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Cold-induced anaphylaxis: new insights into clinical and genetic characteristics

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Introduction: The pathogenesis of cold urticaria (ColdU) and cold-induced anaphylaxis (ColdA) remains poorly understood, and ColdA is underrepresented in anaphylaxis literature. Laboratory features to guide management are largely unknown. This study evaluated basal serum tryptase (BST) and total immunoglobulin E (IgE) levels in ColdU and ColdA, their associations with clinical features, and the utility of testing for the *KIT* p.D816V variant in blood leukocytes and hereditary α-tryptasemia (HαT).

Methods: Ninety-two adults with ColdU were enrolled. ColdA was defined as a reaction involving skin and/or visible mucosal tissue with cardiovascular, respiratory, or gastrointestinal manifestations. Evaluations included patient history, standard cold stimulation testing (sCST) using an ice cube and TempTest®, and laboratory tests.

Results: ColdA was diagnosed in 35.9% of patients. ColdU phenotypes based on sCST included typical ColdU (52.2%), localized cold-reflex urticaria (5.4%), and ColdU with negative sCST (42.4%). Negative sCST, compared to typical ColdU, was associated with fewer ColdA cases ($p = 0.004$) but more spontaneous wheals ($p < 0.001$). ColdA patients more frequently exhibited generalized wheals ($p = 0.047$), skin angioedema ($p = 0.007$), oropharyngeal/laryngeal manifestations ($p < 0.001$), and itchy earlobes ($p = 0.002$) than non-ColdA patients. Elevated BST levels (>11.4 ng/mL) in 9.8% of patients were attributed to *KIT* p.D816V and/or HαT. *KIT* p.D816V was detected in 6.6% of ColdU and 6.3% of ColdA patients. HαT prevalence was higher in ColdU (10.9%) and ColdA (15.2%) than the general population (estimated at 5.7%; $p = 0.041$ and $p = 0.038$). Total IgE levels were significantly higher in ColdA than non-ColdA ($p = 0.021$).

Discussion: This study confirmed clinical features linked to ColdA previously identified by the multicenter COLD-CE study, including generalized wheals, skin angioedema, oropharyngeal/laryngeal manifestations, and itchy earlobes. We identified new high-risk features. ColdA is more frequently associated with typical ColdU than with ColdU with negative sCST, the latter being linked to

spontaneous wheals. ColdA is additionally associated with higher total IgE levels. Furthermore, patients with ColdU and ColdA exhibit higher prevalence of *KIT* p.D816V and HαT compared to general population data, a finding not previously reported. Further research is needed to explore their clinical implications.

KEYWORDS

anaphylaxis, cold urticaria, hereditary α-tryptasemia, KIT p.D816V, mast cell, total IgE, tryptase

1 Introduction

Cold-induced anaphylaxis (**ColdA**) is a potentially life-threatening systemic reaction triggered by cooling in patients with cold urticaria (**ColdU**) (1–3). It requires the urgent administration of adrenaline but is often underrecognized and undertreated (1, 4). The condition remains poorly understood, with no universally accepted definition and limited insight into its underlying mechanisms (1, 5). Additionally, literature on anaphylaxis frequently overlooks ColdA (1).

The clinical evaluation of patients with ColdU and ColdA presents significant challenges. Standard cold stimulation testing (**sCST**) on the volar forearm, which involves a 5-minute test using an ice cube melting in a small amount of water and/or a TempTest® device with a 4–44°C electrode, often produces negative results (2, 6). In the large multicenter COLD-CE study, sCST was negative in 25% (139/551) of enrolled patients (2). The following phenotypes of ColdU can be diagnosed based on sCST: typical ColdU (i.e., whealing directly over the stimulated area), cold-reflex urticaria (i.e., papular wheals adjacent to the stimulated area) and ColdU with negative sCST (i.e., no whealing within 10 minutes). To further define ColdU with negative sCST, alternative provocation methods are required, such as total body cooling (5, 7). While ColdA has been reported in over a third of patients with typical ColdU (1, 2), its prevalence in other ColdU subtypes remains unknown (5, 8).

Anaphylaxis results from the sudden systemic release of mediators from mast cells (**MCs**) and basophils (9). MCs are distributed throughout vascularized tissues (10), especially in the skin, airways, and gastrointestinal tract (11). Basophils circulate in the blood. The release of MC-derived mediators has been well-documented in ColdU (12–18).

Tryptase, predominantly expressed by MCs, exists in four isoforms, with α and β being the most prevalent (19). Pro-α- and β-tryptases, which are constitutively secreted into the serum, account for the majority of measured basal serum tryptase (**BST**) levels in healthy individuals (20). Elevated BST levels can be observed in hereditary α-tryptasemia (**HαT**), clonal MC diseases, or chronic kidney disease (21). The upper limit of normal BST levels in individuals without HαT is 11.4 ng/mL (21–23), while the upper normal level for individuals with HαT is 15 ng/mL (24).

