of these chemokines. CCR2 is virtually undetectable on human eosinophils[28], and thus CCL2 fails to induce eosinophil migration, which is not the case for the CCR3 ligands CCL5 and CCL11.[29] Therefore, CCL2-mediated migration may represent a unique mechanism for the selective migration of human basophils in allergic reactions. However, in the present study we could not determine the cellular sources of CCL2 during acute reactions.

We could not answer the question of whether anaphylaxis is associated with extensive activation and degranulation of circulating basophils. Patients with anaphylaxis present to ED up to hours after onset of symptoms, and it takes additional time to obtain informed consent and perform venipuncture. In our study, the median time between the onset of symptoms and sample collection was 105 minutes, which is comparable to previous ED studies.[4,30,31] Plasma histamine levels, which correlate with anaphylactic symptoms,[32,33] typically peak within 5-10 minutes after the onset of anaphylaxis and subsequently decrease to baseline within one hour as a result of rapid catabolism. Consequently, the relatively modest increase in CD63 expression on basophils (a marker of basophil degranulation) may represent an underestimate

of the peak basophil activation during acute reactions. In a recent open food challenge study of delayed responses to meat in patients sensitized to galactose-alpha-1,3-galactose, expression of CD63 on was reported for >15% of basophils in 9/12 patients at the onset of symptoms.[34] This is consistent with our data, which also supports more extensive basophil activation (typically up to 20% of basophils expressing CD63 and CD203c) during peanut-allergic reactions.[35] In our ED study, only one of 31 predominantly venom-allergic patients had >15% CD63-activated basophils, despite the fact that the majority (24/31) experienced anaphylactic reactions of Mueller grade III-IV severity (with bronchospasm, airway obstruction, hypoxemia or hypotension, and collapse). Whether this difference is due to the unavoidable delay in sampling following onset of symptoms in the ED compared to the challenge setting, or a difference in the extent of basophil activation for venom *versus* food-induced allergic reaction, is unknown. It is most likely that we detected only those basophils that remained in the circulation following the acute reaction (approximately 20% of the normal level of basophils), and not the basophils that had migrated out of the circulation.

Interpretation

Recent reports have implicated a specific effector role for basophils in acute allergic responses.[21,36–38] Studies which used oral food or nasal allergen challenge responses in omalizumab-treated adults with peanut[37] or cat allergies[36] have suggested that acute reactions may be basophil, rather than mast cell, dependent. Decreases in the basophil allergen responses following venom immunotherapy reflect the induction of tolerance to sting challenges.[21] A recent study in peanut allergic children suggested that an *in vitro* basophil activation test at baseline may correlate with reaction severity at subsequent food challenge.[38] However, these *in vitro* studies could not confirm whether basophil activation actually contributes to the acute allergic reactions, or is a surrogate marker of mast cell or overall IgE responsiveness. Thus, studies investigating human basophils during allergic reactions *in vivo*

are required. However, such studies in a controlled challenge setting are difficult due to the general consensus that patients who may experience severe anaphylactic reactions should be excluded. Moreover, reaction severity at challenge is generally limited by the controlled nature of the challenge (where allergen exposure is stopped at onset of objective symptoms). We therefore combined an ED-based study in venom allergy, which focused on basophil migration and/or activation during more severe anaphylaxis, with a study of peanut-allergic reactions during DBPCFC in which patients tended to experience less severe reactions. Data from this latter study in peanut-allergic subjects corroborated the findings from the ED study.

One interesting question which remains unanswered is when and where basophil activation occurs. Anti-IgE, anti-FcεRI or allergen stimulation of basophils also promote their migration and adherence to endothelial cells.[39,40] However, these stimuli may enhance basophil adherence to the vascular endothelium and migration at concentrations which are lower than the threshold required for basophil degranulation and histamine release.[39,40] Therefore, IgE-mediated basophil migration may be induced without basophil degranulation. This suggests that basophils may be activated after migration, or partly in circulation and partly after migration, or may even migrate without activation. The different clinical severities and end-organ patterns of anaphylaxis[1,2] and the finding that serum mast cell tryptase is often within normal limits[3,4] suggest that local rather than generalized mast cell and/or basophil degranulation may predominate in some individuals. Additional studies are required to confirm these speculations.

The short time frame within which the reduction in circulating basophils occurred, coupled with previous findings that basophils are the granulocytes most resistant to apoptosis,[41] suggest that anaphylaxis induces a prompt basophil migration rather than elimination by apoptosis. We did not observe a change in serum IL-3 or TSLP. This suggests that it is unlikely that basophil migration during anaphylaxis is related to changes in basophil

development or homeostasis, a process which is IL-3-elicited for basophils that operate in an IgE-dependent manner, or TSLP-elicited for basophils that operate in a non-IgE-dependent manner.[6] Our results are consistent with a recent study which demonstrated no changes in CCL11 or IL-3 during anaphylaxis.[30]

Risk assessment of individuals with anaphylaxis is hampered by limitations in laboratory tests to confirm the diagnosis, and predict its severity.[42,43] Currently, the only readily available laboratory test to confirm the diagnosis of anaphylaxis is the measurement of total tryptase in serum/plasma.[1,2] However, even when blood sampling is optimally timed, tryptase levels are often within the normal limits, particularly for food-induced reactions.[3,4] In our study of predominantly venom-induced reactions, a diagnostic increase in the total tryptase was seen in 71% of the individuals with anaphylaxis, which is comparable to other reports.[30] While other mediators have been proposed as potential biomarkers,[30,31,44–46] these have not exhibited sufficient diagnostic utility or technical reproducibility to be routinely used.[1,2] Our results indicate that CCL2, *FcεRI* expression and basophil counts may potentially be useful biomarkers of anaphylaxis. However, a substantially broader assessment is required to validate these methods and replicate the findings.

Conclusions

Our data suggest a substantial migration of circulating basophils during anaphylaxis, which correlates with a significant increase in serum concentration of the major basophil chemotactic factor CCL2. These findings suggest an important and specific role for basophils in the pathophysiology of human anaphylaxis.

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551 **LEGEND FOR FIGURES**

552

553 **Figure 1.** Basophil CD63 activation **(A)**, absolute basophil count **(B)**, whole blood FcεRI **(C),**
554 CPA3 **(D)** and HDC **(E)** gene expression, serum tryptase **(F)**, PMNs **(G)** and lymphocytes **(H)**
555 absolute counts in emergency department patients during the acute anaphylactic reactions to
556 *hymenoptera* venom, and 7 and 30 days after the anaphylactic episode. Horizontal lines
557 represent median values with IQR.

558 **Figure 2.** Comparison of basophil CD63 activation **(A)**, absolute basophil count **(B)**, whole
559 blood *FcεRI* gene expression **(C)**, CCL2 serum concentration **(D)** and serum tryptase levels
560 **(E)** between patients with acute anaphylactic reactions to *hymenoptera* venom upon ED
561 presentation, and venom-allergic or healthy controls. Horizontal lines represent median values
562 with IQR.

563 **Figure 3.** Correlation between absolute basophil counts and whole blood *FcεRI* **(A)**, *CPA3*
564 **(B),** *HDC* **(C)** gene expression, and serum CCL2 concentration **(D)** in patients with acute
565 anaphylactic reactions presenting to the ED.

566 **Figure 4.** Serum CCL2 **(A)**, CCL5 **(B)**, CCL11 **(C)**, IL-3 **(D)** and TSLP **(E)** levels in
567 emergency department patients during the acute anaphylactic reactions to *hymenoptera*
568 venom, and 7 and 30 days after the anaphylactic episode. Horizontal lines represent median
569 values with IQR.

570 **Figure 5.** Absolute basophil count **(A)** and whole blood *FcεRI* gene expression **(B)** in peanut
571 allergic patients undergoing DBPCFC to peanut. Horizontal lines represent median values
572 with inter-quartile ranges (IQR).

573 **Figure 6.** Serum CCL2 levels in allergic patients undergoing controlled DBPCFC to peanut:
574 **(A)** absolute CCL2 levels, **(B)** % change in CCL2 from baseline. Horizontal lines represent
575 median values with inter-quartile ranges (IQR).

