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LETTER TO THE EDITOR

Severe cold urticaria can point to an underlying clonal mast cell disorder

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KEYWORDS

basal serum tryptase, clonal mast cell disorder, cold urticaria, systemic mastocytosis

To the Editor:

Gain-of-function mutations of KIT, most frequently D816V, cause clonal mast cell disorders (CMCD) and drive severe anaphylaxis in patients with systemic mastocytosis (SM). The connection between CMCD and chronic inducible urticaria (CIndU) has not been investigated before.

Cold urticaria (ColdU) is characterised by the appearance of wheals and/or angioedema in response to body cooling and rewarming. In cold-induced anaphylaxis (ColdA) there is a sudden cold-induced onset of at least two of the following: (a) involvement of the skin and/or mucosal tissue, (b) respiratory involvement, (c) reduced blood pressure or associated symptoms, or (d) gastrointestinal

symptoms. Clinical presentations of ColdU depend on the potency and duration of exposure to different cold triggers (air, fluids, surfaces), individual cold sensitivity thresholds and other yet to be defined factors. The diagnosis of ColdU relies on the patient's history and positive cold stimulation testing (CST) with an ice cube and/or *TempTest*[®] or other less commonly used methods. Critical temperature threshold (CTT) is the highest *TempTest*[®] temperature and the critical stimulation time threshold (CSTT) is the shortest time required to induce a wheal in CST.¹

Here, we describe detailed characteristics of a 65-year old female patient who presented with a 19-year history of ColdU with increasing severity two years before evaluation and compare them to characteristics of 35 other subsequent ColdU patients comprehensively evaluated at the Urticaria Center of Reference and Excellence (UCARE) at the University Clinic Golnik². The following data indicated a severe ColdU (Table 1): (a) wheals induced by air temperatures of 24°C or lower, (b) an unusual 5-minute episode of dyspnea after drinking cold water, and (c) high disease activity with a 30 second CSTT and a 28°C CTT, with ice cube- and *TempTest*[®]-induced pseudopodial whealing, and whealing beyond the *TempTest*[®]-stimulated skin area (Figure 1A, Figure 1B). Because these findings pointed to unusually severe ColdU as compared to 35 control ColdU patients (Table 1), we determined the patient's basal serum tryptase levels, which were elevated twice in a four months period (17.3, 23.0 ng/mL). An underlying CMCD was suspected and confirmed by a positive KIT D816V mutation test in circulating blood leukocytes (1.0% mutational burden) and in the bone marrow. No cytomorphological features of SM were found in the bone marrow.

The diagnosis of SM is established if: (a) at least a major and one minor or (b) three minor criteria defined by the World Health Organization are fulfilled.³ Our patient did not meet these criteria. Since she presented with symptoms of mast cell activation, the KIT D816V mutation, and lacked signs of cutaneous mastocytosis, she was diagnosed with a monoclonal mast cell activation syndrome (MMAS).⁴ MMAS has not been previously described in patients with ColdU, probably because they are hardly ever assessed for a CMCD.

Our findings suggest that severe reactivity to cold triggers, in patients with ColdU, can point to comorbid MMAS and the possibility that MMAS is a risk factor for severe reactions. Our patient's reaction to drinking cold water, a single, short-lived episode of dyspnea with spontaneous resolution, may have been ColdA or, more likely, a severe local response. Screening CIndU patients with severe

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disease for an elevated tryptase and the presence of the KIT D816V mutation could help to clarify the rates and role of activating KIT mutations as a potential driver for severe reactions. It could also improve our approach to the management and treatment of patients with severe KIT D816V-positive ColdU, for example by the use of selective tyrosine-kinase inhibitors, such as avapritinib or other KIT-targeting therapies, and of omalizumab, which has been shown to benefit patients with CIndU and effectively prevent anaphylaxis in patients with MMAS and SM.^{5,6}

