

Autologous Whole Blood Injections to Patients with Chronic Urticaria and a Positive Autologous Serum Skin Test: A Placebo-Controlled Trial

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Key Words

Autohemotherapy · Autoimmune · Autologous serum skin test · Urticaria · Whole blood injection

Abstract

Background: Patients with chronic urticaria (CU) frequently exhibit positive skin test reactions to autologous serum (ASST). Therapies aimed at inducing tolerance to circulating histamine-releasing factors in ASST+ CU patients, e.g. by treatment with autologous whole blood (AWB), have not yet been tested. **Objective:** To test whether ASST+ CU patients can benefit from repeated low-dose intramuscular injections of AWB. **Methods:** We characterized CU severity and duration, anti-Fc_εRI and anti-IgE expression, use of antihistamines, and quality of life in 56 CU patients (ASST+: 35, ASST–: 21) and assessed the therapeutic effects of 8 weekly AWB injections in a randomized, placebo-controlled, single-blind, parallel-group trial. **Results:** Numbers, size, intensity, and/or duration of CU symptoms, quality of life, as well as expression of anti-Fc_εRI or anti-IgE were similar in

ASST+ and ASST– CU patients. However, CU in ASST+ patients was of longer duration and required markedly more antihistaminic medication. Interestingly, ASST+ patients, but not ASST– patients, showed significantly (1) reduced CU activity, (2) decreased use of antihistamines, and (3) improved quality of life after AWB treatment. Placebo treatment was ineffective in both groups, but differences of AWB and placebo treatment responses did not achieve statistical significance in either group, most likely due to the limited number of patients treated. **Conclusion:** Our findings suggest that ASST+ CU is clinically different from other CU subforms and that ASST+ CU patients can benefit from AWB therapy.

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Introduction

Chronic urticaria (CU), a common dermatosis characterized by spontaneously occurring short-lived and itching weal and flare type skin reactions, can be caused by a large number and a variety of distinct conditions including intolerance to food or drugs as well as infectious and autoimmune diseases [1, 2]. About 10 years ago, Hide et al. [3] reported that the intracutaneous injection of serum, a procedure also known as autologous serum skin test

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(ASST), results in immediate hypersensitivity type skin reactions in a subpopulation of CU patients. This observation has been confirmed by several other reports showing that ASST-positive (ASST+) patients account for 41–67% of all CU patients [4–7]. Today, CU in ASST+ patients is widely regarded to represent a distinct subgroup of CU termed ‘autoimmune’ CU [8]. This notion is supported by several independent lines of evidence: (1) ASST+ CU patients have been shown to express autoantibodies directed against the high-affinity receptor for IgE (anti-Fc_εRI) or IgE (anti-IgE) [6]. (2) These circulating antibodies can release histamine from mast cells and basophils [9], and injections of anti-Fc_εRI IgG in healthy volunteers cause urticarial reactions [8]. (3) Disease activity in ASST+ patients has been shown to decrease after plasmapheresis [10]. (4) ASST+ patients have been reported to develop other autoimmune diseases more often than ASST-negative (ASST–) CU patients [11]. It remains unknown whether or not CU activity in ASST+ CU patients can be decreased by tolerization, i.e. by repeated treatment with the mast cell secretagogues that are present in the sera of these CU patients [8].

As circulating histamine-releasing factors including autoantibodies are responsible for the induction of urticarial symptoms in ASST+ CU patients [9], autohemotherapy, a historic form of CU treatment [12], may be regarded as a promising specific and potentially curative therapeutic option for this subgroup of CU patients. Autohemotherapy, i.e. repeated intramuscular, intravenous, or oral applications of autologous whole blood (AWB) sometimes treated with ozone or UV light, was commonly used to treat CU patients before the development and introduction of antihistamines [12–14]. AWB injections, which are also claimed to have therapeutic value in allergic diseases, circulatory disorders, viral diseases, and cancer [15,16], are still popular in central Europe, where they are mostly performed in private clinics, even though there is little evidence from rigorous clinical trials to suggest that they are effective [17].

Speculating that ASST+ CU patients can benefit from repeated low-dose applications of the circulating histamine-releasing factors responsible for their urticarial symptoms, we conducted a randomized, placebo-controlled, single-blind, parallel-group trial to test the efficacy of intramuscular injections of AWB. 35 ASST+ and 21 ASST– CU patients treated with weekly AWB or placebo injections for 8 weeks were examined for treatment effects using CU severity, requirement of antihistaminic rescue medication, and life quality as outcome measures.

Subjects and Methods

Patients

Fifty-three of a total of 135 CU patients (≥ 6 weeks duration of disease) subjected to skin tests with their own serum (see below) exhibited positive ASST reactions. 35 (females: 26; age: 42.1 ± 2.9 years) of these 53 ASST+ patients were compared with 21 ASST– CU patients (females: 15; age: 45.5 ± 4.0 years) for differences in clinical parameters and were then treated with repeated injections of AWB or placebo. Each patient gave written informed consent. Exclusion criteria were: CU due to predominantly physical causes, pregnancy, lactation or risk of pregnancy without medically approved contraception, severe systemic diseases, as well as anticoagulation or systemic corticoid therapy. All patients underwent a pretreatment evaluation consisting of clinical assessment including routine blood screening for undetected systemic illnesses.

