

D-Dimer Plasma Levels Parallel the Clinical Response to Omalizumab in Patients with Severe Chronic Spontaneous Urticaria

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Key Words

Chronic urticaria · Omalizumab · D-dimer · Coagulation

Abstract

Omalizumab is very effective in the majority of patients with severe chronic spontaneous urticaria (CSU), but its mechanism of action is still unclear. In CSU the coagulation cascade is activated with an intensity that parallels the disease severity, and elevated plasma D-dimer levels are associated with a poor response to both antihistamines and cyclosporin. We measured D-dimer plasma levels before and after the first administration of omalizumab in 32 patients with severe CSU. A number of clinical and laboratory parameters were recorded, including the urticaria activity score, presence of angioedema, disease duration, C-reactive protein, anti-nuclear, and anti-thyroid antibodies. Baseline D-dimer levels were elevated in 19 (59%) cases. Omalizumab induced a complete response in 25 patients (78%), in most cases already after the first administration. At baseline, 14/25 responders had increased D-dimer plasma levels versus 5/7 non-responders. All responders showed a dramatic decrease of D-dimer plasma levels after the first administration of the drug (from $1,024 \pm 248$ [mean $\pm$ SE] to 251 ± 30 ng/mL; $p = 0.003$). In contrast, non-responders did not show any reduc-

tion in D-dimer levels after omalizumab administration (from 787 ± 206 to $1,230 \pm 429$ ng/mL; $p = \text{ns}$). In conclusion, plasma levels of D-dimer are frequently elevated in patients with severe CSU before omalizumab administration and decrease according to the clinical response of the disease to the drug, suggesting a possible effect of omalizumab on coagulation activation and fibrin degradation in a subset of CSU patients.

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Introduction

During the last 10 years a number of case reports and case series along with 3 phase III studies [1–3] and a real-life study [4] have shown that the humanised monoclonal anti-IgE antibody omalizumab is effective in patients with severe chronic spontaneous urticaria (CSU) not responding to antihistamines at higher than licensed doses. However, despite its often prompt efficacy, there are a proportion of patients ranging between 7 and 48% who do not show a satisfying response to the drug [5]. Interestingly, most of these omalizumab-unresponsive patients were corticosteroid-responsive before, whereas in other cases patients may become omalizumab-resistant over time [6, 7]. The reasons why some patients fail to

respond to anti-IgE treatment are still unclear, but this highlights the fact that several distinct pathways, all eventually leading to histamine release from mast cells and basophils, might play a role in the pathogenesis of the disease and that the relative relevance of such pathways may change from one patient to another or even in the same patient in different phases of the disease. Recent studies found that the coagulation cascade is activated via the extrinsic pathway in CSU, and that the intensity of such activation parallels disease severity [8–10]. Furthermore, it has been shown that elevated plasma D-dimer levels are associated with a poor response to both antihistamines and cyclosporine [11, 12]. In the present study, we measured D-dimer plasma levels before and after the first administration of omalizumab in patients with severe CSU and analysed the results in the light of clinical response to the treatment.

Patients and Methods

Patients

Thirty-two subjects (mean age 50 years, range 17–78 years; M/F 11/21) with severe CSU (urticaria activity score [UAS 7] >30; mean disease duration 43.1 months, range 2–120) unresponsive to second generation antihistamines at higher than licensed doses [13] were studied. A course of subcutaneous omalizumab 300 mg every 4 weeks for 3 months was started in all patients. The presence of angioedema and disease exacerbations induced by non-steroidal anti-inflammatory drugs (NSAIDs) were recorded, and thyroid autoantibodies, anti-nuclear antibodies (ANA), C-reactive protein (CRP), and the fibrin fragment D-dimer were measured before the start of omalizumab therapy. D-dimer plasma levels were remeasured 3 weeks after the first dose of omalizumab, and the results were analysed in terms of the clinical response to the drug.

