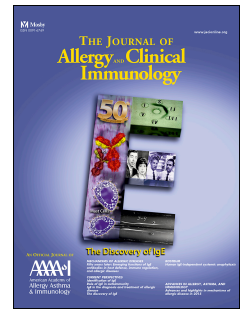


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Antihistamine updosage reduces disease activity in difficult-to-treat cholinergic urticaria patients

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Antihistamine up dosing reduces disease activity in difficult-to-treat cholinergic urticaria patients.

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Short title:

Antihistamine treatment in CholU

Keywords:

Cholinergic urticaria, urticaria activity score, pruritus7 score, antihistamine, therapy

24 *Abbreviations:*

25	CholU	Cholinergic urticaria
26	CholUAS7	Cholinergic urticaria activity score 7
27	CholUSI	CholU severity index
28	DLQI	Dermatology life quality index
29	PGA	Physician global assessment
30	PCE	Puls-controlled ergometry
31	UAS	Urticaria activity score
32	sgAH	Second generation antihistamines

33

34

35 **To the Editor:**

36 Cholinergic Urticaria (CholU) is a frequent skin disorder characterized by itchy
37 wheals induced by physical exercise and passive warming. Although CholU affects
38 up to 20% of young adults ¹, its etiopathogenesis is still not fully understood ².
39 Second generation antihistamines (sgAHs) are the first-line therapy in CholU, but
40 many patients do not respond to standard dosed sgAHs ^{3, 4}. In these patients, the
41 EAACI/GA²LEN/EDF/WAO urticaria guideline recommends sgAH up dosing ⁵, for
42 which there is very little evidence ^{6, 7}. One of the reasons for this is that CholU used
43 to be hard to study, because of the lack of validated outcome measures. To address
44 this gap, we developed a CholU severity index (CholUSI) ⁸ and a CholU activity score
45 (CholUAS7; see the Methods section in this article's Online Repository at
46 www.jacionline.org). Very recently, we also developed and published a standardized
47 specific puls-controlled ergometry (PCE) protocol for the diagnosis of CholU as well
48 as to objectively evaluate disease activity ⁸.

49 Here, we assessed the efficacy of sgAH up dosing in CholU patients refractory to
50 standard treatment by using the CholUSI, the CholUAS7, and PCE, as well as a skin
51 health-related quality-of-life instrument, the Dermatology Life Quality Index (DLQI).

52 CholU patients that had been treated with at least one sgAH at the licensed dose,
53 without achieving sufficient symptom control were enrolled (Study design see Figure
54 E1 in this article's Online Repository at www.jacionline.org). Their severity of disease
55 was assessed at baseline by using the CholUSI and a physician global assessment
56 (PGA). Patients documented their symptoms for 7 days without taking
57 antihistamines, completed the DLQI and performed the puls-controlled ergometry
58 (PCE) provocation testing (30 minute bicycle challenge with controlled increase of 3
59 pulse beats/minute) ⁸. Patients were subsequently treated with a sgAH at fourfold the
60 licensed dose for 7 to 28 days (mean treatment duration $\pm$ SD: 9.8 ± 4.9 days) and

61 reassessed by PCE and DLQI at the end of the treatment phase. Throughout the
62 study, patients documented their CholU signs and symptoms as well as exposure to
63 eliciting triggers in a daily diary. The 7 day score CholUAS7 was calculated with
64 these data and used to determine changes in disease activity in response to
65 treatment. (Detailed description of the study and the applied methods see the
66 Methods section in this article's Online Repository at www.jacionline.org). In total, 32
67 CholU patients were screened for this study, and 28 of them (20 females, mean age
68 34.0 years, mean duration of disease 7.6 years, mean duration of whealing episodes
69 1.5 hours) were included. (The detailed clinical characteristics of the patients are
70 listed in the Online Repository Table E1)

71 Analyzing the diary recorded disease activity, sgAH treatment at fourfold the
72 standard dose overall significantly ($p=0.01$) reduced the disease activity score
73 CholUAS7 by -41% (CholUAS7 components: Wheal7: -38%, $p=0.01$; Pruritus7: -
74 41%, $p=0.002$, Elicitor7: -3.5%, $p=$ n.s. Table 1), as compared to baseline.

75 UAS score based PCE provocation test results at fourfold standard dose sgAH
76 treatment also improved, albeit not significantly (UASprovo: -13%, $p=0.08$, Table 1).
77 Interestingly, PCE threshold levels, i.e. time to onset of whealing, time to onset of
78 sweating, and change in body core temperature before onset of whealing were not
79 reduced by high dose sgAH treatment (Table 1).

80 Health-related quality-of-life as determined by the DLQI showed an improvement of
81 21% during high dose sgAH treatment as compared to baseline ($p=0.09$, Table 1).

82 In the detailed responder analysis only 11 of 28 (39%) patients treated with high
83 dosed sgAHs reported an improvement in the disease activity score CholUAS7 of $\geq$
84 50% (CholWheal7: 43% of patients; CholPruritus7: 46% of patients, Figure 1A).
85 Complete response, as defined by CholUAS7 improvement $>90\%$, was achieved by
86 only one patient (Figure 1A). In the PCE responder analysis, only 2 of 28 patients

87 (7%) showed an improvement of their UASprovo score of $\geq 50\%$ (Whealprovo 4%,
 88 Itchprovo 36%, Fig. 1B). None of the patients, in PCE, had a $>90\%$ reduction of the
 89 UASprovo score.