Autologous Serum Skin Test

Venous blood was taken and serum was obtained by centrifugation (5,000 g, 10 min, 21 °C). Samples (100 μ l) of autologous serum, histamine (1 mg/ml, ALK-Scherax, Hamburg, Deutschland) and human serum albumin (0.3 mg/ml, ALK-Scherax) were separately injected intracutaneously (lower arm). Intervals of at least 5 cm were left between injection sites, and areas known to have been involved in spontaneous wealing during the last 24 h were avoided. Short-acting antihistamines were withdrawn at least 2 days prior to the skin tests. No subject had taken long-acting antihistamines or immunosuppressants for at least 2 months before testing.

Weal and flare responses were measured at 30 min and the ASST response was taken to be positive when the serum-induced weal had a diameter at least 1.5 mm greater than the HSA-induced weal [18].

Clinical Assessment

CU patients recorded expression of urticarial symptoms using score values from 0 to 3 and the need for rescue medication (short-acting antihistamines required per day) every day for 1 week. The weekly aggregate urticaria activity score (UAS), based on the UAS used by O'Donnell et al. [9] and Grattan et al. [19], was calculated for week 0 (before treatment), week 8 (immediately after end of treatment) and week 12 (4 weeks after end of treatment). Briefly, score values for ‘weal numbers’ (range: 0–3) and ‘pruritus intensity’ (range: 0–3) were added (range: 0–6) and the sums of all days of week 0, 8 or 12 were totaled to obtain the weekly UAS (range: 0–42) (table 1).

Construction of the Fc_εRI-cDNA

Amplification and cloning procedures were performed as described previously [20]. Briefly, primers for polymerase chain reactions (PCR) were synthesized by Applied Biosystems (Foster City, Calif., USA). The 622-bp Fc_εRI cDNA (nt 107–718) encoding the 239 amino acid residues of the soluble extracellular fragment of the α -chain of the high-affinity IgE receptor were generated by applying PCR on a cDNA derived from mast cells. Restriction sites for *Bam* HI and *Hind* III were introduced by PCR using primer pair p183 gatcgatccatggctcctgcatggaatcccc and p184F gatcgtcgaccgcttgacagcaatactctctctctc. The cDNA was ligated into linearized pQE30 (Qiagen, Hilden, Germany) resulting in the recombinant expression vector pQE-FCER. The presence of the correct insert was verified by DNA sequence analysis following the dideoxy method [21]

Table 1. Description of scoring system

Symptoms	Score value			
	0	1	2	3
Weal number (daily average)	none	<10	10–50	>50
Weal size (average diameter)	none	<1 cm	1–3 cm	>3 cm
Weal duration (average, h)	none	<1 h	1–24 h	>24 h
Pruritus intensity (daily average)	none	mild	moderate	severe
Pruritus duration (average, h)	none	<1 h	1–24 h	>24 h
Erythema number (daily average)	none	<10	10–30	>30
Erythema size (average diameter)	none	<1 cm	1–3 cm	>3 cm
Erythema duration (average, h)	none	<1 h	1–24 h	>24 h
Angioedema number (daily average)	none	1	2	3
Angioedema size (average diameter)	none	<1 cm	1–3 cm	>3 cm
Angioedema duration (average, h)	none	<1 h	1–24 h	>24 h

using a DyeDideoxy Terminator kit and a 377 DNA sequence analyzer (Applied Biosystems). The DNA sequences were compared with the DNA sequence data retrieved from EMBL DNA data bank (Cambridge, UK) using the most recent accession number (X06948).

Quantification of Anti-Fc ϵ RI and Anti-IgE Antibodies

Anti-Fc ϵ RI antibodies were assessed by immunoblotting as previously described [20]. Briefly, recombinant Fc ϵ RI was fractionated by 14% reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose. Blots were blocked for 1 h in 1% (v/v) Tween-20 in PBS buffer (154 mM NaCl, 20 mM KH₂PO₄, pH 7.6). For detection of auto-antibodies, sera were diluted 100-fold in PBS. Monoclonal antibody directed to the N-terminal tag RGSH₄ (Qiagen) was diluted 2,000-fold in PBS. After a 12-hour incubation at room temperature with diluted sera, the blots were washed and incubated with rabbit anti-human IgG or rabbit anti-mouse (Jackson ImmunoResearch Laboratories, West Grove, Pa., USA) conjugated with alkaline phosphatase. The NBT/BCIP staining system was used as chromogenic substrate.

Analysis of Anti-IgE Antibodies

Anti-IgE antibodies were assessed by ELISA. Briefly, micro titer plates (MaxiSorb™, Nunc) were coated by incubation overnight at 4°C with IgE (10 µg/ml in PBS). Unbound protein was removed by three times washing with PBS. Free binding sites were blocked by incubation with PBS/0.5% Tween-20/0.5% bovine serum albumin/0.5% casein for 3 h at room temperature and plates were washed three times with PBS. Next, patient sera were incubated in a dilution of 1:50 in blocking solution for 1 h at room temperature. Rabbit anti-human IgG (H+L) (Jackson ImmunoResearch Laboratories) conjugated with horseradish peroxidase was added in a dilution of 1:3,000 in blocking solution for 1 h at room temperature. Freshly prepared OPD solution was used as the chromogenic substrate. After incubation for 15 min at room temperature in the dark, the reaction was stopped by adding 100 µl 1 M sulfuric acid. Extinctions at 490 nm >0.2 were considered positive.