TABLE 1 Characteristics of study participants

Characteristics	Described patient	Comparative group (N = 35)
A. Demographics and baseline characteristics		
Age (years)	65	Median (range): 40 (18-73)
Female gender	Yes	66% (23/35)
BMI (kg/m ²)	24.0	Median (range): 24.9 (18.0-36.0)
B. Patient history data		
Age at onset of ColdU (years)	46	Median (range): 33 (9-60)
Early onset of ColdU (≤ 18 years old)	No	9% (3/35)
Duration of ColdU before evaluation (months)	228 [‡]	Median (range): 60 (2-384)
Wheals induced by cold	Yes	100% (35/35)
Induced by air temperatures of $\leq 20^{\circ}\text{C}$	Yes	66% (23/35)
Induced by air temperatures of $> 20^{\circ}\text{C}$	Yes [‡]	11% (4/35)
Induced by contact with solid surfaces	Yes	37% (13/35)
Induced by localized contact with cold liquids	Yes	43% (15/35)
Maximal duration of wheals (min)	45	Median (range): 60 (5-720)
Itch induced by immersion in the sea	Yes	63% (22/35)
The shortest time to onset of itch (minute/s)	1 [‡]	Median (range): 5 (1-20), N=18
Angioedema induced by cold	Yes	34% (12/35)
Maximal duration of angioedema (minutes)	45	Median (range): 60 (20-720), N=11
Oropharyngeal or laryngeal symptoms induced by cold	Yes [‡]	31% (11/35)
Gastrointestinal symptoms induced by cold	No	14% (5/35)
Respiratory symptoms induced by cold	Yes	37% (13/35)
Induced by ingestion of cold drinks	Yes [‡]	0% (0/35)
Induced by air temperatures of $\leq 20^{\circ}\text{C}$	No	9% (3/35)
Induced by air temperatures of $\leq 20^{\circ}\text{C}$, no asthma	No	3% (1/35)
Immersion in the sea	No	11% (4/35)
Symptoms of reduced blood pressure induced by cold	No	29% (10/35)
Wind is an aggravating factor	Yes	60% (21/35)
Increased humidity is an aggravating factor	Yes	49% (17/35)
Asthma	No	34% (12/35)
At least one: asthma, allergic rhinitis, allergic conjunctivitis	No	40% (14/35)

or atopic dermatitis

Thyroid disease	No	17% (6/35)
Systemic reaction to a hymenoptera sting	No	3% (1/35)
Positive family history of ColdU	No	6% (2/35)

C. Cold stimulation testing (CST) results^a

Positive ice cube test	Yes	66% (23/35)
The shortest CSTT ever determined (seconds)	30 [‡]	Median (range): 300 (30-300), N=22
Ice cube-induced pseudopodial whealing	Yes [‡]	9% (2/23)
Positive TempTest [®]	Yes	43% (15/35)
The highest CTT ever determined (°C)	28 [‡]	Median (range): 17 (6-25), N=15
TempTest [®] -induced wheal diameter	10 [‡]	Median (range): 9 (5-15), N=11
TempTest [®] -induced pseudopodial whealing	Yes [‡]	0% (0/11)
Whealing beyond stimulated skin area	Yes [‡]	0% (0/11)

D. Patient reported outcome

ACUSI score (5-18)	13 [‡]	Median (range): 10 (6-17)
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E. Diagnosis

Cold contact urticaria ^b	Yes	60% (21/35)
Ice cube and TempTest [®] positive	Yes	43% (15/35)
Ice cube positive at least once, TempTest [®] never positive	No	17% (6/35)
Ice cube never positive, TempTest [®] positive at least once	No	0% (0/35)
Localized ColdU	No	3% (1/35)
Localized cold reflex urticaria	No	6% (2/35)
Systemic atypical ColdU suspected (negative local CST)	No	31% (11/35)
ColdA	No	34% (12/35)
Comorbid other form of CIndU	No	17% (6/35)
Comorbid CSU	No	17% (6/35)

F. Laboratory findings

Total IgE level (IU/mL)	113	Median (range): 97 (9-2050), N=30
Basal serum tryptase (ng/mL) ^c	23.0	Median (range): 5.5 (2.9-15.9), N=33
Elevated basal serum tryptase (> 11.4 ng/mL)	Yes	15% (5/33)
Basal serum tryptase > 8.0 ng/mL ^d	Yes	18% (6/33)
KIT D816V mutation in circulating blood leukocytes	Yes	0% (0/3)
KIT D816V mutation in the bone marrow	Yes	0% (0/2)

Positive cryoglobulin test	No	28% (9/32)
Positive cold agglutinin test at 4°C	Yes	44% (15/34)
Cold agglutinin titer at 4°C ^e	2	Mean ± SD: 1.4 ± 2.9, N=34

Comparative group: A total of 35 cold urticaria patients represent a comparative group. Results are shown as percentage (%) of total for categorical variables and median (range) or mean ± SD for numerical variables.

Described patient: [‡]Values indicating a more severe disease.
Values exceeding the range of a comparative group are in **bold**.

All patients: ^aCST results from past evaluations were also obtained.

^bWhealing on cold-stimulated area on the forearm is obligatory for diagnosis.

^cThe highest level of basal serum tryptase ever determined.