90 In the responder analysis, less than half of all patients had a substantial improvement
 91 in the DLQI during high dose sgAH treatment (Fig. 1C). Patients who improved in the
 92 diary based disease activity score CholUAS7 score typically also improved in their
 93 health-related quality-of-life DLQI score ($r_s=0.55$, $p=0.002$). Interestingly, the DLQI
 94 improved significantly in CholU patients with mild disease (Baseline PGA = 1; mean
 95 reduction of DLQI = 4.3 points; $n = 7$), but not in severely affected patients (Baseline
 96 PGA = 3; mean reduction of DLQI = 0.4 points; $n = 14$; $p<0.05$). (Supplemental
 97 results of the study see the Supplemental Results section in this article's Online
 98 Repository at www.jacionline.org).

99 In this study, we report, for the first time, that difficult-to-treat CholU patients can
 100 benefit from high dosed sgAHs, as recommended by the current guideline ⁵. The
 101 efficacy of sgAH up dosing was significant but overall moderate, with a reduction in
 102 disease activity of about 40%. Two older studies using cetirizine up dosing ^{6, 7} also
 103 reported significant symptom reduction in CholU, both in much smaller patient
 104 groups. The first of these studies did not provide information on the effect size ^{6, 7}.
 105 The second one, with 11 CholU patients, found that disease activity was reduced by
 106 80% ^{6, 7}. This difference in efficacy is most likely explained by the differences in the
 107 patient populations studied and the outcome measures that were used.

108 Importantly, less than half of our CholU patients showed a clinically meaningful
 109 reduced disease activity, i.e. more than 50% reduction, in response to high dose
 110 sgAH treatment. Improvement as assessed by diary-derived data (CholUAS7)
 111 correlated with improvements in the DLQI. Furthermore, in a distinct subgroup of
 112 patients who improved in the PCE provocation (at maximum elicitation level)

113 regarding the UASprovo, this improvement also correlated with improvements in the
114 DLQI. These results indicate that it is a distinct subgroup of patients that benefits
115 from sgAH up dosing. The fact that only one of 28 patients became virtually symptom-
116 free on high dose sgAH treatment confirms that ChIU is a hard-to-treat form of
117 chronic urticaria and that additional and more effective treatment options are needed
118 for this condition.

119 Surprisingly, PCE provocation testing did not detect changes in disease activity in
120 response to high dose sgAH treatment. This is different from other forms of inducible
121 urticaria (e.g. cold urticaria), where responses to antihistamine treatment can be
122 assessed by measuring changes in trigger thresholds (e.g. critical temperature
123 thresholds)⁹. In contrast to sgAHs, treatment with an anticholinergic drug was found,
124 in a previous case, to change PCE trigger thresholds, i.e. to increase the time to
125 onset of PCE-induced whealing¹⁰. It may be that sgAH can protect from ChIU
126 symptoms induced by mild to moderate elicitors (daily life situation) but not those
127 induced by strong elicitors (PCE).

128 Although our study was not performed or powered to compare different sgAHs our
129 analysis did not find gross differences in the efficacy of different sgAHs (Online
130 Repository at www.jacionline.org, Table E3). However, this does not mean that such
131 differences do not exist or could not be detected in future studies with a higher
132 patient number.

133 In conclusion, this study addresses a common situation in the treatment of ChIU
134 patients, i.e. the need of sgAH up dosing. The use of multiple methods to analyze the
135 outcome of up dosing, including the use of diary-based activity scores (patient-
136 centered methods), supports our findings and makes them relevant for routine
137 patient management. Limitations of our study are that it was not placebo-controlled
138 and not blinded and that the study design did not allow for direct comparisons of the

139 effects of standard dosed and high dosed sgAHs or of different types of used sgAHs
140 in the same patients. Overall, sgAH up dosing in ChOIU patients refractory to standard
141 doses substantially improves disease activity, but only in less than half of all patients.
142 Accordingly, further randomized double-blind placebo controlled trials in stratified
143 patients on single sgAH and more or new effective treatment options are needed.

144

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154 *The authors state no conflict of interests.*

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183

Table 1 High dose antihistamine treatment reduces disease activity in ChIU-patients refractory to standard doses

		Baseline Mean $\pm$ SD (median, range)	End of high dose sgAH treatment Mean $\pm$ SD (median, range)	Improve- ment %	p- value
Diary-documented disease activity and quality of life impairment					
Disease activity	CholUAS7 ^{a)}	43.6 $\pm$ 28.5 (37.0, 9 – 114)	25.9 $\pm$ 15.4 (24.0, 0 – 68)	40.6	0.01
	Wheal7	8.4 $\pm$ 5.8 (9.0, 0 – 21)	5.2 $\pm$ 5.7 (4.0, 0-21)	38.1	0.01
	Pruritus7	10.2 $\pm$ 5.1 (9.0, 2 – 21)	6.0 $\pm$ 4.6 (5.5, 0 – 20)	41.2	0.002
Intensity of elicitors	Elicitor7 ^{b)}	20.1 $\pm$ 4.8 (21.0, 7 – 28)	20.8 $\pm$ 4.3 (21.0, 7 – 28)	-3.5	0.44
Quality of life	DLQI score ^{c)}	9.7 $\pm$ 6.3 (8.5, 1 – 26)	7.6 $\pm$ 7.2 (5.5, 0 – 27)	21.6	0.09
Pulse controlled ergometry (PCE) provocation test results					
PCE- induced CholU activity	UASprovo score	4.7 $\pm$ 1.1 (5.0, 2 – 6)	4.1 $\pm$ 1.2 (4.0, 2 – 6)	12.8	0.08
	Whealprovo score	2.8 $\pm$ 0.5 (3.0, 1 – 3)	2.7 $\pm$ 0.6 (3.0, 1 – 3)	3.6	0.22
	Pruritusprovo score	1.9 $\pm$ 0.9 (2.0, 1 – 3)	1.5 $\pm$ 1.0 (1.0, 0 – 3)	21.1	0.15
PCE- induced onset of whealing	Time to onset of whealing (in minutes)	11.3 $\pm$ 10.0 (12.5, 0-28)	12.2 $\pm$ 10.4 (13.0, 0-29)	7.4	0.78
	Increase in HR at onset of whealing (in bpm)	33.3 $\pm$ 32.7 (26.5, -16-109)	35.1 $\pm$ 31.6 (38.5, 0-99)	5.1	0.53
	Increase in BCT at onset of whealing (in °C)	0.4 $\pm$ 0.7 (0.1, -0.3-2.7)	0.6 $\pm$ 1.0 (0.2, -0.5-3.3)	33.3	0.48
PCE- induced onset of sweating	Time to onset of sweating (in minutes)	14.9 $\pm$ 6.2 (15.5, 0 – 26)	13.3 $\pm$ 7.0 (13.5, 0 – 28)	-12.0	0.63
	Increase in HR at onset of sweating (in bpm)	41.9 $\pm$ 28.5 (44.0, -1 – 99)	32.9 $\pm$ 30.4 (37.5, 0 – 91)	-27.4	0.28
	Increase in BCT at onset of sweating (in °C)	0.4 $\pm$ 0.5 (0.3, -0.2 – 1.7)	0.6 $\pm$ 0.8 (0.3, -0.5 – 3.4)	33.3	0.64