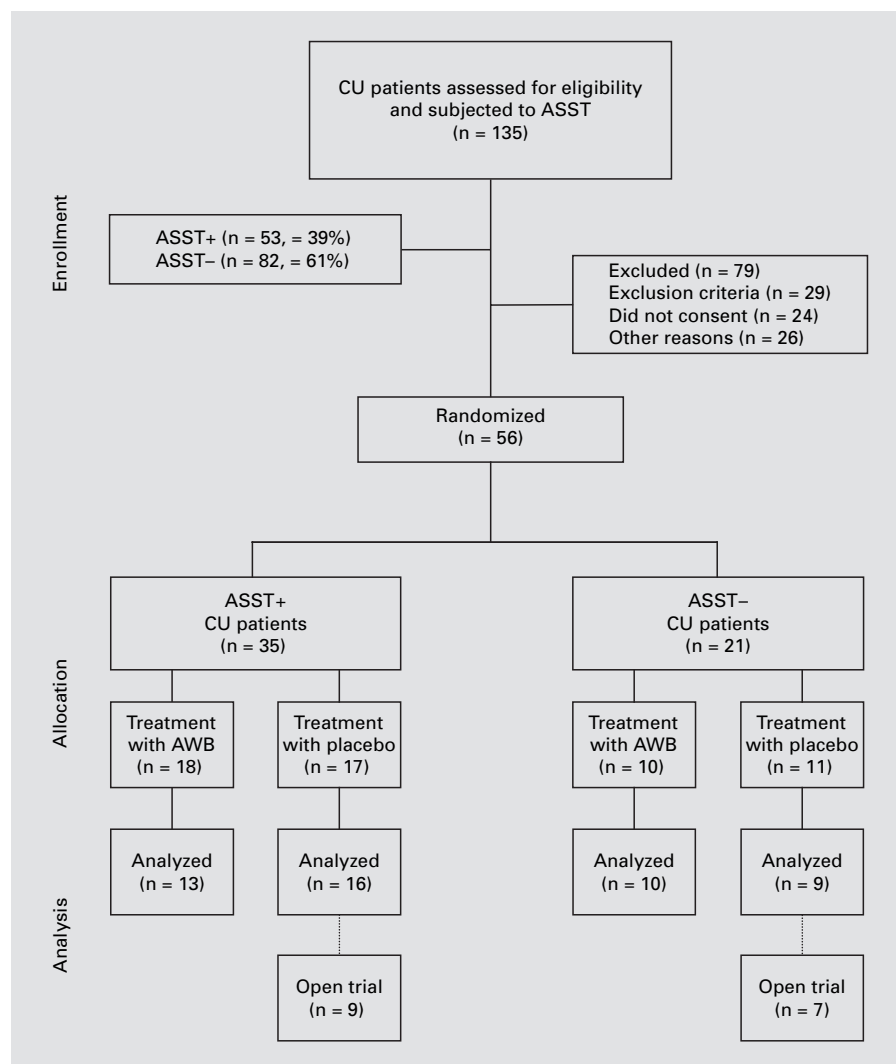
Study Design

A prospective, randomised, single-blind, placebo-controlled parallel group study was designed to evaluate the efficacy of AWB injections in the treatment of ASST+ and ASST– CU patients. Approval of the Ethics Committee Ärztekammer Rheinland-Pfalz was obtained prior to the start of the trial and the study was performed in accordance with the Helsinki Declaration and German and European Good Clinical Practice Guidelines at the Urticaria Clinic of the Department of Dermatology of the University of Mainz.

Stratification of Patients and Treatment

Hundred and thirty-five consecutive CU patients of the Urticaria Clinic Mainz were stratified (ASST+ vs. ASST–) based on the presence or absence of immediate-type hypersensitivity reactions to intracutaneous injections of autologous serum. Seventy-nine patients failed to meet the inclusion criteria, did not provide written consent, or were excluded from the study for other reasons (most common reason: patients lived too far from treatment center). Consequently, 56 patients were enrolled in the study (fig. 1) and were randomized into two parallel groups (ASST+: n = 35; ASST–: n = 21) receiving either repeated injections of AWB or placebo (sterile saline). Randomization was performed by blocks of 10 using the ranuni random number generator in SAS Software (SAS Institute, Cary, N.C., USA). The treatment duration and method of administration were the same in both groups: (1) run-in-period of 1 week to record baseline symptoms; (2) 8 weekly intramuscular gluteal injections (alternating sides; blood was taken immediately before reinjection from a cubital vein; blood was not subjected to anticoagulation or other treatment). This frequency/number of injections and the volume of injections (1st injection: 2.5 ml, injection 2–8: 5 ml) were chosen after detailed consultation with practitioners experienced in AWB therapy and because earlier reports from the literature and initial pilot treatments performed had shown that this regimen can be effective in the treatment of CU patients [22]; (3) 4 weeks of observation after discontinuation of treatment (follow-up). Patients were blinded to the treatment until after completion of the last study visit and handing in their diaries (week 12): (1) by drawing blood before each injection (even in patients who received placebo, where blood was taken but discarded), (2) by preparing sets of two identical non-see-through syringes (one filled with

