

Effectiveness of Autologous Whole-Blood Injections in Patients with Refractory Chronic Spontaneous Urticaria

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Keywords

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Abstract

Background: Nonsedating antihistamines are the treatment of choice for chronic spontaneous urticaria (CSU), while omalizumab and immunosuppressants have also been approved as an add-on treatment. Autologous whole-blood injection (AWBI) has been used in previous studies with ambiguous results. The aim of our study was to evaluate changes in the Urticaria Activity Score (UAS7), Dermatology Life Quality Index (DLQI), and Chronic Urticaria Quality of Life (CU-Q2oL) score, and also the association of serologic markers with disease severity measures after AWBI. **Methods:** In this observational study, AWBIs were performed (8 courses on a weekly basis) in adults with refractory CSU, who refused an add-on treatment with either omalizumab or immunosuppressants. UAS7, DLQI, and CU-Q2oL questionnaires and serum concentrations of total IgE, C-reactive protein (CRP), and D-dimer were evaluated before and after the intervention. **Results:** Nineteen patients (12 females; mean age 54 ± 20.8 years) completed the protocol. Following AWBI, signifi-

cant improvements in the UAS7 (34.26 ± 8.04 vs. 12.52 ± 10.83 , $p < 0.001$), DLQI (11.63 ± 5.51 vs. 3.47 ± 2.85 , $p < 0.001$), and CU-Q2oL score (32.97 ± 18.71 vs. 10.94 ± 7.71 , $p < 0.001$) were recorded. A negative correlation between the baseline D-dimer levels and UAS7 and DLQI variations ($p = 0.002$ and $p = 0.001$, respectively) was noted. D-dimer levels ≥ 292 ng/mL have been associated with poor responsiveness (sensitivity 75%; specificity 83.3%). No correlation with either total immunoglobulin E or CRP levels was observed. **Conclusion:** AWBI appears to be a safe, alternative, add-on therapeutic option in refractory CSU, particularly in patients with low plasma levels of D-dimer.

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Introduction

Chronic spontaneous urticaria (CSU) is a skin disorder characterized by the spontaneous eruption of short-lived (<24 h), cutaneous wheals accompanied by redness and itching, with or without concomitant angioedema, for a period of >6 weeks [1].

All data including the informed consent and the approval by the ethics committee are available upon request.

Several treatment options are available for CSU patients. H₁-antihistamines are considered the “gold-standard” therapeutic approach, although refractory cases, even with quadruple doses, have been reported [1]. Omalizumab is a chimeric anti-immunoglobulin E (IgE) monoclonal antibody that binds to free IgE and downregulates the function of mast cells and basophils. Recent meta-analysis has provided high-level evidence for the efficacy and safety of omalizumab in patients with refractory CSU [2]. Nevertheless, several issues with respect to anti-IgE treatment are still of concern, such as the lack of evidence for CSU immunomodulation or symptomatology recurrence following discontinuation [3, 4]. Although leukotriene antagonists are proposed in the European Academy of Allergy and Clinical Immunology/Global Allergy and Asthma European Network (EAACI/GA²LEN) guidelines in cases of refractory chronic urticaria, well-designed randomized controlled trials about their role in CSU treatment are lacking [5]. In addition, corticosteroid treatment is proposed only as a “short-bursts” intervention (maximum 10 days) in cases of unresponsiveness [1]. Finally, for other proposed anti-inflammatory agents (dapsone) or immunosuppressants (cyclosporine), safety issues of dose-related adverse side effects are of concern [6].

Autologous whole-blood injection (AWBI) has been proposed as a therapeutic intervention for the treatment of CSU; there are conflicting results concerning its effectiveness, but it does not have severe side effects [7, 8].

Various laboratory markers have occasionally been associated with CSU severity. Data from earlier studies are suggestive of an association of disease severity measures with serum levels of total IgE, D-dimer and C-reactive protein (CRP) [8–13].