PCE, puls controlled ergometry; BCT, body core temperature; bpm, beats per minute; HR, heart rate; n = 28

a) CholUAS7 = 7 day sum of [(Whealday + Itchday) x Intensity of elicitor day]; Range 0-168

- 7 b) **Elicitor7** = 7 day sum of Intensity of elicitor/day [no exposure to elicitors = 4, exposure to mild
8 elicitor = 3, exposure to moderate elicitor = 2, exposure to strong elicitor = 1 point]; Range 0-28
9 c) **DLQI**; Range 0-30; no impairment = 0-1 points; mild impairment = 2-5 points; moderate impairment
10 = 6-10 points; very large impairment = 11-20 points; extremely large impairment = 21-30 point

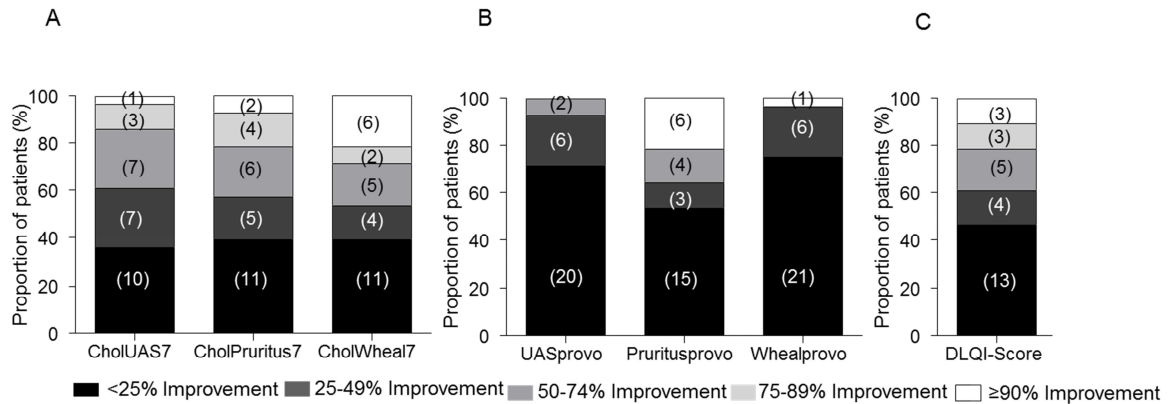


Figure 1: Less than half of all ChIU patients treated with high dosed antihistamines experience complete protection.

Five categories of improvement are depicted, and proportions of patients in each category are shown. Absolute numbers of patients are given in parentheses. (A) 39%, 43% and 46% of ChIU patients showed $\geq 50\%$ improvement to high-dosed antihistamines in the cholinergic urticaria activity score 7 (ChIUAS7^a), the composite pruritus score 7 (ChIUpruritus7^b), and the composite wheal score 7 (ChIUWheal7^c), respectively. (B) Only 7% of ChIU patients showed $\geq 50\%$ improvement of the UASprovo^d score. Improvement was primarily seen in itch severity (36%), but not in whealing intensity (4%). (C) 39% of the ChIU patients reported a DLQI^g score reduction of $\geq 50\%$.

- a) **ChIUAS7** = 7 day sum of [(Whealday + Itchday) x Intensity of elicitor day]; Range 0-168
- b) **ChIUpruritus7** = 7 day sum of (Pruritusday x Intensity of elicitor day); Range 0-84
- c) **ChIUWheal7** = 7 day sum of (Whealday x Intensity of elicitor day); Range 0-84
- d) **UASprovo** = Whealprovo + Itchprovo; Range 0-6
- e) **Pruritusprovo**; Range 0-3
- f) **Whealprovo**; Range 0-3
- g) **DLQI**; Range 0-30; no impairment = 0-1 points; mild impairment = 2-5 points; moderate impairment = 6-10 points; very large impairment = 11-20 points; extremely large impairment = 21-30 points

1 Online Repository: Methods

2 *Patients*

3 Patients with ChIU were recruited from the urticaria specialty clinic of the
4 Department of Dermatology and Allergy, Charité - Universitätsmedizin Berlin. This
5 specialized outpatient clinic serves patients referred by office-based dermatologists
6 or allergologists (tertiary referral center). All patients had been treated before the
7 study with at least one sgAH at the licensed dose, without achieving sufficient
8 symptom control, and none had taken systemic corticosteroids for at least 2 weeks or
9 applied topical antihistamines, corticosteroids or mast-cell stabilizers for 1 week
10 before study enrollment. Patients below 18 years of age, pregnant or breast feeding
11 women, with known iodine allergy, with severe skin diseases that might not tolerate
12 skin sensors, and patients who participated in other clinical trials were excluded from
13 the study. Patients' characteristics and baseline data are given in Table E1.

