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Serum levels of 9 α ,11 β -PGF₂ and apolipoprotein A1 achieve high predictive power as biomarkers of anaphylaxis

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Keywords

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Abbreviations

ANA: Anaphylaxis

Apo: Apolipoprotein

HDL: High density lipoprotein

ROC: Receiver operating characteristic

AUC: Area under the curve

PSA: Passive systemic anaphylaxis

Abstract

Anaphylaxis is a life-threatening hypersensitivity reaction. To identify biomarkers for the condition, we assessed serum levels of apolipoprotein (Apo)A and ApoE. We found a reduction of both lipoproteins in anaphylactic mice as well as in orally challenged food allergic patients. We then compared patients after acute anaphylaxis with several control groups (non-allergic, history of

allergen-triggered anaphylaxis, acute cardiovascular/febrile reactions). In this unpaired setting, ApoE levels were unaltered, while ApoA1 was reduced in the anaphylactic group. Though unable to discriminate between anaphylaxis and cardiovascular/febrile reactions, ROC curve analysis revealed a reasonably high area under the curve (AUC) of 0.91 for ApoA1. Serum $9\alpha,11\beta$ -PGF₂, recently identified as a suitable biomarker for anaphylaxis, outperformed ApoA1 with AUC=0.95. Intriguingly however, its power further increased upon combination of both mediators reaching AUC=1. Our data suggest that ApoA1 combined with $9\alpha,11\beta$ -PGF₂ represents a useful composite biomarker of anaphylaxis, achieving superior diagnostic power over either factor alone.

Introduction

Anaphylaxis is a rapid potentially life-threatening allergic reaction (1, 2). Preformed and de novo synthesized mediators (3), predominantly released by activated mast cells (MCs) or basophils (4) determine the outcome of this response. The diagnosis of anaphylaxis is based on the clinical reaction patterns since reliable biomarkers are sparse. Serum tryptase is the clinically available biomarker for anaphylaxis so far, but it is unable to detect all anaphylactic responses (5, 6). We recently demonstrated (6) that serum levels of $9\alpha,11\beta$ -PGF₂, a metabolite of PGD₂, increases profoundly during anaphylaxis and displays a higher sensitivity and specificity for anaphylaxis than tryptase. To identify additional diagnostic markers for anaphylaxis, we determined proteins with substantial changes during oral food challenge in sera of food allergic patients. Based on this protein identification report and the existing literature (7), we analyzed mouse and human serum apolipoprotein (Apo)E and ApoA1 levels and assessed whether these mediators could serve as suitable biomarkers of anaphylaxis. Both belong to the high density lipoproteins (HDL) and are involved in cholesterol transport and metabolism (8, 9). It has been shown previously that HDL particles can be degraded by chymases and tryptases, released upon MC activation (10-12).

Material and Methods

Protein identification report

Sera derived from food allergic patients with a history of anaphylaxis were analyzed prior to and after oral challenge with the respective allergen. Proteins with substantial changes during the allergic reaction were identified using an MS/MS ion search (13) of the Mascot search engine (www.matrixscience.com) and a protein database (www.ncbi.nlm.nih.gov), Taxonomy: Homo sapiens (218214 sequences), Variable modifications: Oxidation (HW), Oxidation (M), Propionamide (C), Peptide Mass Tolerance: ± 5 ppm, Fragment Mass Tolerance: ± 0.6 Da, Max 1 Missed Cleavages. Ion charge in search parameters for ions from ESI-MS/MS data acquisition were set to "1+, 2+ or 3+" according to the instrument's and method's common charge state distribution (Proteome Factory, Berlin, Germany).

Blood collection and analysis

We compared serum samples derived from anaphylactic or non-anaphylactic Balb/c mice (n=10/group for ApoE; n=8/group for ApoA1). Passive systemic anaphylaxis was induced as described previously (14).