The aim of our study was to determine the effectiveness of AWBI in patients with CSU unresponsive to treatment with H₁-antihistamines, and also identify potential associations of IgE, D-dimer, and CRP with disease severity.

Material and Methods

Patients

We evaluated adult patients with physician diagnosed CSU, unresponsive to treatment with second-generation H₁-antihistamines up to quadruple doses or reported side effects (somnolence, anxiousness, and reduced work performance), who visited the outpatient Dermatology Clinic at the General University Hospital of Larissa. CSU diagnosis was based on continuous or recurrent hives with or without angioedema, lasting ≥6 weeks. Omalizumab and/or immunomodulatory therapy were suggested to patients as a

first-choice add-on treatment, according to EAACI guidelines; when this was refused, AWBI was offered as a second-choice add-on therapy, following a detailed written and verbal description of the procedure.

Exclusion criteria were: a personal history of reported food or drug IgE-mediated reactions, chronic diseases, pregnancy or lactation during the study period, and the use of anticoagulants. Topical or systemic corticosteroid treatment or other immunosuppressants were withheld 4 weeks prior to treatment.

Study Design

This was an observational study, performed between August 2013 and August 2015. At baseline, patients included in the AWBI protocol were assessed by means of detailed questionnaires, clinical evaluation, and laboratory screening for secondary causes of CSU. CSU severity was assessed by the Urticaria Activity Score 7 (UAS7), while for patients' quality of life Dermatology Life Quality Index (DLQI) and the validated Greek version of Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL) [3, 14, 15] were applied. The aforementioned questionnaires were filled in by patients at baseline (week 0) and upon completion of the AWBI treatment.

Further evaluation included blood sampling for complete blood count, including eosinophil count, and erythrocyte sedimentation rate. A serum sample was obtained at baseline for renal, liver, and thyroid function tests, and levels of total protein, albumin, C3, and C4. D-dimer, total IgE, and C-reactive protein (CRP) levels were assessed both at baseline and following ABWI intervention. Moreover, evaluation for autoimmune diseases (rheumatoid factor, antinuclear antibodies, anti-double-stranded DNA) and hepatitis infections was performed. An autologous serum skin test (ASST) was performed at baseline, according to standardized EAACI/GA²LEN protocol [16]. Urine/stool microscopy and culture and chest X-ray were performed in all cases, while anti-*Helicobacter pylori* antibodies (IgM, IgG) were determined in patients with chronic gastric complaints. For atopy assessment, total IgE levels and skin prick tests (ALK-Abelló) were used, using the standard proposed panel by GA²LEN [17]. H₁-antihistamines were withheld 7 days prior to skin prick testing, wherever possible.

Within 1 week after screening, AWBIs were administered; treatment duration was 8 weeks, including 1 intramuscular gluteal injection of autologous whole blood per week (alternating sides), according to the proposed methodology [7, 8]. Specifically, blood was taken from the cubital vein and was injected intramuscularly without further processing. The initial volume injected was 2.5 mL, and 5 mL was administered subsequently. Antihistamines were administered on a per-needed basis.

In order to assess disease activity, reevaluation by telephone questionnaires was planned for 8 months after completion of the intervention.

All patients provided written consent, and study procedures were in accordance with the standards of the General University Hospital of Larissa ethics committee.

Statistical Analysis

For statistical analysis, we used the Spearman ρ and the Pearson r for bivariate correlations, the Pearson χ^2 test to compare qualitative variables, the Student t test for comparing means, and the Mann-Whitney U test for nonparametric testing. For the assessment of paired samples we used the paired-samples t test and the

Table 1. Patients' sex, age, ASST result, and reported antihistamine treatment before and after AWBI intervention