14 A total of 32 patients were enrolled into the study. One patient withdrew his consent
15 during the study and 3 patients did not adhere to the study protocol.

16

17 *Study design*

18 The study design is depicted in Figure E1. After enrollment at visit 0, patients
19 documented their symptoms in a daily diary for 7 days without taking antihistamines,
20 and they completed the DLQI at the last of these 7 days. On day seven (visit 1), we
21 performed puls controlled ergometry (PCE) provocation testing, and we determined
22 the severity of disease with the ChIU severity index (ChIUSI) as described
23 previously¹ and by physician global assessment (PGA). Patients were subsequently
24 treated with a sgAH at fourfold the licensed dose for 7 to 28 days (mean treatment
25 duration $\pm$ SD: 9.8 ± 4.9 days) and reassessed by PCE and DLQI at visit 2, at the

end of the treatment phase. For 7 days before visit 2, patients again documented their symptoms in a daily diary.

The study was approved by the Ethics Committee of the Charité - Universitätsmedizin Berlin (EA4/124/10), registered in the German Clinical Trials Registry (DRKS-ID: DRKS00004277), and conducted according to the Declaration of Helsinki and the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, national laws and regulations as well as to the Standard Operating Procedures (SOPs) of the Allergie-Centrum-Charité. All participants gave signed informed consent before entering the study.

Outcome measures

Severity of disease: The severity of CholU was assessed at baseline by using the CholU severity index (CholUSI) and a physician global assessment (PGA). The CholUSI¹ is a sum score of 1) the frequency of CholU symptoms (less than once a month = 0; once a month = 1; more than once a month = 2; once a week = 3; more than once a week = 4; daily = 5; more than once daily = 6 points), 2) eliciting factors (1 point for each: physical exercise, hot bath, hot shower, emotional stress, hot food, sauna, other), 3) the duration of skin lesions (less than 5 min = 0; 5 to 10 minutes = 1; 10 to 20 min = 2; 20 to 30 min = 3; 30 to 60 min = 4; longer than 1 hour = 5 points), and 4) itch (none = 0; mild = 1; moderate = 2; severe = 3 points). Accordingly, the CholUSI score ranges from 0 to 21 points: 1-4 points: very mild CholU; 5-9 points: mild CholU; 10-15 points: moderate CholU; >16 points: severe CholU. The physician global assessment (PGA) is a subjective measure of the patient's overall CholU severity ranging from 0 to 3 points (none = 0, mild = 1, moderate = 2, severe = 3).

Patient diary: In the diary, patients documented their ChIU signs and symptoms as well as trigger factors during the baseline and treatment phase. Specifically, patients recorded, once a day, their itch intensity (Itch_{day}: none = 0, mild = 1, moderate = 2, severe = 3 points), and their whealing intensity (Wheal_{day}: none = 0, mild = 1, moderate = 2, severe = 3 points) in order to assess disease activity. Because ChIU patients usually exhibit pinpoint sized wheals that are difficult to count, patients globally rated their whealing intensity instead of counting wheal numbers as it is done in chronic spontaneous urticaria ². Furthermore, the patients were advised to continue their daily routine, avoid extreme trigger situations and were held to document elicitors in their daily dairies once daily. Patients documented the exposure to elicitors of symptom development, e.g. physical exercise, and the intensity of the elicitor they were exposed to (Intensity of elicitor_{day}: no exposure to elicitors = 4, exposure to mild elicitor = 3, exposure to moderate elicitor = 2, exposure to strong elicitor = 1 point).

Quality of life: Health-related quality-of-life impairment was assessed at the day before the PCE, both in the baseline and treatment phase, by use of the DLQI ³, a patient reported outcome instrument specifically designed for dermatological disorders. The DLQI has a recall period of 7 days and consists of 10 questions, with a minimum and maximum possible score of 0 and 30, respectively. The commonly used interpretation of DLQI scores is: no impairment = 0-1 points; mild impairment = 2-5 points; moderate impairment = 6-10 points; very large impairment = 11-20 points; extremely large impairment = 21-30 points.

Puls controlled ergometry provocation test: PCE provocation testing was performed as previously described to (A) verify the diagnosis of ChIU (at visit 1) and to (B) assess post PCE whealing intensity and disease activity as well as trigger thresholds (at visit 1 and 2) ¹. In brief, patients performed a 30 minute bicycle ride in a puls controlled manner, i.e. patients were instructed to speed up or slow down, for 30

minutes, their pedaling speed to achieve an increase in pulse rate of 3 beats per minute to a final maximum increase of 90 beats per minute above the starting level. PCE provocation test-induced urticaria activity was assessed by use of the UASprovo score, i.e. the sum score of the highest itch intensity (Pruritusprovo: none = 0, mild = 1, moderate = 2, severe = 3 points) and the highest whealing intensity (Whealprovo: none = 0, mild = 1, moderate = 2, severe = 3 points) that occurred during PCE and up to 15 minutes thereafter, with a minimum and maximum possible score of 0 and 6 points, respectively. In addition, time to sweating by Minor's sweat test (iodine-starch-reaction) ⁴ and individual CholU trigger thresholds, i.e. time to first wheal, were assessed and documented during and up to 15 minutes after PCE testing.