In a matched setting, we compared human serum samples derived from food allergic patients with a history of anaphylaxis, taken before and within 15 min after oral allergen challenge ("before/after challenge", n=11, Table E1). In addition, we analyzed sera derived from patients with acute anaphylactic symptoms, admitted to the Emergency department. Blood was obtained within a maximum of 2 hours after the anaphylactic reaction ("after ANA", n= 10, Table E2). Anaphylaxis severity was assessed based on their clinical reaction patterns according to Ring and Messmer (15). The control groups comprised of non-allergic subjects without any history of allergy, confirmed by negative skin prick test ("non-allergic", n=11, Table E3), patients with a history of anaphylaxis but

without any symptoms at the time of blood withdrawal (“ANA prone”, n=19, Table E4) and patients with acute cardiovascular/febrile reactions (“other diseases”, n=12, Table E5). Serum levels of ApoE and ApoA1 were assessed by ELISA following the manufacturers’ instructions (ApoE, human samples: Assaypro LLC, St Charles, USA; ApoA1, human samples: Abcam plc, Cambridge, UK; ApoE, mouse samples: Cusabio, Hubei, China; ApoA1, mouse samples: Cloud-Clone Corp., Houston, USA).

Statistical analysis

Statistical analysis was performed with PRISM 6.0 (GraphPad Software, La Jolla, CA, USA) using (non-) parametric ANOVA with Dunn’s or Holm-Sidak’s posttest or the Mann-Whitney test. Paired samples were analyzed using the Wilcoxon matched-pairs test. A p-value of 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves and areas under the curve (AUCs) were calculated to determine the predictive power using the “pROC” package for the R statistic computing platform (www.R-project.org). An AUC equal to 1.0 indicates that the diagnostic test discriminates perfectly between anaphylaxis and “no anaphylaxis”. An AUC equal to 0.5 indicates that the results are purely random.

Results

Initially, we analyzed mouse serum ApoE and ApoA1 levels after PSA induction and compared them with those of a control group without anaphylaxis. ApoE and ApoA1 decreased after anaphylaxis in the mouse (Fig. 1A+B). To investigate the validity in human samples, we analyzed matched serum samples from food allergic patients with a history of anaphylaxis, taken before and after a positive oral allergen challenge (Table E1). Consistent with the data from mice, ApoE and ApoA1 levels decreased in patients with objective allergic responses (Fig. 1 C+D). In addition, we compared sera from anaphylactic patients (Table E2) with sera of several control groups (non-allergic subjects, Table

E3; patients with a history of anaphylaxis in the past but without symptoms at the time of blood withdrawal, Table E4 and patients with acute cardiovascular/febrile reactions, Table E5) with respect to the levels of both proteins. In this unrelated setting, ApoE levels were unaltered among groups. In contrast, ApoA1 was significantly decreased in the post-anaphylactic group and in patients with other diseases (Fig. 1E+F). To determine the sensitivity and specificity of ApoE and ApoA1 as biomarkers of anaphylaxis, ROC curve analysis and their corresponding AUC values (Table E6) were computed, resulting in AUC=0.63 for ApoE and AUC=0.91 for ApoA1. We have recently identified serum $9\alpha,11\beta$ -PGF₂ as a suitable biomarker for anaphylaxis with an AUC=0.95 (6). Subsequently, we analyzed whether its predictive power increased when viewed together with ApoE or ApoA1. Combination with $9\alpha,11\beta$ -PGF₂, indeed, improved the sensitivity and specificity for both lipoproteins. The highest diagnostic power was observed with ApoA1 together with $9\alpha,11\beta$ -PGF₂ (AUC=1.0, Fig.2).

Discussion

This study was designed to determine suitable biomarkers of anaphylaxis as up to now there is no such marker available to allow a reliable diagnosis. Based on a protein identification report and on literature (7) we analyzed serum levels of ApoE and ApoA1 in mice and in humans. We found a significant decrease in serum ApoE and ApoA1 levels after anaphylaxis in mice (Fig. 1A+B). Lower levels of both proteins have been found in mice upon IgE-independent MC degranulation before (7) and might be explained by the amount of MC derived proteases (16). It has been shown, indeed, that MC proteases are able to degrade HDL particles (10, 12, 17, 18).