Fig. 1. Study design, stratification of patients, and progress summary flow chart. 135 CU patients were subjected to autologous serum skin test (ASST) to identify patients that exhibit immediate type hypersensitivity reactions to intracutaneous injections of their own serum. 53 CU patients (39%) were found to be ASST+ (diameter of serum-induced weal 1.5 mm > HSA-induced weal). Patients that did not meet inclusion criteria, did not give written consent, or were unlikely to complete treatment (most common problem: patients lived too far from treatment center) were not enrolled in the study. Out of 56 randomized patients (intention-to-treat population) 48 patients completed the study (per protocol population). Reasons for drop out included requirement for systemic treatment with glucocorticoids and failure to document symptoms and/or antihistaminic medication. Sixteen of 25 patients that had received placebo were treated with AWB after completion of the study.



sterile saline and one containing AWB). We abstained from blinding treating physicians to rule out the possibility of injections of nonautologous whole blood.

Outcome Measures

Outcome measures included (1) the improvement of urticarial symptoms and (2) the requirement for antihistaminic rescue medication, which patients recorded daily for 12 weeks as described above, i.e. comparisons of week 0 (before treatment) to week 8 (last week of treatment) and week 12 (week 4 after termination of treatment), respectively. (3) Quality of life was assessed before as well as 8 and 12 weeks after the initiation of treatment using the Dermatology Life Quality Index (DLQI). DLQI, an instrument commonly used to measure disability in CU [23], is a 10-item questionnaire rating daily functioning on a four-point scale (very much, a lot, a little, and not at all). Patients are asked to recall within the past week symptoms and feelings, daily activities, leisure, work and school, personal relationships and treatment. The index is scored from a minimum of 0 to a maximum of 30 [24].

Statistical Analysis

All statistical analyses were primarily based on all randomized patients (intent-to-treat population). As some patients in all treatment groups did not show any urticarial symptoms during the week before they received the study medication for the first time, we defined a modified intention-to-treat-population, i.e. all patients who showed symptoms during this baseline phase (defined by an average urticaria activity score of ≥ 1). All analyses presented here are based on data from this modified intention-to-treat-population, as patients excluded from this group did not suffer from CU during the baseline period and thus were unable to improve under treatment. However, analyses of the intention-to-treat population and the modified intention-to-treat population did not differ significantly, i.e. intergroup differences in the improvement of AWB- and saline-treated CU patients were not statistically different in these populations. Missing values were imputed with the last observation carried forward method. Between-group comparisons were performed using two-sided asymptotic Friedman tests or asymptotic Cochrane-Mantel-Haenszel tests, each employing the ASST status

Table 2. Clinical profiles of ASST+ and ASST– patients with chronic urticaria

	ASST+ CU patients	ASST– CU patients	Statistical significance
ASST, mm ²			
Serum weal	91.6 ± 22.2	0.2 ± 0.2	p < 0.0001
Histamine weal	173.3 ± 44.5	116.5 ± 17.2	n.s.
Albumin weal	0.9 ± 0.8	3.7 ± 3.7	n.s.
UAS, range: 0–42			
Urticaria activity score	20.8 ± 2.0	19.7 ± 2.2	n.s.
Weal (scores), range: 0–3			
Number, daily average	1.3 ± 0.2	1.2 ± 0.2	n.s.
Size, average diameter, cm	1.4 ± 0.2	1.3 ± 0.2	n.s.
Duration, average, h	1.4 ± 0.2	1.2 ± 0.2	n.s.
Erythema (scores), range: 0–3			
Number, daily average	1.0 ± 0.1	1.2 ± 0.2	n.s.
Size, average diameter, cm	1.2 ± 0.2	1.5 ± 0.2	n.s.
Duration, average, h	1.2 ± 0.2	1.4 ± 0.2	n.s.
Angioedema (scores), range: 0–3			
Number, daily average	0.5 ± 0.1	0.3 ± 0.1	n.s.
Size, average diameter, cm	0.7 ± 0.2	0.5 ± 0.2	n.s.
Duration, average, h	0.6 ± 0.2	0.5 ± 0.2	n.s.
Pruritus (scores), range: 0–3			
Intensity, daily average	1.7 ± 0.1	1.6 ± 0.2	n.s.
Duration, average, h	1.7 ± 0.1	1.6 ± 0.1	n.s.
Quality of life, DLQI score, range: 0–30	8.5 ± 1.0	9.3 ± 1.6	n.s.
Antihistamines, week	11.1 ± 2.5	4.5 ± 1.1	p < 0.05
Duration of CU, months	108.7 ± 31.0	29.7 ± 9.2	p = 0.06
Expression of anti-Fc _ε RI	6 of 34	4 of 21	n.s.
anti-IgE	2 of 34	0 of 21	n.s.
Total IgE, kU/l	162.2 ± 42.9	197.2 ± 66.6	n.s.
All data are presented as mean ± SEM. n.s. = Not significant.			

as a block factor. Analysis of within-group differences were done by asymptotic one-sample Wilcoxon tests. p < 0.05 was considered to indicate statistical significance of differences.