Plasma from 30 healthy subjects (10 men and 20 women, median age 54 years, range 20–76) undergoing routine check-up investigations served as normal controls. For blood sampling, sodium citrate anti-coagulated plasma samples taken from all patients and normal subjects were stored in Falcon tubes at –20°C until in vitro assay.

All patients agreed with the treatment regimen and signed a written consent form. Control subjects provided written consent to the use of their serum/plasma in an anonymous form for research purposes. Since all treatments and investigations were carried out within the standard routine management of CSU patients, in view of the purely observational nature of the study, no formal approval by the local ethical committee was required.

Methods

D-dimer levels were measured by means of ELISA (Zymutest D-Dimer; Hyphen BioMed, Neuville-sur-Oise, France) in accordance with the manufacturer's instructions. The intra- and inter-assay coefficients of variation were 10 and 15%, respectively. Levels below 500 ng/mL were regarded as normal. The CRP plasma concentration was measured using a sandwich enzyme immunoassay

(Zymutest CRP, Hyphen BioMed). Intra- and interassay variability was lower than 11%.

ANA were tested by indirect immunofluorescence on HEp2 cells; samples were considered positive at a dilution $\geq 1:160$. Anti-thyroglobulin and anti-thyroid peroxidase antibodies were measured with commercial immunoenzymatic methods (NovaTec, Dietzenbach, Germany).

Statistics

We assessed differences using the paired sample *t* test and associations using the χ^2 test and logistic regression analysis. The level of significance was set at $p < 0.05$. The data were analysed using the SPSS PC statistical package, version 22.00 (IBM SPSS Inc., Chicago, IL, USA).

Results

Table 1 summarises the demographic, clinical, and laboratory features of the 32 CSU patients enrolled in the study. Twelve patients (37.5 %) had a history of angioedema episodes associated with urticaria, and 4 (12.5%) had a history of severe urticaria exacerbations after taking cyclooxygenase-1 (COX-1)-inhibiting NSAIDs. At baseline (before omalizumab treatment) 5 patients showed positive ANA (speckled pattern in 4 cases, nucleolar in 1 case), 8 (25%) showed thyroid autoimmunity, and 10 showed elevated CRP. Baseline D-dimer levels were elevated (>500 ng/mL) in 19 (59%) cases. Omalizumab induced a complete (UAS 7 = 0) or almost complete (UAS 7 = 1–14) response in 24 patients (75%), in most cases already after the first administration of the drug. Most of these patients reported a marked improvement as quickly as 3–7 days after the shot. One patient reported a partial response (UAS dropped to 15–21) after the first administration, whereas 7 patients (22%) showed a poor response or did not show any response to the drug after the first administration and did not improve thereafter. The lack of response to omalizumab was not associated with any specific clinical or baseline laboratory parameter (age, sex, disease duration, NSAID hypersensitivity, thyroid autoimmunity, or positive ANA). When examining omalizumab response as a function of baseline D-dimer plasma levels, it turned out that 15/19 (79%) of those showing elevated D-dimer and 9/13 (69%) of those showing normal baseline D-dimer levels showed a complete response to omalizumab ($p = \text{ns}$). In all responders, D-dimer plasma levels showed a dramatic decrease after only the first administration of omalizumab (from $1,024 \pm 248$ [mean $\pm$ SE] to 251 ± 30 ng/mL; $p = 0.003$), and in the majority of patients D-dimer levels fell within the normal range (Fig. 1). In contrast, non-responders did not

Table 1. Demographic, clinical, and laboratory features of 32 patients with CSU undergoing omalizumab treatment