HαT is characterized by an increased number of α-tryptase-encoding copies at the *TPSAB1* locus (25). Its reported prevalence in the general populations of the US, UK, and EU is 5.7% (26). These individuals have increased levels of mature α/β tryptase heterotetramers (22, 27), which may increase endothelial cell permeability, as demonstrated *in vitro* (22), or induce vibration-triggered degranulation of skin MCs (27). It has been proposed that HαT may augment symptoms associated with clonal MC diseases (22, 28–31), but several questions remain regarding its clinical relevance (24, 28, 32).

The term clonal MC disease includes cutaneous mastocytosis, systemic mastocytosis (**SM**), and monoclonal MC activation syndrome (**MMAS**) (33). SM is diagnosed if the major and one minor criterion or at least three of four minor World Health Organization criteria are present. The major criterion is the presence of infiltrates of ≥15 aggregated MCs in sections obtained from the extracutaneous organ(s). The minor criteria include: (a) ≥25% spindle-shaped MCs in histological sections or ≥25% atypical MCs in a BM smear; (b) *KIT* p.D816V missense variant at codon 816 (***KIT* p.D816V**) in the BM, blood, or extracutaneous organ; (c) MCs in the BM, blood, or extracutaneous organ express one or more of CD2, CD25, or CD30; and (d) BST level persistently >20 ng/mL (29, 34, 35). MMAS includes cases with the presence of the *KIT* p.D816V and/or aberrant CD25 expression on MCs (36–38). Novel, ultrasensitive methods allow the detection of *KIT* p.D816V in blood leukocytes, with the ability to quantify as few as 0.001% of *KIT* p.D816V-encoding alleles (31, 39–42).

In the COLD-CE study, *Hymenoptera* venom-triggered anaphylaxis (**HVA**) was identified as a risk factor for ColdA (2). Severe HVA has been associated with clonal MC disease (31, 43), and severe manifestations of ColdU in a patient with a *KIT* p.D816V have been reported (44). Patients with ColdU were observed to have higher total immunoglobulin E (**IgE**) levels compared to those with chronic spontaneous urticaria (45). Therefore, this study aimed to evaluate BST and total IgE levels

Abbreviations: *BST*, basal serum tryptase; *ColdA*, cold-induced anaphylaxis; *ColdA*^{Cardio}, cold-induced anaphylaxis with cardiac involvement; *ColdU*, cold urticaria; *CRP*, C-reactive protein; *ddPCR*, multiplex droplet digital PCR; *FcεRI*, high-affinity receptor for IgE; *HαT*, hereditary α-tryptasemia; *HVA*, *Hymenoptera* venom-triggered anaphylaxis; *IgE*, immunoglobulin E; *KIT p.D816V*, *KIT* p.D816V missense variant at codon 816; *sCST*, standard cold stimulation testing; *MC(s)*, mast cell(s); *MMAS*, monoclonal mast cell activation syndrome; *qPCR*, allele-specific quantitative PCR; *SM*, systemic mastocytosis.

in patients with ColdU and ColdA, analyze their associations with clinical features, and determine whether testing for the *KIT* p.D816V in blood leukocytes and HαT is useful in ColdU and ColdA.

2 Materials and methods

2.1 Study design and participants

This cross-sectional study included consecutive adult patients with signs and symptoms of ColdU (≤12 months prior to enrollment) who were evaluated at the University Clinic of Respiratory and Allergic Diseases Golnik between May 2019 and December 2022. No patients were excluded from the study. Ethical approval was obtained from the National Medical Ethics Committee of Slovenia (KME0120-62/2019/4), and written informed consent was obtained from all participants. Demographic data and patient history were collected (Table 1), and a full-body examination for cutaneous mastocytosis lesions was conducted.

2.2 Standard 5-minute cold stimulation testing on the volar forearm

The sCST was performed on the volar forearm using an ice cube melting in a small amount of water within a non-latex glove and a TempTest® 4.0 device, which has a 4–44°C electrode. A 5-minute stimulation period was used. Skin responses were assessed 10 minutes post-stimulation. H_1-antihistamines and systemic glucocorticoids were discontinued at least 3 and 7 days prior to testing, respectively (6).

2.3 Definition of clinical phenotypes

Typical ColdU, localized cold-reflex urticaria, and ColdU with negative sCST were defined in accordance with above described criteria. The diagnosis of ColdU with negative sCST was established based on a reliable medical history of reactivity to cold stimuli and patients' photographs of cold-induced wheals and/or angioedema in real life. ColdA was defined as a reaction involving the skin and/or visible mucosal tissue, along with at least one additional systemic manifestation: (a) cardiovascular (syncope [loss of consciousness] or near syncope [dizziness, weakness]), (b) respiratory (difficulty breathing [dyspnea]), or (c) gastrointestinal (crampy abdominal pain or vomiting) (2). The term ColdA with cardiac involvement (**ColdA^{Cardio}**) referred to reactions involving the skin and/or visible mucosal tissue accompanied by syncope or near syncope. Cold-induced oropharyngeal and laryngeal manifestations (i.e., swelling in the oral cavity, painful swallowing [odynophagia], or hoarse voice) were not regarded as part of the signs and symptoms of ColdA; data on these manifestations were reported separately.