Results

Clinical Profiles of ASST+ and ASST– Patients with CU

When we assessed CU activity in all ASST+ and ASST– CU patients suitable for further analyses (n = 35 and 21, respectively; see Subjects and Methods section and fig. 1), we found no significant differences between ASST+ and ASST– CU patients comparing numbers, size, intensity, and/or duration of urticarial symptoms such as weals, erythemas, angioedema or pruritus (ta-

ble 2). Nor did ASST+ and ASST– CU patients differ with respect to total IgE levels or quality of life scores (table 2). Notably, only 6 of 34 ASST+ CU patients were found to exhibit antibodies against Fc_εRI (vs. 4 of 21 ASST– CU patients) and only 2 of 34 ASST+ CU patients showed anti-IgE (vs. 0 of 21 ASST– CU patients), confirming that such antibodies are not required for the induction of cutaneous immediate-type hypersensitivity reactions to autologous serum (table 2).

In contrast, ASST+ CU patients were found to require significantly more antihistaminic medication as compared to ASST– CU patients when instructed to only use short-acting antihistamines in cases of severe bouts of weal and flare type skin reactions (11.1 vs. 4.5 antihistamines/week; p = 0.02; table 2). ASST+ CU patients also reported longer duration of CU (109 ± 31 months) than

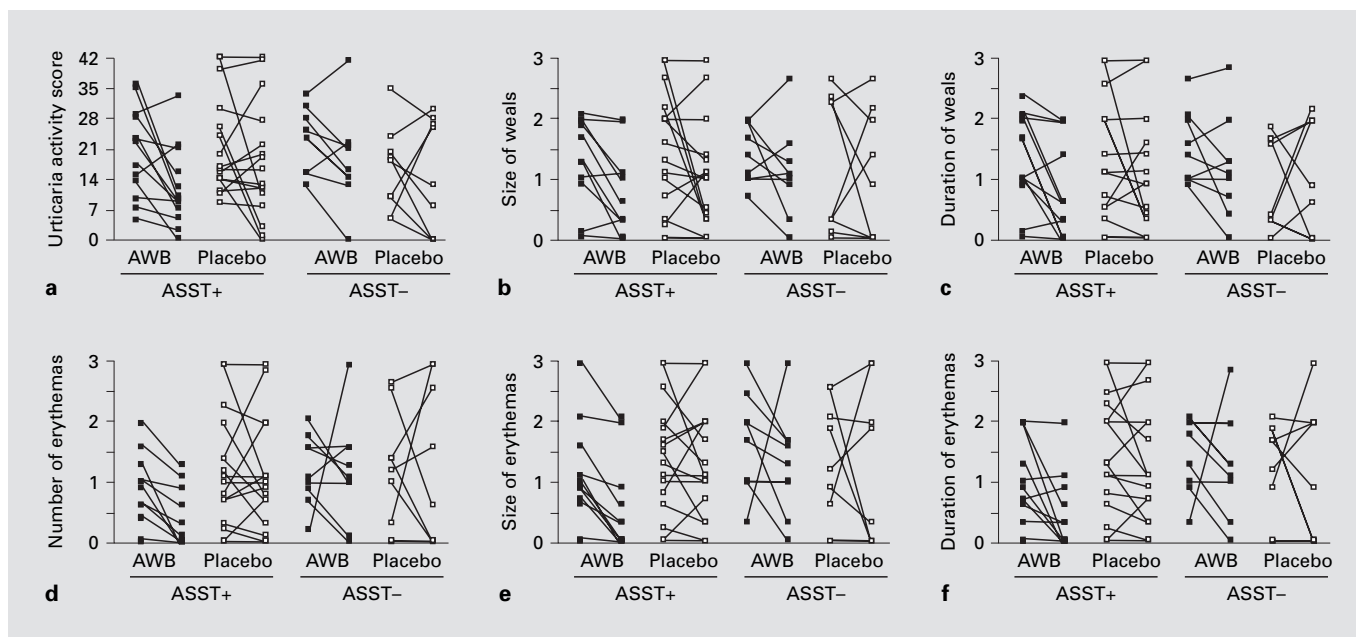


Fig. 2. ASST+ CU patients exhibit improvement of urticarial symptoms after treatment with AWB. Individual changes of weekly UAS scores (**a**; number of weals + intensity of pruritus) and daily scores for weal size and duration (**b**, **c**) and numbers (**d**), size (**e**), and duration (**f**) of erythemas from week 0 (before treatment) to week 12 (4 weeks after termination of treatment) in ASST+ or ASST- patients treated with 8 weekly intramuscular injections of AWB or placebo (sterile saline).

ASST- CU patients (30 ± 9 months). However, this difference failed to achieve statistical significance ($p = 0.06$), most likely due to the large variations of disease duration in the limited number of patients studied.

Repeated Injections of Autologous Whole Blood Significantly Reduce Disease Activity and Increase Life Quality in ASST+ CU Patients, but Not in ASST- CU Patients

On the basis of the modified intention-to-treat population (fig. 1), 9 of 13 ASST+ CU patients showed significant improvement of UAS after repeated injections of AWB (week 0 vs. 12, improvement by $>30\%$), calculated from daily numbers of weals (0–3) and intensity of pruritus (0–3; UAS maximum: 42/week; fig. 2a). In contrast, only 4 of 16 ASST+ CU patients that had received saline injections exhibited significant UAS improvement after treatment (fig. 2a). Interestingly, no differences in the percentage of responders were found comparing UAS improvements of ASST- CU patients that had received AWB or placebo (5 of 9 in both treatment groups; fig. 2a).