576

Fig.1

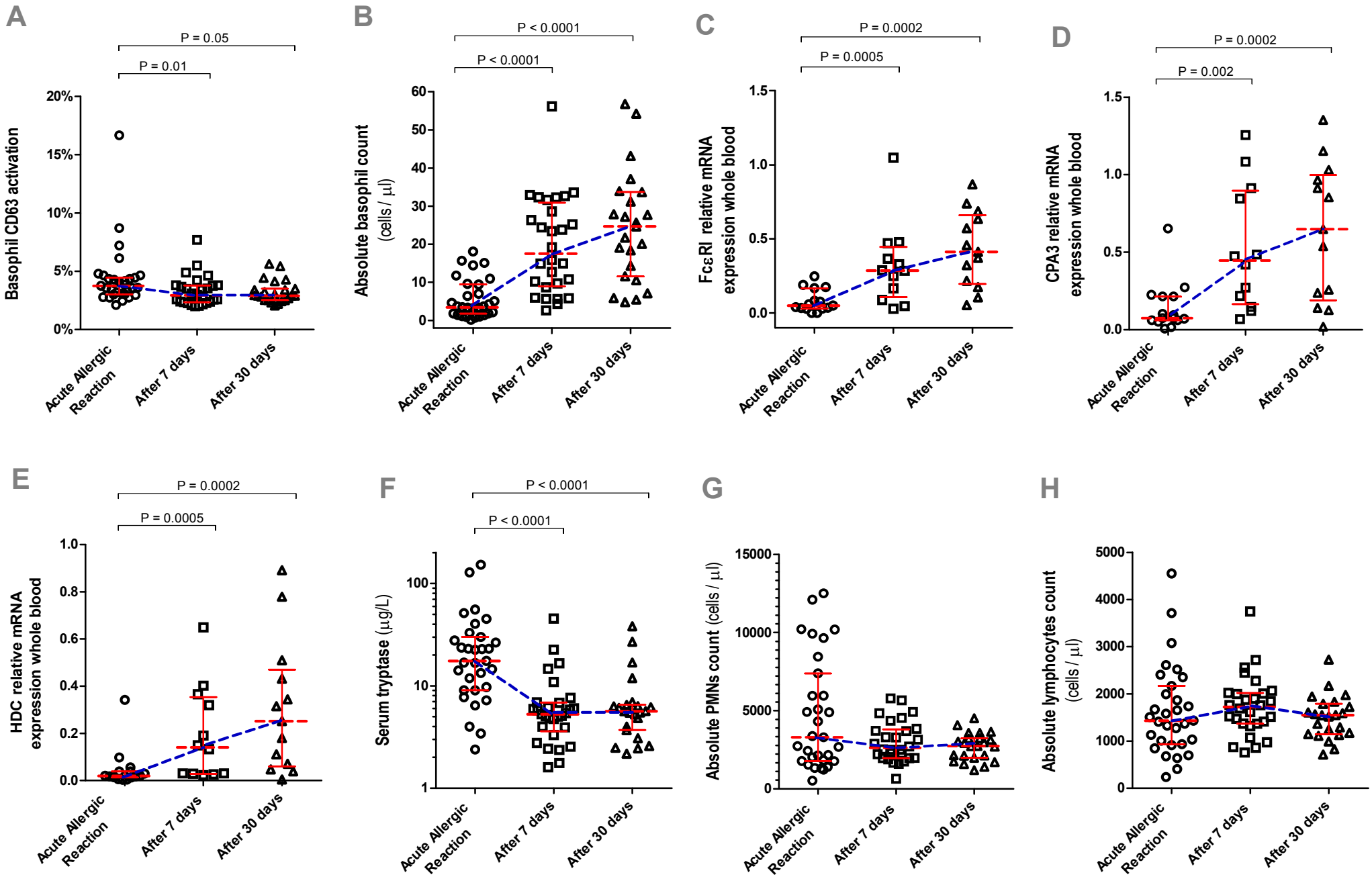

A
P = 0.05
P = 0.01
Basophil CD63 activation
20%
15%
10%
5%
0%
Acute Allergic Reaction
After 7 days
After 30 days
B
P < 0.0001
P < 0.0001
Absolute basophil count (cells / µl)
60
50
40
30
20
10
0
C
P = 0.0002
P = 0.0005
FcεRI relative mRNA expression whole blood
1.5
1.0
0.5
0.0
D
P = 0.0002
P = 0.002
CPA3 relative mRNA expression whole blood
E
P = 0.0002
P = 0.0005
HDC relative mRNA expression whole blood
0.8
0.6
0.4
0.2
F
P < 0.0001
P < 0.0001
Serum tryptase (µg/L)
100
10
1
G
Absolute PMNs count (cells / µl)
15000
10000
5000
0
H
Absolute lymphocytes count (cells / µl)
5000
4000
3000
2000
1000

Fig. 2

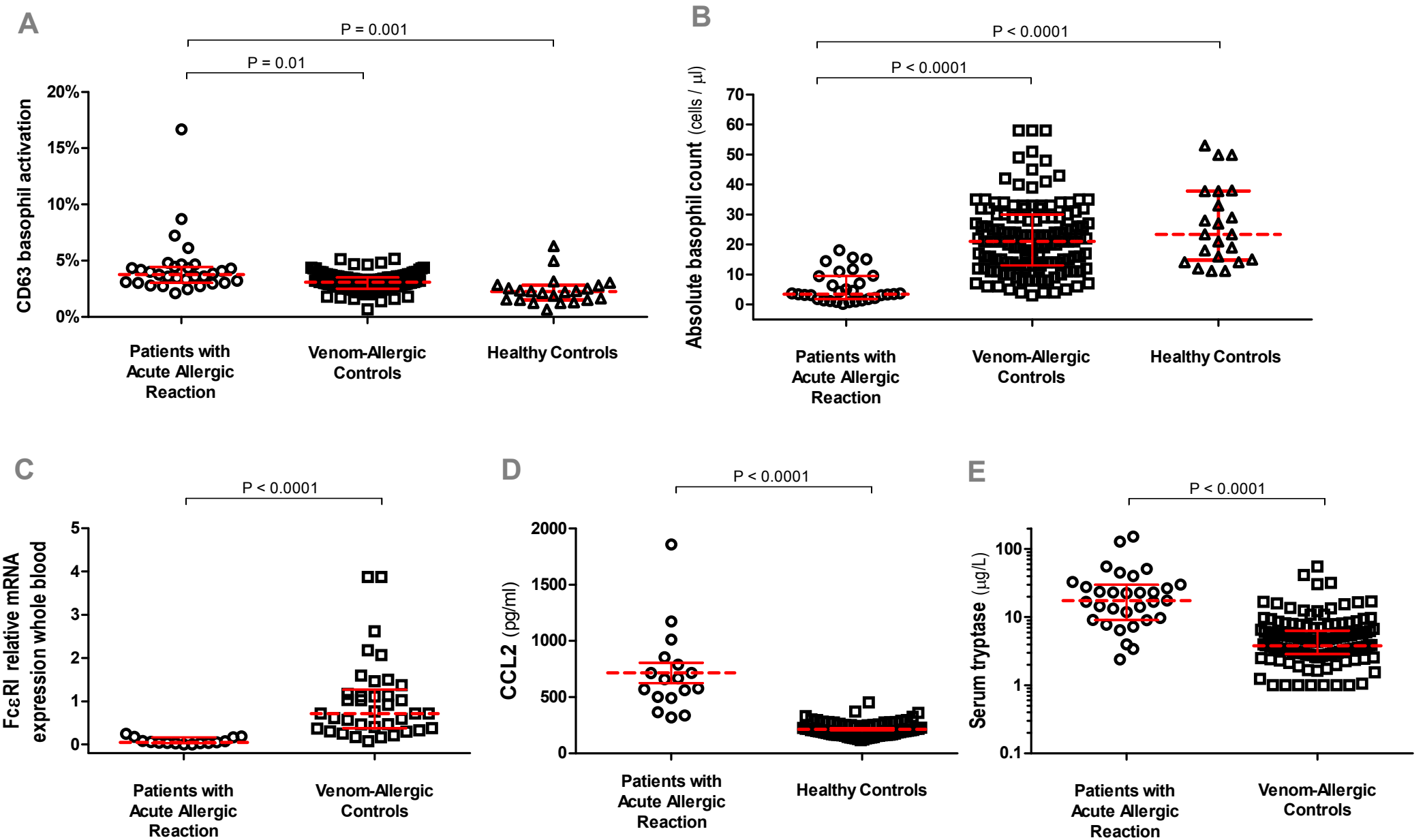

A
P = 0.001
P = 0.01
CD63 basophil activation
20%
15%
10%
5%
0%
Patients with Acute Allergic Reaction
Venom-Allergic Controls
Healthy Controls
B
P < 0.0001
P < 0.0001
Absolute basophil count (cells / µl)
70
60
50
40
30
20
10
0
Patients with Acute Allergic Reaction
Venom-Allergic Controls
Healthy Controls
C
P < 0.0001
FcεRI relative mRNA expression whole blood
5
4
3
2
1
0
Patients with Acute Allergic Reaction
Venom-Allergic Controls
D
P < 0.0001
CCL2 (pg/ml)
2000
1500
1000
500
0
Patients with Acute Allergic Reaction
Healthy Controls
E
P < 0.0001
Serum tryptase (µg/L)
100
10
1
0.1
Patients with Acute Allergic Reaction
Venom-Allergic Controls