2.4 Laboratory workup

Blood samples were collected during >24-hour symptom-free intervals and before sCST. The laboratory workup included: (a) BST level (n = 92) by ImmunoCAP 100 (Thermo Fisher Scientific, Uppsala); (b) total serum IgE level (n = 92) by Immulite 2000Xpi (Siemens); (c) C-reactive protein (**CRP**) level (n = 92) by Cobas 6000 (Roche); (d) specific IgE levels to honeybee (i1; n = 90) and wasp (i3; n = 91) by Immulite 2000Xpi (Siemens Healthcare Diagnostics, Erlangen); (e) blood *KIT* p.D816V analysis (n = 91); and (f) tryptase genotyping (n = 29).

2.4.1 Blood KIT p.D816V assay

Genomic DNA was extracted from 400 µL of EDTA-containing whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The *KIT* c.2447A>T, p.D816V missense variant (p.Asp816Val) and quantification of allele burden were assayed with allele-specific quantitative PCR (**qPCR**) (31, 39, 46). The ABI 7500 Fast Real-Time PCR system and SDS 2.3 software (Thermo Fisher Scientific, Uppsala) were used.

2.4.2 Tryptase genotyping

Genotyping of *TPSAB1* and *TPSB2* was performed using multiplex droplet digital PCR (**ddPCR**) in individuals with BST level ≥6 ng/mL (n = 29), as previously described (25, 47). No individual with HαT has ever been reported with BST level <6.0 ng/mL (39). A manual droplet generator (Bio-Rad), QX200 droplet reader (Bio-Rad), associated QX Manager software (Bio-Rad), and custom primers and probes targeting specifically α- and β-tryptase sequences, along with primers and probes targeting *AP3B1* or *AGO1* as a reference gene, were used. Patients were arranged into two groups: unaffected and HαT genotypes.

2.5 Hematological assessment

Patients testing positive for *KIT* p.D816V in blood leukocytes were referred to the University Medical Centre Ljubljana for hematological evaluation, including bone marrow biopsy and aspiration (33, 48).

2.6 Statistical analysis

IBM SPSS version 25 was used for statistical analysis. Descriptive statistics included frequencies and proportions for categorical variables; ranges, means with 95% confidence intervals (CI), and standard deviations (SD) for normally distributed numerical variables; and medians and interquartile ranges (IQR) for non-normally distributed numerical variables. A p-value <0.05 was considered statistically significant. Categorical variables were assessed by Fisher's exact test. Numerical variables with normal distribution and those not normally distributed were analyzed using

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TABLE 1 Comparison of clinical characteristics between patients with ColdA and those without.

	Total n = 92	ColdA n = 33 (35.9)	Non-ColdA n = 59 (64.1)	p-value
Demographics and baseline clinical characteristics				
Age (years)[a]	40.4 ± 13.7	42.2 ± 14.0	39.4 ± 13.6	0.361
Female gender[b]	64 (69.6)	24 (72.7)	40 (67.8)	0.814
Duration of ColdU (months)[c]	60.0 (14.3–129.0)	72.0 (32.5–162.5)	36.0 (13.0–120.0)	0.065
Age at onset of ColdU (years)[c]	33.0 (20.0–42.0)	33.0 (18.0–45.5)	32.0 (20.0–41.0)	0.935
Pediatric onset of ColdU (<18 years)[b]	15 (16.3)	8 (24.2)	7 (11.9)	0.147
Positive family history of ColdU[b]	5 (5.4)	2 (6.1)	3 (5.1)	1.000
Maximal wheal duration (minutes)[c]	60 (30–120), n = 89	60 (30–90)	60 (30–120), n = 56	0.727
Cold-induced clinical signs and symptoms				
Generalized wheals[b]	37 (40.2)	18 (54.5)	19 (32.2)	**0.047***
Skin angioedema, any location[b]	33 (35.9)	18 (54.5)	15 (25.4)	**0.007****
Oropharyngeal/laryngeal manifestations[b]	25 (27.2)	18 (54.5)	7 (11.9)	**<0.001*****
Itchy earlobes[b]	41 (44.6)	22 (66.7)	19 (32.2)	**0.002****
Fever or arthralgia[b]	0	0	0	NA
Loss of consciousness or dizziness/weakness (= ColdA^{Cardio})[b]	25 (27.2)	25 (75.8)	0	NA
Dyspnea[b]	26 (28.3)	26 (78.8)	0	NA
Crampy abdominal pain or vomiting[b]	2 (2.2)	2 (6.1)	0	NA
Triggers of ColdU				
Cold foods or drinks[b]	25 (27.2)	18 (54.5)	7 (11.9)	**<0.001*****
Whole-body water immersion[b]	67 (72.8)	30 (90.9)	37 (62.7)	**0.003****
Cold air[b]	66 (71.7)	27 (81.8)	39 (66.1)	0.148
Transition cold outdoors to warm indoors[b]	47 (51.1)	19 (57.6)	28 (47.5)	0.390
Cold surfaces[b]	39 (42.4)	20 (60.6)	19 (32.2)	**0.015***
Wind[b]	39 (42.4)	22 (66.7)	17 (28.8)	**0.001****
Summer rain[b]	34 (37.0)	18 (54.5)	16 (27.1)	**0.013***
ColdU phenotype based on sCST				
Typical ColdU[b]	48 (52.2)	25 (75.8)	23 (39.0)	**0.001****
Localized cold-reflex urticaria[b]	5 (5.4)	0	5 (8.5)	0.156
ColdU with negative sCST[b]	39 (42.4)	8 (24.2)	31 (52.5)	**0.009****
Comorbidities (medical diagnosis)				
Chronic spontaneous urticaria[b,d]	18 (19.6)	3 (9.1)	15 (25.4)	0.098
Atopic disease[b]	30 (32.6)	11 (33.3)	19 (32.2)	1.000
Systemic reaction to *Hymenoptera* venom[b]	6 (6.5)	3 (9.1)	3 (5.1)	0.663
Thyroid disease[b]	8 (8.7)	2 (6.1)	6 (10.2)	0.707
Connective tissue disease[b]	2 (2.2)	1 (3.0)	1 (1.7)	1.000