Patient No.	Sex	Age, years	ASST	Daily antihistamine dose before AWBI	Daily antihistamine dose after AWBI
1	M	16	P	desloratadine 20 mg	none
2	F	80	P	desloratadine 20 mg	desloratadine 10 mg
3	M	43	N	ebastine 10 mg + rupatadine 10 mg	none
4	F	52	N	hydroxyzine 100 mg	none
5	F	64	N	levocetirizine 20 mg	none
6	F	46	P	ebastine 20 mg	ebastine 10 mg
7	F	19	P	ebastine 20 mg	occasional use
8	F	57	P	ebastine 10 mg + rupatadine 10 mg	occasional use
9	F	60	N	levocetirizine 20 mg	none
10	F	18	N	cetirizine 20 mg	cetirizine 10 mg
11	F	57	P	ebastine 20 mg	occasional use
12	M	54	N	cetirizine 20 mg	cetirizine 20 mg
13	F	46	N	desloratadine 20 mg	desloratadine 20 mg
14	F	83	P	bilastine 40 mg	occasional use
15	M	76	P	levocetirizine 20 mg	levocetirizine 20 mg
16	M	79	P	bilastine 40 mg	bilastine 40 mg
17	F	78	P	bilastine 40 mg	none
18	M	50	N	levocetirizine 20 mg	occasional use
19	M	40	N	bilastine 40 mg	none

M, male; F, female; P, positive; N, negative.

Wilcoxon test, while the receiver operating characteristics (ROC) curve was performed to determine patients' D-dimer levels, which corresponded to the highest specificity and sensitivity in relation to patients' treatment response. Normality of our data was assessed by the Kolmogorov-Smirnov test and the Shapiro-Wilk test. SPSS v20.0 statistical software was used for all analyses and α was set to 0.05 to all calculations.

Results

Thirty patients were invited to participate in the study. Nine of them refused the first-choice add-on treatment due to skepticism for recently approved interventions, while 2 dropped out due to needle-phobia.

Nineteen patients (12 females; mean age of 54 ± 20.8 years) proceeded and completed the AWBI protocol. Investigation for underlying pathologies was negative in all cases. Regarding the ASST results, 10 patients were ASST positive. Patients' baseline characteristics are listed in Table 1. Eight patients were able to discontinue antihistamines prior to treatment; 11 reported occasional use.

A significant improvement in patients' UAS7 following AWBI treatment (34.26 ± 8.04 vs. 12.52 ± 10.83 before and after AWBI, respectively, $p < 0.001$) was noted, while

Table 2. Patients' UAS7, DLQI, and CU-Q₂oL scores before and after AWBI, corresponding to the differentiation of disease severity and patients' quality of life before and after intervention

Patient No.	UAS7 before/after AWBI	DLQI before/after AWBI	CU-Q ₂ oL before/after AWBI
1	42/7	12/4	51.38/14.23
2	42/7	11/1	65.27/12.15
3	35/0	19/0	52.08/10.41
4	42/0	14/4	46.18/15.97
5	21/7	9/4	18.05/8.67
6	35/21	16/10	53.47/32.98
7	35/7	23/3	52.77/4.86
8	42/14	12/5	37.5/18.4
9	28/0	9/0	24.3/0
10	28/14	11/8	22.91/11.11
11	42/7	17/3	36.8/13.19
12	21/14	3/2	19.09/11.8
13	28/14	6/1	19.09/2.08
14	21/14	4/1	5.2/1.04
15	42/42	6/6	15.62/15.62
16	35/28	7/7	13.54/13.54
17	42/7	8/2	22.91/11.1
18	28/14	15/5	10.76/10.76
19	42/0	19/0	59.59/0