Similar results were observed for other parameters of treatment efficacy such as the reduction in the size or duration of weals or the improvement in size, numbers, and duration of erythemas (fig. 2b–f). Most notably, in the modified intention-to-treat population, all but 1 of 13 ASST+ CU patients treated with AWB reported improvement of quality of life (assessed by using the DLQI questionnaire) 8 and 12 weeks after the initiation of therapy, whereas only 2 of 9 placebo-treated ASST+ CU patients showed significant reductions ($>30\%$ improvement) of DLQI scores (week 0 vs. weeks 8 or 12). On the other hand, the percentage of ASST- CU patients that reported improved quality of life scores was higher in the group that had received placebo than in the active therapy group.

When we assessed treatment groups for average changes in disease activity, we found that ASST+ patients showed markedly improved UAS after AWB therapy (-41% , $p < 0.05$) as compared to UAS levels before therapy, whereas UAS did not improve significantly in ASST+ CU patients that had received placebo (-18%) or in ASST- patients that had been treated with AWB (-21%) or placebo (-11% ; fig. 3a). ASST+ AWB-treated CU pa-

tients also showed significant improvements of duration and size of weals (-46% , $p < 0.05$ and -48% , $p < 0.05$, respectively), whereas placebo-treated ASST+ CU patients as well as ASST- placebo- or AWB-treated CU patients only showed marginal and statistically not significant improvements (fig. 3b, c). In addition to these improvements of the leading CU symptoms weals and pruritus, ASST+ CU patients also developed less erythemas, which were also of smaller size and shorter duration after therapy with AWB (fig. 3d–f). In these patients, numbers, size, and duration of erythemas were reduced by up to 56% ($p < 0.05$), whereas placebo-treated ASST+ CU patients and placebo- or AWB-treated ASST- CU patients only showed negligible reductions ($<20\%$, $p > 0.05$) of these parameters (fig. 3d–f).

The quality of life in ASST+ AWB-treated CU patients was markedly improved after therapy, i.e. by 70 and 66% in weeks 8 ($p < 0.005$) and 12 ($p < 0.05$), respectively. Also, drops in DLQI scores in ASST+ AWB-treated subjects (reflecting quality of life improvement) were significantly greater than in ASST+ placebo-treated CU patients ($p < 0.05$), where quality of life did not improve significantly (by 20 and 28% in weeks 8 and 12; fig. 4a). ASST- CU patients treated with AWB did not show increased improvement of life quality 8 or 12 weeks after start of treatment as compared to placebo-treated ASST- CU patients (fig. 4a).

Interestingly, 12 weeks after the initiation of therapy ASST+ patients treated with AWB required less than half of the antihistaminic rescue medication they had taken before therapy (-52% , $p = 0.005$). In contrast, usage of antihistamines by ASST+ placebo-treated CU patients and placebo- or AWB-treated ASST- patients improved only slightly or not at all (fig. 4b).

As all placebo-treated study patients were offered open trial AWB treatment after completion of the study, 9 ASST+ and 7 ASST- CU patients that had received saline