Categorical variables are presented as counts (percentages), while numerical variables are expressed as mean ± SD for normally distributed data and median (IQR) for non-normally distributed data. If data were not obtained for all patients, the number of patients is displayed as "n." Statistical significance of differences between groups was assessed using the Student's t-test ([a]), Fisher's Exact test ([b]), and Mann-Whitney test ([c]). Statistically significant p-values are highlighted in bold. Significance levels are indicated by *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$). [d]ColdU was the predominant subtype of chronic urticaria as determined from patient history.

ColdA, cold-induced anaphylaxis; *ColdA^{Cardio}*, cold-induced anaphylaxis with cardiac involvement; *ColdU*, cold urticaria; *NA*, not applicable; *non-ColdA*, absence of cold-induced anaphylaxis; *sCST*, standard cold stimulation testing.

Student's t-test and Mann-Whitney test, respectively. Prevalence of HαT was compared to the hypothetical prevalence of the parameter in the general population, acquired from the literature, using the exact binomial test.

3 Results

3.1 Demographic and baseline clinical characteristics

A total of 92 ColdU patients were enrolled, 69.6% of whom were female. Patient ages ranged from 18 to 73 years (mean: 40.4 years). Key clinical features are summarized in Table 1. Pediatric onset of ColdU and a positive family history of ColdU were identified in 16.3% and 5.4% of patients, respectively. The following ColdU phenotypes were diagnosed based on sCST: typical ColdU in 52.2% (n = 48), localized cold-reflex urticaria in 5.4% (n = 5), and ColdU with negative sCST in 42.4% (n = 39) of patients. ColdA was diagnosed in 35.9% (n = 33) of patients, including ColdA^{Cardio} in 27.2% (n = 25). Concomitant chronic spontaneous urticaria was diagnosed in 19.6% (n = 18) of patients, with ColdU being the predominant subtype of chronic urticaria in these cases. Cold-induced oropharyngeal and laryngeal manifestations were reported by 27.2% (n = 25) of patients (Table 1).

3.2 Triggers of ColdU and ColdA

Patients reported the following triggers of ColdU (i.e., wheals and/or angioedema): cold foods or drinks (27.2%), whole-body water immersion (72.8%), cold air (71.7%), transitioning from cold outdoors to warm indoors (51.1%), contact with cold surfaces (42.4%), wind (42.4%), and summer rain (37.0%) (Table 1). ColdA was triggered by whole-body water immersion (n = 27), cold air (n = 8), or cold drinks (n = 2). Compared to non-ColdA patients, ColdA patients more frequently reported the following triggers of their wheals and/or angioedema: cold foods or drinks ($p < 0.001$), whole-body water immersion ($p = 0.003$), contact with cold surfaces ($p = 0.015$), wind ($p = 0.001$), and summer rain ($p = 0.013$) (Table 1).

3.3 Clinical features associated with ColdA

Patients with ColdA had a higher frequency of generalized wheals ($p = 0.047$), skin angioedema ($p = 0.007$), oropharyngeal/laryngeal manifestations ($p < 0.001$), itchy earlobes ($p = 0.002$), and typical ColdU ($p = 0.001$) compared to those without ColdA (Table 1). Patients with ColdA^{Cardio} had a higher frequency of generalized wheals (64.0% [16/25] vs. 31.3% [21/67], $p = 0.008$), skin angioedema (56.0% [14/25] vs. 28.4% [19/67], $p = 0.026$), oropharyngeal/laryngeal manifestations (52.0% [13/25] vs. 17.9% [12/67], $p = 0.003$), itchy earlobes (72.0% [18/25] vs. 34.3% [23/67], $p = 0.002$), and typical ColdU (80.0% [20/25] vs. 41.8% [28/67], $p = 0.002$) compared to those without.

3.4 Elevated BST levels were attributed to *KIT* p.D816V and/or HαT

BST levels ranged from 1.19 to 27.80 ng/mL (median: 4.70 ng/mL). Elevated BST levels (>11.4 ng/mL) were identified in 9.8% (9/92) of ColdU patients (Table 2) and attributed to *KIT* p.D816V (n = 3) or HαT (n = 8) (Figure 1). Two patients had both *KIT* p.D816V and HαT (Figure 1, Table 2). The prevalence of elevated BST levels was higher in ColdA patients compared to non-ColdA patients, with the difference approaching statistical significance (18.2% vs. 5.1%, $p = 0.065$; Table 2).