^dCut-off value for likelihood of hyper-alpha-tryptasemia.

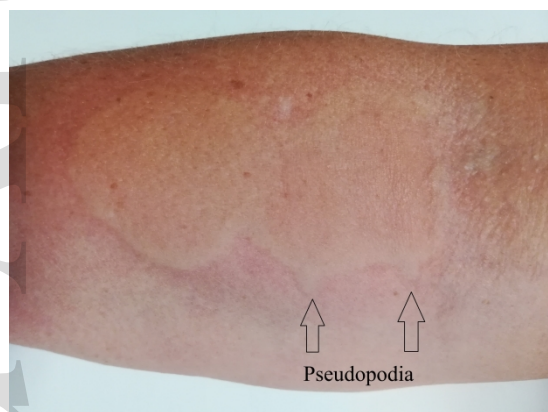
^eThe numbers are a reciprocal expression of cold agglutinin titers (i.e. 2 = agglutination at 1:2 dilution).

Abbreviations: ACUSI, Acquired Cold Urticaria Severity Index; BMI, body mass index; CIndU, chronic inducible urticaria; ColdA, cold-induced anaphylaxis; ColdU, cold urticaria; CST, cold stimulation testing; CSTT, critical stimulation time threshold; CSU, chronic spontaneous urticaria; CTT, critical temperature threshold; IgE, immunoglobulin E; N, number of patients; SD, standard deviation.

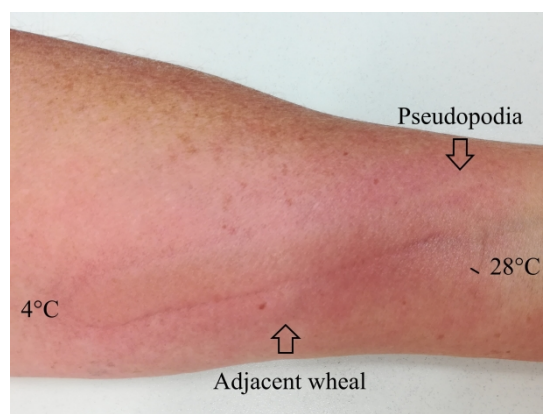
FIGURE 1 Cold stimulation test (CST) results on the volar forearms

Panel A shows wheal and flare reactions with pseudopodia after stimulation with a melting ice cube placed in a non-latex glove. Panel B shows an unusually strong wheal and flare reaction after stimulation with TempTest®: 10 millimeter wide wheal on the scale from 4°C to 28°C, pseudopodia and an additional wheal in proximity to stimulated area.

A Ice cube test result



B TempTest® test result



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CONFLICT OF INTEREST

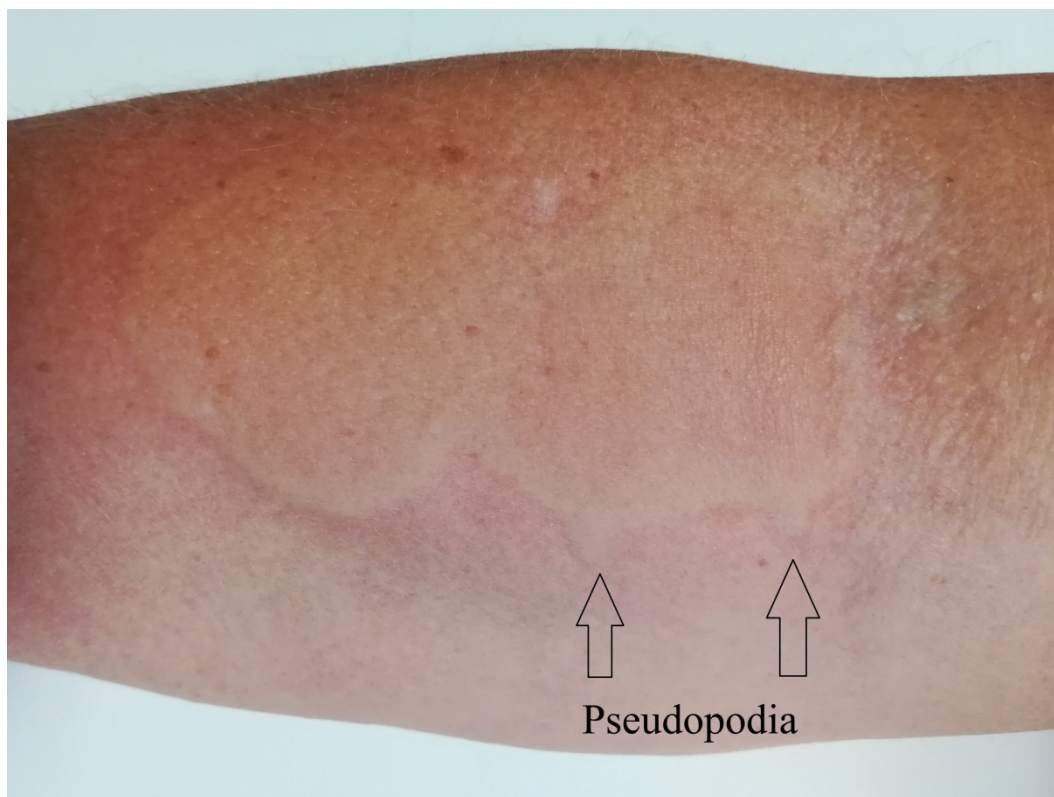
MB has been a speaker and an advisor for Novartis. MM is or recently was a speaker and/or advisor for and/or has received research funding from Allakos, Aralez, ArgenX, AstraZeneca, Celldex, Centogene, CSL Behring, FAES, Genentech, GIInnovation, Innate Pharma, Kyowa Kirin, Leo Pharma, Lilly, Menarini, Moxie, MSD, Novartis, Roche, Sanofi/Regeneron, Third HarmonicBio, UCB, and Uriach. MK, DTM, SZ and TM have nothing to disclose. FS reports non-financial support from Allakos, grants and personal fees from Blueprint, grants and personal fees from Novartis, personal fees from Sanofi-Aventis, outside the submitted work.

