Features of chronic urticaria after COVID-19 mRNA vaccine, a real-life cohort study

Yannick Muller

Yannick.Muller@chuv.ch

CHUV https://orcid.org/0000-0003-0513-7156

Johan Schwab1

CHUV

Mathilde Foglierini

CHUV https://orcid.org/0000-0001-7538-4262

Eva Pescosolido

CHUV

Inès Pacheco

Lausanne University Hospital https://orcid.org/0009-0005-4720-9658

Gustavo Ruiz Buendía

Swiss Institute of Bioinformatics https://orcid.org/0000-0002-4935-1480

Natacha Madelon

Celine Pellaton

Véronique Monzambani

Lausanne University Hospital

Camillo Ribi

Marcel Bergmann

Arnaud Didierlaurent

Craig Fenwick

Service of Immunology and Allergy, Lausanne University Hospital, University of Lausanne
https://orcid.org/0000-0002-9435-0110

Olivier Duperrex

https://orcid.org/0000-0002-0932-6846

Article

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Johan Schwab[1], Mathilde Foglierini[1,2], Eva Pescosolido[3], Ines Pacheco[1], Gustavo A. Ruiz Buendía[4,5,6] Natacha Madelon[4], Celine Pellaton[1], Véronique Banderet[1], Camillo Ribi[1], Marcel M. Bergmann[3,7,8], Arnaud M. Didierlaurent[4], Craig Fenwick[1], Olivier Duperrex[9], Yannick D. Muller[1,10]

[1]Division of Immunology and Allergy, Lausanne University Hospital (CHUV) and University of Lausanne, Switzerland.
[2]Biomedical Data Science Centre (BDSC) Lausanne University Hospital (CHUV) and University of Lausanne, Switzerland
[3]Centro Pediatrico del Mendrisiotto, Mendrisio, Switzerland
[4]Centre of Vaccinology, University of Geneva, Switzerland.
[5]Translational Data Science Facility, AGORA Cancer Research Center, Lausanne, Switzerland.
[6]Swiss Institute of Bioinformatics, Lausanne, Switzerland.
[7]Faculty of Biomedical Science, Università della Svizzera Italiana (USI), Lugano, Switzerland
[8]Pediatric Allergy Unit, Department of Women, Children and Adolescents, University Hospitals of Geneva, Geneva, Switzerland
[9]Center for Primary Care and Public Health (Unisanté), Lausanne, Switzerland
[10]Centre for Human Immunology Lausanne, Switzerland.

Correspondence
Yannick Daniel Muller – MD-PhD – Assistant Professor
Service d'immunologie et d'allergie, Département de médecine, BH010-511
Rue du Bugnon 46, CH-1011 Lausanne
Mob: +41(0)795569438 ; Email : Yannick.muller@chuv.ch.

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Conflict of interest

Dr Fenwick report having a patent pending (application No. EP20205298.1) for a SARS-Cov2 neutralization assay. Prof. Muller has received grant support/consulting income from AstraZeneca, Sanofi and GSK. Prof. Didierlaurent received research grants from Moderna, GSK and Sanofi outside the scope of this study. The research was conducted without any other commercial or financial relationships that could be construed as a potential conflict of interest to this study.

Abbreviations

BAT: basophil activation test
CIU: chronic inducible urticaria
COVID-19: coronavirus disease
CSU: chronic spontaneous urticaria
CU: chronic urticaria
EAACI: European Academy of Allergology and Clinical Immunology
FcεRI: high-affinity IgE receptor
Spikevax: The mRNA-1273 Moderna vaccine
NSAID: non-steroidal anti-inflammatory drugs

PEG: polyethylene glycol
Comirnaty : BNT 162b2 vaccine from BioNtech/Pfizer
SARS-CoV2: severe acute respiratory syndrome coronavirus 2
UCT: urticaria control test

Author's contribution

YDM, OD, AD, CR and MB contributed in the design of the study. YDM and JS wrote the manuscript. JS, MF, EP, IP, GR, NM, contributed in the analysis of the study. CP and VB contributed in the recruitment of patients. YDM, CF, AD supervised laboratory testing. All authors have revised and approved the final version

Abstract

Background

New onsets of chronic urticaria (CU) have been reported after repeated immunizations, mainly with the Moderna mRNA-1273 vaccine (Spikevax)

Objective

This study aims to evaluate patients with CU after COVID-19 mRNA vaccination. The contribution of SARS-Cov2 infection, atopy and IgE against the vaccine was analyzed.

Methods

We monitored the features of patients who developed CU after vaccination in the Canton of Vaud through two surveys conducted in 2022 and 2023. Fifty individuals with CU underwent blood tests, and their results were compared with individuals without a history of urticaria (N=135). The presence of anti-vaccine IgE was detected with basophil activation tests (BAT). We assessed anti-SARS-Cov2 humoral response, and the presence of IgEs against common respiratory allergens (Phadiatop) as a surrogate for atopy.

Results

Post-vaccination CU occurred after a median interval of 10 days and significantly more after the Spikevax booster, affecting middle-aged individuals (median 41, 66% females). In 2023, CU was still active in 53% of the cases. Inducible forms of CU, primarily dermographism, were reported in 54% (2022) and 61% (2023) of the cases. BAT positivity was not specific to CU, anti-nucleocapsid positivity, or atopy but was significantly associated with higher anti-spike neutralizing activities and younger age. Four CU patients tolerated an additional dose of mRNA vaccine with no disease exacerbation/recurrence.

Conclusion

The Spikevax booster induced anti-vaccine IgE independently of CU, the latter being not directly associated with COVID-19 infection nor atopy. The tolerance to a new booster in 4/4 patients suggests that the Spikevax vaccine indirectly triggered CU in predisposed individuals.

Introduction

A major contribution to reducing the burden of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) pandemic was the rapid development of an efficient vaccination strategy (1). The two mRNA vaccines, the mRNA-1273 (Spikevax®) from Moderna and BNT 162b2 (Comirnaty®) from Pfizer-BioNTech were authorized in January 2021(2) and December 2020(3) and were the most commonly given vaccines in Switzerland (4–6). Yet, these COVID-19 vaccines were associated with several adverse effects with up to 17'000 reports of suspected adverse drug reactions collected in Switzerland by February 2023 (7, 8). In particular, new onsets of chronic urticaria (CU) have been reported after repeated immunizations, mainly with the Spikevax vaccine (9–11).