Pa-tient No.	Sex	Age, years	Disease duration, months	AE	a-TPO	ANA	CRP, µg/mL	NSAID	Previous treatments	Response	Latency, days	D-dimer, baseline, ng/mL	D-dimer, treatment, ng/mL
1	M	77	36	0	1	1	11.90	0	CS, CyA	0	–	808	847
2	F	47	18	1	1	1	14.20	0	CS, CyA	4	3	1,200	140
3	M	47	9	0	0	0	7.80	0	CS, CyA	4	3	787	170
4	M	65	18	1	0	0	12.80	1	CS	4	2	1,815	657
5	F	51	15	0	1	0	1.20	0	None	4	3	260	240
6	F	51	120	0	0	0	8.25	0	CS, CyA	4	4	670	194
7	F	71	60	0	0	0	16.10	0	CS	0	–	805	3,446
8	F	39	4	1	0	0	0.83	1	CS	4	4	280	237
9	M	76	24	0	0	0	3.20	0	CS	0	–	1,080	1,271
10	F	47	4	1	1	0	4.90	0	CS	4	5	3,929	419
11	F	17	2	1	0	0	0.89	0	CS	4	4	320	260
12	M	41	6	0	1	0	1.23	0	CS, CyA	3	7	280	210
13	F	54	120	0	0	0	3.90	0	CS, CyA	4	5	563	144
14	F	76	120	0	0	0	5.90	0	None	0	–	1,800	1,880
15	F	78	80	1	0	0	5.20	0	CyA	4	4	1,428	184
16	M	24	4	1	0	0	1.20	0	CS	0	–	230	240
17	F	61	48	1	0	0	4.00	0	CS	4	1	564	159
18	M	39	100	1	1	0	5.80	1	CS	4	2	2,096	200
19	F	75	6	0	1	0	6.50	0	CS	4	7	260	220
20	F	21	2	0	0	0	0.76	0	None	3	10	180	160
21	F	50	131	1	1	1	1.00	0	CS, CyA	3	6	194	168
22	F	70	36	0	0	0	0.65	0	CS	2	7	210	187
23	F	37	60	0	0	0	1.17	0	None	3	4	912	306
24	M	51	18	0	0	0	13.69	0	CS	4	1	1,330	117
25	F	60	33	1	0	0	8.87	0	CS, anti-H2	3	5	1,296	431
26	F	21	46	0	0	0	4.78	0	CS, anti-H2	4	1	348	230
27	F	31	39	0	0	0	0.48	0	None	0	–	554	656
28	M	54	19	0	0	1	1.58	0	CS	4	1	234	103
29	F	15	28	0	0	1	0.23	0	None	4	1	288	84
30	M	59	62	0	0	0	3.48	0	None	3	1	752	428
31	F	39	47	0	0	0	12.30	0	None	0	–	234	268
32	M	69	66	1	0	0	13.63	1	CS, anti-H2	4	1	5,403	631

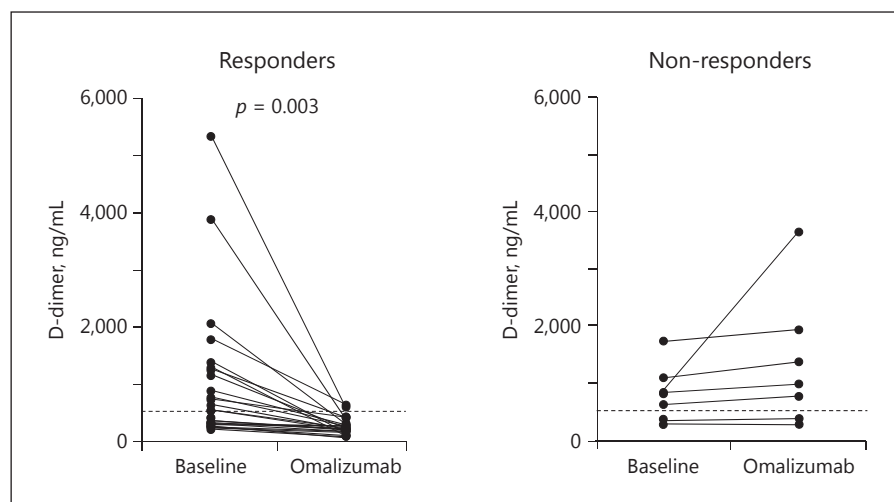
AE, angioedema; a-TPO, anti-thyroid peroxidase antibodies; ANA, anti-nuclear antibodies; CRP, C-reactive protein; NSAID, non-steroidal anti-inflammatory drug-induced exacerbation (0, absent; 1, present); CS, corticosteroids; CyA, cyclosporin A; anti-H2, H2 antihistamines; Response, response to omalizumab (0, UAS-7 unchanged; 1, UAS-7 reduced to 22–28; 2, UAS 7 reduced to 15–21; 3, UAS-7 reduced to 8–14; 4, UAS-7 reduced to 0–7).