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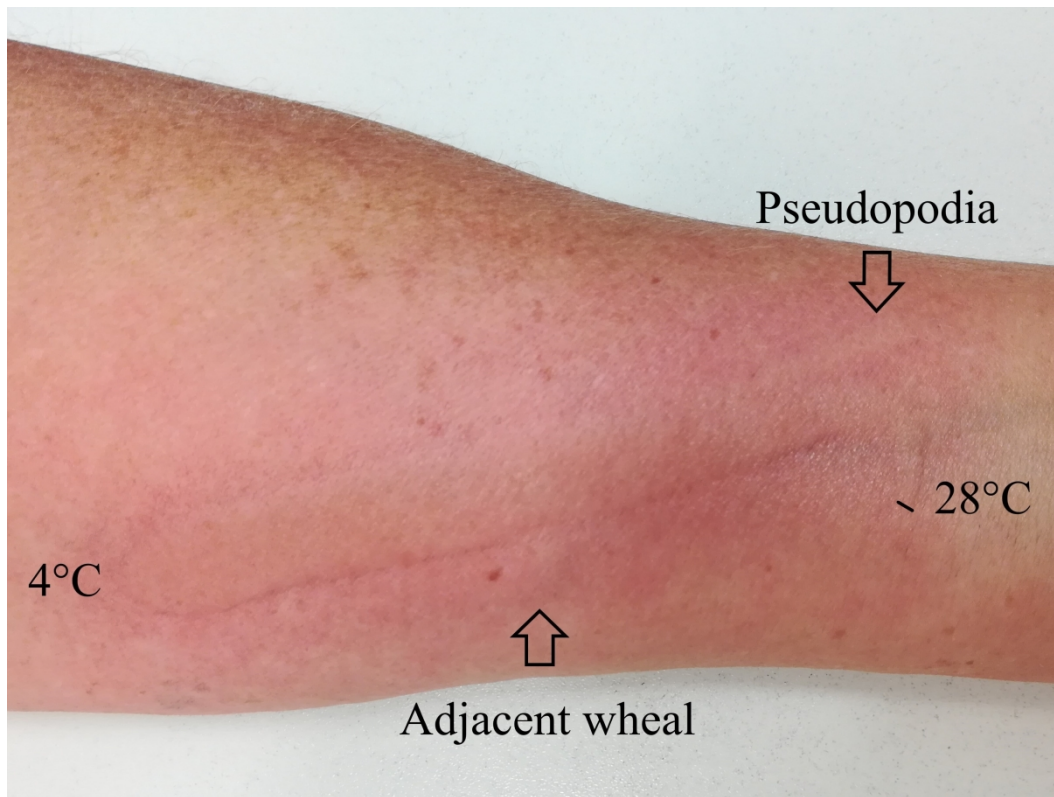
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.