CU is defined by the European Academy of Allergology and Clinical Immunology (EAACI) as the development of wheals (hives), angioedema, or both for more than six weeks (12). It can be classified as spontaneous, inducible, or both. Chronic inducible urticaria is triggered by physical factors such as pressure, contact, vibration, temperatures, sun, or cholinergic activity. In Switzerland, we observed an outbreak of CU starting in December 2021 (9, 11). In a first analysis, we collected pharmacovigilance data from the Swiss Agency for Therapeutic Products (Swissmedic), and we estimated the overall crude incidence rate of CU after a COVID-19 booster at 19/100’000 from 2021-01-21 to 2022-08-31. The relative risk of new-onset CU after Spikevax compared to Comirnaty was 16.1 (95%CI, 10.8-24.0) (11). Immunological data in seven patients revealed a systematic sensitization against the mRNA lipid nanoparticles but not against the linear polyethylene glycol-2000 nor the tromethamine (9). The contribution of this IgE dependent sensitization to the pathogenesis and persistence of CU remains undetermined (13). Notably, the contribution of infections with the omicron variant could also have been a confounding factor.

In the present study, we analyzed the features of patients who developed CU in the Canton of Vaud through two separate surveys sent in 2022 and 2023. We recruited 50 patients for blood tests and compared the results to 135 individuals not suffering of CU but either infected with SARS-Cov2 (COSED) or vaccinated with COVID-19 mRNA vaccines (ImmunoVax).

Results

Among the 111 identified CU patients, we were able to contact 110, and 88 responded to our 2022 survey. One patient did not consent, one response was duplicated and excluded (Figure 1A). Of these 88 patients, 66% were middle-aged female (median age 41, IQR 35-48, Figure 1B). In 89% of cases, CU started after the booster shot and not after primary vaccination, predominantly with Spikevax (93%). The median interval time between vaccination and CU onset was 10 days. As of June 2022, CU remained active in 81% of these cases. Only 14% of the patients reported a previous history of urticaria, with the majority being cases of acute urticaria (92%). Inducible factors, mainly dermographism, were reported in 55% of the cases. The Urticaria Control Test (UCT) score, the number of lesions, and the severity of pruritus at disease onset indicated poor disease control. Although disease activity improved over time, control remained largely insufficient, possibly due to suboptimal antihistamine therapy (Table 1). Notably, only one-third of the patients reported pollinosis, and a mere 2% reported asthma, suggesting that the disease is unrelated to atopy.

A year later, we contacted the same patients for a follow-up survey, to which 61 patients responded (Table 2). Similar to the previous survey, 64% were middle-aged females (median age 41.5); 92% developed CU after the booster shot with Spikevax. CU was still active in 53% of these cases. In 41% (13/32) of cases (compared to 42% in 2022), patients reported inducible

86 factors, primarily dermographism (68% compared to 77% in 2022). The UCT score, number of
87 lesions, and pruritus severity showed clear improvement compared to 2022. Yet the disease was
88 still insufficiently controlled in 50% of the patients. Only four patients received omalizumab,
89 which was discontinued in three cases. Worsening of CU by non-steroidal anti-inflammatory
90 drugs was reported by 10% of cases (Tables 1 and 2). Importantly, mRNA vaccine was
91 readministered in four CU patients – two in remission and two with persistent symptoms
92 (Comirnaty in 3 and Spikevax in one) (Table 3). Subsequent immunization was not associated
93 with CU re-occurrence or worsening.
94
95 We further explored the potential association between COVID infection and CU. Based on our
96 surveys, only 34% and 44% of patients reported a formal SARS-CoV-2 infection in 2022 and
97 2023, respectively. CU exacerbation after infection occurred in one-third of the cases in 2022
98 and 15% in 2023. We also compared the CU onset dates with official COVID infection reports
99 and vaccination dates in the population of the canton of Vaud. Interestingly, the peak of booster
100 vaccinations preceded the peak of CU cases, which in turn preceded the peak of COVID cases
101 (Figure 2). Antibodies against the nucleocapsid were negative in 21/50 (42%) of subjects tested.
102 Importantly, seropositivity to the nucleocapsid as a surrogate for past COVID infection did not
103 influence the UCT (Supplemental figure 1). These findings suggest that, in contrast to the
104 vaccine, there is not association between COVID infection and CU.
105
106 We then explored the potential link between vaccine sensitization and CU. To do this, we
107 conducted basophil activation tests (BAT) using a cryopreserved batch of the Spikevax vaccine,
108 which we previously validated (14). Out of 50 blood samples tested, two patients had no
109 basophils, and four were excluded due to basophil areactivity. BAT was positive in 64% of the
110 cases. To further understand the relevance of this sensitization, we included patients without a

history of CU from two separate cohorts monitored by our division. The first cohort (n=105) consisted of 59 patients with long COVID and 46 patients with an acute COVID infection yet without persistent symptoms. The second cohort comprised 30 healthy vaccinated volunteers. We were able to subgroup these patients according to the type of vaccine received (Spikevax versus BNT 162b2) and the number of doses (0-1-2-booster) (Figure 3A). Notably, sensitized patients were predominantly those vaccinated with the Spikevax booster, regardless of their CU status. Females were sensitized in 60% compared to 44% of males. Younger age was associated with a higher rate of sensitization (Figure 3B). Sensitization didn't predict the duration of CU (Figure 3C). No significant difference in CD63 levels on basophils, an activation marker, was observed in sensitized patients when comparing the two vaccines (Figure 3D).

It was previously suggested that control patients who recovered from COVID infection are more likely sensitized against the vaccine (15). Thus, we wanted to evaluate the frequency and level of anti-nucleocapsid antibodies in patients with positive and negative BAT against the vaccine. Anti-nucleocapsid antibodies did not correlated with higher CD63 expression. In fact, sensitized patients exhibited significantly lower level of nucleocapsid antibodies arguing against a direct link between COVID infection and vaccine sensitization (Figure 3E-F). On the other hand, we found that sensitized patients had higher levels of anti-Spike antibodies, which correlated with a better neutralization against the wild-type but not the Omicron variant (Figure 3H). Intriguingly, CU patients also had significantly higher anti-Spike neutralizing activity against the wild-type compared to patients from the two control cohorts (Immunovax, COSEDH) (Figure 3I). Thus, our results suggest that younger females with good vaccine immuno-reactivity are at a higher risk of developing CU and getting sensitized against the vaccine. However, vaccine sensitization does not appear to be associated with the onset of CU.