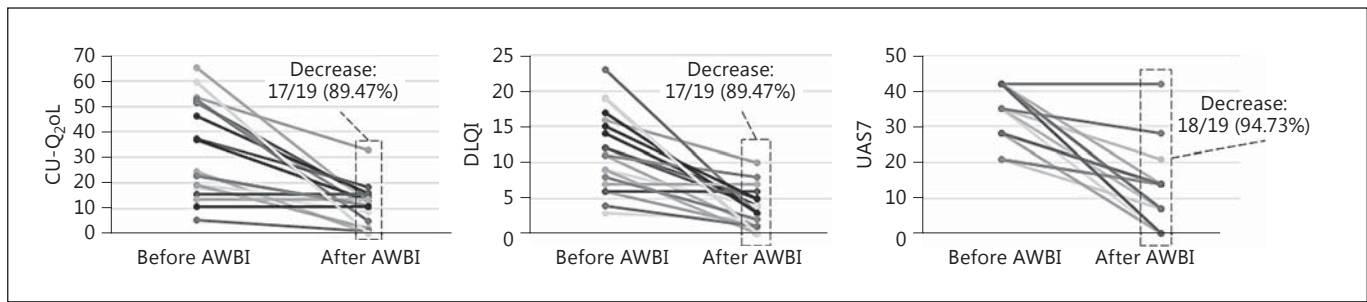


Fig. 1. Individual variations in CU-Q₂oL, UAS7, and DLQI scores before and after AWBI intervention.

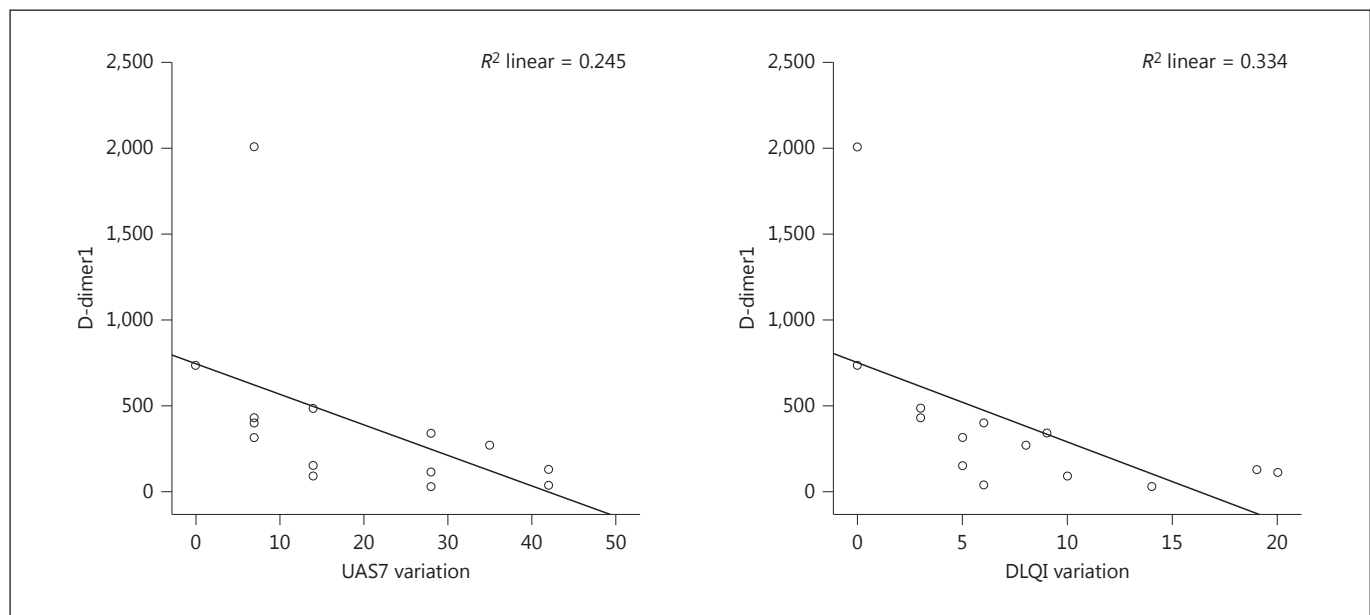


Fig. 2. Negative correlation between patients' baseline D-dimer (D-dimer1) levels and variations in UAS7 and DLQI scores, before and after AWBI intervention.