3.5 *KIT* p.D816V was detected in ColdU and ColdA

Analysis revealed that *KIT* p.D816V was present in 6.6% (6/91) of all ColdU patients, 4.2% (2/48) of typical ColdU patients, and 6.3% (2/32) of ColdA patients (Table 2, Supplementary Tables S1, S2). None of the enrolled patients had clinical signs of cutaneous mastocytosis. One patient fulfilled the diagnostic criteria for SM, four met the criteria for MMAS, and one remained unclassified due to declining a bone marrow biopsy (Table 3). BST levels for individual patients with *KIT* p.D816V are detailed in Table 3. Patients with this variant had higher BST levels compared to individuals without it (median [IQR]: 10.87 [7.90–24.20] vs. 4.56 [3.39–6.04] ng/mL, $p < 0.001$). Interestingly, half of the patients with *KIT* p.D816V had elevated BST levels, while the other half had normal levels. No statistically significant associations were found between the presence of *KIT* p.D816V and the clinical characteristics of ColdU or ColdA. However, a trend was observed, with *KIT* p.D816V-positive patients experiencing disease onset at an older age compared to those without this variant (median [IQR]: 47.0 [28.3–54.0] vs. 33.0 [20.0–41.0] years, $p = 0.074$; Supplementary Table S2).

3.6 Prevalence of HαT in ColdU and ColdA was higher than in the general population

Tryptase genotyping identified a significantly higher prevalence of HαT in ColdU (10.9% [10/92]) compared to the estimated 5.7% prevalence in the general population (26) ($p = 0.041$). Similarly, the prevalence of HαT was higher in ColdA (15.2% [5/33]) than in the general population ($p = 0.038$) (Table 2, Figure 2). Among the HαT-positive patients, three (30%) had a duplication of the *TPSAB1* gene (genotype αα/β), while the remaining seven (70%) had the genotype αα/α (Supplementary Table S1). Patients with HαT had significantly higher BST levels than those without the condition (median [IQR]: 13.40 [11.48–16.20] vs. 4.50 [3.34–5.84] ng/mL, $p < 0.001$). Eighty percent of HαT-positive individuals had BST levels exceeding 11.4 ng/mL, and 30% had BST levels above 15.0 ng/mL. HαT was not associated with clinical parameters of ColdU or ColdA (Supplementary Table S3).

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TABLE 2 Comparison of laboratory characteristics between patients with ColdA and those without.

	Total n = 92	ColdA n = 33 (35.9)	Non-ColdA n = 59 (64.1)	p-value
BST				
BST level (ng/mL)[a]	4.70 (3.46–6.45)	5.02 (3.64–7.03)	4.63 (3.23–6.37)	0.476
Elevated BST level (>11.4 ng/mL)[b]	9 (9.8)	6 (18.2)	3 (5.1)	0.065
Elevated BST level (>15.0 ng/mL)[b]	4 (4.3)	3 (9.1)	1 (1.7)	0.130
Genetic tests				
KIT p.D816V[b]	6 (6.6), n = 91	2 (6.3), n = 32	4 (6.8)	1.000
HαT[b]	10 (10.9)	5 (15.2)	5 (8.5)	0.486
KIT p.D816V and HαT[b]	2 (2.2), n = 91	1 (3.1), n = 32	1 (1.7)	1.000
KIT p.D816V or HαT[b]	14 (15.4), n = 91	6 (18.8), n = 32	8 (13.6)	0.552
Total serum IgE				
Total serum IgE level (IU/mL)[a]	96.0 (31.0–212.3)	113.0 (43.0–389.0)	63.0 (24.0–164.0)	**0.021***
Total serum IgE level ≥100 IU/mL[b]	43 (46.7)	18 (54.5)	25 (42.4)	0.284
High total serum IgE level (≥75th percentile; ≥212.3 IU/mL)[b]	23 (25.0)	13 (39.4)	10 (16.9)	**0.024***
***Hymenoptera* sensitization**				
Wasp sensitized (sIgE >0.35 IU/mL)[b]	37 (40.7), n = 91	13 (40.6), n = 32	24 (40.7)	1.000
Honeybee sensitized (sIgE >0.35 IU/mL)[b]	23 (25.6), n = 90	6 (19.4), n = 31	17 (28.8)	0.447
Wasp or honeybee sensitized[b]	46 (50.5), n = 91	15 (46.9), n = 32	31 (52.5)	0.664

Categorical variables are presented as counts (percentages), while numerical variables are expressed as median (IQR). If data were not obtained for all patients, the number of patients is displayed as "n".

Statistical significance of differences between groups was assessed using the Mann-Whitney test ([a]) and Fisher's Exact test ([b]). Statistically significant p-values are highlighted in bold. Significance levels are indicated by *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$).

BST, basal serum tryptase; *ColdA*, cold-induced anaphylaxis; *HαT*, hereditary α-tryptasemia; *IgE*, immunoglobulin E; *KIT p.D816V*, KIT p.D816V missense variant at codon 816 detected in blood leukocytes; *non-ColdA*, absence of cold-induced anaphylaxis; *sIgE*, specific immunoglobulin E antibodies.