Body core temperature: To assess the body core temperature, twenty minutes acclimatization time was allowed before PCE during which patients were fitted with a Double Sensor device attached to the forehead ⁵⁻⁷ (Double Sensor T_{DS}, Drägerwerk AG, Lübeck, Germany) using a two-sided adhesive ring tape. A chest strap pulse monitor (Polar Electro GmbH, Büttelborn, Germany) was used to monitor pulse rate. All sensors were operated by Health Lab System (Koralewski Ind., Hambühren, Germany).

96

97 *Calculation of diary scores*

98 To determine which diary score is best suited to assess changes of disease activity
99 in response to treatment, we calculated and compared different scores (Table EM1):

100

Score-Name	Calculation	max. points
weekly UAS: <u>UAS7</u>	7 day sum of (Wheal _{day} + Itch _{day})	42

weekly wheal score: <u>Wheal7</u>	7 day sum of Wheal _{day}	21
weekly pruritus score: <u>Pruritus7</u>	7 day sum of Itch _{day}	21
cholinergic UAS7: <u>CholUAS7</u>	7 day sum of [(Wheal _{day} + Itch _{day}) x Intensity of elicitor _{day}]	168
cholinergic wheal7: <u>CholWheal7</u>	7 day sum of (Wheal _{day} x Intensity of elicitor _{day})	84
cholinergic pruritus7: <u>CholPruritus7</u>	7 day sum of (Itch _{day} x Intensity of elicitor _{day})	84
alternative cholinergic UAS7: <u>aCholUAS7</u>	7 day sum of (Wheal _{day} + Itch _{day} + Intensity of elicitor _{day})	70
alternative cholinergic wheal7: <u>aCholWheal7</u>	7 day sum of (Wheal _{day} + Intensity of elicitor _{day})	49
alternative cholinergic pruritus7: <u>aCholPruritus7</u>	7 day sum of (Itch _{day} + Intensity of elicitor _{day})	49
weekly intensity of elicitors: <u>Elicitor7</u>	7 day sum of Intensity of elicitor _{day}	28

101 Table EM1

102 The three composite diary scores (UAS7, CholUAS7, aCholUAS7) at baseline were
 103 correlated with provocation-based assessments of disease activity (UAS_{provo},
 104 Wheal_{provo}, Pruritus_{provo}), CholU trigger thresholds, i.e. the time to onset of
 105 whealing in PCE), and HRQoL (DLQI) as anchors (Table EM2).

		UAS provo score	Wheal provo score	Pruritus provo score	DLQI score	Time to onset of whealing (in PCE)
		correlation coefficient (p-value)	correlation coefficient (p-value)	correlation coefficient (p-value)	correlation coefficient (p-value)	correlation coefficient (p-value)
	UAS7 score	0.30 (0.12)	0.14 (0.48)	0.30 (0.12)	0.31 (0.10)	-0.39 (0.04)
	Wheal7 score	0.15 (0.44)	0.20 (0.30)	0.12 (0.55)	0.18 (0.35)	-0.29 (0.13)

Disease activity (Diary based scores)	Pruritus7 score	0.49 (0.008)	0.14 (0.48)	0.50 (0.007)	0.36 (0.06)	-0.40 (0.03)
	CholUAS7 score	0.36 (0.06)	0.05 (0.82)	0.39 (0.04)	0.30 (0.12)	-0.42 (0.03)
	CholWheal7 score	0.16 (0.40)	0.08 (0.68)	0.17 (0.38)	0.16 (0.43)	-0.35 (0.07)
	CholPruritus7 score	0.48 (0.01)	0.009 (0.97)	0.53 (0.004)	0.32 (0.09)	-0.40 (0.03)
	aCholUAS7 score	0.28 (0.14)	0.09 (0.64)	0.29 (0.13)	0.30 (0.13)	-0.38 (0.05)
	aWheal7 score	0.004 (0.98)	0.05 (0.80)	0.01 (0.95)	-0.04 (0.82)	-0.19 (0.33)
	aPruritus7 score	0.25 (0.21)	-0.12 (0.53)	0.32 (0.10)	0.13 (0.50)	-0.33 (0.08)

Table EM2: Correlations of diary scores with different anchors; $n = 28$, r_s Spearman's correlation coefficient, $p < 0.05$ depicted in bold

This identified the CholUAS7 as the best-suited composite diary score to assess disease activity, and this score was, therefore, used to determine changes in disease activity in response to treatment.

114 *Statistical Analyses*

115 Statistical analyses were performed using Graph Pad Prism Version 6 for Windows,
 116 2012. The statistical significance of differences between patient groups was
 117 calculated using the Mann-Whitney U-test and the Chi-squared test. Correlations
 118 were calculated using the Spearman's rank test. $P < 0.05$ was considered to indicate a
 119 significant difference.

120

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- 134

135 Online Repository: Supplemental Results

136 Additional PCE results:

137 At baseline we observed a statistically significant correlation between time to onset of
 138 whealing in PCE provocation and the CholUSI score ($r_s = -0.39$, $p=0.04$). In line with
 139 previous observations ¹, we found no correlation between the change in body core
 140 temperature and the time to onset of whealing during PCE provocation, the
 141 UASprovo score, the CholUSI score, or the PGA. PCE threshold levels, i.e. time to
 142 onset of whealing, time to onset of sweating, and change in body core temperature
 143 before onset of whealing were not reduced by high dose sgAH treatment (Table 1).
 144 SgAH treatment at fourfold the standard dose improved provocation test results, i.e.
 145 reduced the development of urticaria symptoms in response to PCE provocation,
 146 albeit not significantly (UASprovo: -13%, $p=0.08$). Interestingly, no improvement was
 147 seen in the UASprovo wheal score (-4%, $p=0.22$), whereas the UASprovo itch score
 148 decreased by 21% ($p=0.15$).