Fig. 3

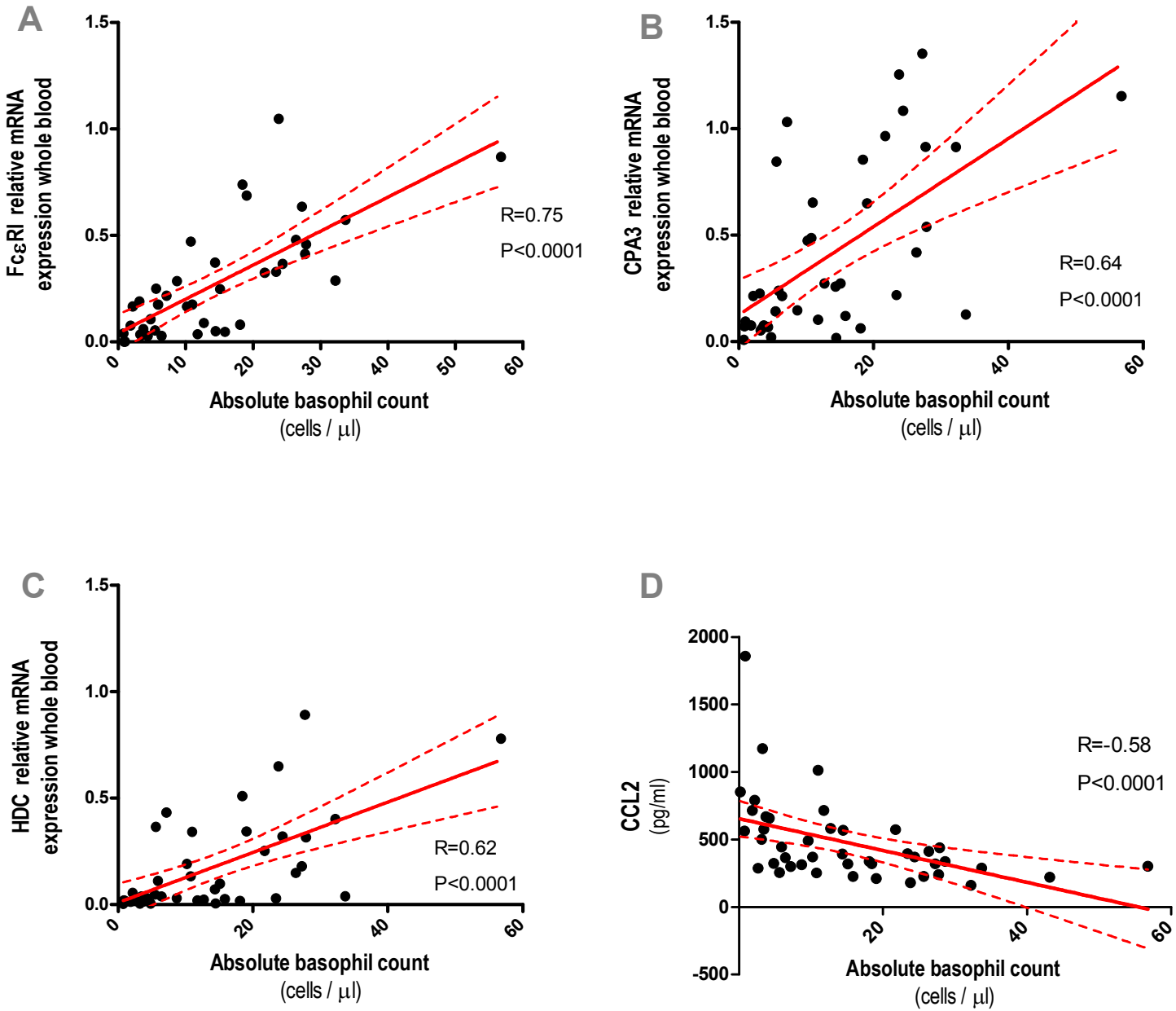
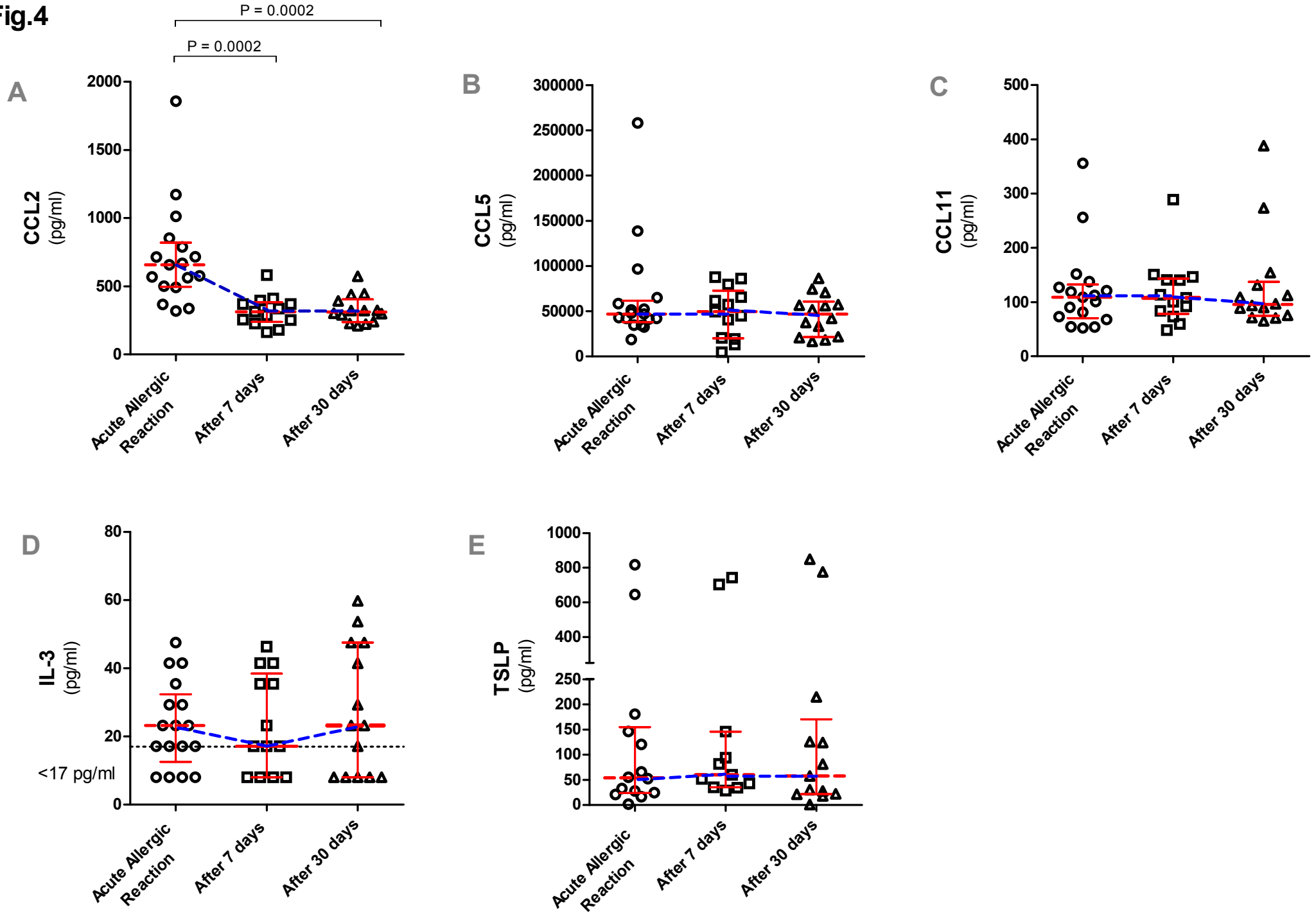
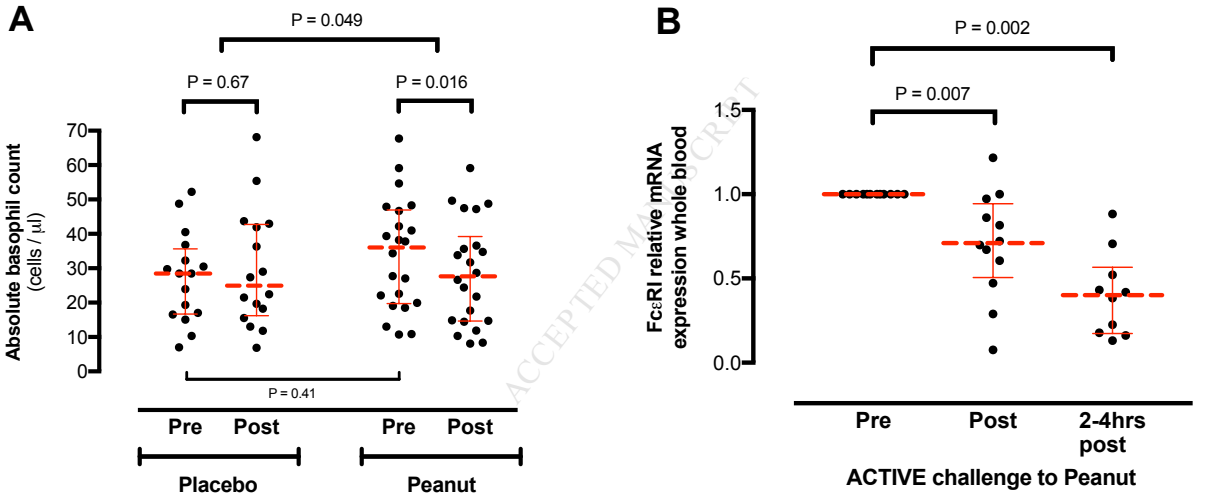
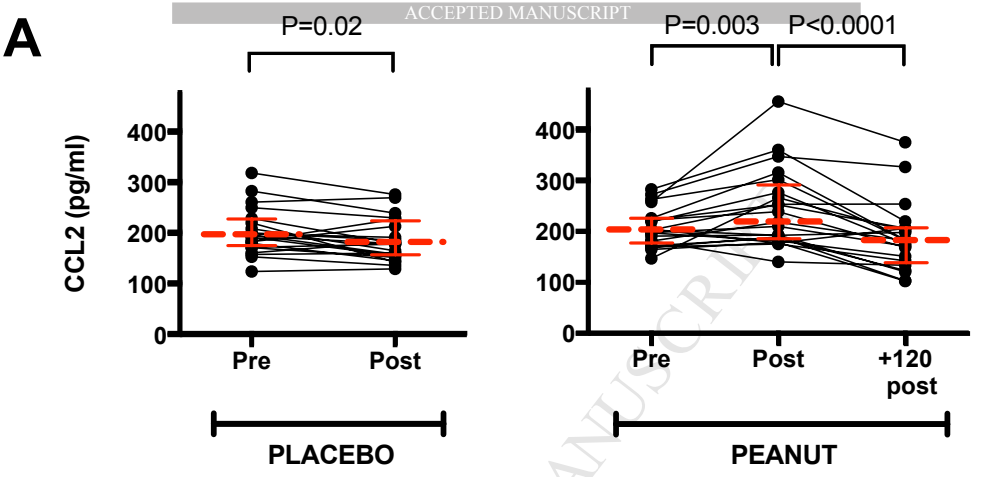
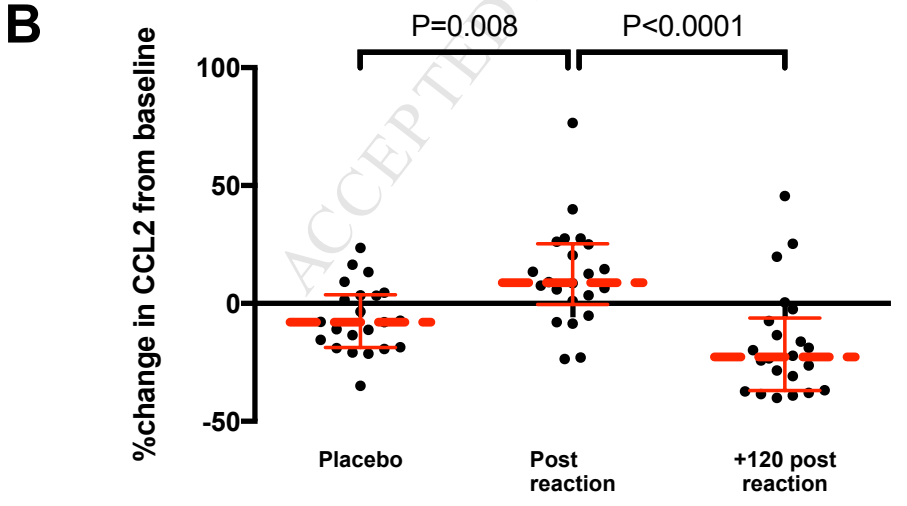

A
FcεRI relative mRNA expression whole blood
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
Absolute basophil count
(cells / µl)
R=0.75
P<0.0001
B
CPA3 relative mRNA expression whole blood
1.5
1.0
0.5
0.0
0
20
40
60
Absolute basophil count
(cells / µl)
R=0.64
P<0.0001
C
HDC relative mRNA expression whole blood
1.5
1.0
0.5
0.0
0
20
40
60
Absolute basophil count
(cells / µl)
R=0.62
P<0.0001
D
CCL2
(pg/ml)
2000
1500
1000
500
0
-500
20
40
60
Absolute basophil count
(cells / µl)
R=-0.58
P<0.0001

Fig.4


P = 0.0002
P = 0.0002
A
CCL2 (pg/ml)
2000
1500
1000
500
0
B
CCL5 (pg/ml)
300000
250000
200000
150000
100000
50000
0
C
CCL11 (pg/ml)
500
400
300
200
100
0
D
IL-3 (pg/ml)
80
60
40
20
0
<17 pg/ml
E
TSLP (pg/ml)
1000
800
600
400
250
200
150
100
50
0
Acute Allergic Reaction
After 7 days
After 30 days







Basophils, high-affinity IgE receptors and CCL2 in human anaphylaxis

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ONLINE DATA SUPPLEMENT

METHODS

DBPCFC to peanut

DBPCFC were conducted according international consensus criteria (PRACTALL)[1]. In brief, subjects underwent double blind placebo controlled peanut challenge (DBPCPC) over two separate days, at least 7 days apart. On each day, subjects received increasing doses, every 30 minutes, of peanut protein (or placebo) at the following doses: 3mg, 10mg, 30mg, 100mg, 300mg, 1000mg and 3000mg until stopping criteria were met (as per PRACTALL consensus[1]). Blood samples were collected from a venous cannula sited prior to challenge, and immediately snap-frozen or transferred without delay for flow cytometry.

