

LETTER TO THE EDITORS

Research Letter

Dear Editor,

Higher mean platelet volume is associated with paediatric chronic spontaneous urticaria and with a more severe disease in the affected children

Chronic spontaneous urticaria (CSU) is a mast cell-driven disease characterised by recurrent wheals, angioedema or both, persisting most days of the week, for 6 weeks or longer.¹ Chronic autoimmune urticaria is a form of CSU mediated by IgG against the high-affinity IgE receptor on the mast cells and basophils or against membrane-bound IgE and is diagnosed by a positive autologous serum skin test (ASST).² Pathophysiology of chronic urticaria is complex and includes an interplay between inflammatory cellular responses, autoimmunity, vasoactive mediators, platelet activation and coagulation.^{3,4} Mean platelet volume (MPV) is considered as a marker of platelet reactivity as larger platelets are metabolically and enzymatically more active.^{5,6} Reports in adults suggest that higher MPV is associated with CSU.^{7,8} To our knowledge, only one small study in children with CSU has been published indicating lower MPV in the affected *vs* healthy children (small, unadjusted difference).⁹ Due to this discrepancy (adults *vs* children), we conducted a case-control study: cases ($n = 50$) were children (<18 years of age) with CSU (autologous serum skin test positive or negative) and were age and sex-matched to two types of hospital-based controls (1:1:1) – healthy children and children with non-CSU recurrent diseases (atopic, recurrent infections or non-allergic, non-infectious conditions; see Data S1). MPV at presentation was closely similar in autologous serum skin test positive and negative cases, and appeared numerically higher than in both control groups (Table 1; individual raw data in Fig. S1A), although all individual values across the groups were within the normal range (Table 1). With adjustment for age, sex, cell blood counts, haemoglobin, haematocrit, C-reactive protein and whether condition was immunologic or not, MPV was consistently by around 1.8 fL higher in cases than in healthy controls, non-CSU controls and all controls taken together (Table 2; adjusted means in Fig. S1B). In a reverse analysis, MPV well differentiated between cases and all types of controls (areas under the receiver-

operating characteristics curves were 0.85 or 0.86). With the same adjustments, by 1 fL higher MPV was associated with 3.3–4.2 higher odds of CSU (*vs* different controls; Table 2). There were no remarkable findings regarding complement components, specific IgE, auto-antibodies or thyroid function in children with CSU (Table S1 gives details on diagnostic work-up results). Stool was positive for *Candida* in 19/50 children and serological analysis indicated active immune responses to different pathogens in 11/50 children. The commenced treatments were mainly anti-allergy treatments (in 33/50 children) and systemic azoles (in 13/50 children). After 4 weeks of treatment, MPV was lower than at presentation by around 0.55 fL and only one child with autologous serum skin test negative CSU experienced a mild increase in MPV (by 0.1 fL; Table 1). With adjustment for age and sex, these MPV values were consistently higher (across subsets based on autologous serum skin test, *Candida* colonisation or anti-allergy treatment) than in healthy or non-CSU controls: differences were around 1.0–1.4 fL, and somewhat higher in 19 cases with *Candida* colonisation (around 1.65 fL; Fig. S2A). In reverse, by 1 fL higher MPV was associated with 2.5–4.1 fold higher odds of CSU (ORs were around 7.0 to around 11.3 in the *Candida* positive subset; Fig. S2B). The reduction in MPV coincided with a considerable reduction in urticaria activity score in all children with CSU: severity of the disease was reduced from on average moderate (autologous serum skin test negative children) or severe (autologous serum skin test positive children) to mild (Table 1). Based on paired data (at presentation and after a 4-week treatment), when urticaria activity score was considered a dependent variable, higher MPV was independently associated with higher score, comparably in patients with a positive and a negative autologous serum skin test (insignificant autologous serum skin test *MPV interaction; Table 3). In reverse, when MPV was considered a dependent variable, higher urticaria activity score was associated with higher MPV, comparably in patients with a positive and a negative autologous serum skin test (insignificant autologous serum skin test *urticaria activity interaction; Table 3).

Although derived from a limited single-centre sample, the present observations strongly suggest that in paediatric patients (just as it has been suggested in adults^{7,8}) higher MPV is associated with presence of CSU/chronic autoimmune urticaria, and also with a more severe clinical picture in the affected children. They also indicate a possibility that *Candida* colonisation of the gastrointestinal tract might moderate this relationship. There is mechanistic data to support an active role of (particularly

Conflict of interest: None.