149

150 Additional dairy/disease activity results:

151 The reduction of several calculated scores as described in the online repository
 152 Methods section are shown in Online Repository at www.jacionline.org, Table E2.
 153 Notably, up dosing of sgAHs improved the CholUAS7 components wheal and itch
 154 (Wheal7: -38%, $p=0.01$; Pruritus7: -41%, $p=0.002$), whereas the CholUAS7 elicitor
 155 component, i.e. exposure to eliciting triggers, remained unchanged (Table 2A).

156 SgAH up dosing for more than 7 days did not significantly improve disease activity as
 157 compared to 7 days of treatment (CholUAS7 improvement: >7 days of treatment
 158 (mean $\pm$ SD: 14.2 $\pm$ 5.4 days of treatment ($n=11$): 8.5 $\pm$ 12.9 points vs. 7 days of
 159 treatment ($n=17$): 6.6 $\pm$ 7.8 points; $p=0.67$).

160

161 Gender differences to sqAH treatment:

162 Interestingly, more women (53%) exhibited an improved Pruritusprovo score (≥ 1
163 point improvement), as compared to men (33%, Chi-Squared test: $p=0.004$). In
164 contrast, 44% of the men improved in the Whealprovo score (≥ 1 point improvement),
165 but only 16% of the women (Chi-Squared test: $p<0.001$).

166 Online Repository: Supplemental Discussion

167 Effect size of antihistamine up dosing as assessed by use of the CholUAS7

168 In our study, the efficacy of sgAH up dosing in difficult-to-treat CholU patients was
 169 significant but overall moderate, with a reduction in disease activity defined by
 170 CholUAS7 of about 40%. The difference reported in comparison to two older studies
 171 using cetirizine up dosing^{8,9}. In one of these earlier studie, the mean reductions of
 172 single symptom items (wheals, erythema, itch) were calculated based on diary-
 173 derived data from selected days, i.e. days when eliciting factors were present. In
 174 contrast, our study included only patients who had previously failed standard dosed
 175 sgAH treatment, and we assessed disease activity before and during high dose
 176 sgAH treatment by use of the CholUAS7, which reflects the symptoms of a whole
 177 week. In our study, the CholUAS7 showed good correlation with other outcome
 178 measures such as the DLQI, the UASProvo score and the time to onset of PCE
 179 provocation-induced symptoms, which corroborates the effect size determined by
 180 this score.

181

182 In CholU, high dosed sgAHs affect the itch more than the wheals

183 Of all single item scores, the Pruritus7 score showed the most significant
 184 improvement in response to high dosed sgAHs. Also, 36% of patients reported an
 185 improvement (> 50%) in their PCE-induced itch, while this was only true for 4% with
 186 regard to PCE-induced whealing. Zuberbier et al, using Cetirizine 20mg, also
 187 reported itch to show the most significant improvement of all outcomes assessed^{8,9}.
 188 These findings provide evidence for what sgAH treated CholU patients often report in
 189 daily clinical practice ("I still get wheals, but they don't itch so much anymore"). The
 190 mechanisms of this differential response of itch and wheals to sgAH treatment
 191 remain unknown. Critical histamine thresholds may be higher for wheal than for itch

page 10

192 induction in CholU, or mast cell mediators other than histamine may be more
193 important for wheal formation than for pruritus development. Interestingly, wheal and
194 itch were differently affected by sgAH up dosing in women and men, with better
195 improvement of itch in women and better improvement of wheals in men. To our
196 knowledge, these gender specific differences have not been reported before in
197 CholU.

198

199 Provocation testing is a valuable tool for assessing CholU activity, but not suitable for
200 detecting moderate changes of CholU activity in response to treatment

201 The good correlation of disease activity assessed by the CholUAS7 and by PCE
202 provocation testing found in our study confirms that PCE is a valid and valuable
203 instrument for the diagnostic work up of CholU patients. Provocation testing is
204 recommended in patients with chronic inducible urticaria such as CholU to confirm
205 the diagnosis and to assess trigger thresholds. PCE does both.

206 In contrast, PCE provocation testing did not detect moderate changes in disease
207 activity in response to high dose sgAH treatment. In other words, up dosing of sgAH
208 did not alter the time to onset of PCE-induced whealing. This is different from other
209 forms of inducible urticaria (e.g. cold urticaria), where treatment responses to
210 antihistamine treatment can be assessed by measuring changes in trigger thresholds
211 (e.g. critical temperature thresholds)¹⁰. There are two likely explanations for this: 1)
212 disease activity, in our patients, was rather high, their trigger thresholds were low (i.e.
213 not measurable in more than one third, due to whealing at onset of PCE), and the
214 effects of sgAH treatment were moderate. Low trigger thresholds in CholU may be
215 less susceptible to treatment effects, especially suboptimal treatment effects, than
216 higher thresholds.

217 In a previous case report we demonstrated that treatment can change PCE trigger

218 thresholds, i.e. increase the delay in the onset of PCE-induced whealing¹¹. But in
 219 this patient the treatment worked very well, with significantly reduced disease activity
 220 and markedly improved quality of life. 2) PCE may have a lower sensitivity than diary
 221 based activity scores such as the CholUAS7 for picking up moderate treatment
 222 effects, because, in daily life, the strength of triggers is usually lower than in PCE.
 223 Antihistamines are likely to better protect from CholU symptoms induced by mild to
 224 moderate elicitors (daily life situation) as compared to strong elicitors (PCE).