To understand whether new-onset CU following mRNA vaccination was associated with atopy, i.e., a genetic predisposition to produce IgE against common respiratory allergens, we performed a Phadiatop analysis. This test quantifies the presence of IgE against various allergens including grass, birch, olive, mugwort, parietaria, dog, cat, horse, house dust mite, flour mite, and Cladosporium in all groups. Patients with CU were not more frequently atopic compared to those in the two control cohorts (Figure 3J). In addition, IgE sensitization to the vaccine was not associated with atopy, nor was it correlated with the level of IgE against common respiratory allergens (Figure 3K-L). Finally, we did not find any specific signature for CU based on a pilot bulk RNA study comparing the transcriptional profile of 15 patients with CU and 17 vaccinated heathy volunteers recruited at the university hospital of Geneva (Supplemental figure 2).

Discussion

This study represents the first comprehensive analysis of a large cohort of patients who developed CU following mRNA vaccination, mostly the Moderna vaccines, an observation als made by others (16). The majority of patients were middle-aged individuals with in overall 54-61% suffering from an inducible form of CU. We demonstrated that CU was unrelated to the Omicron Wave, atopic predisposition, and vaccine sensitization. Importantly, 4/4 CU patients re-exposed to the mRNA vaccine did not exacerbate CU and tolerated the vaccine well. These results expand a series cases of another four patients with CU who received a subsequent COVID-19 booster vaccine without disease exacerbation at a military academy (17).

In our study, we observed a substantial number of patients who were sensitized to mRNA vaccines independently of known allergies nor active CU. These findings are consistent with the higher prevalence of positive skin tests in patients vaccinated with Spikevax (13). This

sensitization is mediated through specific IgE against the spherical polyethylene glycol (PEG) conformation of the lipid nanoparticle (18). The clinical relevance of those IgE remains undefined. On the one hand, they could contribute to protective immunity as previously suggested in the context of flu vaccines (19) and corroborating the positive association we observed between the anti-spike titer and anti-vaccine IgE. On the other hand, they could predispose individuals to developing allergic reactions (20). At this stage, this remains speculative as it has been repeatedly shown that the majority of sensitized patients can tolerate the vaccine (18). Thus, there is growing evidence showing that immediate reactions are primarily non-IgE dependent, due to complement activation (21), and that C5a could be a relevant biomarker of anaphylaxis (22). In conclusion, IgE against PEG molecules on lipid nanoparticles (LNP) are frequently produced after multiple exposures to mRNA-based vaccines. Their clinical relevance requires further investigation and careful monitoring.

We did not observe a direct link between CU and atopy. This is corroborated by the rate of allergic rhinitis (28%) in CU patients which is comparable to the general population and confirmed by the Phadiatop analysis, which was positive in one-third of CU patients, a rate not higher than that observed in controls. Thus, the relationship between atopy and CU, while frequently discussed, is currently recognized as a co-occurring condition without a clear pathogenetic link (23, 24). Even in cases of auto-allergic or type 1 CU, conditions associated with self-antigen IgEs like anti-TPO or anti-IL24 (25, 26), atopic disease affects less than half of the patients (25).

While the WHO declared Omicron a variant of concern on November 26, 2021, and the virus rapidly spread in Europe, the incidence of CU reported to the Swiss national pharmacovigilance database was significantly higher than in other countries. This could be related to the notably

higher proportion of Spikevax administered in Switzerland as compared to other European countries (Figure 4). The temporal gap between the administration of the booster dose, the onset of new CU cases, and the subsequent COVID-19 wave suggests a lack of direct connection between viral infection and the onset of CU, which would have led to more CU cases in western countries. The lack of direct link with COVID is also supported by the low infection rate reported in our initial survey as well as by the anti-nucleocapsid data and neutralizing activities against the Omicron (BA.1 and BA.2) variants. Finally, reinfection with SARS-CoV-2 only led to CU exacerbation in a minority of cases (15%) corroborating the data from the UCARE COVAC-CU study who found a rate of COVID-19 vaccination-induced CU exacerbation of 9% (27).

As of June 1st 2022, in Switzerland, 43.53% and 26.44% of the population were fully vaccinated with Spikevax and Comirnaty respectively (28). Yet, over 90% of CU occurred after the Spikevax booster. Several hypotheses might explain this observation. Firstly, the mRNA content in the Spikevax vaccine is higher (100 µg) compared to Comirnaty (30 µg). Secondly, the Spikevax vaccine seems more stable in solution than Comirnaty after reconstitution (14). Thus, we recently demonstrated that cell lines become spike protein positive in culture when exposed to Spikevax but not to Comirnaty (14). Apart from the dosage differences, the Pfizer and Moderna platforms have few distinctions, with some variations in the structures of LNP carriers. Both contain PEG-2000, albeit in different forms and quantities (ALC-0519 and ALC-0315 in Comirnaty, PEG2006-DMG in Spikevax (8,20,52,53)) potentially also contributing to the immunogenicity of the vaccine. Thus, it has been repeatedly shown that the mRNA-1273 vaccine elicits higher and more persistent antibody production (29–31). Future research should explore the contribution of vaccine intervals and prior COVID-19 infection as risk factors for the development of new-onset CU.

This study has several limitations. First, there is a selection bias toward CU associated with the COVID-19 vaccine. Thus, we did not include CU patients unrelated to the vaccine as a control group. As the study started after the booster doses, there could also be a selection bias towards patients who received multiple doses. Yet, the data from the Swissmedic showed that CU occurred in 81% of the cases after the booster (11). Secondly, we did not investigate the presence of type IIb autoimmune mechanisms by performing autologous serum skin tests, immunoassays for IgG autoantibodies, or indirect basophil activation tests (32). Thirdly, several measures, such as total IgE, IgG anti-thyroid peroxidase, and complete blood count, were not available for this study. Indeed, CU is associated with an increased odds ratio for antithyroid antibodies and a higher incidence of autoimmune diseases including rheumatoid arthritis, Sjögren's syndrome, celiac disease, type I diabetes mellitus, and systemic lupus erythematosus (33). Given that only 4 out of 58 required omalizumab, of which 75% were able to discontinue the treatment, one might speculate that type IIb autoimmune CU, which is typically more refractory to anti-IgE therapies (32), is less prevalent in our CU population.

