

EXPERT OPINION

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D-dimer levels decreased in severe allergic asthma and chronic urticaria patients with the omalizumab treatment

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Background: D-dimer (DD), a fibrin degradation product formed during the lysis of a thrombus, is also detected in high levels in patients with active chronic urticaria (CU). Severe persistent allergic asthma (SPA) is associated with a procoagulant state in the bronchoalveolar space, further aggravated by impaired local activities of the anticoagulant protein C/protein S, antithrombin III system and fibrinolysis. This was demonstrated as massive fibrin depositions found in the alveoli of a SPA patient who died from a SPA attack and who did not respond to treatment.

Objectives: For this reason, we investigated the effect of omalizumab both in bronchial and systemic vascular areas and evaluated SPA (group I) and CU (group II) patients before and after therapy period.

Methods: Blood samples were taken before treatment (A), on 4th month (B), on 8th month (C) and on 12th month (D) post treatment in both groups.

Results: We compared DD levels between groups: the significant DD difference was observed between group-IA and group-IC ($p = 0.031$); between group-IA and group-ID ($p = 0.003$); between group-IB and group-ID ($p = 0.049$) and between group IIA-1 and group-IID ($p = 0.015$). In the IIA-1 group, there was a significant positive correlation between DD and age ($p = 0.008$, $r = 0.848$).

Conclusion: In conclusion, mediators and cells classically involved in procoagulant and anticoagulant pathways together play a role in SPA and CU pathophysiology, where omalizumab has its effect.

Keywords: anti-immunoglobulin E, antithrombin III, chronic urticaria, d-dimer, factor V Leiden and prothrombin G20210A mutation, omalizumab, protein C, severe persistent allergic asthma

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1. Introduction

Omalizumab, a humanized monoclonal antibody that binds to the CH3 domain, near the binding site for the high-affinity type-I immunoglobulin E (IgE) Fc receptors of human IgE, can neutralize free IgE and inhibit the IgE allergic pathway without sensitizing mast cell and basophils [1]. Omalizumab has been approved in over 100 countries for treating patients with severe persistent allergic asthma. These pharmaceutical developments have validated the IgE pathway as an effective therapeutic target for treating IgE-mediated allergic disease. More interestingly, as we have observed in some of our cases, one severe persistent asthmatic (SPA) patient who had protein C (pC)/protein S (pS) deficiency history (who was also a heterozygous carrier of factor V Leiden and prothrombin G20210A mutation) and multiple massive pulmonary embolus, systemic subacute thrombosis determined in vena saphena parva and in left venae perforantes cruris, underwent omalizumab

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treatment and after a long-term (20 months) treatment with omalizumab, he had a decreased fractional exhaled nitric oxide concentrations (FENO), d-dimer (DD), sTRAIL, proinflammatory IL-1 β and OX-2 and had an increased CXCL8, activated pC (APC), antithrombin III (AIII), pS and pC levels [2]. In this patient's blood, levels of APC, AIII, pS and pC were found to be increased (74, 128, 102 and 86%, respectively), and DD level (412 U/l) was found to be decreased at 30th month under omalizumab therapy and this result was significant. Tissue factor (TF) is the main initiator of coagulation and is found exclusively in the respiratory epithelium. Recent reports revealed that patients with chronic urticaria (CU) show signs of thrombin generation and activation of the TF pathway of the coagulation system [3]. DD, a fibrin degradation product formed during the lysis of a thrombus, is also detected in high levels in patients with active CU [3,4]. SPA is associated with a procoagulant state in the bronchoalveolar space, further aggravated by impaired local activities of the anticoagulant pC/pS, AIII system and fibrinolysis, as demonstrated by massive fibrin depositions in the alveoli of a SPA patient who died from a SPA attack and who did not respond to treatment [2,5].

For considering the effect of omalizumab both in bronchial and systemic vascular areas, we evaluated SPA (group I) and CU (group II) patients before and after therapy period.

2. Materials and methods

2.1 Experimental procedures

All patients have been informed and consent was acquired for this study. Group I consisted of SPA patients and group II consisted of CU patients. Both groups were also divided into four category: before treatment (A), 4th month (B), 8th month (C) and 12th month (D) post treatment. This study was also approved by the local ethics committee. DD plasma level was measured by enzyme-linked fluorescence assay kits (VIDAS® DD Exclusion II) from bioMérieux, France. The cut-off level is 500 ng/ml. Total IgE levels were enumerated by fluoroenzyme immunoassay (ImmunoCAP-FEIA) using an ImmunoCAP (Pharmacia, Uppsala, Sweden) kit. The data were presented as mean $\pm$ SD.

2.2 Statistical analysis

All statistical analyses were carried out using the SPSS 18.0 software (SPSS, Inc., New York, USA). Data were analyzed for normality of distribution by using the Kolmogorov-Smirnov test. Results of normally distributed data are expressed as mean $\pm$ SD. Comparison of parameters between the groups was performed using independent-samples *t*-test. Correlation between variables was assessed by Pearson's correlation coefficient. A *p*-value < 0.05 indicated statistical significance.