Basophil activation and absolute cell count

A precise volume of whole heparinized blood (100 µL) was incubated with FITC-conjugated anti-CD63 mAb, PE-conjugated anti-CD123 mAb, and PerCP-conjugated anti-HLA-DR mAb (BD Biosciences, San Jose, CA, USA) and thereafter the samples were lysed, washed, fixed, and analyzed within 2 hours on a FACSCalibur flow cytometer (BD Biosciences). In a proportion of samples, we also added APC-conjugated CD203c (Miltenyi Biotec, Auborn, CA, USA) for an additional activation analysis. The basophils were identified as low side-scatter, CD123-positive, and HLA-DR-negative cells. The quantitative percentage determination of activated basophils (CD63-positive) was measured in FL1. To evaluate unspecific staining, FITC mouse IgG1 isotype control (BD Biosciences) was also tested.

For the absolute basophil count (CD123+ HLA-DR- cells), 50 µL of AccuCount Fluorescent microbeads (7.7 µm, 51,011 particles per 50 µL; Spherotech, Lake Forest, IL, USA) were added to the fixed samples prior to flow cytometric analysis. The lymphocytes and polymorphonuclear

leukocytes (PMNs) were gated according to lysed whole blood FSC/SSC characteristics. The absolute numbers of basophils, lymphocytes and PMNs per µL of whole blood were calculated using the following equation: (number of cells / number of events per microbead region) × (number of microbeads used in test / volume of the whole blood sample). In samples from peanut-allergic donors undergoing DBPCFC, absolute basophil count was determined using a similar methodology using 50 µL of CountBright microbeads (7 µm, 0.45 - 0.55 x 10^5 beads/50 µL; ThermoFisher Scientific Inc, USA), with basophils identified as CRTh2+CD303-CD123+ cells.[2] In a selection of samples, basophil counts were determined using both microbeads, in order to confirm equivalency.

Gene expression

We analyzed gene expressions of the α-subunit of the high-affinity IgE receptor (*FCER1A*, Hs00175232_m1, presented as *FcεRI* in manuscript), carboxypeptidase A3 (*CPA3*, Hs00157019_m1) and histidine decarboxylase (*HDC*, Hs00157914_m1). *FcεRI* is expressed on mast cells and basophils as tetramers ($\alpha\beta\gamma_2$) and on antigen presenting cells, although at substantially lower levels, as trimers ($\alpha\gamma_2$).[3] *CPA3* is expressed in mast cells and basophils and may be expressed in populations of T-cell progenitors and thymic T cells and in some hematopoietic progenitor cells.[4] *HDC* catalyzes the formation of histamine from L-histidine, and in hematopoietic cell lineages, the gene is expressed only in mast cells and basophils.[5]

Total RNA was isolated from whole blood samples using the PAXgene Blood miRNA Kit (PreAnalytiX GmbH, Switzerland) and quantified by Qubit® fluorometer (Thermo Fisher Scientific, Waltham, MA USA). Following reverse transcription, cDNA was quantified by real-time PCR (ABI PRISM 7500 Real-Time PCR System) at standard conditions using TaqMan Universal PCR Master Mix (Thermo Fisher Scientific). Expression levels were normalized

against ribosomal 18s RNA Endogenous Control (Thermo Fisher Scientific). All measurements were performed in triplicate for each sample and time point and relative expressions were analyzed using the ΔΔCt method.

IL-3 spiking experiments

For IL-3 measurements we performed spiking experiments with *E. coli*-derived recombinant human IL-3 protein (from R&D Systems) in which a known amount of recombinant protein was spiked into a sera sample with undetectable intrinsic IL-3 concentration (thus bellow 17 pg/mL according to our detection limit) and run in the ELISA. We successfully recovered samples spiked with 250, 125, 62.5 or 32.5 pg/mL of recombinant human IL-3 protein, but not samples spiked with known concentrations of 15.6, 7.8 or 3.9 pg/mL recombinant human IL-3 protein. This sensitivity is within the range of the minimum detectable concentration of IL-3 (from 3.46-57.4 pg/mL) evaluated by the commercial kit manufacturer (R&D Systems; Human IL-13 Quantikine ELISA Kit).

Table E1. Demographic and clinical data of subjects with acute anaphylactic reactions recruited from the hospital emergency department (ED)

No.	Sex	Age	Culprit	Mueller grade	Emergency treatment	Time from onset of reaction to blood collection	Previous anaphylaxis or venom immunotherapy (VIT)
1	M	41	Honeybee	4	aH1 (*2 mg iv*), ST (*80 mg iv*)	2 h, 7 d, 30 d	No
2	F	39	Honeybee	4	Epi (*0.5 mg im*), aH1 (*2 mg iv, 1 tbl*), ST (*64 mg po, 250 mg iv*)	4 h, 7 d	1 y honeybee VIT in 2005
3	M	63	*Vespula*	4	Epi (*1.5 mg im*), ST (*32 mg po, 80 mg iv*)	2 h, 7 d, 30 d	5 y *Vespula* VIT finished in 1999
4	F	54	*Vespula*	2	Epi (*0.5 mg sc*), aH1 (*2 mg iv*), ST (*125 mg iv*)	2 h 30 min, 7 d, 30 d	No
5	F	54	*Vespula*	3	aH1 (*2 mg iv*), ST (*125 mg iv*)	1 h 30 min, 7 d, 30 d	No
6	M	49	*Vespula*	2	aH1 (*2 mg iv, 1 tbl*), ST (32 mg *po, 125 mg iv*)	2 h. 7 d, 30 d	No
7	M	32	Unknown *Hym.*	2	aH1 (*2 mg iv*), ST (*250 mg iv*)	5 h, 7 d	No
8	M	49	*Vespula*	3	aH1 (*2 mg iv, 2 tbl*), ST (*64 mg po, 300 mg iv*)	1 h 15 min, 7 d, 30 d	*Vespula* VIT from 2009
9	F	40	*Vespula*	3	aH1 (*2 mg iv*), ST (*250 mg iv*)	3 h, 7 d, 30 d	2010 *Vespula* – grade 1

10	M	74	Honeybee	4	Epi (*0.1 mg iv*), aH1 (*2 mg iv*), ST (*125 mg iv*)	1 h, 7 d, 30 d	No
11	M	51	Eu. Hornet	4	aH1 (*2 mg iv*), ST (*165 mg iv*)	2 h, 7 d	No
12	M	28	*Vespula*	3	aH1 (*4 mg iv*), ST (*64 mg po, 40 mg iv*)	1 h 30 min, 7 d	No
13	M	18	Honeybee	1	aH1 (*4 mg iv*), ST (*80 mg iv*)	1 h 45 min, 7 d	No
14	M	42	Unknown *Hym.*	2	aH1 (*2 mg iv*), ST (*80 mg iv*)	1 h 30 min, 24 h, 7 d, 30 d	No
15	F	61	Unknown *Hym*.	3	aH1 (*2 mg iv, 1 tbl*), ST (*32 mg po, 125 mg iv*)	1 h 30 min, 24 h, 7 d, 30 d	No
16	F	20	Eu. Hornet	4	aH1 (*2tbl, 2 mg iv*) ST (*64mg po, 125 mg iv*)	30 min, 7 d, 30 d	2012 *Vespula* – grade 1
17	F	70	Unknown *Hym*.	3	aH1 (*2 mg iv*), ST (*500 mg iv*)	2 h25 min, 30 d	No
18	M	71	Vespula	3	aH1 (*2 mg iv*), ST (*125 mg iv*)	2 h 30 min, 7d, 30 d	No
19	M	57	Eu. Hornet	4	aH1 (*2 mg iv*), ST (*40 mg iv*)	2 h 45 min, 30 d	No
20	F	33	Eu. Hornet	1	No drugs administered	4 h, 7 d, 30 d	*Vespula* – multiple times as child – grade 3
21	M	50	*Vespula*	4	Epi (*0.3 mg im)*, aH1 (*2tbl, 2 mg iv*), ST (*64 mg po, 125 mg iv*), bronchodilator (fenoterol *0,5mg*, ipratropium bromide *0,2mg*)	1 h 20 min, 7 d, 30 d	*Vespula* – 4x since 2002 - grade 4

22	M	48	Honeybee	4	Epi (*0.3-0.5 mg im*), aH1 (*2 mg iv*), ST *(>40 mg iv*)	1 h 20 min, 7 d, 30 d	Honeybee – 2009, 2011 – grade 2
23	M	47	*Vespula*	3	aH1 (*2 mg iv*), ST (*80 mg iv*)	2 h, 7 d, 30 d	No
24	M	62	Eu. Hornet	4	Epi (*0.5 mg im*), aH1 (*2 mg iv*), ST (*125 mg iv*)	55 min, 30 d	Since 2007 VIT *Vespula*, since 2009 VIT honeybee
25	F	56	Unknown	4	Epi (*0.3-0.5 mg*), aH1 (*2mg iv*), ST (*125 mg iv*)	< 1 h, 7 d, 30 d	2 previous anaphylaxis - unknown trigger – grade 4
26	F	56	*Vespula*	4	Epi (*2x 0.5 mg im*), aH1 (*2 mg iv*), ST (*125 mg iv*)	2 h, 7 d, 30 d	2010 – *Vespula* – grade 1
27	M	79	Eu. Hornet	3	aH1 (*2 mg iv*), ST (*80 mg iv*)	1 h, 7 d, 30 d	No
28	F	66	Iv analgesic	4	Epi (0.3 mg im), aH1 (*2 mg iv*), ST (*80 mg iv*)	20 min, 7 d, 30 d	No
29	F	56	Honeybee VIT	4	Epi (*0.3 mg im*), aH1 (*2 mg iv*)	55 min, 7 d	2012 - Unknown *Hym.* 4 grade
30	F	55	Honeybee	3	Epi (*0.3 mg iv*), aH1 (*2tbl, 2 mg iv*), ST (*64 mg po, 500 mg iv*)	3h 10 min, 7 d	Honeybee VIT started in 2008, but stopped the same year
31	M	68	Eu. Hornet	4	Epi (*0.3 mg im*), aH1 (*2 mg iv*), ST (*125 mg iv*)	1 h 30 min, 7 d	No

Epi = epinephrine, aH1 = clemastine, ST = methylprednisolone, min = minutes, d = days, Hym. = *Hymenoptera*

Table E2. Demographic data and sampling of healthy subjects after a single dose of oral methylprednisolone

ST: 64 mg of oral methylprednisolone

No.	Sex	Age	Single dose oral methylprednisolone	Time of blood collection
1	F	41	64 mg	just before ST, after 3 h
2	M	29	64 mg	just before ST, after 3 h
3	F	28	64 mg	just before ST, after 3 h
4	M	42	64 mg	just before ST, after 5 h
5	F	32	64 mg	just before ST, after 3 h
6	F	44	64 mg	just before ST, after 2.5 h
7	F	37	64 mg	just before ST, after 2.5 h
8	F	24	64 mg	just before ST, after 2.5, 5 and 24 h
9	M	28	64 mg	just before ST, after 2.5, 5 and 24 h
10	M	30	64 mg	just before ST, after 2.5, 5 and 24 h
11	F	24	64 mg	just before ST, after 2.5, 5 and 24 h
12	F	24	64 mg	just before ST, after 2.5, 5 and 24 h
13	F	35	64 mg	just before ST, after 2.5, 5 and 24 h
14	F	39	64 mg	just before ST, after 2.5, 5 and 24 h
15	F	35	64 mg	just before ST, after 2.5, 5 and 24 h
16	M	30	64 mg	just before ST, after 2.5, 5 and 24 h
17	F	28	64 mg	just before ST, after 2.5, 5 and 24 h

Table E3: Demographic and clinical data relating to peanut-allergic subjects undergoing double-blind, placebo-controlled food challenge (DBPCFC) to peanut

	Overall cohort	Epinephrine administered at DBPCFC*
n	22	5
Age (years) median [range]	14.8 [8-36]	21.5 [12-26]
% male	64%	40%
SPT to peanut (mm) median [range]	9 [5-16]	11 [9-11]
sIgE to peanut (kUA/L) median [range]	18.1 [3.1 - >100]	27.6 [13.5-61.4]
sIgE to r Ara h2 (kUA/L) median [range]	12.2 [0.23 - >100]	13.1 [12.2-52.9]
Grade of reaction at DBPCFC:		
Mueller I/II	16	0
Mueller III	6	5

SPT = skin prick test; sIgE = specific IgE.