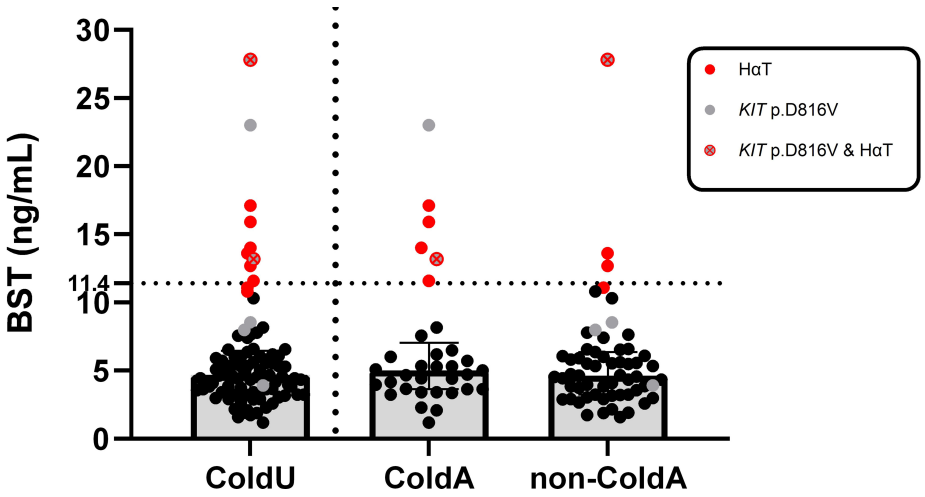

FIGURE 1
Relationship between BST levels and the presence of HαT and *KIT* p.D816V. The BST levels measured in ColdU patients ranged from 1.19 to 27.80 ng/mL. Elevated BST levels (>11.4 ng/mL) were identified in 9 ColdU patients, with elevations attributed to either HαT (n = 8, indicated by red circles) or the presence of *KIT* p.D816V (n = 3, represented by grey circles). Two patients had both conditions (marked by crossed red and grey circles). Three patients positive for *KIT* p.D816V demonstrated BST levels ≤11.4 ng/mL. *BST*, basal serum tryptase; *ColdA*, cold-induced anaphylaxis; *ColdU*, cold urticaria; *HαT*, hereditary α-tryptasemia; *KIT p.D816V*, *KIT* missense variant at codon 816; *non-ColdA*, absence of cold-induced anaphylaxis.

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TABLE 3 Clinical and laboratory characteristics of patients with the *KIT* p.D816V variant.

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6
Diagnosis: SM or MMAS	MMAS	MMAS	SM	*Nd*	MMAS	MMAS
Age (years)	65	35	47	58	56	70
Gender	f	m	f	f	m	f
BM biopsy analysis						
Infiltrates of ≥15 MCs in aggregates[a*]	*Nr*	–	–	*Nd*	–	–
≥25% spindle-shaped MCs[b]	*Nr*	–	+	*Nd*	–	–
KIT p.D816V[b]	+	+	+	*Nd*	*Nd*	–
MCs with ≥1: CD2, CD25, CD30[b]	*Nr*	–	+	*Nd*	–	–
Blood analysis						
KIT p.D816V[b]	+	+	+	+	+	+
BST level persistently >20 ng/mL[b]	+	–	+	–	–	–
BST level (ng/mL)	23.00	8.53	27.80	13.20	7.99	3.90
KIT p.D816V allele burden (%)	0.415	0.027	0.086	0.001	0.001	0.001
HαT	–	–	+	+	–	Nd
ColdA phenotype						
ColdA	+	–	–	+	–	–
ColdA with cardiac involvement	–	–	–	+	–	–
Systemic reactions to *Hymenoptera* venom[c]	–	–	+ (IV)	–	+ (I)	–

The BM sample from patient #1 was not representative; MCs were slightly multiplied but did not form aggregates, and a few MCs were spindle-shaped. Patient #4 declined BM biopsy.
SM is diagnosed if the major[a] criterion and one minor[b] criterion, or at least three of four minor[b] criteria are present. MMAS is defined by the presence of one or two minor clonality criteria: the *KIT* p.D816V variant and/or aberrant CD25 expression on MCs. [c]Reaction severity grades were assigned according to the Mueller grading system (grades I–IV).
BM, bone marrow; *BST*, basal serum tryptase; *ColdA*, cold-induced anaphylaxis; *ColdA*^{Cardio}, cold-induced anaphylaxis with cardiac involvement; *f*, female; *HαT*, hereditary α-tryptasemia; *KIT* p.D816V, *KIT* missense variant at codon 816; *m*, male; *MCs*, mast cells; *MMAS*, monoclonal mast cell activation syndrome; *Nd*, not determined; *Nr*, not representative; *SM*, systemic mastocytosis; +, yes/positive; -, no/negative.

3.7 ColdA was associated with higher total serum IgE

Patients with ColdA had higher total serum IgE levels (median [IQR]: 113.0 [43.0–389.0] vs. 63.0 [24.0–164.0] IU/mL, p = 0.021, Table 2, Figure 3A) and a higher frequency of high total IgE, defined as ≥75th percentile (39.4% [13/33] vs. 16.9% [10/59], p = 0.024; Table 2, Figure 3B), compared to non-ColdA patients. Patients with ColdA^{Cardio} also had higher total serum IgE levels (median [IQR]: 207.0 [33.5–408.0] vs. 79.0 [30.0–153.0] IU/mL, p = 0.022) and a higher frequency of high total IgE (48.0% [12/25] vs. 16.4% [11/67], p = 0.003, Figure 3C) compared to those without cardiac involvement.