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Severe cold urticaria can point to an underlying clonal mast cell disorder

Mojca Bizjak, Marcus Maurer, Mitja Košnik, Dorothea Terhorst-Molawi, Samo Zver, Thomas Burmeister, Frank Siebenhaar

Patients

A total of 36 consecutive patients with cold urticaria (ColdU) who were referred to the Urticaria Center of Reference and Excellence (UCARE) at the University Clinic Golnik from May 1, 2019 to March 31, 2020 (11-months period) were recruited to participate in this study. It was approved by the National Medical Ethics Committee of the Republic of Slovenia. All patients gave written informed consent.

A diagnosis of ColdU was confirmed by a dermatologist based on patient history and results of local cold stimulation testing (CST), which was performed in all (N = 36) patients. If CST was negative, the diagnosis was established based on reliable medical history of cold-induced reactions and patients' photographs of these reactions. General body cooling to confirm systemic atypical ColdU was not performed in this study.

Methods

We placed an ice cube measuring 25x25 mm (melting in a small amount of water in a non-latex glove) on the volar forearm for five minutes, three minutes, one minute, and 30 seconds (Figure S1A). Skin response was assessed ten minutes after the end of cold stimulation to determine the critical stimulation time threshold (CSTT).^{S1-3}

We also used a standardised TempTest® thermoelectric device which consists of a single U-shaped piezoelectric element that generates a continuous temperature gradient ranging from 4°C

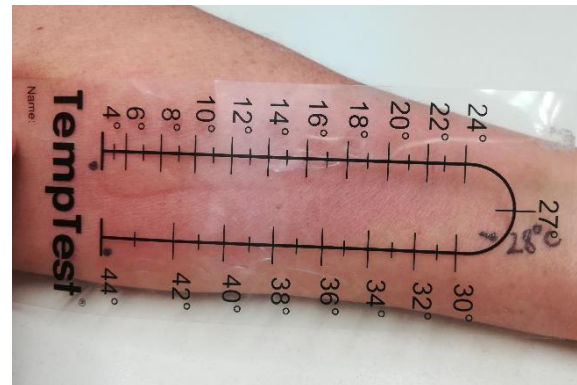
to 44°C (Figure S1B) to determine the critical temperature threshold (CTT).^{S1,S3,S4} The TempTest® technology is based on the Peltier effect, comprising heating or cooling of plastic embedded thermoelectric elements according to the polarity and voltage of an electric current passing through two semiconductors.^{S4,S5} It allows measurements of CTTs with an accuracy of $\pm 1^\circ\text{C}$.^{S3} Importantly, CTT testing determines the critical skin temperature rather than the critical exposure temperature, e.g. air or water temperature. These are not the same, as thermoregulatory mechanisms allow the skin to counteract the adoption of environmental temperatures.^{S4} We placed the patient's volar forearm on the device for five minutes (Figure S1C), and assessed her skin response ten minutes thereafter (Figure S1D).

Due to the severe and potentially life-threatening reactions our patient had experienced, her diagnostic workup at initial consultation included complete blood count with differential and C-reactive protein (CRP) as recommended by the current international urticaria guideline^{S6} and, additionally, basal serum tryptase (ImmunoCAP 100 Thermo Fisher Scientific, Uppsala, Sweden). The blood sample was taken more than 24 hours after the complete resolution of any cold-induced clinical signs and symptoms. We then analyzed circulating blood leukocytes for KIT D816V mutation using a highly sensitive quantitative PCR technique.^{S7} A bone marrow biopsy was also taken and investigated for systemic mastocytosis (SM) criteria as defined by the World Health Organization (WHO) criteria.^{S8} Other laboratory tests included routine blood biochemistry, total IgE level, cold agglutinin test at 4°C, and cryoglobulin test. We performed a physical examination including a whole body check for typical cutaneous mastocytosis lesions. The patient completed the qualitative Acquired Cold Urticaria Severity Index (ACUSI) questionnaire, which was developed for cold urticaria severity grading.^{S3}

Laboratory findings are presented in Table 1 of the manuscript. Additionally, no relevant abnormalities of complete blood count with differential and routine blood biochemistry were found. CRP level was normal. Clinical examination did not reveal cutaneous mastocytosis lesions.

FIGURE S1 Cold stimulation testing (CST) procedure on the volar forearm

Panel A shows skin testing with a melting ice cube in a glove. Panel B shows the TempTest® device with a U shaped aluminum element (length 350 mm, width 2 mm) providing a continuous gradient of temperatures ranging from 4 to 44°C. Panel C shows the TempTest® provocation procedure. Panel D shows skin response evaluation using the foil stencil.

A Ice cube test**B** TempTest® device**C** TempTest® testing procedure**D** Critical temperature threshold reading

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88 **Data availability statement**

89 The datasets generated for this study are available on request to the corresponding author.

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