*IM epinephrine was given for any lower respiratory and/or cardiovascular symptoms

Table E4. Detailed information on the number of participants in whom we assessed basophil activation, absolute cell count, gene expression and soluble markers

	Basophil absolute count	**Basophil activation** (CD63)	**Basophil activation** (CD 203c)	***FcεRI***	***CPA3***	***HDC***	**CCL2**	**CCL5**	**CCL11**	**IL-3**	**TSLP**	**Serum tryptase**	**PMN & Ly absolute count**
ED patients (n=31)	31	31	9	15	15	15	17	17	17	17	14	31	31
Venom-allergic controls (n=134)	134	134	*	37	*	*	*	*	*	*	*	134	*
Healthy controls (n=76)	22	22	*	*	*	*	71	*	*	*	*		*

RESULTS

Inter-assay coefficient of variation

We estimated an inter-assay coefficient of variation of 6.7% for the absolute basophil count and 4.8% for basophil CD63 activation by repeated measurements in five healthy control subjects.

The effect of oral corticosteroid on basophil markers and other blood cells

We followed 17 healthy subjects up to 24 hours after a single dose of 64 mg of oral methylprednisolone (Table E2).

Basophil activation: There was no significant effect of the treatment with oral corticosteroids on basophil (CD63) activation (Figure E4A).

Circulating basophils: We identified a small, but statistically significant decrease in the absolute number of blood basophils (from a median of 23.4 to 19.7 cells/μL; median decrease 19%, P=0.006). However, a major decrease (to 8 cells/μL, median decrease 67%, P=0.004) was observed 5 hours after methylprednisolone administration (Fig. E4B). Basophils numbers returned to normal values after 24 hours (to 22 cells/μL).

Gene expression: We observed a small and non-significant decrease *FcεRI* expression 2.5-3 hours after methylprednisolone intake, followed by a substantial decrease after 5 hours which corresponded to a major decrease in basophils (median decrease 63%, P=0.006, Fig. E4C). *FcεRI* expression did not differ between the baseline level and 24 hours after methylprednisolone.

Other blood cells: There was a significant increase in the absolute number of blood PMNs 2.5-3 hours after the methylprednisolone intake (>two-fold increase, median 2070 to 4585 cells/μL,

P=0.0005, Fig. E4D). This increase was also seen after 5 hours (4853 cells/µL, P=0.001) and 24 hours (4422 cells/µL, P=0.002, Fig. E4D).

After 2.5-3 hours, there was a small, but statistically significant decrease in the number of blood lymphocytes (median 960 to 768 cells/µL, P=0.004, Fig. 4E). There was no difference in lymphocyte counts 5 and 24 hours after methylprednisolone compared to baseline (Fig. E4E).

Serum markers: There was no significant effect of the treatment with oral corticosteroids on CCL2, CCL5, CCL11 or IL-3 (Fig. E5A-D).

ED patients: In two ED patients (No. 14 and 15, Table E1) in whom we collected samples during the acute anaphylactic episode and 24 hours later, and who received emergency treatment with systemic corticosteroids, during the acute allergic reaction we observed changes in basophils, CCL2 and tryptase, but not in PMNs and lymphocytes (Fig E6). The increase in the PMNs and the decrease in the lymphocytes became evident only at the 24-hour sampling point (Fig. E6). The decrease in basophil count and *FcεRI* expression, as well as the increase in tryptase and CCL2 level were also observed in two ED patients (No. 20 and 29, Table E1) who did not receive treatment with corticosteroids (Fig. E7).

Predictors of anaphylactic reactions

We compared the performance of basophil counts, basophil activation, tryptase levels, as well as CCL2 and FcεRI expressions in discriminating between patients with anaphylactic reactions and those without using a ROC curve analysis. For the control groups, we used the patients with confirmed venom allergy from whom samples were obtained at least two months after the last sting reaction, and before venom immunotherapy was initiated (134 controls for basophil counts,

basophil activation and tryptase level, and 37 controls for *FcεRI* expression) or healthy controls (54 controls for CCL2).

When we compared values at the time of the reaction with those one month later, the estimated areas under the ROC curve (95% CI) were 0.92 (0.83-1), 0.93 (0.84-1) and 0.92 (0.86-0.99) for CCL2, *FcεRI* expression and basophil counts, respectively.

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LEGEND FOR FIGURES

Figure E1. Time between the onset of symptoms to the collection of blood sample in ED patients with acute anaphylactic reactions.

Figure E2. Correlation between basophil CD63 and CD203c activation in ED patients with acute anaphylactic reactions.

Figure E3. Absolute basophil count **(A)**, whole blood *FcεRI* gene expression **(B)**, serum tryptase **(C)** and CCL2 serum concentration **(D)** in ED patients divided according to severity of acute allergic reactions (Mueller grade I and II vs. grade III and IV) and then 7 and 30 days after the episode. The threshold for diagnostically positive tryptase measurement was set at 11.2 µg/L. Data are presented as a person-to-person scatter plot.

Figure E4. Basophil CD63 activation **(A)**, basophil absolute count **(B)**, whole blood *FcεRI* gene expression **(C)**, lymphocytes **(D)** and PMNs **(E)** absolute count in healthy control subjects 2.5-3 hours, 5 hours and 24 hours after the single dose of oral methylprednisolone (64 mg). Horizontal lines represent median values with IQR.

Figure E5. Serum concentrations of CCL2 **(A)**, CCL5 **(B)**, CCL11 **(C)** and IL-3 **(D)** in healthy control subjects 2.5-3 hours, 5 hours and 24 hours after the single dose of oral methylprednisolone (64 mg). Horizontal lines represent median values with IQR.

Figure E6. Basophil CD63 activation, absolute basophil count, serum tryptase levels, PMNs and lymphocytes absolute counts, and CCL2 serum concentration in two ED patients (No. 14 and 15; Table E1) sampled 1.5 hours, 24 hours, 7 days and 1 month after the onset of symptoms. Both patients were treated with methylprednisolone. Data are presented as a before-after scatter plot.

Figure E7. Basophil absolute count, whole blood *FcεRI* gene expression, serum tryptase and CCL2 serum concentration during the acute anaphylactic reactions to hymenoptera venom, and 7 and 30 days after the anaphylactic episode in ED patients divided according methylprednisolone treatment (patients No. 20 and 29 were not treated with methylprednisolone; Table E1). Data are presented as a person-to-person scatter plot.

Figure E8. Receiver operating characteristic (ROC) curve analysis of basophil CD63 activation, absolute basophil count, whole blood *FcεRI* gene expression, CCL2 concentration and serum tryptase levels between patients with acute anaphylactic reactions to insect venoms upon ED presentation, and venom-allergic or healthy controls. AUC: area under the curve.

Fig E1

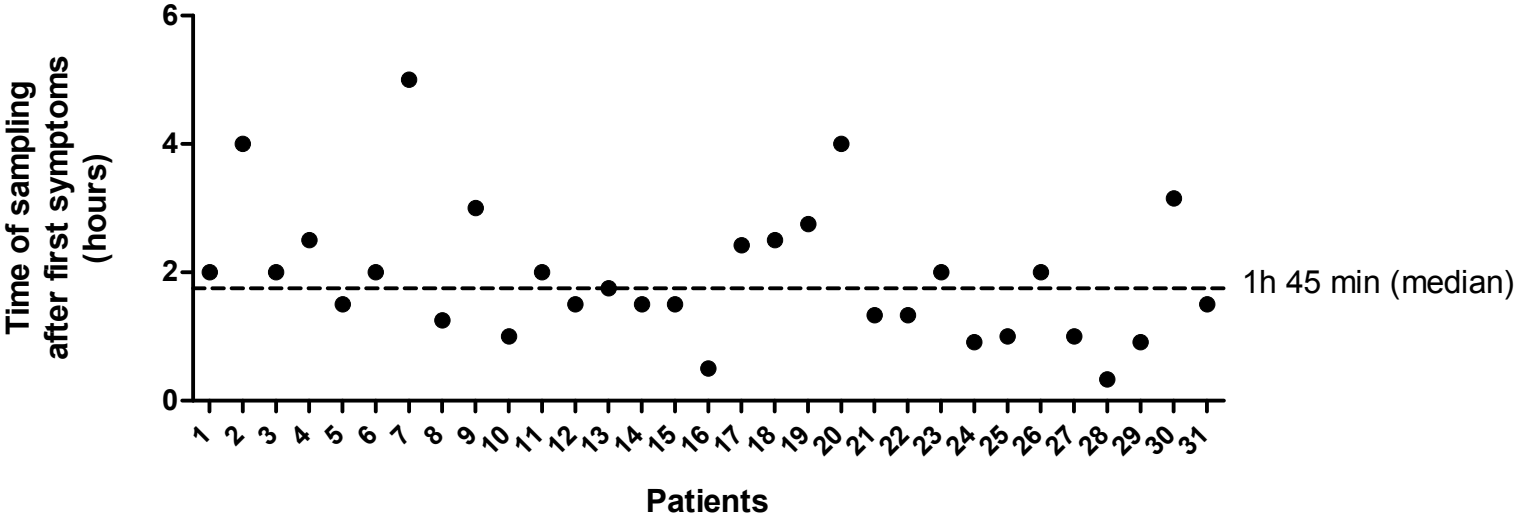

Time of sampling after first symptoms (hours)
6
4
2
0
1h 45 min (median)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Patients