In conclusion, our one-year survey revealed that CU remained active in about 50% of the cases, with the inducible form of CU being quite common. There was no direct correlation between the onset of CU, PEG sensitization, atopy, and the concurrent Omicron virus infection. The fact that several individuals were able to tolerate an additional dose of the COVID mRNA vaccine without disease exacerbation, and considering that new onset CU remains a relatively rare event following vaccination, suggests that the mRNA vaccine may indirectly reveal a predisposition in certain individuals to develop CU. However, repeated exposure to the vaccine appears to be necessary in most cases to trigger CU, indicating that a vaccine-specific pre-existing immunity may provide a favorable condition and environment for recruitment of a CU-specific B cell

repertoire. Therefore, future research should focus on characterizing the nature of the auto-antibody response and comparing it to CU cases that are temporally unrelated to mRNA vaccines.

Methods

Ethical approval

The local ethical commitee approved the study "Commission cantonale d'éthique de la recherche sur l'être humain" CER-VD which registered in the Swissethics database (BASEC 2021-00735 (COVURT), Lausanne, Switzerland, https://swissethics.ch/en/basec). This study followed the STROBE reporting guideline.

Study population

We assembled the CU-VAUD cohort with the help of local allergists, contacted trough their association ("Groupement Vaudois des allergologues et immunologues") as previously described (11). Sixteen allergists contributed in identifying eligible patients with CU. The University Hospital of Lausanne (CHUV) contacted patients who gave their consent and sent them a link to an online questionnaire and included cases which were previously reported (11). Study data of the first survey were collected by participants between April 14th and January 5th 2023 and managed using REDCap electronic data capture tools hosted at Unisanté (Lausanne, Switzerland). All patients received a link to a second online questionnaire in 2023. Study data of the second survey were collected by participants between June 12th and September 4th 2023. Blood tests were performed from May 16th until January 23rd 2023.

As controls for the blood testing, we included patients from two observational cohorts without CU. The first study cohort regrouped patients with a formal diagnosis of COVID infection and who developed persistent symptoms in 56% (59/105) of the cases. Median age was 45 (IQR

35.5-54). 78/105 (74%) were females. Blood testing was performed between May 20 2022 and January 13 2023. The second group consisted of healthy collaborators from our hospital who systematically received a primary vaccination and a booster. Median age was 41 (IQR 35-48). 21/30 (70%) were females. Blood testing was performed between August 30th and October 4th 2022.

The third group consisted of heathy volunteers (n=17) recruited at the Geneva University Hospitals between Dec 2021 and Feb 2022 willing to receive their dose of mRNA COVID-19 vaccine (Comirnaty or Spikevax). Blood samples were collected before the third vaccine dose. Nine out of 17 (53%) were females and median age was 44.

Whole blood RNA sequencing

Blood samples were collected in PAXgene Blood RNA Tube (BD Biosciences). RNA extraction was performed using the PAXgene Blood miRNA Kit (BD) on the QIAcube instrument (QIAGEN) following the manufacturer's instructions. RNA concentration and quality were assessed by using the Qubit instrument (Invitrogen) and the Agilent 2100 Bioanalyzer, respectively. The Stranded Total RNA Ribo-Zero Plus kit from Illumina was used for the library preparation with 100 ng of total RNA as input. Library molarity and quality were assessed with the Qubit and Tapestation using a DNA High sensitivity chip (Agilent Technologies). Libraries were pooled at 2 nM for clustering and sequenced on an Illumina HiSeq4000 sequencer for a minimum of 30 million single-end 100 reads per sample. The RNA-sequencing libraries were aligned to the human genome (GRCh38.96) using STAR ((34). Only uniquely mapped reads were kept for downstream steps. Gene expression quantification was performed with featureCounts (35) for reads overlapping protein-coding genes. Low-count genes were filtered out with the filtered.data() function from the NOISeq R package (36) using the following parameters: method = 1, norm = FALSE, cv.cutoff = 100, cpm = 1.

286

287 ***Basophil activation test***

288 As previously reported vaccine-sensitization could be assessed by means of CD63 upregulation
289 with Spikevax or Comirnaty in an interchangeable way, as a surrogate of intra-dermal skin test
290 (14). Briefly, blood samples were collected in 3ml EDTA tubes and were used up to 24h of
291 blood collection using the Flow CAST® from Bühlmann Labs according to manufacturer's
292 instructions (FK-CCR). Vaccines were tested at up to 3 different concentration (1%-0.1% and
293 0.01%) as previously reported (Stehlin et al., 2022, #127677). A threshold of 10% in the
294 αFcεRI-stimulated or FMLP condition was used to define non-responders (=areactivity). The
295 same threshold was applied to the stimulated condition with mRNA vaccine to defined
296 positivity. For this study, two subjects were classified as non-responder (both from the cohort
297 CU). Results were analyzed using the FlowJo software (FLowJo LLC, Becton Dickinson,
298 Ashland, OR).

299

300 ***Serological analyses***

301 All analyzes were performed retrospectively on frozen serum samples. Regarding the Phadiatop
302 (detecting IgE against a mixture of common respiratory allergens, including grass, birch, olive,
303 mugwort, parietaria, dog, cat, horse, house dust mite, flour mite, and *Cladosporium*) was
304 measured using ImmunoCAP technology (Phadia 250, Thermo Fischer Scientific, Waltham,
305 Massachusetts) as previously reported (37). The lower detection limit was 0.35 kU/L for the
306 Phadiatop assay. Patients with a positive Phadiatop (≥ 0.35 kU/L) were considered atopic.
307 Serum IgG anti-S and anti-nucleocapsid antibody levels and neutralizing antibody levels were
308 determined using two Luminex bead-based binding assays recently developed in our laboratory
309 (38, 39). Neutralizing activity was assessed by monitoring the ability of anti-S antibodies to
310 prevent S-trimer protein binding to the angiotensin-converting enzyme 2 (ACE2) entry

receptor, which is essential for the viral infection of a target cell. Half maximal inhibitory concentration (IC_{50}) dilution values in the Spike-ACE2 surrogate neutralization assay and binding IgG anti-S antibody ratios were log_{10} transformed for visualization and statistical modeling as previously described (29).

Statistics

The neutralization assay was analyzed with a two-way ANOVA test using the software package GraphPad PRISM v9. Two-tailed unpaired T tests were performed for comparing group with a positive versus negative BAT. Mean and standard deviation is shown. A value of $P < 0.05$ was considered statistically significant. Using a Fisher exact test, statistical analysis evaluated associations between vaccination parameters (type and doses), cohorts, gender, and BAT or PhadiatTop results. Unvaccinated donors served as the reference group for each specific vaccine dose. Analyses were conducted using R Statistical Software (v4.2.1).

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Figure legends

Figure 1. Flowchart of the patients included in the COVURT study.

Figure 2. Peak incidence of the first booster, new-onset chronic urticaria, and COVID-19 cases over time. Only patients who developed CU after November 1st, 2021, were included in the analysis.