225

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259 **Repository tables**260 **Table E1:** Patient characteristics

Cholinergic urticaria patients (n=28)		Mean $\pm$ SD (median, range)
Age (in years)		34.0 $\pm$ 20.0 (29.5, 19 – 76)
BMI		24.2 $\pm$ 5.2 (24.0, 19.4 – 37.2)
Duration of disease (in years)		7.6 $\pm$ 8.5 (5.0, 1-43)
Duration of whealing (in hours)		1.5 $\pm$ 2.3 (1.0, 0.25 – 12)
Severity of disease	CholUSI^{a)}	14.6 $\pm$ 2.4 15.0 (9 – 18)
	PGA score^{b)}	2.3 $\pm$ 0.8 2.5 (1 – 3)

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a) CholUSI = sum score of 1) the frequency of CholU symptoms (less than once a month = 0; once a month = 1; more than once a month = 2; once a week = 3; more than once a week = 4; daily = 5; more than once daily = 6 points), 2) eliciting factors (1 point for each: physical exercise, hot bath, hot shower, emotional stress, hot food, sauna, other), 3) the duration of skin lesions (less than 5 min = 0; 5 to 10 minutes = 1; 10 to 20 min = 2; 20 to 30 min = 3; 30 to 60 min = 4; longer than 1 hour = 5 points), and 4) itch (none = 0; mild = 1; moderate = 2; severe = 3 points). Range 0 - 21: 1-4 points: very mild; 5-9 points: mild; 10-15 points: moderate; >16 points: severe CholU.

b) PGA score: Range 0-3 (none = 0, mild = 1, moderate = 2, severe = 3)

Table E2: High dose antihistamine treatment reduces disease activity in CholU-
patients refractory to standard doses

		Baseline Mean $\pm$ SD (median, range)	End of treatment Mean $\pm$ SD (median, range)	Improve- ment %	p-value
Disease activity (diary based scores)	UAS7	18.6 $\pm$ 9.3 (18.0, 4 – 42)	11.3 $\pm$ 9.0 (8.5, 1 – 39)	39.8	0.002
	Wheal7	8.4 $\pm$ 5.8 (9.0, 0 – 21)	5.2 $\pm$ 5.7 (4.0, 0-21)	38.1	0.01
	Pruritus7	10.2 $\pm$ 5.1 (9.0, 2 – 21)	6.0 $\pm$ 4.6 (5.5, 0 – 20)	41.2	0.002
	CholUAS7 ^{b)}	43.6 $\pm$ 28.5 (37.0, 9 – 114)	25.9 $\pm$ 15.4 (24.0, 0 – 68)	40.6	0.01
	CholWheal7 ^{c)}	18.9 $\pm$ 15.1 (17.5, 0 - 56)	11.5 $\pm$ 11.5 (11.0, 0 – 54)	39.2	0.03
	CholPruritus7 ^{d)}	20.7 $\pm$ 16.1 (20.5, 6 - 60)	14.4 $\pm$ 9.5 (15.5, 0 – 36)	30.4	0.01
	aCholUAS7 ^{e)}	38.7 $\pm$ 8.6 (38.0, 20 – 59)	32.1 $\pm$ 6.2 (31.0, 23 – 52)	17.1	0.0009
	aCholWheal7 ^{f)}	28.5 $\pm$ 5.4 (28.0, 14 – 42)	26.0 $\pm$ 3.9 (27.5, 20 – 39)	8.8	0.02
	aCholPruritus7 ^{g)}	30.0 $\pm$ 5.6 (29.0, 20 – 42)	26.9 $\pm$ 3.5 (27.5, 21 – 34)	10.3	0.006
Intensity of elicitors	Elicitor7 ^{h)}	20.1 $\pm$ 4.8 (21.0, 7 – 28)	20.8 $\pm$ 4.3 (21.0, 7 – 28)	-3.5	0.44
Quality of life	DLQI score ⁱ⁾	9.7 $\pm$ 6.3 (8.5, 1 – 26)	7.6 $\pm$ 7.2 (5.5, 0 – 27)	21.6	0.09

n = 28, significant changes marked in bold.

- a) **CholUAS7** = 7 day sum of [(Whealday + Itchday) x Intensity of elicitor day]; Range 0-168
- b) **CholWheal7** = 7 day sum of (Whealday x Intensity of elicitor day); Range 0-84
- c) **CholPruritus7** = 7 day sum of (Pruritusday x Intensity of elicitor day); Range 0-84
- d) **aCholUAS7** = 7 day sum of [(Whealday + Itchday) + Intensity of elicitor day]; Range 0-70
- e) **aCholWheal7** = 7 day sum of (Whealday + Intensity of elicitor day); Range 0-49
- f) **aCholPruritus7** = 7 day sum of (Pruritusday + Intensity of elicitor day); Range 0-49
- g) **Elicitor7** = 7 day sum of Intensity of elicitor day [no exposure to elicitors = 4, exposure to mild elicitor = 3, exposure to moderate elicitor = 2, exposure to strong elicitor = 1 point]; Range 0-28
- h) **DLQI**; Range 0-30; no impairment = 0-1 points; mild impairment = 2-5 points; moderate impairment = 6-10 points; very large impairment = 11-20 points; extremely large impairment = 21-30 points

Table E3: Comparison of responder (CholUAS7 $\geq$ 50% improvement) and non-responder patients (CholUAS7 less than 50% improvement)