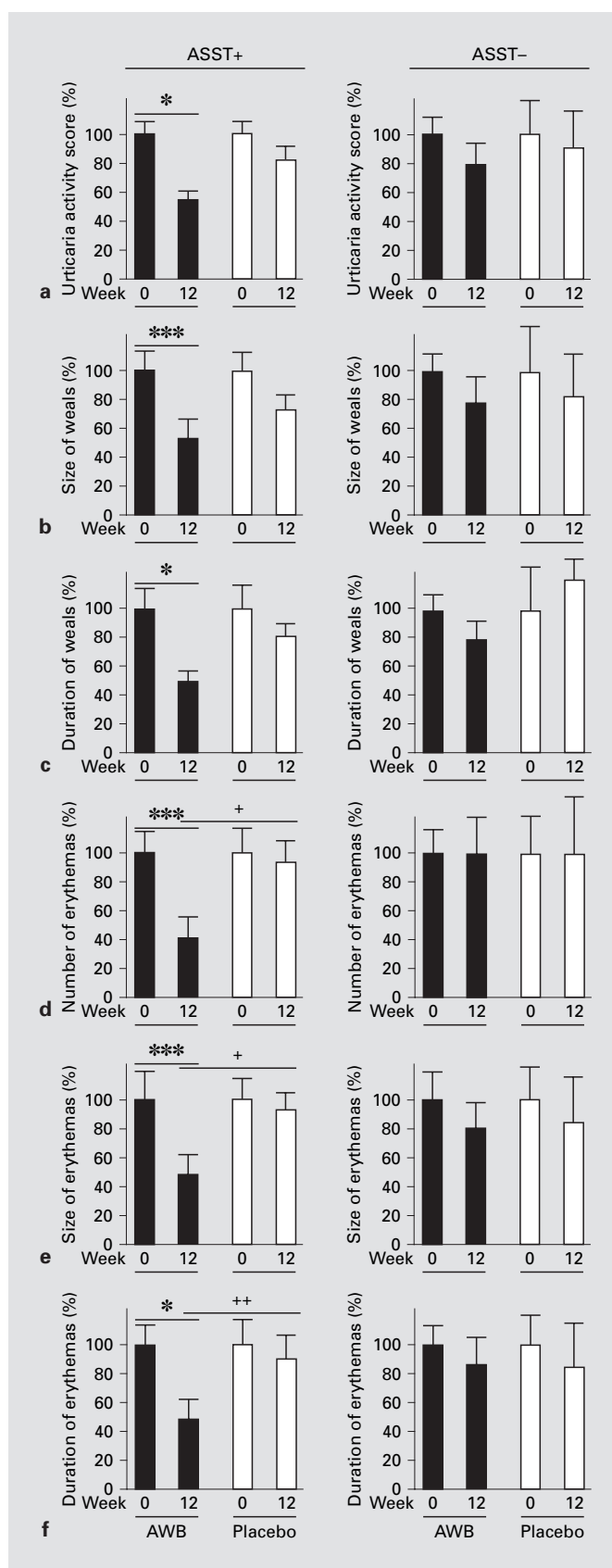


Fig. 3. AWB injections reduce disease activity in ASST+ CU patients. Changes of average weekly UAS scores (a; number of weals + intensity of pruritus) and average daily scores for weal size (b) and duration (c) and numbers (d), size (e), and duration (f) of erythemas from week 0 (before treatment) to week 12 (4 weeks after termination of treatment) in ASST+ or ASST- patients treated with 8 weekly intramuscular injections of AWB or placebo (sterile saline). Data are presented as means $\pm$ SEM; * $p < 0.05$, *** $p < 0.001$ for week 0 vs. week 12; + $p < 0.05$, ++ $p < 0.005$ for AWB- vs. placebo-treated patients at the same time point.

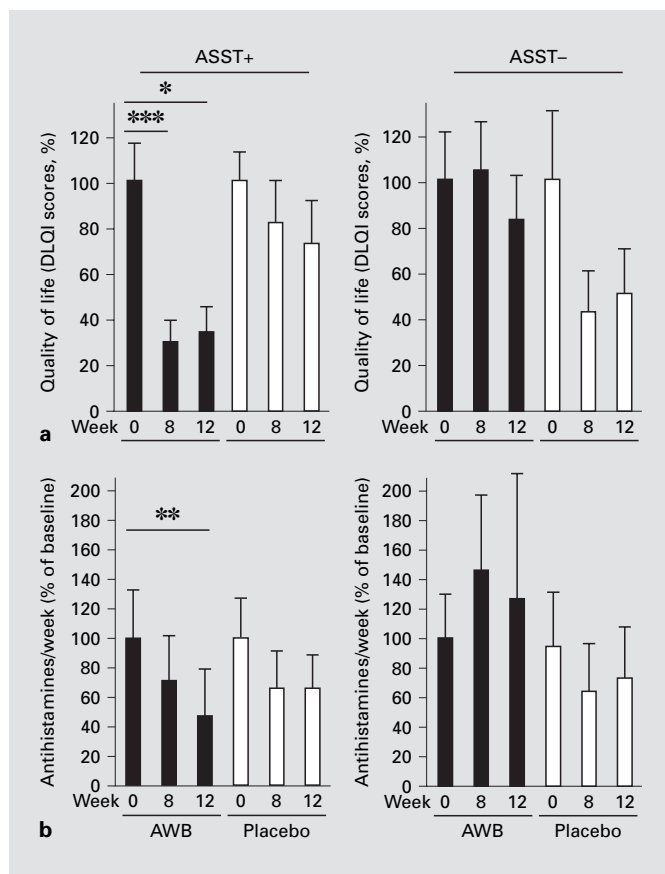


Fig. 4. ASST+ patients exhibit improved quality of life and reduced requirement for antihistaminic medication after injections with AWB. Changes in (a) the quality of life as assessed by the DLQI questionnaire and (b) the requirement to use antihistaminic medication from week 0 (before treatment) to week 8 (last week of treatment) or 12 (4 weeks after termination of treatment) in ASST+ and ASST- CU patients treated with AWB or placebo. Data represent means $\pm$ SEM; * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ for week 0 vs. week 8 or week 12.

injections were treated with AWB. Notably, 7 of the 9 ASST+ CU patients, but only 1 of the 7 ASST- patients, reported improvement of CU activity after the completion of treatment. AWB injections were found to be well tolerated and safe, i.e. none of the patients enrolled in the placebo-controlled or open trial reported serious adverse events due to the treatment. Minor adverse events resulting from study treatment were rare, transient, and equally frequent in all groups (mostly bruising and soreness at the injection site).

Discussion

The aim of the present study was to evaluate the effects of AWB injections in ASST+ CU patients. Confirming previous reports, about one third of CU patients (53 of 135, 39%) were found to exhibit positive ASST responses [4, 25]. ASST+ patients did not show significant differences in the expression (duration, size, intensity, and/or number) of urticarial symptoms including weals, pruritus, angioedema, and erythemas as compared to ASST- patients. However, we found that ASST+ patients exhibit a longer, albeit not significantly longer, duration of disease and require markedly more antihistamines (about 2.5-fold) than ASST- patients, suggesting that ASST+ CU patients may be less responsive to routine antihistamine therapy [8]. Our data confirm observations by Nettis et al. [4], who also found similar expression of urticarial symptoms in ASST+ and ASST- CU patients. In line with these results, CU in both ASST+ and ASST- patients had a negative impact on quality of life (determined by the DLQI questionnaire), i.e. DLQI scores were greatly increased, but no significant differences were found between the two groups.