2.3 Results

Group I: SPA, *n* = 20, asthma diagnosis years (ADY): 13.58 $\pm$ 8.64, age (years): 45.37 $\pm$ 12.32. Blood samples were taken

before treatment (group IA), on 4th month (group IB), on 8th month (group IC) and on 12th month (group ID) post treatment. There were five patients with concomitant cardiovascular disease and three patients with concomitant type 2 diabetes in group I. Pulmonary function tests with the measurement of FENO were performed on the same day. FENO were assessed under the GINA guideline as an indicator of disease severity, efficacy of omalizumab therapy and airway inflammation [6]. We observed that FENO concentration (mean $\pm$ SD) level decreased in the follow-up period (group-IA: 65.55 $\pm$ 7.90, group-IB: 53.45 $\pm$ 5.7, group-IC: 51.2 $\pm$ 3.1, group-ID: 39.70 $\pm$ 3.5). Total IgE level measurements were (mean $\pm$ SD) as follows: group-IA: 987.6 $\pm$ 386.5, group-IB: 939.6 $\pm$ 353.7 and total IgE IC: 739.6 $\pm$ 441.7, group-ID: 388.9 $\pm$ 196.6. Group IA, using desloratadine, inhalant forte doses steroid (Best Standard Care, following the recommendations of GINA, included inhaled fluticasone 500 mg twice daily [b.i.d.], inhaled salmeterol 50 mg b.i.d.), and daily 80 – 8 mg oral methyl-prednisolone, montelukast therapy. We compared DD levels between groups: the significant DD difference was observed between group-IA and group-IC (*p* < 0.05 , *p* = 0.031, respectively); between group-IA and group-ID (*p* < 0.05 , *p* = 0.003, respectively) and between group-IB and group-ID (*p* < 0.05 , *p* = 0.049, respectively). In group IA, there was a significant positive correlation between age and ADY (*p* = 0.043, *r* = 0.468, *p* < 0.05). In group-ID, there was a significant positive correlation between DD and age (*p* = 0.034, *r* = 0.488, *p* < 0.05) (Figure 1).

Group II: CU, *n* = 8, urticaria diagnosis years (UDY): 17.50 $\pm$ 8.19, age (years): 47.50 $\pm$ 10.04. There were two patients with concomitant cardiovascular disease. We subgrouped these patients as group IIA-1, who had active generalized urticarial plaques taking no medication. At the time of blood sampling, autolog serum skin test and disease activity assessment, all patients discontinued short-acting antihistamines for at least 4 days and long-acting antihistamines and systemic corticosteroids for at least 10 days. Disease activity was assessed by EAACI/GA2LEN/EDF activity score [7]. Diagnosis score for all patients were severe. Total IgE level measurements were as follows (mean $\pm$ SD): group IIA-1: 757.9 $\pm$ 156.7.9, group IIB: 457.3 $\pm$ 63.2, group IIC: 428.3 $\pm$ 71.8 and group IID: 238.9 $\pm$ 96. Group IIA-2 consisted of patients whose lesions were in passive stage. Patients were under steroid (40 – 16 mg oral methyl-prednisolone) and desloratadine 2 $\times$ 2 medications. We observed that DD level decreased. Omalizumab was given to groups IIB and IIC (225 – 375 mg) and was gradually ceased and continued with desloratadine 1 $\times$ 1. Group IID patients were under omalizumab medication only. The significant DD difference was observed between group IIA-1 and group-IID (*p* < 0.05 , *p* = 0.015, respectively). In the IIA-1 group, there was a significant positive correlation between DD and age (*p* = 0.008, *r* = 0.848, *p* < 0.005) (Figure 2).

For IgE: The significant IgE level difference was observed between group-IA and group-ID (*p* = 0.002, *p* < 0.005) and between group IIA-1 and group IID (*p* = 0.021, *p* < 0.05).

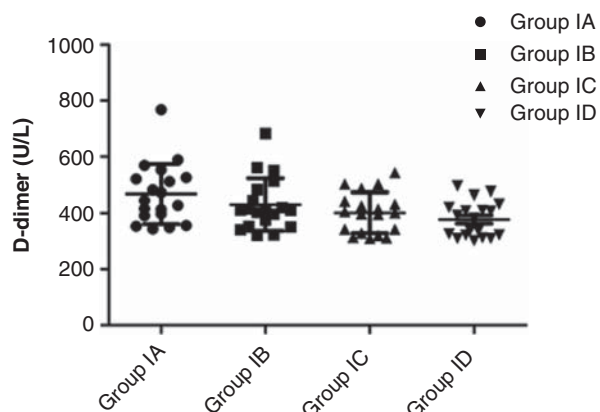


Figure 1. (Group I: severe persistent asthmatic, n = 20) D-dimer levels (mean ± SD). Group-IA: 467.63 ± 106.39. Group-IB: 430.00 ± 92.90. Group-IC: 401.21 ± 72.19. Group-ID: 377.37 ± 62.33.