We and others have shown previously (5, 6) that tryptase is a suitable marker for anaphylaxis, when the baseline level of the patient is known. In fact, using matched serum samples from food allergic patients with objective symptoms upon challenge, tryptase levels were likewise increased in the current study (Suppl Fig. E1). Simultaneously, ApoE and ApoA1 decreased significantly (Fig. 1A-D),

although ApoE failed to discriminate between anaphylactic and non-anaphylactic conditions when comparing unrelated groups of patients. In contrast, ApoA1 was significantly decreased in the post-anaphylactic group, likely resulting from increased proteolytic activity upon mast cell activation. However, ApoA1 did not discriminate between anaphylaxis and cardiovascular/febrile reactions. Low levels of ApoA1 have been found in patients with chronic cardiovascular reactions (8, 20) but interestingly also in patients with acute conditions like sepsis (19). Our data suggest that ApoA1 degradation may occur in diverse acute disorders, including cardiovascular, febrile and anaphylactic.

Since the focus of our study was to identify whether ApoA1 and ApoE could serve as biomarkers of anaphylaxis, we determined their predictive power to discriminate between anaphylactic and non-anaphylactic patients by ROC curve analysis. ApoE showed poor sensitivity/specificity in discriminating between anaphylactic and non-anaphylactic patients (AUC=0.63). In contrast, ApoA1 showed high sensitivity/specificity (AUC=0.91) but was inferior to $9\alpha,11\beta$ -PGF₂ alone (AUC=0.95). This constellation implied that it may be worthwhile to assess the investigated biomarkers for complementarity. Combination with $9\alpha,11\beta$ -PGF₂ indeed improved the sensitivity/specificity of both lipoproteins. The highest predictive power was observed with ApoA1 and $9\alpha,11\beta$ -PGF₂ (AUC=1.0), suggesting that both mediators together represent a highly suitable composite biomarker of anaphylaxis. It should be noted, however, that only small cohorts of patients were analyzed in our study, warranting further studies on the utility of these biomarkers.

Conclusion

Serum levels of ApoA1 decrease during anaphylaxis. ApoA1 and $9\alpha,11\beta$ -PGF₂ combined have high diagnostic power as a composite biomarker of anaphylaxis.