10/19 patients presented a reduction of UAS7 ≥ 28 units. Corresponding significant improvements were noted for DLQI (11.63 ± 5.51 vs. 3.47 ± 2.85 , $p < 0.001$) and CU-Q₂oL (32.97 ± 18.71 vs. 10.94 ± 7.71 , $p < 0.001$) before and after treatment (Table 2). Individual variations in UAS7, DLQI, and CU-Q₂oL scores are shown in Figure 1.

A statistically significant negative correlation concerning patients' D-dimer baseline values and UAS7/DLQI variation ($r_s = -0.745$, $p = 0.002$ and $r_s = -0.797$, $p = 0.001$, respectively, Fig. 2) was observed, but no such association was observed for the CU-Q₂oL variation ($r_s = -0.440$, $p = 0.116$).

Patients were grouped as "good responders" when the improvement in UAS7 score following AWBI ranged from 14 to 42 units, while patients with lesser improve-

ments were grouped as "non-responders." In a ROC analysis of the ability of baseline D-dimer levels to discriminate between "good and non-responders," values of 292 ng/mL yielded an area of 0.854, $p = 0.028$, sensitivity 75%, specificity 83.3% (Fig. 3).

A trend between disease activity prior to AWBI intervention and treatment response was also noted ($p = 0.086$).

No significant associations were observed in D-dimer (394.8 ± 506.1 ng/mL vs. 285.35 ± 237.84 ng/mL, $p = 0.966$), IgE (194.95 ± 138.35 IU/mL vs. 192.95 ± 141.78 IU/mL, $p = 0.909$), and CRP levels (0.58 ± 0.79 mg/dL vs. 0.85 ± 1.32 mg/dL, $p = 0.826$), before and after AWBI. Moreover, variations in DLQI, CU-Q₂oL, and UAS7