Fig. E2

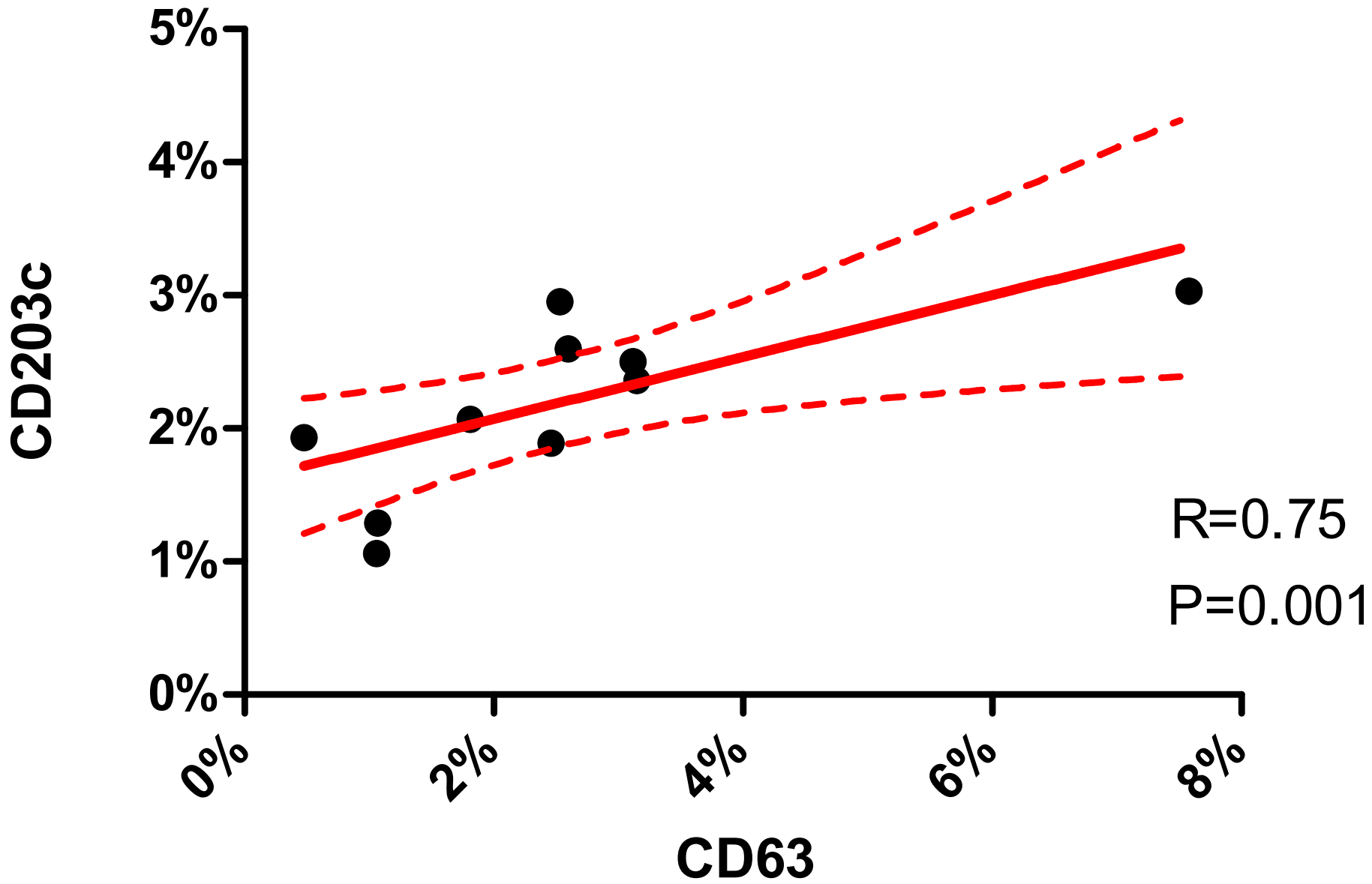


Fig. E3

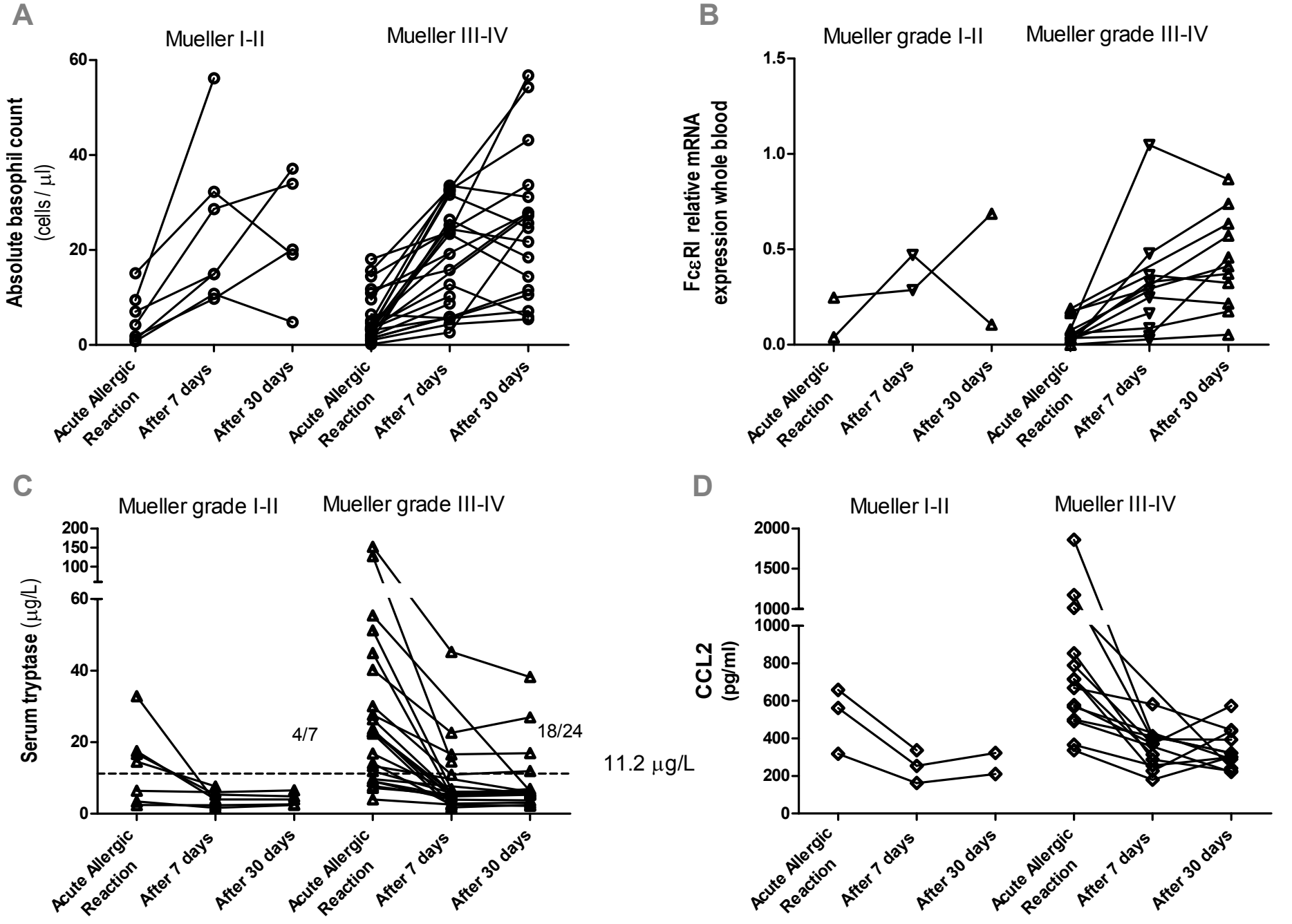
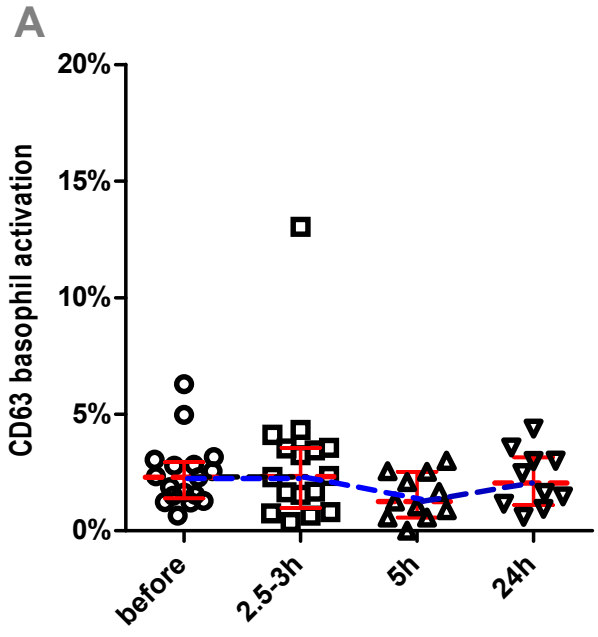
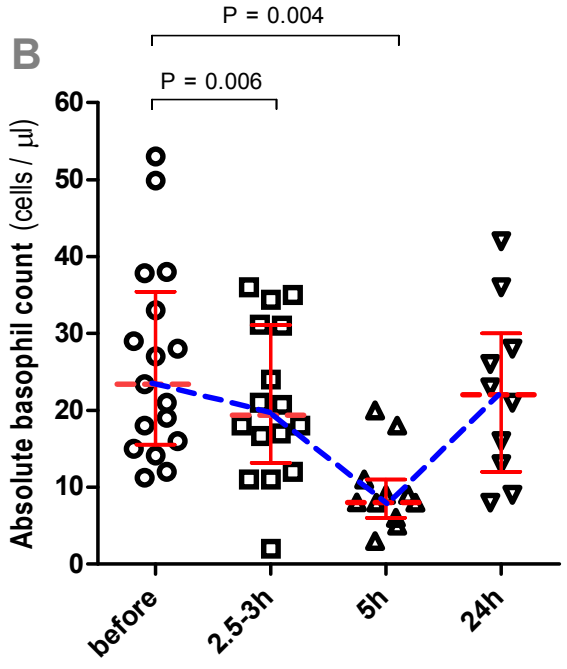
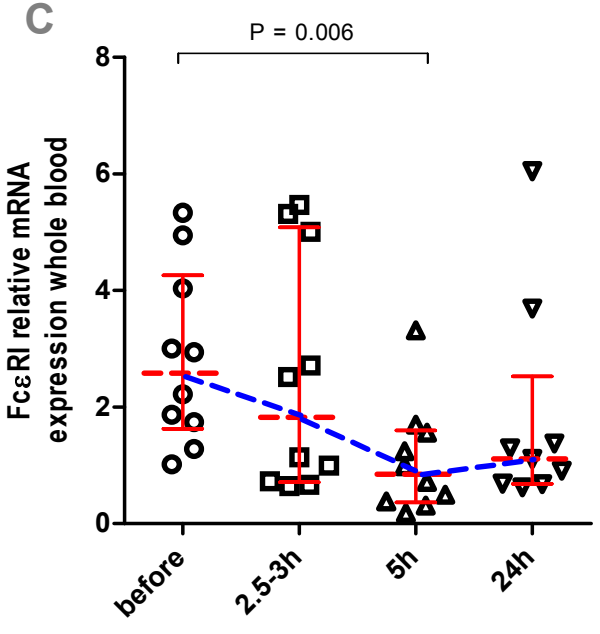
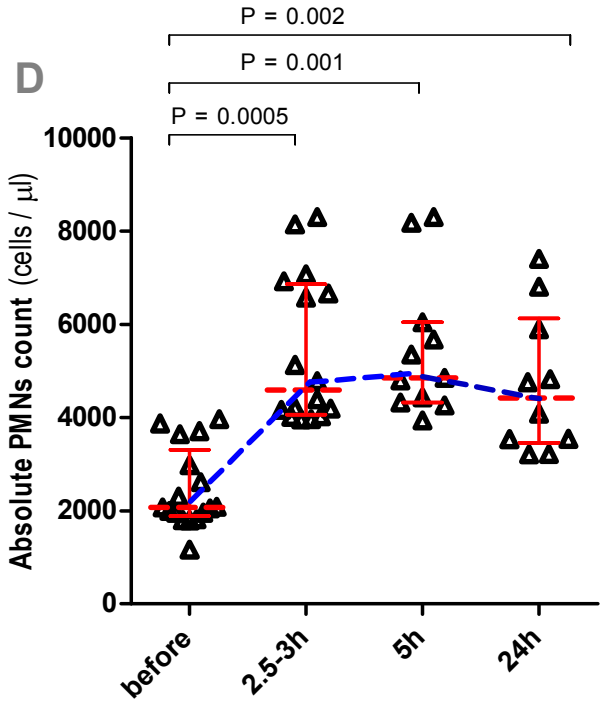