3.8 Negative sCST was associated with lower ColdA frequency

ColdU with negative sCST (n = 39), compared to typical ColdU (n = 48), was associated with a lower frequency of ColdA (20.5% [8/39] vs. 52.1% [25/48], p = 0.004) and a higher frequency of spontaneous wheals (38.5% [15/39] vs. 4.2% [2/48], p < 0.001).

3.9 Negative sCST was not linked to features of autoinflammatory diseases

No significant differences were found between patients with negative sCST and those with typical ColdU in rates of recurrent fever or arthralgia (n = 0 for both groups), high CRP levels (≥5 mg/L; 10.3% [4/39] vs. 10.4% [5/48], p = 1.000), or CRP levels (median [IQR]: 0.90 [0.60–3.00] vs. 1.35 [0.73–3.48] mg/L, p = 0.225).

3.10 Sensitization to wasps or honeybees in ColdU patients

Elevated specific IgE levels to wasps (40.7%, 37/91) and honeybees (25.6%, 23/90) were detected among ColdU patients, with no significant differences in sensitization rates between ColdA and non-ColdA patients (Table 2). Among wasp- or honeybee-sensitized ColdU patients, 13% (6/46) experienced HVA, classified as Mueller grades I (n = 2), III (n = 1), and IV (n = 3) (Supplementary Table S1).

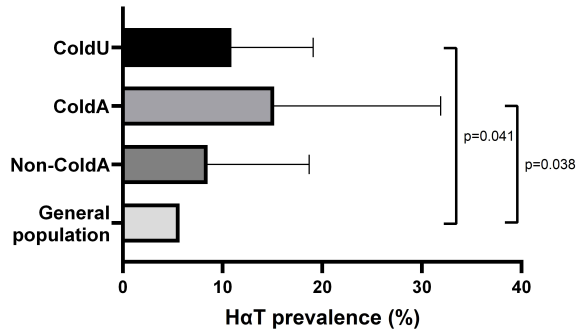

FIGURE 2
Prevalence of HαT. The prevalence of HαT was significantly higher in ColdU (10.9%) and ColdA (15.2%) compared to an estimated 5.7% prevalence in the general population, as determined using the exact binomial test. *ColdA*, cold-induced anaphylaxis, *ColdU*, cold urticaria; *HαT*, hereditary α-tryptasemia; *non-ColdA*, absence of cold-induced anaphylaxis.

4 Discussion

To the best of our knowledge, this is the first study to systematically analyze BST levels, *KIT* p.D816V, HαT, and IgE levels in patients with ColdU and ColdA. Our findings provide new insights into the clinical and laboratory characteristics of these conditions.

Patients with typical ColdU were significantly more likely to experience ColdA than those with ColdU and negative sCST, a finding not previously reported. While negative sCST has been linked to hereditary systemic autoinflammatory diseases (49, 50), our study found no association with elevated CRP or other typical features of these disorders. Instead, negative sCST was associated with spontaneous wheals, suggesting that chronic spontaneous urticaria may be exacerbated by cold stimuli, a phenomenon not easily confirmed through sCST. ColdA patients exhibited a higher frequency of clinical features previously associated with ColdA in the COLD-CE study, including generalized wheals, skin angioedema, oropharyngeal/laryngeal manifestations, and itchy earlobes (2).

The *KIT* p.D816V variant, which is considered absent in the general population (51), was identified in 6.6% of ColdU patients and 6.3% of those with ColdA. Valent et al. highlighted that cold exposure might trigger anaphylaxis in individuals with SM, suggesting that temperature fluctuations can influence MC activity (52). Additionally, Akin et al. noted that hypotension during anaphylaxis raises suspicion of underlying clonal MC disease (36). Investigating clonal MC disease is standard practice in severe HVA cases (21, 53, 54). In our study, the presence of *KIT* p.D816V was not linked to increased ColdU severity, and thus it cannot currently be recommended as a routine test for assessing ColdA risk. Some patients with *KIT* p.D816V had normal BST levels, reinforcing the conclusion that normal BST levels do not exclude underlying clonal MC disease (39).

We observed a higher prevalence of HαT in ColdU and ColdA compared to the general population (24, 26, 32). Interestingly, the prevalence of HαT in ColdA exceeded that reported in patients with severe HVA (8.7%), where HαT has been linked to increased reaction severity (31). However, in our cohort, HαT was not associated with greater ColdU severity. The co-occurrence of HαT and *KIT* p.D816V in two out of six (33%) patients with *KIT* p.D816V is unlikely to be coincidental. Prior studies have reported this overlap (22, 31, 32, 43).