	CholUAS7		
	responder (n=11)	non-responder (n=17)	p-value
Cetirizine 40mg daily	4	2	0.22
Rupatadine 40mg daily	5	10	0.70
Loratadine 40mg daily or Desloratadine 20mg daily	0	3	0.18
Ebastin 40mg daily	2	1	0.36
Fexofenadine 480mg daily	0	1	0.43
Age in years (mean $\pm$ SD)	38.1 $\pm$ 25.0	31.1. $\pm$ 4.7	0.35
Sex (n females : n males)	9:2	10:7	0.20
Duration of disease in years (mean $\pm$ SD)	5.6 $\pm$ 4.9	9.9 $\pm$ 10.1	0.55
CholUSI^{a)} (mean $\pm$ SD)	13.9 $\pm$ 3.5	15.1 $\pm$ 2.2	0.29
PGA score^{b)} (mean $\pm$ SD)	1.8 $\pm$ 1.2	2.5 $\pm$ 0.0	0.03
DLQI^{c)} (mean $\pm$ SD)	9.6 $\pm$ 6.6	9.8 $\pm$ 6.3	0.98
Erlanger Atopy Score (mean $\pm$ SD)	11.5 $\pm$ 4.2	12.7 $\pm$ 2.8	0.38

n = 28; Chi-Square, p <0.05 depicted in bold

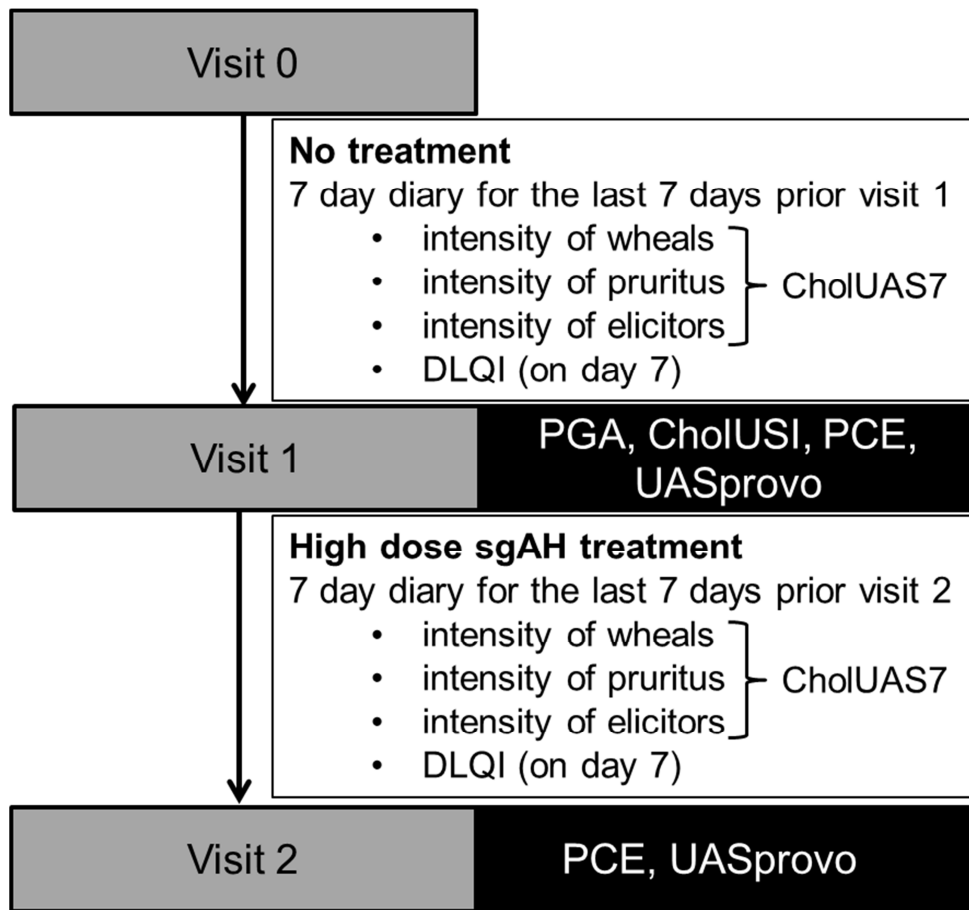
a) CholUSI = sum score of 1) the frequency of CholU symptoms (less than once a month = 0; once a month = 1; more than once a month = 2; once a week = 3; more than once a week = 4; daily = 5; more than once daily = 6 points), 2) eliciting factors (1 point for each: physical exercise, hot bath, hot shower, emotional stress, hot food, sauna, other), 3) the duration of skin lesions (less than 5 min = 0; 5 to 10 minutes = 1; 10 to 20 min = 2; 20 to 30 min = 3; 30 to 60 min = 4; longer than 1 hour = 5 points), and 4) itch (none = 0; mild = 1; moderate = 2; severe = 3 points). Range 0 - 21: 1-4 points: very mild; 5-9 points: mild; 10-15 points: moderate; >16 points: severe CholU.

b) PGA score; Range 0-3 (none = 0, mild = 1, moderate = 2, severe = 3)

c) DLQI; Range 0-30; no impairment = 0-1 points; mild impairment = 2-5 points; moderate impairment = 6-10 points; very large impairment = 11-20 points; extremely large impairment = 21-30 points

1 **Figure E1: Study flow chart**

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3

4 Abbreviations: CholUSI (CholU severity index), DLQI (Dermatology life quality index),
 5 PGA (Physician global assessment), PCE (Puls controlled ergometry), UASprovo
 6 (Urticaria activity score after PCE provocation testing), sgAH (Second generation
 7 antihistamines)

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