The ASST is claimed to be a sensitive and specific test for screening CU patients for the presence of anti-Fc ϵ RI [18] and previous reports have shown functional anti-Fc ϵ RI to be present in ~40% of ASST+ CU patients and in only <2% of ASST- CU patients [6]. However, we found that less than 20% of ASST+ patients express anti-Fc ϵ RI (anti-IgE: 6%). These differences may be explained in part by differences in the sensitivities of the methods employed to detect anti-Fc ϵ RI, i.e. histamine release assays vs. immunoblotting. Nonetheless, our findings support the concept that positive cutaneous responses to autologous serum characterize a subgroup of patients suffering from 'autoreactive' rather than 'autoimmune' CU as most ASST+ skin reactions were found to occur in CU patients that did not express Fc ϵ RI/IgE-targeting autoantibodies [26].

On the other hand, we detected anti-Fc ϵ RI about as often in sera of ASST- patients as in ASST+ patients. Because mast cell activation by anti-Fc ϵ RI from some CU patients has been shown to be impaired when IgE is bound to its high-affinity receptor [27, 28], we screened ASST+ and ASST- patients for total serum IgE levels. However, we did not find significantly higher levels of IgE in ASST- patients, which may have accounted for the lack of mast cell degranulation in anti-Fc ϵ RI+ ASST- CU patients after intracutaneous injection of their own serum. At this point, we cannot explain why anti-Fc ϵ RI is detected al-

most exclusively in ASST+ CU patients by some groups [6], whereas other studies, including ours, find both ASST+ and ASST- CU patients and even healthy controls to express anti-Fc ϵ RI [29]. Whether these discrepancies are caused by differences in patient populations or due to the diversity of detection methods employed will have to be determined in further studies (1) combining functional tests (histamine release assays, ASST) and detection of anti-Fc ϵ RI, (2) examining sound numbers of CU patients, and (3) characterizing patients at multiple treatment centers.

Independent of the likely heterogeneous identity of serum factors responsible for degranulating MC after intracutaneous injections in ASST+ patients, it appeared feasible to attempt to tolerize such CU patients to their respective circulating MC secretagogues. To test this, we subjected ASST+ and ASST- CU patients to 8 weekly intramuscular injections of their own blood and assessed disease activity before and after treatment. The results of this randomized, placebo-controlled, single-blind, parallel-group trial provide direct evidence that urticarial symptoms can be decreased by repeated challenge with circulating histamine-releasing factors. Most notably, we found that AWB injections significantly reduced CU activity by >40% in ASST+ CU patients and that 70% of all ASST+ CU patients reported relevant improvement after repeated AWB injections (vehicle treated: 25%). Therapeutic effects of AWB injections required the presence of circulating histamine releasing factors, as only ASST+ patients, but not ASST- patients showed significantly (1) reduced CU activity (weals, pruritus, and erythemas), (2) decreased use of antihistaminic medication, and (3) increased quality of life after AWB treatment. These findings suggest that ASST+ CU patients can benefit from a simple, inexpensive, effective, potentially curative, and safe therapy with AWB injections.

Our findings encourage us to try and confirm the efficacy of AWB treatment in ASST+ CU patients in larger and double-blind trials and to clarify two important questions that remain unanswered: (1) AWB-treated ASST+ CU patients, in whom all outcome measures improved significantly, clearly exhibit responses different from those of AWB-treated ASST- CU patients and placebo-treated CU patients, in whom none of the outcome measures improved significantly. However, intergroup differences were not statistically significant for some of these outcome measures including 'disease activity', most likely due to the limited numbers of patients per treatment group. Thus, subsequent trials will have to assess greater numbers of ASST+ CU patients, ideally at multiple treat-

ment centers. (2) Our data suggest a sustained benefit of AWB injections in ASST+ CU patients as score values for virtually all outcome measures remained unchanged for 4 weeks after cessation of treatment or even showed further improvement. This suggests that the study period may have been too short and that future trials should include follow-up assessments at later time points after the end of treatment.

To the best of our knowledge, this is the first prospective randomized placebo-controlled study that assesses therapeutic effects of AWB injections in CU patients. As AWB injections induced significant improvement of CU only in ASST+ patients, this traditional form of autohemotherapy may represent a means of inducing specific tolerizing immune responses, similar to those seen in specific immunotherapy [30, 31]. While the precise underlying processes of therapeutic AWB effects remain to be identified, possible mechanisms include the induction of anti-idiotypes, which have recently been shown to inhibit the function of disease-inducing antibodies in pemphigus, another autoimmune dermatosis [32]. Alternatively, AWB could skew CU Th2 cytokine patterns that promote the induction of urticarial symptoms towards Th1, as ASST+ CU patients, but not ASST- CU patients have recently been reported to express significantly less IFN γ (Th1 cytokine) than normal controls [7].

Taken together, these data indicate that ASST+ CU, i.e. autoreactive CU, exhibits a clinical profile different from that of other subforms of CU, and ongoing studies are aimed at better defining these differences. Our findings also indicate that ASST+ CU patients can benefit from autohemotherapy and, therefore, call for further investigation of the effects and underlying mechanisms of AWB injections in ASST+ CU patients.

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