Figure 3. A. Table summarizing the percentage of patients across the different cohort studies with positive versus negative basophil activation tests (BAT). Associations between the different variables were assessed using a Fisher exact test. B. Age (mean and SD) of patients with a positive (+) or negative (-) BAT. C. CU duration in patients with a positive (+) or negative (-) BAT. D. CD63 expression in patients with a positive BAT who received the

Spikevax and the BNT 16b2. E. CD63 expression in patients a positive versus negative serology for the nucleocapsid. Anti-nucleocapsid (F) and anti-spike (G) titers in patients with positive (+) or negative (-) BAT. H. Neutralizing activities against the different SARS-COV2 variants in patients with a positive (+) or negative (-) BAT or with/without CU (I). J. Table summarizing the percentages of patients across the different cohort studies with positive or negative Phadiatop results. K. Phadiatop titer in patients with a negative (-) or positive (+) BAT. Associations between the different variables were assessed using a Fisher exact test. L. Correlation of the Phadiatop titer and BAT results. Mean and SD are shown. Unpaired two-sided T-tests or two-way ANOVAs were used for statistical analysis. Abbreviation: BAT basophil activation tests.

Figure 4. The map of Europe shows the proportion of individuals who received Spikevax (black) and the BNT 16b2 (blue circles) vaccines for each country. The larger the circle is, the larger the frequency is. Data were downloaded from the European Centre for Disease Prevention and Control (ECDC) and Federal Office of Public health (FOPH) of Switzerland on November 27th. Bivalent vaccines were not included in the analysis.

Table 1. 2022 survey of patients with chronic urticaria identified in the Canton the Vaud. Missing data for age n=4.

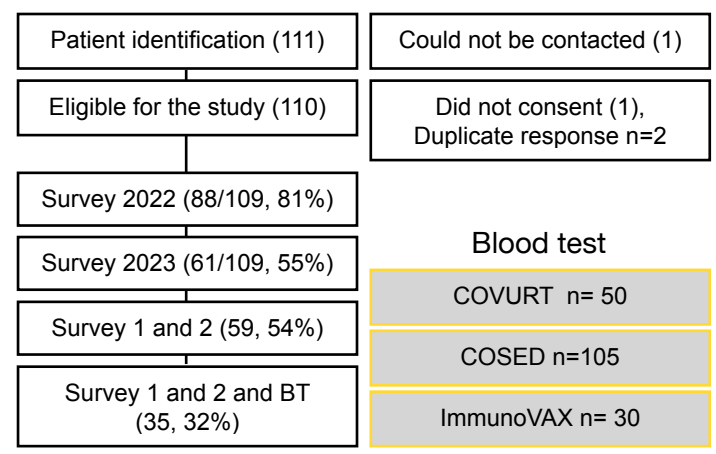
Table 2. 2023 survey of patients with chronic urticaria identified in the Canton the Vaud.

Table 3. Detailed features of the patients with CU who successfully received a new dose of mRNA vaccines after CU onset.

493 Supplementary figure 1. Urticaria control test (UCT). In patients with a negative versus
494 positive nucleocapsid titer. Two-way ANOVA was used for the statistics.
495
496 Supplementary figure 2. Heatmap showing the top 5% highly variable genes from the bulk
497 RNA sequencing results comparing patients with chronic urticaria (n=15) to healthy
498 vaccinated controls (n=17).