Previous research reported higher total IgE levels in patients with ColdU compared to those with chronic spontaneous urticaria (45). Our study found higher total IgE levels in ColdA patients compared to non-ColdA patients. Elevated IgE levels may reflect a type 2 immune response involving Th2 cells, eosinophils, MCs, and basophils, in which Th2 cytokines stimulate B cells to produce IgE (55, 56). However, in our study, atopic diseases, typically associated with Th2 dominance, were not linked to ColdA. While total IgE levels may serve as a potential biomarker for identifying ColdU patients at risk for severe reactions, the low specificity of total IgE testing limits its clinical utility (55).

The hypothesis that MC activation in ColdU is autoallergic and IgE-mediated, with cold triggering new autoallergens detected by IgE bound to MCs, remains compelling but unconfirmed. Supporting evidence includes the efficacy of omalizumab in ColdU (57) and earlier experiments showing that sensitivity to cold in some patients can be passively transferred, with particularly IgE suspected to play a role (14). Nevertheless, direct data validating this hypothesis are lacking.

The binding and cross-linking of the high-affinity receptor for IgE (**FcεRI**) on MCs and basophils is crucial in many cases of

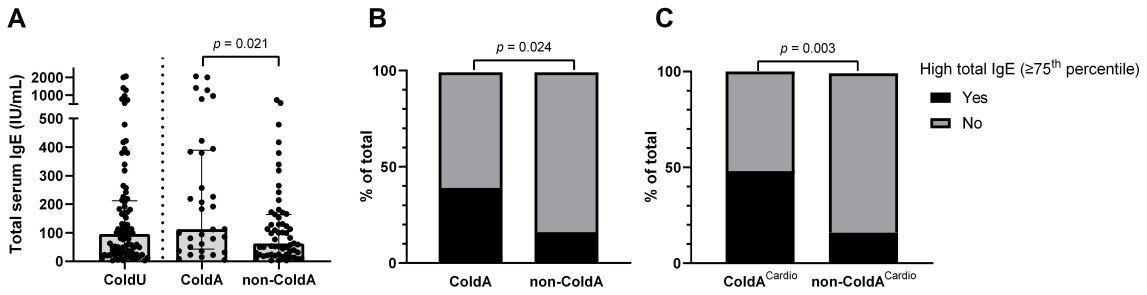

FIGURE 3
Total serum IgE levels and their association with ColdA. **(A)** Patients diagnosed with ColdA had significantly higher total serum IgE levels compared to those without (Table 2). **(B)** High total IgE levels, defined as being in the ≥75th percentile (≥212.3 IU/mL), were associated with a higher frequency of ColdA (Table 2). **(C)** High total IgE levels were also associated with a higher frequency of ColdA with cardiac involvement. *ColdA*, cold-induced anaphylaxis; *ColdA*^{Cardio}, cold-induced anaphylaxis with cardiac involvement; *ColdU*, cold urticaria; *IgE*, immunoglobulin E; *non-ColdA*, absence of cold-induced anaphylaxis.

anaphylaxis (10, 11, 58). Surface expression levels of FcεRI on MCs are known to be positively regulated by IgE (45, 59), and significantly higher FcεRI expression has been reported in patients with chronic inducible urticaria compared to controls (60). This further underscores the potential role of IgE in ColdA. Importantly, both IgE directed against exogenous antigens and autoreactive IgE antibodies have been found to elicit similar cellular responses (61), and IgE itself may enhance MC activation (62–65).

This study has several strengths, including the large cohort evaluated at a specialized academic center. However, limitations must be acknowledged. In patients with negative sCST, additional time-consuming tests with adjusted cold stimuli were not performed. Nonetheless, the diagnosis of ColdU was reliable based on consistent patient histories and documented photographs. Seasonal desensitization (66) may have led to falsely negative sCST results in patients exposed to cold air, and sCST was not repeated across different seasons. The diagnosis of ColdA relied on medical histories, as systematic generalized cold exposure testing would be unethical (1). Additionally, we lacked data on event-related tryptase levels (67), as such evaluations are rarely conducted systematically in this patient population (1). Variability in IgE levels due to demographic factors such as age and gender (45, 68) was also not assessed.

Assessing BST levels may help identify patients with HαT and, to a lesser extent, the *KIT* p.D816V variant. Our findings demonstrate a higher prevalence of HαT and *KIT* p.D816V in ColdU and ColdA compared to the general population. These results raise the hypothesis that HαT and clonal MC disease may contribute to the pathogenesis of ColdU and ColdA in some patients. Additionally, elevated IgE levels could serve as a potential biomarker for ColdA. Further research is needed to clarify the clinical significance of these genetic and immunological findings.

Data availability statement

The datasets presented in this article are not readily available because of privacy or ethical restrictions. Requests to access the datasets should be directed to MB, mojca.bizjak@klinika-golnik.si.

Ethics statement

The studies involving humans were approved by Slovenian National Medical Ethics Committee (KME0120-62/2019/12). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PK: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. MK: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. JŠ: Conceptualization, Methodology, Writing – review & editing. UB-S: Conceptualization, Methodology, Writing – review & editing. MS: Conceptualization, Methodology, Writing – review & editing. SZ: Conceptualization, Data curation, Methodology, Writing – review & editing. DD: Conceptualization, Formal analysis, Methodology, Writing – review & editing. MR: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

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Conflict of interest

MB is or recently was a speaker and advisor for Novartis, outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1558284/full#supplementary-material

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