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Cholinergic urticaria patients of different age groups have distinct features.

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Abbreviations:

CholU.....	Cholinergic urticaria;
AD.....	Atopic dermatitis
AOD.....	Age at onset of disease
DoD.....	Duration of disease
EO.....	Early onset
LO.....	Late onset
CSU.....	Chronic spontaneous urticaria

Abstract – maximum allowable 250 words

Background: Cholinergic Urticaria (CholU) is a common skin disease characterized by the development of pinpoint sized wheals and severe itch upon physical exercise. Little is known about the epidemiology of CholU. CholU can occur at any age and has the highest prevalence among young adults. As of now, it is unclear if patients of different age show differences in the clinical manifestation of CholU, duration of disease, comorbidities or response to treatment.

Methods: Here, we analysed the demographic data and clinical characteristics including disease duration and comorbidities of 200 CholU patients, 12 to 76 years of age.

Results: We identified two distinct types of CholU, one with early onset (EO, 71%) and one with late onset (LO, 29%). EO and LO CholU patients markedly differ in key characteristics: EO patients, who had a disease onset before the age of 36, showed no gender preponderance, and had a significantly higher rate of concomitant atopic dermatitis (16.9 vs. 5.2%; $p=0.028$) and higher IgE-Levels (295.5 vs. 267.1 IU/ml; $p=0.020$) as compared to LO patients, who were mainly female (69%), had a shorter duration of disease (33.3 vs. 63.7

months; $p=0.005$), a higher rate of concomitant other forms of urticaria (48.3 vs. 33.1%; $p=0.044$), and a higher rate of psychiatric comorbidities (12.1 vs 1.4%; $p=0.001$).

Conclusion: There are two subtypes of CholU patients with different gender ratios, disease duration, and comorbidities. These findings suggest that two distinct underlying pathogenetic pathways are relevant in these two subgroups of CholU patients.

Introduction

Cholinergic Urticaria (CholU) is a common skin disease, characterized by pinpoint-sized wheals and severe itch, triggered by physical exercise and passive warming. Other known triggers are the consumption of hot or spicy food and emotional distress. The wheals typically appear on the upper body and usually remain for some minutes up to an hour before they spontaneously disappear (1, 2).

In most cases the disease is self-limited, it usually lasts for several years, and then shows spontaneous remission. Some patients suffer from frequent and strong outbreaks, which severely reduce their quality of life (3). As of yet, the underlying etiology is ill characterized, and there is no cure. Consequently, CholU patient rely on trigger avoidance and symptomatic treatment. Current guidelines recommend the daily intake of a second generation H1-antihistamine (4, 5), but the success in symptom control is often limited (6). In difficult-to-treat cases, up dosing of antihistamines (7) or the off-label use of Omalizumab (8) can be beneficial.

In spite of the typical clinical features of the disease, the pathogenesis of CholU is not yet fully understood. Several studies suggest that CholU patients have allergic reactions against their own sweat (or against fungal proteins contained in their sweat) (9-13). On a molecular level, other studies have found elevated levels of mast cell activation and higher levels of

IgE (13, 14). CholU has also been associated with hypohydrosis (15, 16), suggesting that poral occlusion due to hyperkeratosis or deformed sweat-glands in some CholU patients result in occluded sweat as a cause of their signs and symptoms (15, 17). Other proposed pathomechanisms include aberrations in the acetylcholine/acetylcholinereceptor pathway and dysregulation of sweat induction (18), reduced levels of alpha-1-antichymotrypsin (19), and aberrant systemic responses to body temperature elevation (20).

Previous studies suggest a high prevalence of CholU, and up to 10% of young adults have been claimed to be affected (6, 21, 22). CholU can occur at any age during life, but no studies on larger patient cohorts have characterized and compared the incidence of CholU in defined age groups or reported the distribution and average age of onset of CholU. Also, nothing is known about the impact of age or age at onset of the disease on CholU features.

Here, we analysed the demographic and clinical data of 200 individual CholU patients for the impact of age of onset on the clinical features of CholU. Specifically, we hypothesized that patients who develop CholU during different times in their lives exhibit differences in their CholU duration and severity.

Materials and Methods

The study was approved by the Ethics Committee of the Charité - Universitätsmedizin, Berlin (approval # EA1/241/15), registered in the German Clinical Trials Register (DRKS-ID: DRKS00012387), 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.