A
Mueller I-II
Mueller III-IV
Absolute basophil count (cells / µl)
60
40
20
0
Acute Allergic Reaction
After 7 days
After 30 days
B
Mueller grade I-II
Mueller grade III-IV
FcεRI relative mRNA expression whole blood
1.5
1.0
0.5
0.0
C
Mueller grade I-II
Mueller grade III-IV
Serum tryptase (µg/L)
200
150
100
60
40
20
0
4/7
18/24
11.2 µg/L
D
Mueller I-II
Mueller III-IV
CCL2 (pg/ml)
2000
1500
1000
800
600
400
200
0

Fig. E4


A
CD63 basophil activation
20%
15%
10%
5%
0%
before
2.5-3h
5h
24h


B
P = 0.004
P = 0.006
Absolute basophil count (cells / µl)
60
50
40
30
20
10
0
before
2.5-3h
5h
24h


C
P = 0.006
FcεRI relative mRNA expression whole blood
8
6
4
2
0
before
2.5-3h
5h
24h


D
P = 0.002
P = 0.001
P = 0.0005
Absolute PMNs count (cells / µl)
10000
8000
6000
4000
2000
0
before
2.5-3h
5h
24h

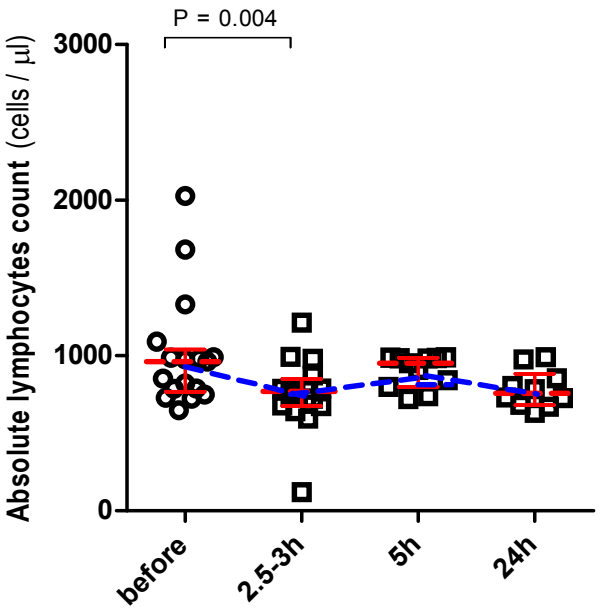

E
P = 0.004
Absolute lymphocytes count (cells / µl)
3000
2000
1000
0
before
2.5-3h
5h
24h

Fig. E5

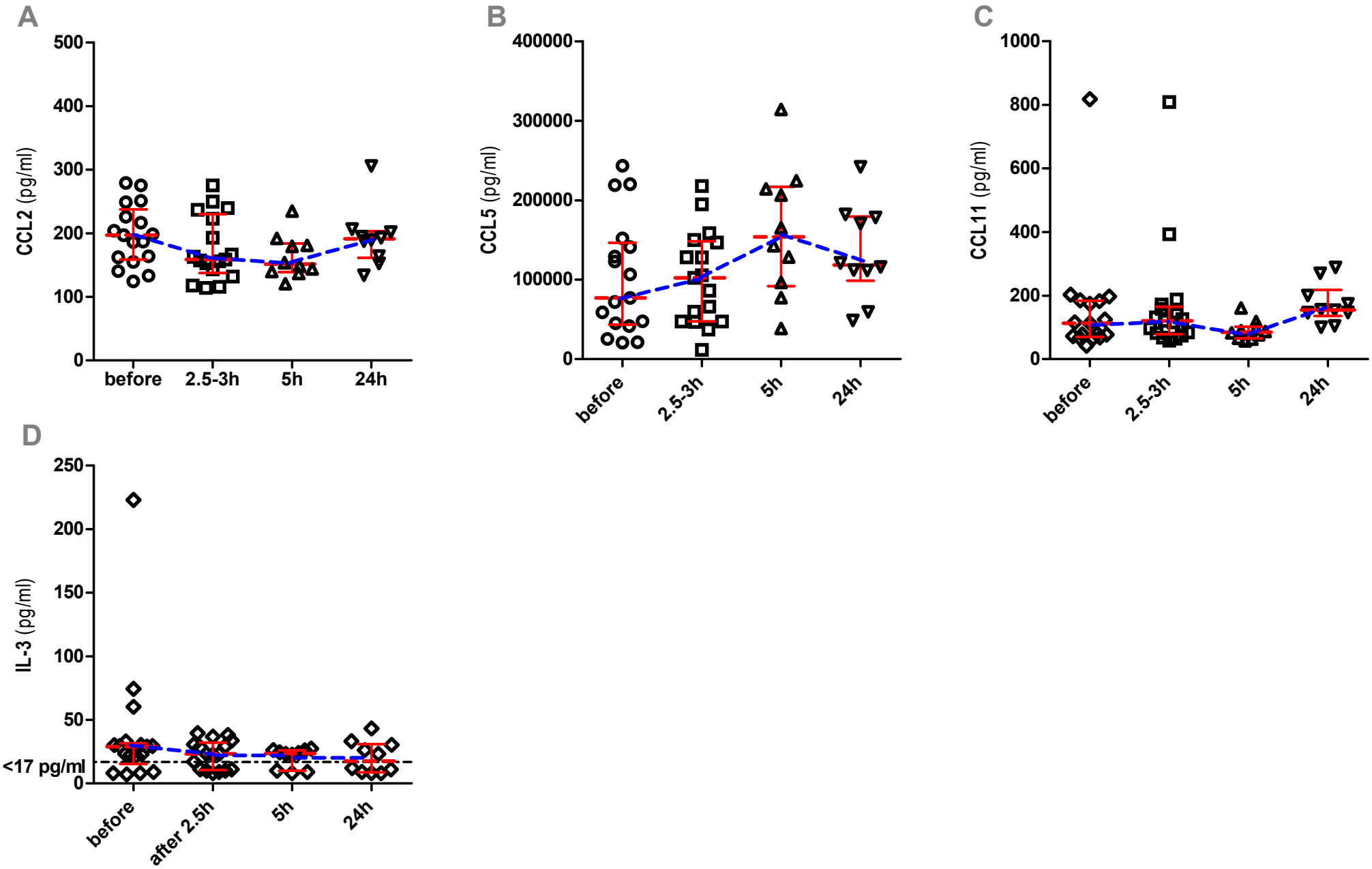
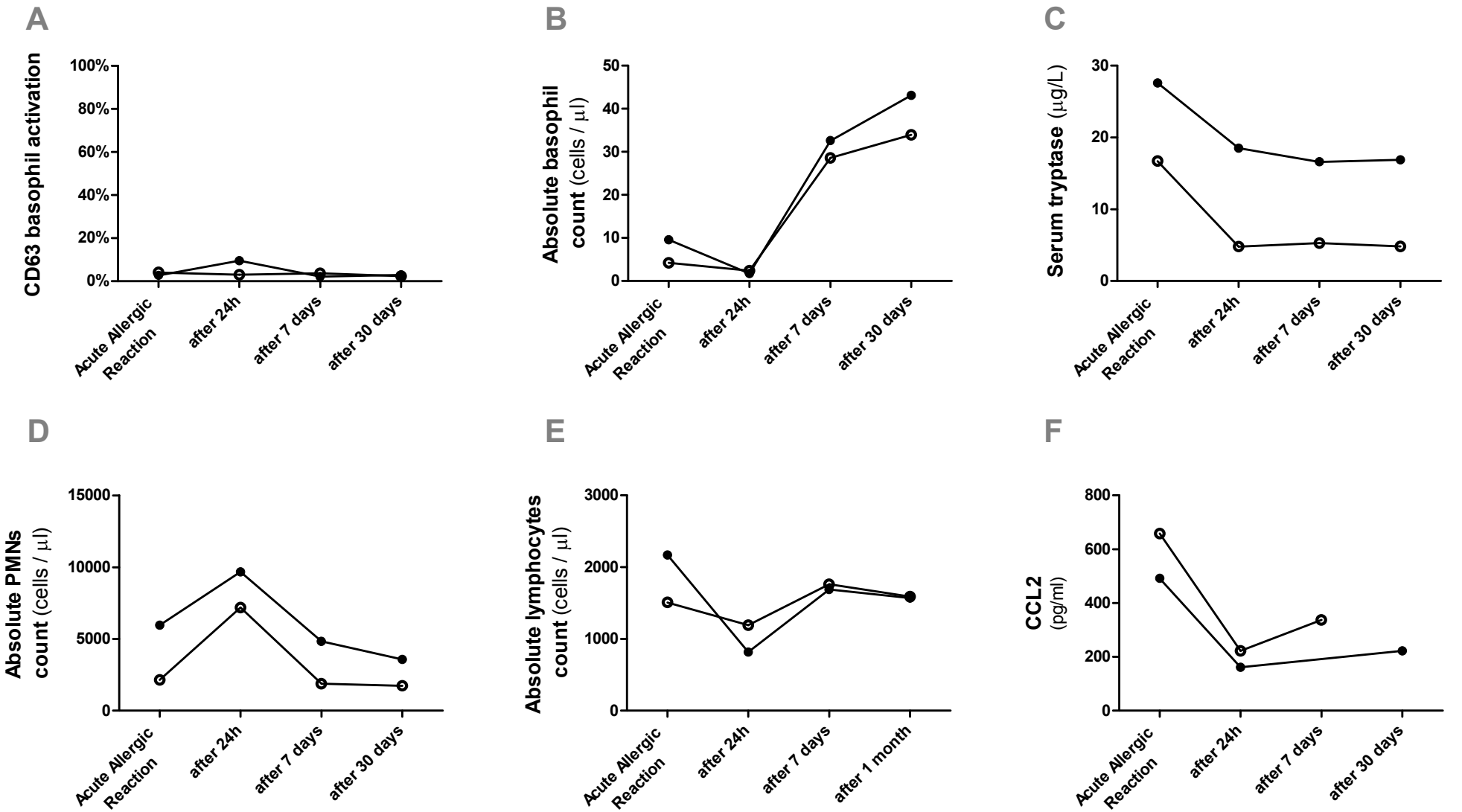