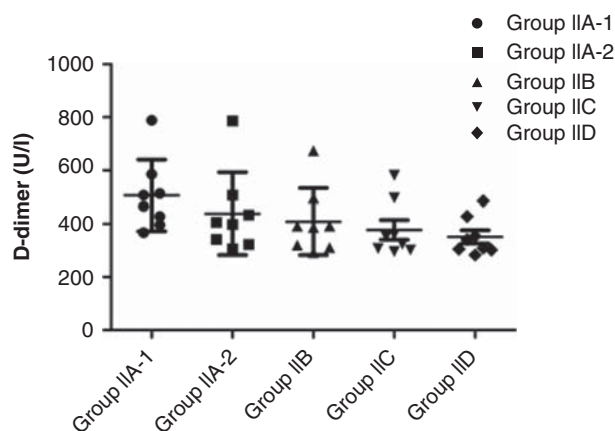


Figure 2. (Group II: chronic urticaria, n = 8) D-dimer levels (mean ± SD). Group IIA-1: 506.25 ± 133.93. Group IIA-2: 437.75 ± 54.83. Group IIB: 408.00 ± 125.39. Group IIC: 377.38 ± 104.50. Group IID: 350.87 ± 70.60.

In group-IA, group IIA and group IIA-2, there were a significant positive correlation between DD and IgE ($r = 0.559$, $p = 0.013$, $p < 0.05$), ($r = 0.764$, $p = 0.027$, $p < 0.05$) and ($r = 0.892$, $p = 0.007$, $p < 0.01$), respectively.

3. Discussion

The biological effects of APC and pC can be divided into anti-coagulant and cytoprotective effects [8]. In patients with SPA, bronchoalveolar levels of APC decreased after a bronchial allergen challenge and were significantly lower than healthy controls and APC:pC ratios were decreased in induced sputum of patients with SPA, thus pointing to an imbalance between coagulation and the pC system [9]. Extrinsic pathway of coagulation is activated in response to high level of circulatory IgE. Best example of this purported relationship is the correlation

between higher TF expression and vasculitis degree that has been seen in hyper IgE syndrome (HIES). One of the complement component C5a also activates extrinsic pathway by increasing TF in a similar way, which was reported in humans [10]. HIES is a heterogeneous group of immune disorders. It is characterized by very high concentrations of the serum antibody IgE. Clinically, eczema-like rash, cold staphylococcal infection, severe lung infection are seen. An IgE level $> 2,000$ IU/ml is often considered diagnostic, except in patients younger than 6 months of age [11]. Several studies reported clinical improvement in patients with severe atopic eczema with high serum IgE level [12]. Additionally, the drugs other than omalizumab that target JAK-STAT cascade or TH17 differentiation may be a potential successful treatment in HIES [13]. Severe staphylococcal infection seen in HIES patients may reflects IgE impacts on ongoing proinflammatory state. The activation of extrinsic pathway by TF then generates thrombin, which leads to DD formation [3]. We think that anti-IgE treatment with omalizumab inhibited activation of extrinsic pathway and lowered DD levels by blocking free IgE. Because of this, we think that omalizumab has a similar effect with heparin. After the injection of heparin, an increase in the percentage of pC/pS has been observed.

Besides its anticoagulant properties, heparin possesses a wide range of anti-inflammatory activities, including inhibition of proinflammatory mediators, such as eosinophil cationic peptid (ECP), peroxidase, neutrophil elastase and inhibition of lymphocyte activation [14]. Anticoagulant treatment with heparin and warfarin had been attempted to reduce the symptoms of CU and SPA; however, inhaled heparin is no longer used in clinical practice as adjunctive therapy for SPA attacks because of equivocal results [3-5].

Our knowledge concerning the use of omalizumab in treatment of SPA and other allergic diseases has improved our understanding that treatment acts on many levels, including affecting levels of oxidative stress markers (copper-containing α -2-glycoprotein, total antioxidant capacity, hydrogen peroxide, malondialdehyde, total nitric oxide concentrations), and regulating levels of inflammatory proteins, including TH1-2 cytokines, PT, PTT, INR, MPV, platelet count, Hs-CRP, ECP, vitamin-D and albumin [2,15-17]. After omalizumab therapy, significant decrease in the levels of DD observed in our study shows the importance of procoagulant state in allergic patients. In group-IA, group IIA and group IIA-2, there were a significant positive correlation between DD and IgE. We suggest that DD may have an important role in the relationship between IgE and extrinsic pathway of coagulation, that is, endothelial cells.

In conclusion, mediators and cells classically involved in pro-coagulant and anticoagulant pathways together play a role in SPA and CU pathophysiology where omalizumab has its effect.

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conceived and designed the study: AD Yalcin. Clinical follow up: AD Yalcin. Analyzed the data: S Gumuslu. Contribution of reagents/materials: AD Yalcin. Writing of the paper: AD Yalcin, B Celik.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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