Patients & Study design:

In this study, we analysed 200 consecutive ChIU patients who visited the urticaria outpatient clinic at the Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, before January 2016. These patients were all referred to us by dermatologists with the suspected clinical diagnosis of ChIU. We confirmed this diagnosis based on a systematic history, in all patients, and on the presence (and/or photodocumentation provided by patients) of the clinical signs and symptoms of ChIU and/or the results of provocation testing, in most patients. Patients were selected for this study based on their main diagnosis (ICD-10-code: L50.5), independently of age, sex and other characteristics. Patients with ChIU, who also had chronic spontaneous urticaria, other forms of chronic urticaria or exercise-induced anaphylaxis, were only included in the study, if ChIU was the dominant disease. The main information concerning age at first presentation at the Department, age at onset of disease, gender, duration of disease until first presentation, main symptoms, trigger factors, concomitant allergies, concomitant other forms of urticaria, psychiatric disorders, treatment and response to treatment were extracted from the patient charts. Treatment outcome was categorized as positive and negative, when patients reported a complete remission or major improvement of ChIU symptoms and when patients reported minor or no improvement of symptoms, respectively. Depression and anxiety diagnosed prior to or during treatment at our outpatient clinic were included in the analysis as concomitant psychiatric disorders. Patients' characteristics at first presentation are given in table 1.

Statistical Analyses

Statistical analysis was performed using IBM SPSS Statistics Version 24 and Graph Pad Prism Version 6 for Windows, 2012. The statistical significance of differences between patient groups was calculated using the Mann-Whitney U-test and the Chi-Square test.

Correlations were calculated using the Spearman's rank test, $P < 0.05$ was considered to indicate a significant difference.

Detailed subgroup analysis was performed using R (Version 3.3.2). CholU patient subgroups with a normal distribution were identified by the method of mixed distribution (23). Histograms were calculated using Library ggplot2 and the option + geom_density (alpha = 0.2). Cutoffs were calculated using github library cutoff by Marc Choisy: <http://marcchoisy.free.fr/fmm/index.html>.

Results

CholU patients have a wide range of age at onset of disease

Of the 200 patients included in the study, 112 (58%) were female (88 male), and the age at onset of CholU ranged from 12 to 76 years. The mean age at onset was 28.1 (SD $\pm$ 14.0) years and was significantly higher in women than in men (30.2 vs 25.2 years, $p = 0.003$). Also, the age at their first presentation in our outpatient consultation centre was significantly higher for women (34.2 vs. 29.9 years, $p = 0.015$) (see Table 1).

CholU patients can be divided in two populations: early onset vs. late onset

Mixed distribution analysis of our CholU patients revealed two populations that differed in their age at onset, with a calculated cut-off at 36 years (Figure 1a, Suppl. Figure 1). Patients with an early onset (EO) of disease (range 12 – 35 years) account for 71% ($n = 142$) of the total population as compared to 29% ($n = 58$) of patients with late onset (LO, range 36 – 76 years). These groups differed significantly in some key disease characteristics (see Table 2).

EO and LO ChIU patients show different gender distribution

In the EO-group, both genders were equally represented (53.5% female), while in the LO-group a majority of 69% were female ($p=0.045$). As demonstrated in figure 1b, female ChIU patients show two distinct peaks of age of onset of ChIU. In contrast, male ChIU patients only show one peak of age of onset, before the age of 36 years (Figure 1c).

EO ChIU patients show longer duration of disease

On average, the duration of ChIU was 55.3 months (SD=74.3 months), with a range from 3 months to 50 years (Table1). EO-patients showed a significantly longer duration of disease (mean 63.7 months SD $\pm$ 82.9) as compared to LO-patients (mean 33.3 months SD $\pm$ 37.2; $p=0.005$).

Rates of atopic dermatitis and levels of IgE in EO-group

About one third of all ChIU patients (37.5%) suffered from concomitant allergic diseases including atopic dermatitis (AD, $n = 27$, 13.5%). AD was significantly more frequent in the EO-group with 16.9% versus 5.2% in the LO-group ($p=0.028$). Also, total IgE levels were higher in the EO-group (295 vs. 267 IU/ml, $p=0.020$).

Concomitant chronic urticaria and psychiatric disorders are more common in patients with LO ChIU

About one in three ChIU patients ($n = 75$, 37.5%) had another form of chronic urticaria, in addition to their ChIU, mostly chronic spontaneous urticaria (CSU, $n=49$). Comorbid CSU was significantly more frequent in patients with LO ChIU (48.3%) than with EO ChIU (33.1%, $p=0.044$). Also, psychiatric disorders were significantly more frequent in the LO-group (12.1 vs. 1.4%, $p=0.001$).