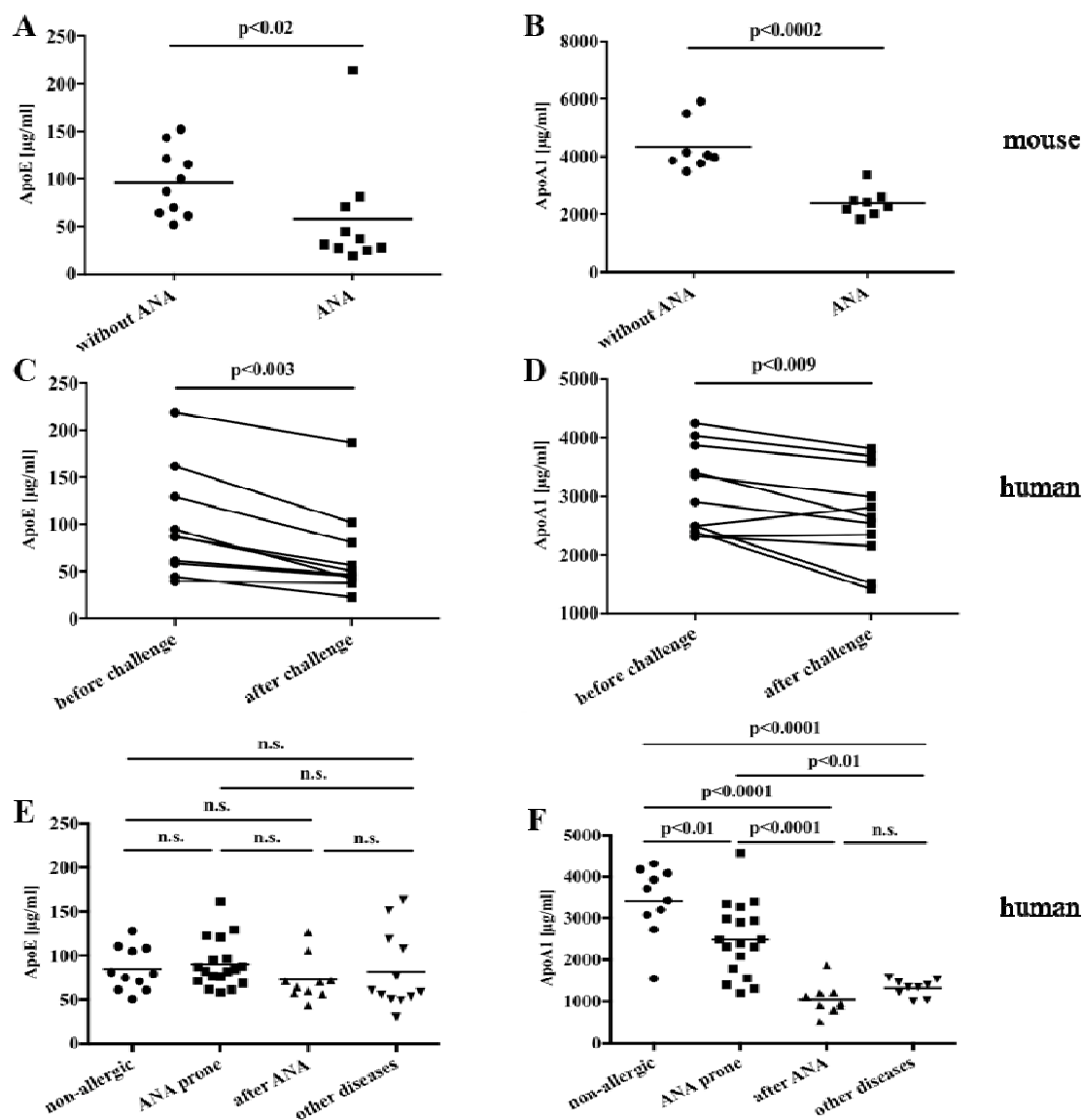


Figure 1:

Serum levels of ApoE (n=10/group, A) and ApoA1 (n=8/group, B) in mice with and without an anaphylactic response. Passive systemic anaphylaxis was induced as described previously (14). Blood was taken 70 min after the onset of anaphylaxis.

Serum levels of ApoE (C) and ApoA1 (D) prior to and after an oral allergen challenge in the same patient (n=11). Blood was taken a maximum of 15 minutes after oral challenge, depending on clinical reactivity. Dots corresponding to the same patient are interconnected. Serum levels of ApoE (E) and ApoA1 (F) in non-allergic patients (n=11) versus those of patients who had experienced anaphylaxis in the past but were not having an anaphylactic reaction at the time of blood withdrawal (“ANA prone”, n=19) versus those of patients with acute anaphylaxis (“after ANA”, blood obtained a maximum of 2 hours after the event, n=10) and patients with cardiovascular or febrile reactions (“other diseases”, n=12). ApoE and ApoA1 were assessed via ELISA. A p-value of 0.05 was considered statistically significant.