Figure 1

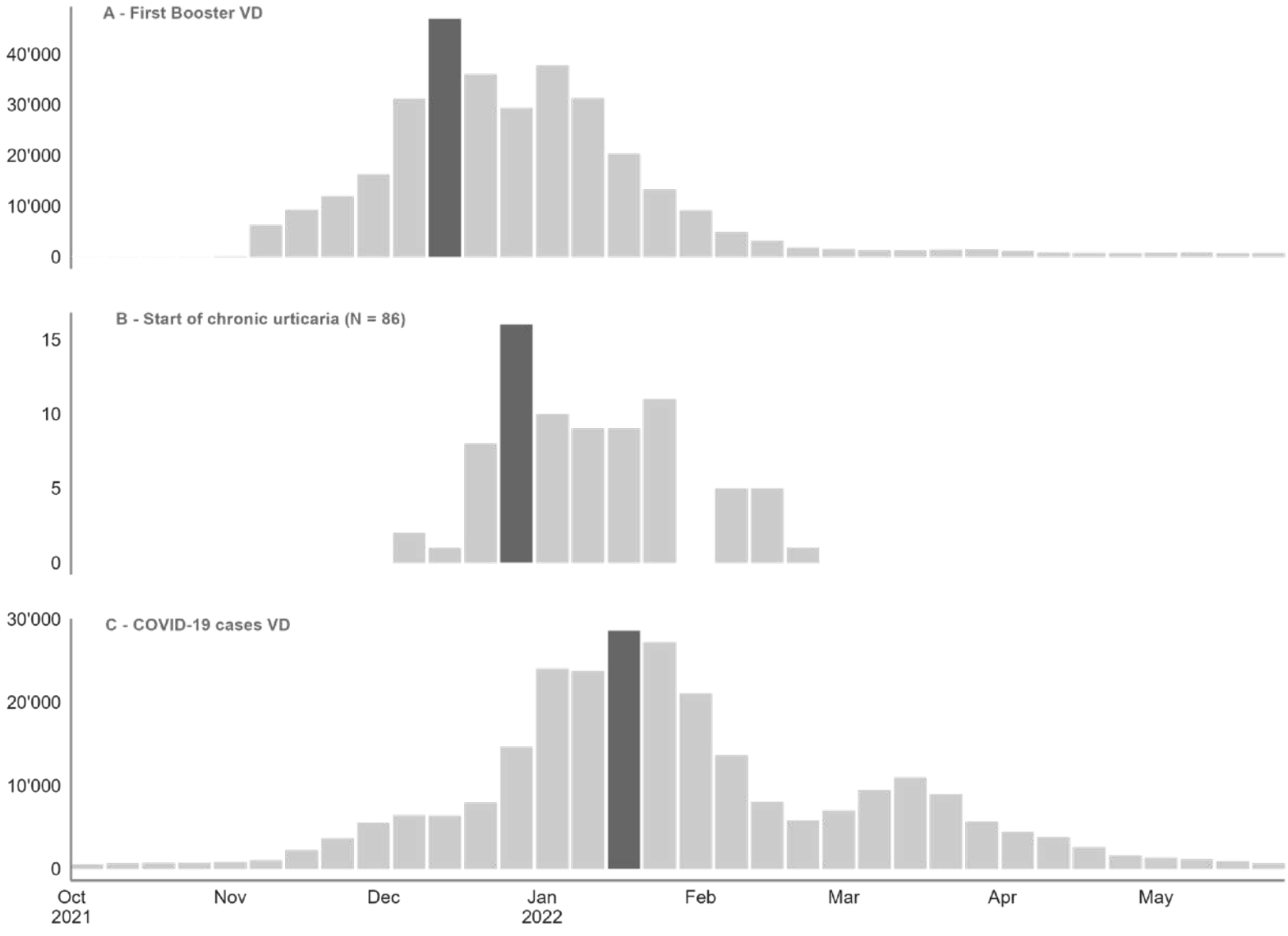
A



B

	COVURT	COSED	ImmunoVAX
Gender			
F	37 (74%)	78 (74%)	21 (70%)
M	13 (26%)	27 (26%)	9 (30%)
Age, years (median)	42 (IQR 36.8-48)	45 (IQR 35.5-54)	41 (IQR 35-48)
Vaccine received			
Yes	50 (100%)	80 (76%)	30 (100%)
No	0	25 (24%)	0
Vaccine Type			
Moderna	46 (92%)	34 (43%)	16 (53%)
Pfizer	4 (8%)	45 (56%)	14 /47%)
Janssen	0	1 (1%)	0
Number of Dose			
1	2	14 (13%)	0
2	3	35 (33%)	0
booster	45 (90%)	31 (39%)	30 (100%)

Figure 2



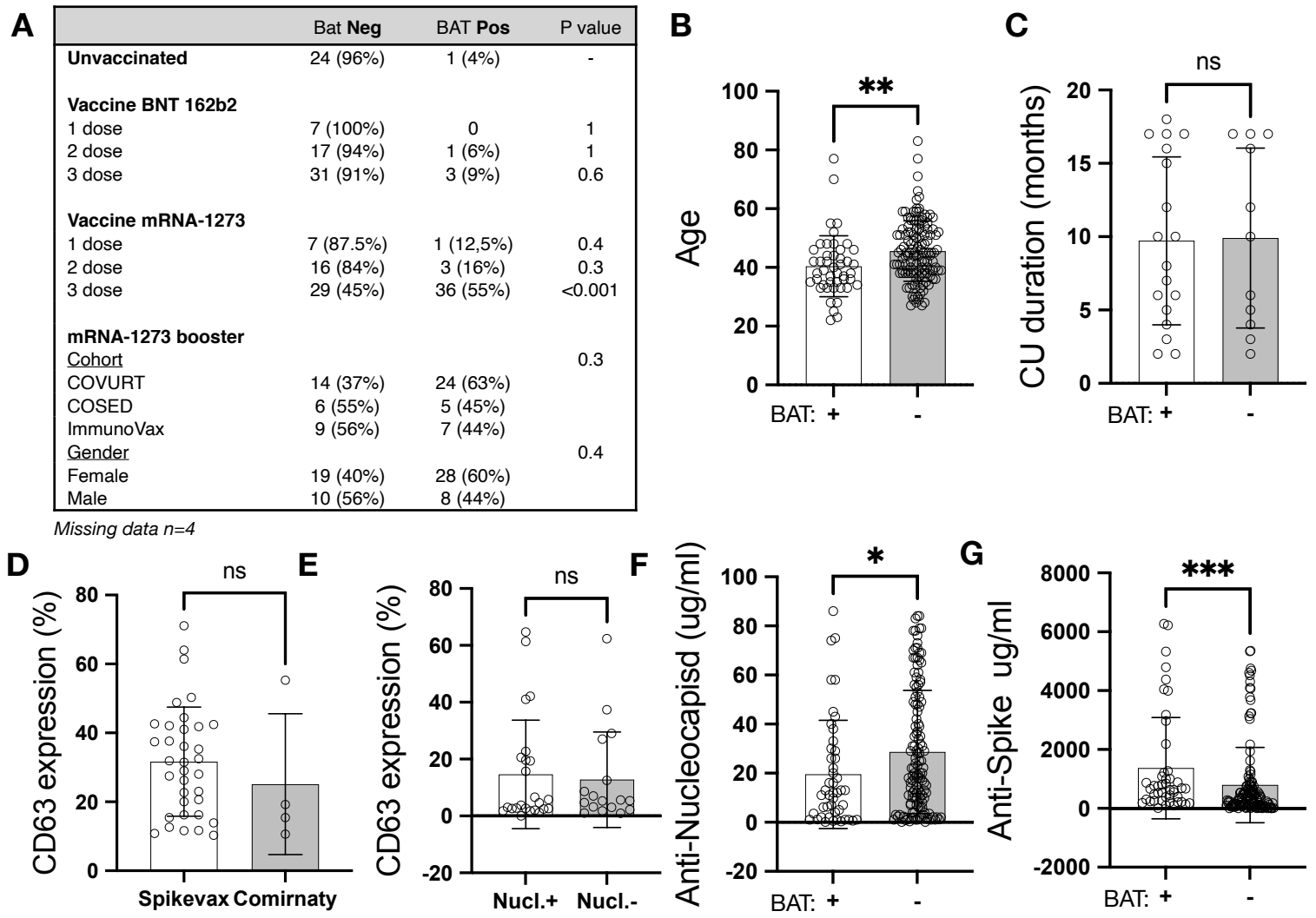
Source : OMC-VD/Unisanté via website OFSP + CHUV-CSU [data on 2023-01-24]

Figure 3

A

	Bat **Neg**	BAT **Pos**	P value
Unvaccinated	24 (96%)	1 (4%)	-
Vaccine BNT 162b2			
1 dose	7 (100%)	0	1
2 dose	17 (94%)	1 (6%)	1
3 dose	31 (91%)	3 (9%)	0.6
Vaccine mRNA-1273			
1 dose	7 (87.5%)	1 (12,5%)	0.4
2 dose	16 (84%)	3 (16%)	0.3
3 dose	29 (45%)	36 (55%)	<0.001
mRNA-1273 booster			
Cohort			0.3
COVURT	14 (37%)	24 (63%)	
COSED	6 (55%)	5 (45%)	
ImmunoVax	9 (56%)	7 (44%)	
Gender			0.4
Female	19 (40%)	28 (60%)	
Male	10 (56%)	8 (44%)	