EO and LO ChIU are comparable in their clinical features and response to treatment

As for symptoms, patients with both, EO and LO showed the typical clinical features of ChIU. Whealing (98%), itch (98.5%) or both were the leading symptoms in all patients, independent of their age. Also, 51% experienced flare type skin reactions and 24.5% had experienced at least one episode of angioedema. Relevant reported triggers for ChIU symptoms were physical activity (93.5%), passive warming (71.5%), emotional distress (37.5%), the consumption of hot food and drinks (5.0%), or a combination of these trigger. There were no statistically significant differences between EO and LO ChIU patients regarding these clinical features.

Discussion

This study shows, to our knowledge for the first time, that ChIU patients fall into two subgroups, i.e. patients with early and patients with late onset of disease. The two groups are markedly different in their gender ratios, their duration of disease, and rates of comorbidity including chronic spontaneous urticaria, thyroid disease, depression and anxiety.

A "typical" ChIU patient is widely held to be a young adult, and in the few previous studies on ChIU, the gender distribution was almost equally female or male, with a slight predominance for women (21, 22). Indeed, the majority of our ChIU patients were young, i.e. in their twenties and thirties. Surprisingly, in our population analysis, we also saw a sizeable cohort of "untypical" ChIU patients aged 36 or older at the time of onset (LO). The mixed distribution model clearly identified two patient groups among ChIU patients: EO and LO ChIU patients.

The "typical" younger ChIU patients had characteristics that were described before for ChIU patients. They were almost equally female or male, showed a long duration of disease, and they had high rates of concomitant atopic dermatitis and elevated total IgE

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levels. The latter findings support the existing hypothesis that atopic dermatitis and ChIU are linked and share pathogenic features (24). Patients who have both, ChIU and AD score higher in disease activity scores and appear to be more severely affected in their quality of life (13). New data suggest a shared hypersensitivity to common antigens in sweat for these two diseases (25). In another study, IgE antibodies against human sweat were significantly elevated in both ChIU and AD patients and linked to higher disease activity than in their control groups (13). Accordingly, ChIU patients with an early onset of disease may have their urticaria because they are type-I-allergic against sweat antigens, as described in former reports (9). It is feasible that this patient cohort could benefit from anti-IgE (Omalizumab) therapy (26) and from desensitization therapy with autologous sweat as described in former reports (27). A better understanding of this link could facilitate the successful treatment of affected patients, as about half of this patient group does not respond to antihistaminergic treatment, even when treated off-label with up to 4 times the licenced dose. This poor response to antihistamines has also been described before (7).

The most striking result of our study is that we identified a distinct and substantial subgroup of “untypical” ChIU patients, aged 36 or older at the time of onset of disease. These ChIU patients were mostly female, had higher rates of concomitant other forms of chronic urticaria and suffered more frequently from psychiatric comorbidities than the younger age group. For female ChIU patients, the distribution in 2 major age at onset groups could point to the importance of hormonal effects, as the first peak coincides with the most fertile period and the second peak with the premenopausal age. Interestingly, ChIU activity ceased completely during pregnancy in two of our patients (data not shown). The most frequent concomitant chronic urticaria form was chronic spontaneous urticaria (CSU). As this link had not been reported before, we recommend that patients of all ages with known other forms of urticaria such as CSU should be screened in their anamnesis for ChIU, especially patients aged 36 years or older.

Females of older age are more prone to autoimmune diseases like Hashimoto's disease, which is linked to CSU (28). A contribution of autoimmune pathomechanisms in LO ChIU patients appears likely, perhaps through autoantibodies directed against sweat glands that lead to secondary poral occlusion (2). Accordingly, it is feasible that LO ChIU patients respond best to immunosuppressive therapy (29). It will be interesting and important to compare EO and LO ChIU patients for differences in their therapeutic responses in future trials.

Interestingly, EO and LO patients cannot be distinguished based on their clinical profile. The eliciting factors and clinical signs and symptoms of their ChIU were comparable. This points towards the fact that the final stretch (wheal formation due to histamine release) of the induction of ChIU signs and symptoms is the same in both subforms, whereas the cause and the signals leading to histamine release may be different. As outline above, future trials will need to clarify if EO and LO ChIU are linked to different underlying pathomechanisms and responses to treatment. As the evaluation of the age at onset of disease is a routine question in clinical practice, patient grouping into EO and LO ChIU patients is very easy and quick and would be an easy tool for optimizing treatment.