A
CCL2 (pg/ml)
500
400
300
200
100
0
before
2.5-3h
5h
24h
B
CCL5 (pg/ml)
400000
300000
200000
100000
0
before
2.5-3h
5h
24h
C
CCL11 (pg/ml)
1000
800
600
400
200
0
before
2.5-3h
5h
24h
D
IL-3 (pg/ml)
250
200
150
100
50
<17 pg/ml
0
before
after 2.5h
5h
24h

Fig. E6



No. 14 : first sampling 1h 30 min after symptom onset, emergency treatment included methylprednisolone 80 mg iv

No. 15 : first sampling 1h 30 min after symptom onset, emergency treatment included methylprednisolone 32 mg po and 125 mg iv

Fig. E7

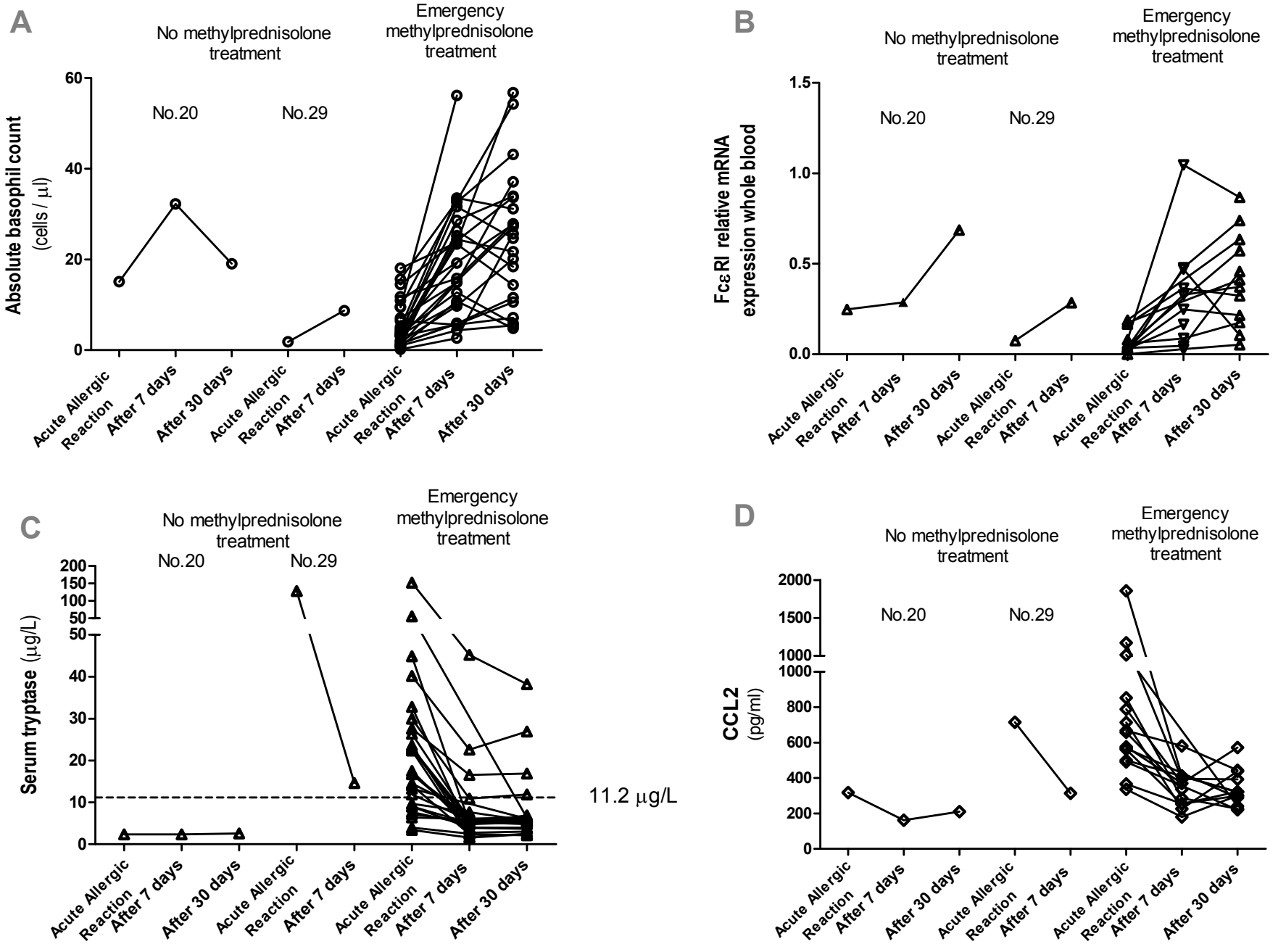
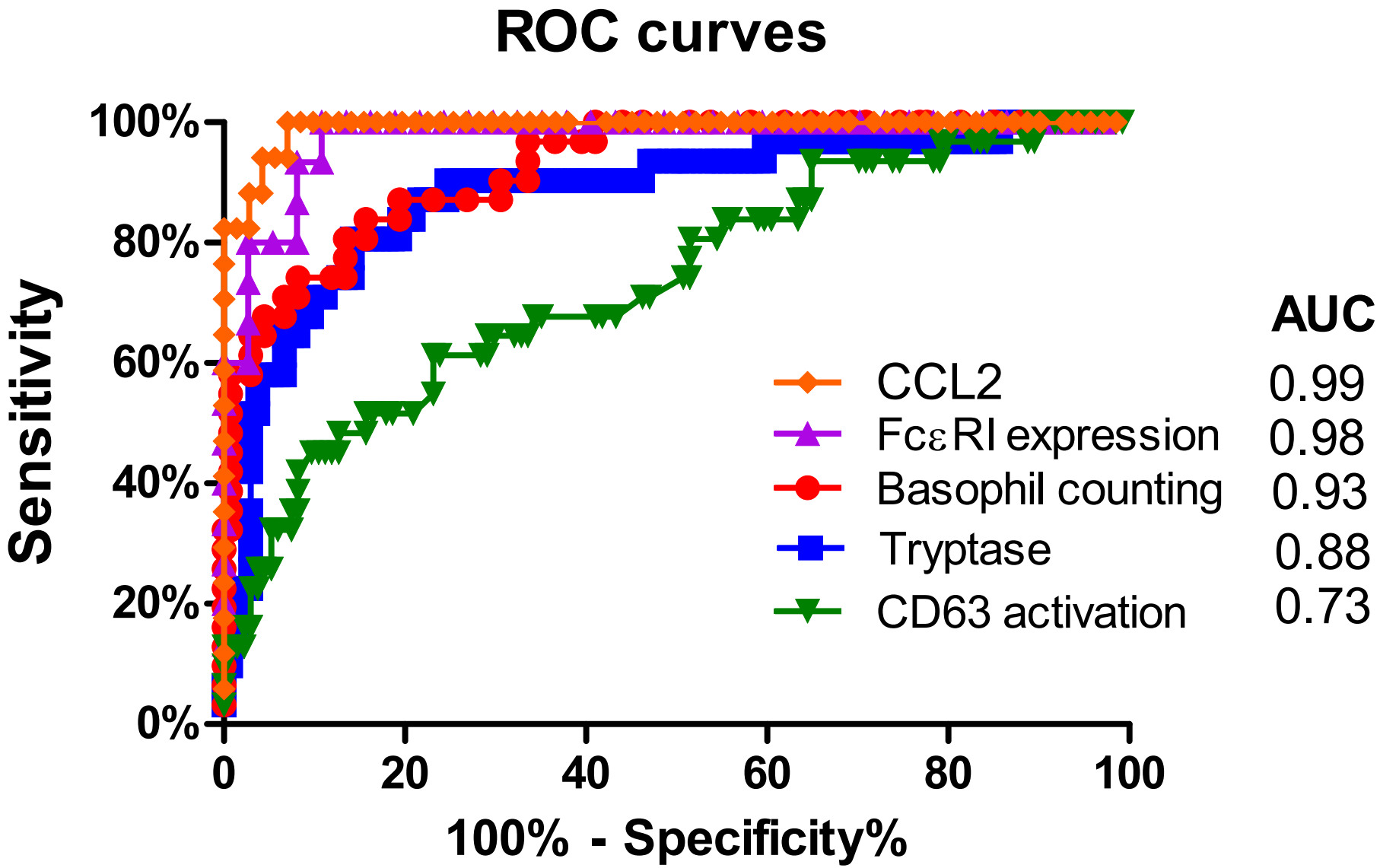

A
No methylprednisolone treatment
Emergency methylprednisolone treatment
No.20
No.29
Absolute basophil count (cells / μl)
60
40
20
0
Acute Allergic Reaction
After 7 days
After 30 days
B
No methylprednisolone treatment
Emergency methylprednisolone treatment
No.20
No.29
FcεRI relative mRNA expression whole blood
1.5
1.0
0.5
0.0
C
No methylprednisolone treatment
Emergency methylprednisolone treatment
No.20
No.29
Serum tryptase (μg/L)
200
150
100
50
40
30
20
10
0
11.2 μg/L
D
No methylprednisolone treatment
Emergency methylprednisolone treatment
No.20
No.29
CCL2 (pg/ml)
2000
1500
1000
800
600
400
200
0

Fig. E8


ROC curves
Sensitivity
100%
80%
60%
40%
20%
0%
0
20
40
60
80
100
100% - Specificity%
AUC
CCL2 0.99
FcεRI expression 0.98
Basophil counting 0.93
Tryptase 0.88
CD63 activation 0.73