Missing data n=4



J

	Phadiatop **Neg**	Phadiatop **Pos**	p value
Cohort	27 (64%)	15 (36%)	
CU	27 (64%)	15 (36%)	0.3
COSED	56 (54%)	48 (46%)	0.3
ImmunoVax	11 (58%)	8 (42%)	1
Unvaccinated	12 (48%)	13 (52%)	0.4
Vaccination type			0.7
Moderna	50 (59%)	35 (41%)	
Pfizer	32 (59%)	22 (41%)	
Janssen	0	1 (100%)	
Vaccination			0.6
1 dose	11 (69%)	5 (31%)	
2 dose	21 (55%)	17 (45%)	
3 dose	51 (59%)	36 (41%)	
BAT			1
Positive	22 (58%)	16 (42%)	
Negative	72 (57%)	55 (43%)	

For 13 patients, not sufficient material to perform the analysis

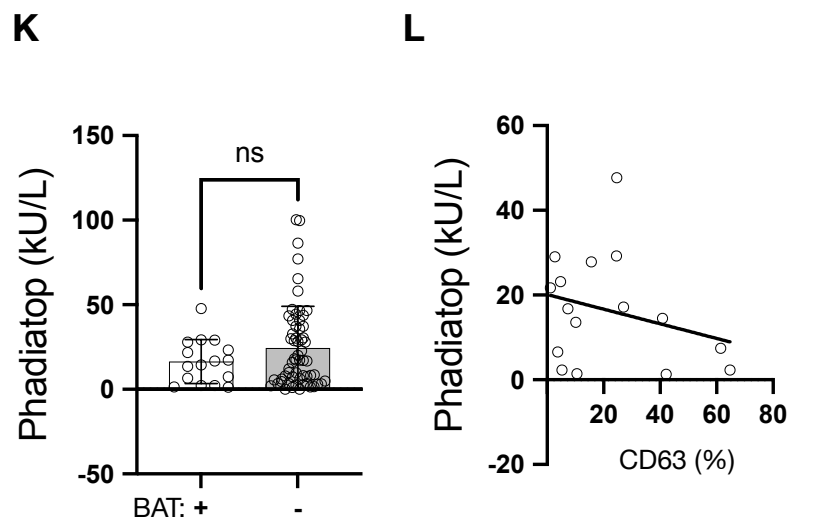


Figure 4

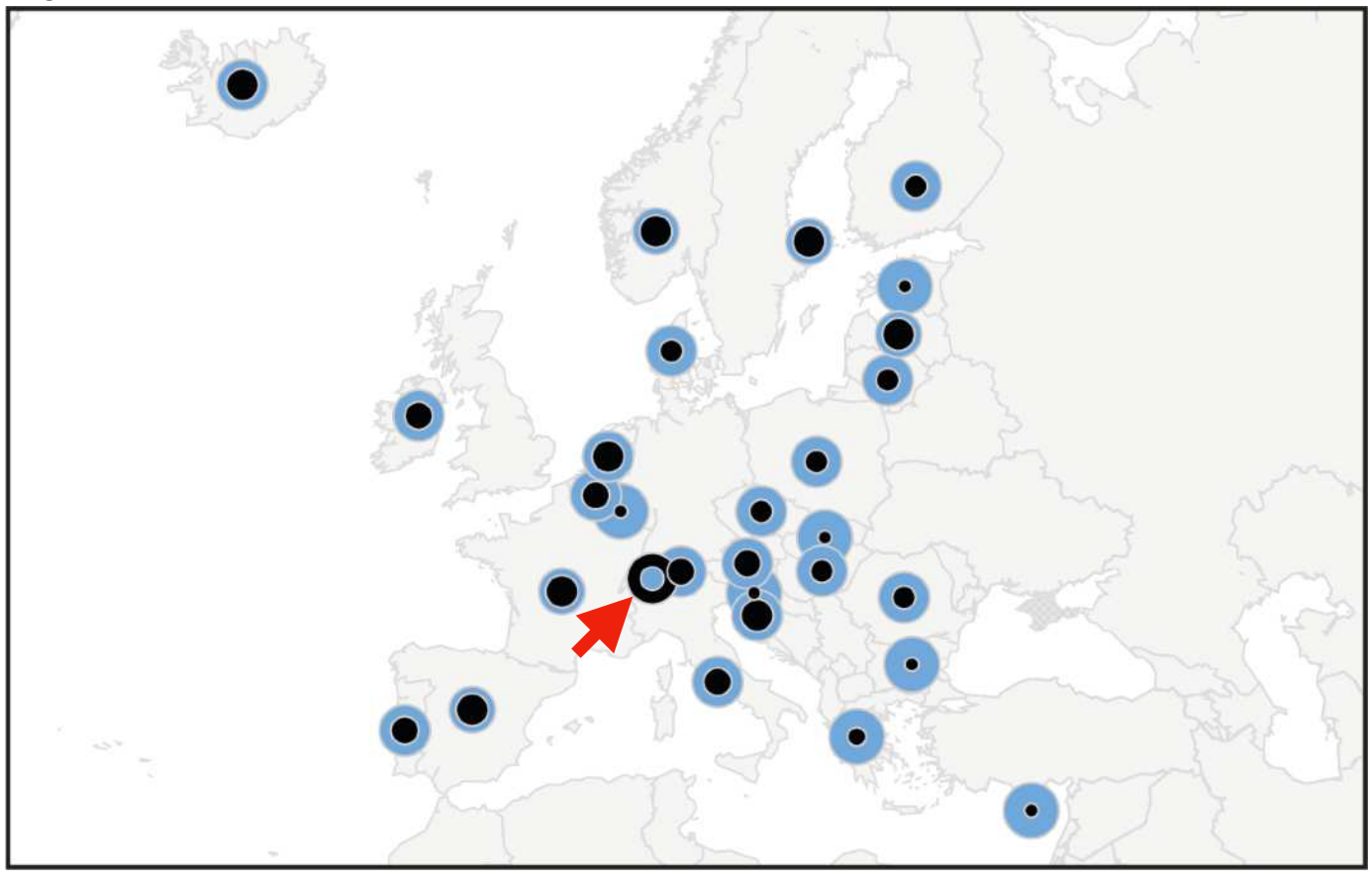


Table 1

Survey 2022 (n=88)	
Gender	
F	58 (66%)
M	30 (34%)
Age, (median IDR) * missing data (n=4)	41 (35-47)
UC after booster	
yes	78 (89%)
no	10 (11%)
Delays between last dose and CU (days)	10 (8,12)
Vaccine received	
Moderna	82 (93%)
Pfizer	6 (7%)
CU active by June 2022	
yes	71 (81%)
no	17 (19%)
Inducible urticaria	
yes	48 (55%)
no	40 (45%)
Inducible factors	
dermographism	37 (77%)
sun	12 (25%)
water	14 (29%)
cold	10 (20%)
sport	7 (15%)
UCT score (first month of activity)	
< 12	86 (98%)
> 12	2 (2%)
UCT score (last month of activity)	
< 12	83 (94%)
> 12	4 (5%)
Unknown	1 (1%)
Mean number of lesion (first week of activity)	
None	2 (2%)
< 20	20 (24%)
20-50	38 (43%)
> 50	28 (31%)
Mean number of lesion (last week of activity)	
None	11 (13%)
< 20	61 (69%)
20-50	11 (13%)
> 50	5 (6%)
Pruritus severity (first week)	
None	1 (1%)
mild (bearable)	0
medium	11 (13%)
severe (interfere with sleep and/or daily activities)	76 (86%)
Pruritus severity (last week)	
None	10 (11%)
mild (bearable)	34 (39%)
medium	29 (33%)
severe (interfere with sleep and/or daily activities)	15 (17%)
Onging anti-histamine therapy	
yes	67 (75%)
no	16 (19%)
missing	5 (6%)
Anti-histamine therapy (maximum)	
not taken	1 (1%)
1 pill/day	22 (25%)
2 pills/day	23 (26%)
3 pills/day	10 (11%)
4 pills/day	27 (31%)
unknown	5 (6%)
Urticaria in the past	
yes	12 (14%)
no	76 (86%)
Duration of previous urticaria	
< 6 weeks	11 (92%)
> 6 weeks	1 (8%)
NDAIDs exacerbating CU	
yes	4 (5%)
no	84 (95%)
COVID infection	
yes	30 (34%)
no	58 (66%)
Did CU get worse after COVID	
yes	11 (12%)
no	20 (22%)
Ashtma	
yes	2 (2%)
no	86 (98%)
Pollinosis	
yes	25 (28%)
no	63 (72%)
Drug allergies	
yes	9 (10%)