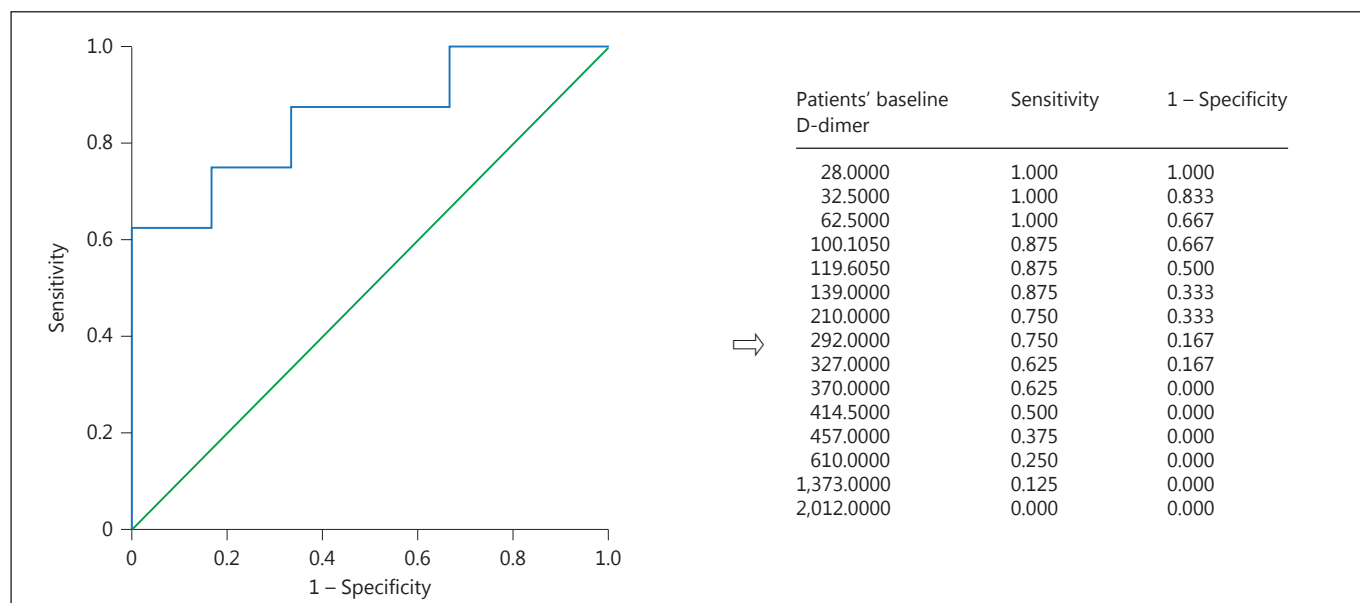


Fig. 3. Comparison of “good responders” (UAS7 variation >14) and “non-responders” (UAS7 variation ≤14), with respect to patients’ baseline D-dimer levels (AUC = 0.854, $p = 0.028$). The arrow points out the D-dimer level (292 ng/mL) with the highest specificity and sensitivity.

scores were not associated with either IgE and CRP levels, or ASST positivity.

No significant associations were recorded for ASST-positive patients with respect to treatment response, sex, or D-dimer/IgE/CRP levels at baseline.

After the completion of AWBI treatment, 15 patients reported complete disease remission, while in 4 patients no notable difference was observed. With respect to antihistamine requirements, 7 patients reported a complete discontinuation, 5 occasional and 3 daily use, but all in significantly reduced doses compared to prior to the intervention (Table 1).

When patients were assessed by telephone questionnaire at 8 months post-intervention, no significant differentiation in the disease’s clinical course (compared to immediately post-intervention) was identified.

Local side effects following the AWBI procedure were reported in 3 patients (such as minimal local bleeding, swelling and discomfort).

Discussion

In our study, no severe adverse side effects were reported for patients following AWBI for the treatment of CSU. Furthermore, a considerable proportion of patients

with refractory CSU to antihistamines presented with a great improvement in their clinical symptoms and quality of life, as assessed by significant improvements in their UAS7, DLQI, and CU-Q2oL scores. In addition, low D-dimer plasma levels correlated with treatment efficacy. Higher disease activity prior to our intervention seemed to be related to greater treatment response. Elevated baseline D-dimer levels in our study were associated with poor outcome, as assessed by the UAS7 variation following AWBI, in line with Asero et al. [18].

We did not observe differences with respect to the treatment responses in ASST-positive and ASST-negative patients, as opposed to previous reported results [8].

The pathogenesis of chronic urticaria involves both the intrinsic and extrinsic coagulation pathway [13, 19, 20], although the precise underlying pathogenetic mechanisms are largely unknown. It is well established that thrombin enhances vascular permeability, and activates and degranulates mast cells, thus generating anaphylatoxin C5a [21].

The mechanism of action of AWBI in patients with CSU is largely unknown. It has been suggested [8] that AWBI could alter the immune response, from a Th2 to a Th1 pattern, based on previously reported efficacy in ASST-positive patients, but we did not confirm such association. Moreover, documentation of a specific antigen

responsible for the induction of Th2 response has not been achieved [22]. A second theory [23] supports the notion of potential induction of anti-idiotypes that might contribute to the release of disease-triggering autoantibodies. Although such an approach is quite interesting, further investigation is needed.

Since normalization of D-dimer values was not observed in the majority of patients after an 8-week follow-up, it is hypothesized that the observed effectiveness of the method cannot be attributed to direct intervention into the coagulation system.

Although a significant proportion of our patients remained asymptomatic after 8 months, this could be attributed to the natural course of the disease and not the AWBI treatment per se.

Weaknesses of this study were the lack of a control group, due to ethical, logistic and financial reasons, and the small sample size. In addition, withdrawal of antihistamines prior to treatment could have been a confounding factor for the final analysis, since any intervention could potentially lead to disease improvement. Nevertheless, the observed high effectiveness of AWBI and the safety of the method underscore the need to perform additional studies to further evaluate its therapeutic role in CSU.

In conclusion, we have observed that AWBI could serve as a safe, alternative, add-on therapeutic option for patients with refractory CSU who are unresponsive to conventional treatment with H₁-antihistamines, especially those with low plasma levels of D-dimer.

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