Our study has additional epidemiological findings of interest. The duration of disease until first presentation was significantly different in the two patient subgroups. It might be shorter for older patients due to their higher disease activity, stronger limitations in daily life at work or at home, or better use of medical services. The higher prevalence of depression and anxiety in LO ChIU patients could be due to the higher incidence of psychiatric disorders in this age group or because of their high disease activity and insufficient treatment of ChIU. On the other hand, depression and anxiety may be risk factors to develop ChIU, and they could influence the perception of ChIU signs and symptoms, as described for other forms of chronic urticaria (30)

The limitations of this study are mainly concerning the monocentric retrospective approach. Not all patients were subjected to provocation testing. Analysed patients might overall belong to the most severely affected patients, as they were referred to a tertiary center (Urticaria Center of Reference and Excellence, UCARE). This could also explain the overall higher female frequency, as there is in general a higher willingness of women to seek specialized medical help (31).

In summary, this study of a large CholU patient cohort describes, for the first time, a distinct subtype of CholU patients, characterized by their late onset of disease. CholU patients of this subgroup are different from “typical” CholU patients in their gender ratio, disease duration, and comorbidities. Our findings suggest that two distinct underlying pathogenetic pathways are relevant in these two subgroups of CholU patients. Further studies are needed to identify and characterize these pathways, which may lead to more targeted and better treatment.

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Table 1: Male vs. Female

	Total (n=200)	Male (n=84)	Female (n=116)	p-Value
Sex (% female)	58.0	0	100	-
Age at onset of disease (MW $\pm$ SD)	28.1 $\pm$ 14.0	25.2 $\pm$ 13.8	30.2 $\pm$ 12.8	0.003
Age at first visit (MW $\pm$ SD)	32.4 $\pm$ 13.4	29.9 $\pm$ 13.2	34.2 $\pm$ 13.3	0.015
Duration of disease (months)	55.3 $\pm$ 74.3	58.0 $\pm$ 81.9	53.1 $\pm$ 68.1	0.462
Total IgE (IU/ml)	287.2 $\pm$ 532.4	339.8 $\pm$ 466.2	256.5 $\pm$ 568.8	0.094
Treatment with AH efficient (% yes)*	55.2	56.6	54.1	0.743
Concomitant diseases	Allergies (% yes)	37.5	37.9	0.882
	Atopic dermatitis (% yes)	13.5	14.7	0.574
	Other forms of chronic urticaria (% yes)	37.5	40.5	0.300
	Thyroid disease (% yes)	13.5	18.1	0.025
	Psychiatric disorders (% Depression/ Anxiety)	4.1	6.9	0.055

* Treatment with AH was considered efficient when patients reported a complete absence or major improvement of ChIU symptoms

Table 2: Younger (<36 years) vs older (≥ 36 years) patients

Age at onset of disease (n=200)		EO < 36 years (n=142)	LO ≥ 36 years (n=58)	p-Value
Sex (% female)		53.5	69.0	0.045
Duration of disease until first presentation (months)		63.7	33.3	0.005
Total IgE (IU/ml)		295.5	267.1	0.020
Treatment with AH efficient* (% yes)		51.6	64.6	0.123
Concomitant diseases	Allergies (% yes)	37.3	37.9	0.936
	Atopic dermatitis (% yes)	16.9	5.2	0.028
	Other forms of chronic urticaria (%) yes)	33.1	48.3	0.044
	Thyroid disease (% yes)	7.0	29.3	0.025
	Psychiatric disorders (% Depression/Anxiety)	1.4	12.1	0.001

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Figure 1a.b.c.:

Histograms depict the age distribution of the CholU patients at the time point of onset of the disease. Age groups were separated in 5 years intervals. Dotted black line depicts the overall density of the combined population. The red and green line depicts the two normal distributed subpopulation as calculated by the mixed distribution model. A) All CholU patients: two subpopulations are distinctable. b) Female CholU patients: two subpopulations are more distinct visible compared to all CholU patients c) Male CholU patients: no clear separation into two subpopulations possible; domination of early onset group

Supplemental material S1:

Calculated cut-off between EO and LO CholU patients. Histogram depicts the age distribution of all CholU patients at the time point of onset of the disease. Age groups were separated in 0.5 years intervals. Black line depicts the overall density of the combined population, the cut-off value (blue line), separates the population into two densities (red dotted and green dotted line). The blue dotted line depicts the 95% confidence interval for the cut-off value.

Female and Male