no	79 (90%)

Table 2

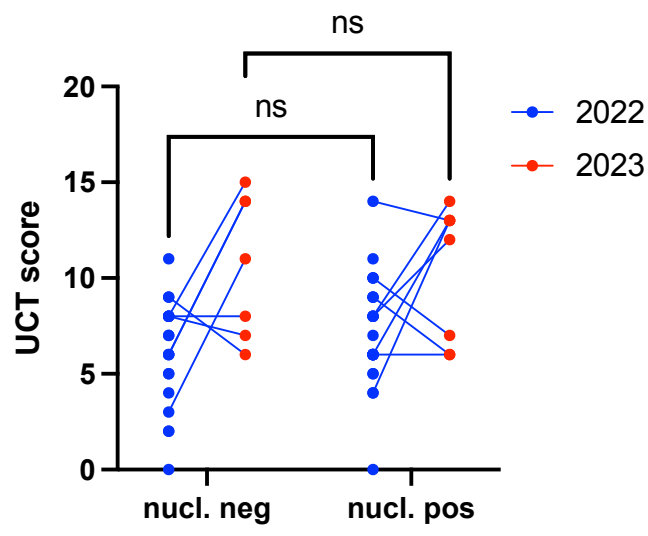
Survey 2023 (n=61)	
Gender	
F	39 (64%)
M	22 (36%)
Age (median, IDR) (missing data n=1)	41.5 (35-50)
Vaccine received	
Moderna	56 (92%)
Pfizer	3 (5%)
missing data	2 (3%)
UC after booster	
yes	56 (92%)
no	4 (7%)
unknown	1 (2%)
CU active by June 2023	
yes	32 (52.5%)
no	29 (47.5%)
Active CU is	
inductible	7 (22%)
spontaneous	13 (42%)
both	12 (39%)
If inducible, triggered by	
dermographism	13 (68%)
sun	7 (37%)
water	2 (11%)
cold	5 (26%)
sport	8 (42%)
vibration	2 (11%)
UCT score	
< 12	16 (50%)
> 12	16 (50%)
Unknown	0
Mean number of lesion during the past week	
None	6 (19%)
< 20	22 (69%)
20-50	4 (13%)
> 50	0
Prurit severity	
None	1 (3%)
mild (bearable)	15 (47%)
medium	10 (31%)
severe (interfere with sleep and/or daily activities)	6 (19%)
Anti-histamine therapy	
< 3 times a week	13 (42%)
> 3 times a week	6 (19%)
1 pill/day	8 (26%)
2 pills/day	1 (3%)
3 pills/day	0
4 pills/day	2 (6%)
missing data	1 (3%)
Omalizumab	
yes ongoing	1 (2%)
yes stopped	3 (5%)
no	54 (89%)
missing data	3 (5%)
Corticosteroids (anytime)	
yes	14 (23%)
no	47 (77%)
NDAIDs exacerbating CU	
yes	6 (10%)
no	53 (87%)
missing data	2 (3%)
New booster after CU onset	
yes	3 (5%)
no	58 (95%)
Did CU get worse after the booster	
yes	0
no	3/3 (100%)
Which vaccine was recevied?	
Pfizer	3/3 (100%)
COVID infection after CU onset	
yes	27 (44%)
no	31 (56%)
Did CU get worse after COVID	
yes	4/27 (15%)
no	23/27 (85%)

Table 3

	Cohort	gender	age	CU still active	CU after	Vaccine received	Timing between vaccine and CU	BAT agasint mRNA (>10%)	Inductible?	NSAID and CU
patient 1	VD	female	82	no	dose 1	Pfizer	8 days	neg	no	no
patient 2	VD	male	41	yes	booster	Moderna	7 days	pos	no	no
patient 3	VD	male	50	yes	booster	Moderna	12 days	neg	yes (sun)	no
patient 4	TI	female	50	no	booster	Moderna	10 days	pos	yes (dermog)	no

	History of urticaria	COVID infection	Asthma	Hay fever	Drug allergy	Vaccine received after CU	Did the vaccine worsened CU?	Anti histmaine	Treated with omalizumab
patient 1	yes	no	no	no	no	Pfizer	no	no	no
patient 2	no	no	no	no	no	Pfizer	no	3x/semaine	no
patient 3	no	yes (no impact on CU)	no	yes	no	Pfizer	no	1 anti histamine/ days	no
patient 4	no	no	no	no	no	Moderna	no	on demand	no

Supplemental Figure 1



Supplemental Figure 2