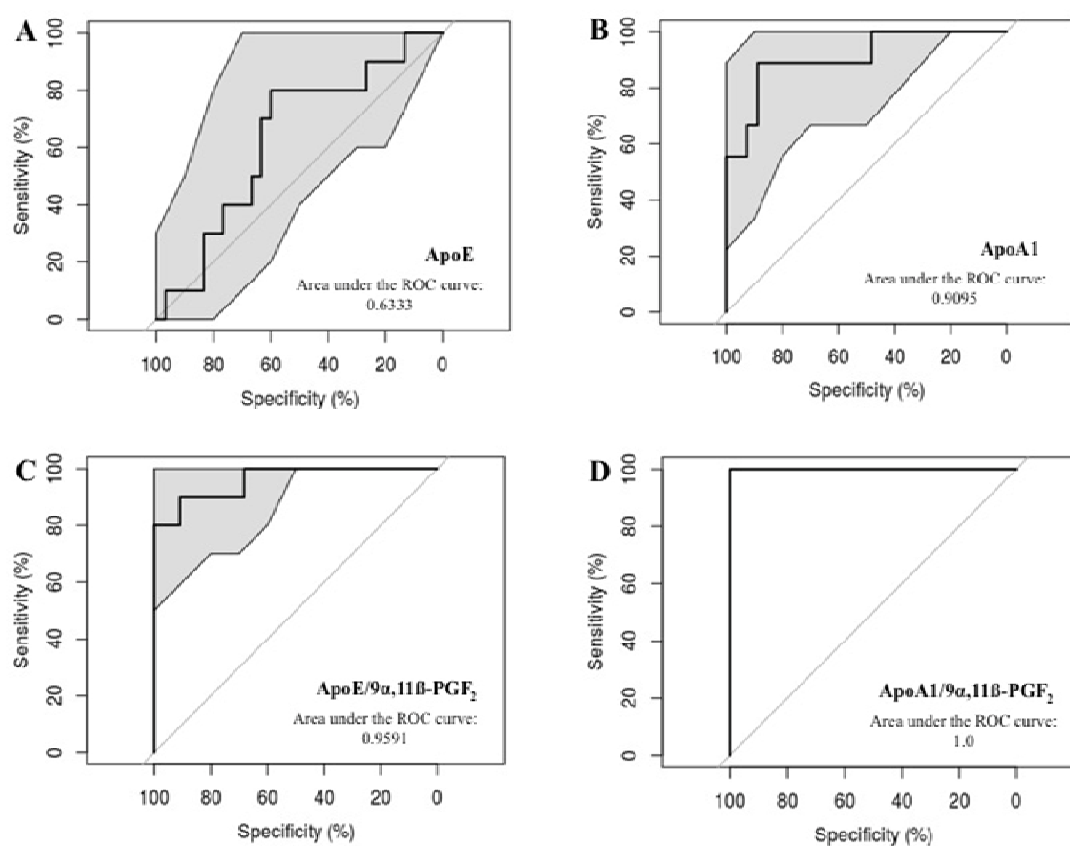


Figure 2

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Comparison of post-anaphylactic patients with non-anaphylactic patients. ROC curves and AUC values are given for ApoE (A) and ApoA1 (B) alone and as a composite measure with $9\alpha,11\beta$ -PGF₂ (C+D). For comparison, the AUC for $9\alpha,11\beta$ -PGF₂ alone=0.95. ROC curve analysis was performed with the post-anaphylactic patients as positive and the non-anaphylactic patients (“ANA prone” and “other diseases”) as negative samples. An AUC equal to 1.0 indicates that the diagnostic test discriminates perfectly between anaphylaxis and no anaphylaxis. An AUC equal to 0.5 indicates that the results are purely random.

Table E1-E5

The study compared 5 groups of patients. Table E1 shows the food allergic patients (n=11), who had at least one anaphylactic reaction in the past (referred to as “initial reaction”) and were orally challenged with the respective allergen. Sera were taken before and within 15 minutes after positive oral allergen challenge (clinical manifestation and anaphylaxis severity are given for the reaction after food challenge). Table E2 shows the post-anaphylaxis cohort (n=10). Blood was obtained within a maximum of 2 hours after an anaphylactic reaction. Table E3 shows the non-allergic subjects (n=11). Table E4 shows patients outside of an acute reaction but who had at least 1 anaphylactic episode in the past (n=19). The clinical manifestations and severity grades for the latter group refer to that previous anaphylactic episode. The culprit elicitor (if known), sex, manifestation profile, and anaphylaxis grades according to Ring and Messmer and mediator levels are given for each patient. Table E5 shows patients with acute cardiovascular symptoms (n = 6) or fever of unknown origin (n = 6).

Table E6

AUCs for the (combination of) mediators were calculated from ROC curves derived by using logistic regression analysis using the “post-anaphylactic” versus the “anaphylaxis-prone” and the “other diseases groups” as positive and negative samples. An AUC equal to 1.0 indicates that the diagnostic test discriminates perfectly between anaphylactic and non-anaphylactic responses.

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