show any reduction in D-dimer plasma levels after omalizumab administration, which, on the contrary, increased from 787 ± 206 to $1,230 \pm 429$ ng/mL ($p = \text{ns}$; Fig. 1). CSU patients with associated angioedema had higher D-dimer plasma levels ($1,563 \pm 467$ ng/mL) than CSU patients without angioedema (618 ± 96 ng/mL; $p = 0.018$) and their rate of response to omalizumab was also higher (92 vs. 70%), but the difference did not reach statistical significance. D-dimer plasma levels did not significantly correlate with CRP plasma levels, anti-nuclear and anti-thyroid antibodies or disease duration.

Discussion

The mechanism of action of omalizumab in CSU is not clearly understood. It has been suggested that most of its clinical effects rely on the downregulation of the high affinity IgE receptor on mast cells and basophils, which eventually leads to a reduced activation and to a decrease or stop of degranulation and release of inflammatory mediators from these cells [14]. Such effects require 3–4 weeks to occur, at least on mast cells, which is far longer than the few days frequently needed to appreciate a

Fig. 1. D-dimer plasma levels in 32 patients with CSU divided into responders ($n = 25$) and non-responders ($n = 7$) to omalizumab. Dotted lines represent the upper limit of normal. Values <500 ng/mL are considered as normal.



marked decrease in urticaria severity score. Thus, other mechanisms with a more rapid action are possibly also involved. In fact, omalizumab exerts a number of other effects [5] at different levels, including those on the coagulation cascade, which may lead to a marked reduction of D-dimer plasma levels [15]. Furthermore, omalizumab has been shown to induce the apoptosis of eosinophils [16], a cell type that we recently found to be the initiator of the activation of the coagulation cascade in chronic urticaria by the hyper-expression of tissue factor [17]. The pathogenic roles of thrombin and other coagulation factors in CSU are still debated. One possibility is that the activation of the coagulation cascade non-specifically mirrors the general inflammatory state that underlies the disease. However, if this were the case, one would expect to find elevated D-dimer plasma levels in all patients with severe, antihistamine-resistant urticaria. In contrast, as the subjects with severe CSU investigated in this study show, D-dimer plasma levels are elevated only in a proportion of cases (60% in our population), which suggests that the activation of the coagulation cascade may represent a potential pathogenic mechanism in a specific subset, but not in all CSU patients. In animal models, thrombin and other coagulation factors have been shown to induce mast cell degranulation with a potency similar to that of FcεRI-mediated activation via the so-called protease-activated receptors 1 and 2 (PAR-1 and PAR-2) [18], and we cannot rule out that such a mechanism is active in a proportion of patients with CSU.

In accordance with previous studies [1–4], omalizumab was effective in the majority of patients with severe CSU but not in the totality of patients. In our hands, the

drug was equally effective in patients showing elevated or normal baseline plasma levels of D-dimer. However, in those showing elevated baseline D-dimer levels the clinical response to the drug was paralleled by a rapid and impressive decrease of coagulation activation [9], whereas in treatment failures D-dimer levels remained unchanged or even increased during the treatment. The longer we investigate CSU, looking at clinical expression, pathophysiological aspects, and response to therapies, the higher is the number of different phenotypes that seemingly characterise this complex disease. It is possible that the activation of the coagulation cascade characterises one specific phenotype strictly paralleling the state of activity of the disease in these subjects and possibly playing a pathogenic role as well.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

The authors contributed to the manuscript by planning the study (R.A.), providing clinical data (R.A., A.V.M, S.F., M.C.) and assembling and analysing the data.

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