Table 1 Subject characteristics. Data are median (minimum-maximum) or count (%) if not stated otherwise

	Cases - children with CSU				Controls 1-healthy children			Controls 2 - children with non-CSU recurrent diseases			
	ASST negative		ASST positive		children	All	Non-allergic	All	Non-allergic	Allergic	
	All										
<i>N</i>	50	28	22	50	50	50	50	50	50	20	
Age (years)	9.1 (1-17)	9.1 (1-17)	8.9 (1-16.7)	8.9 (1-17.1)	8.9 (1-17.1)	9.1 (1-17)	9.1 (1-17)	9.1 (1-17)	9.1 (1-17)	9.7 (1.6-15.9)	
Boys	22 (44.0)	16 (57.1)	6 (27.3)	23 (46.0)	23 (46.0)	22 (44.0)	11 (36.7)	22 (44.0)	11 (36.7)	11 (55.0)	
At presentation											
MPV (fL)	10.7 (7.0-11.9)	10.5 (8.2-11.9)	11.1 (7.0-11.9)	8.8 (7.0-11.2)	8.8 (7.0-11.2)	8.8 (7.1-10.8)	9.0 (7.1-10.2)	8.8 (7.1-10.8)	9.0 (7.1-10.2)	8.6 (7.2-10.8)	
UAS	22.5 (10-40)	20.5 (10-52)	32 (10-40)	-	-	-	-	-	-	-	
Platelets ($\times 10^9/L$)	523 (164-546)	307 (164-546)	343 (198-435)	306 (187-450)	306 (187-450)	318 (180-603)	332 (180-603)	318 (180-603)	332 (180-603)	307 (217-480)	
RBC ($\times 10^{12}/L$)	4.59 (3.52-5.56)	4.48 (3.52-5.56)	4.60 (3.92-5.12)	4.75 (4.01-5.22)	4.75 (4.01-5.22)	4.79 (4.12-5.44)	4.81 (4.12-5.24)	4.79 (4.12-5.44)	4.81 (4.12-5.24)	4.78 (4.19-5.44)	
Haemoglobin (g/L)	123 (97-149)	129 (97-145)	122 (109-149)	131 (103-151)	131 (103-151)	128 (95-145)	126 (95-145)	128 (95-145)	126 (95-145)	128 (117-142)	
Haematocrit (L/L)	0.37 (0.29-0.42)	0.35 (0.29-0.42)	0.37 (0.32-0.41)	0.32 (0.15-0.45)	0.32 (0.15-0.45)	0.37 (0.30-0.42)	0.37 (0.30-0.42)	0.37 (0.30-0.42)	0.37 (0.30-0.42)	0.38 (0.34-0.41)	
Leukocytes ($\times 10^6$)	9.2 (4.7-15.9)	9.1 (4.7-15.9)	10.6 (6.0-15.2)	8.5 (4.6-15.8)	8.5 (4.6-15.8)	6.9 (4.5-18.5)	6.6 (4.5-18.5)	6.9 (4.5-18.5)	6.6 (4.5-18.5)	7.2 (5-13.5)	
CRP (mg/L)	1.8 (0.3-27.5)	1.7 (0.3-7.7)	2.7 (0.4-27.5)	2.1 (0.1-7.5)	2.1 (0.1-7.5)	2.1 (0.1-5.1)	2.1 (0.1-5.9)	2.1 (0.1-5.1)	2.1 (0.1-5.9)	2.2 (0.3-5.1)	
CRP ≥ 10 mg/L	1 (2.0)	0	1 (4.5)	0	0	0	0	0	0	0	
At +4-weeks											
MPV (fL)	10.2 (6.7-11.7)	9.9 (8.2-11.7)	10.3 (6.7-11.7)	-	-	-	-	-	-	-	
Change (95%CI)	-0.55 (-0.70, -0.45)	-0.55 (-0.75, -0.35)	-0.55 (-0.85, -0.40)	-	-	-	-	-	-	-	
Range of change	-1.8 to 0.1	-1.8 to 0.1	-1.7 to -0.1	-	-	-	-	-	-	-	
UAS	8 (2-38)	6 (2-18)	14 (2-58)	-	-	-	-	-	-	-	
Change (95%CI)	-12 (-14, -12)	-12 (-15, -9)	-12 (-15, -10)	-	-	-	-	-	-	-	
Range of change	-34 to -2	-26 to -4	-34 to -2	-	-	-	-	-	-	-	

ASST, autologous serum skin test; CRP, C-reactive protein; CSU, chronic spontaneous urticaria; MPV, mean platelet volume; RBC, red blood cells; UAS, urticaria activity score.

Table 2 Independent association between higher mean platelet volume (MPV) and chronic spontaneous urticaria (CSU). (A) A general linear model (GLM) was fitted to MPV to determine adjusted (for age, sex, cell blood counts, haemoglobin, haematocrit, C-reactive protein and whether condition was immunologic or not) mean differences between children with CSU (cases) and different controls (healthy or non-CSU disease). Differences (Δ) are given with 97.5%CI to account for comparisons with two types of controls. (B) A separate logistic model (defined by different controls) was fitted to probability of CSU to determine adjusted (as above) odds ratios (OR) associated with 1 fL increase in MPV

(A) Difference between CSU and controls			(B) Change in odds of CSU with 1 fL increase in MPV		
Comparison	Mean Δ (97.5%CI)	P-value	Probability of	OR (95.0%CI)	P-value
CSU <i>vs</i> healthy	1.78 (1.19, 2.58)	<0.001	CSU (<i>vs</i> healthy)	5.33 (1.99–5.55)	<0.001
CSU <i>vs</i> non-CSU	1.85 (1.26, 2.40)	<0.001	CSU (<i>vs</i> non-CSU)	4.23 (2.25–7.98)	<0.001
CSU <i>vs</i> any controls	1.81 (1.30, 2.31)	<0.001	CSU (<i>vs</i> any controls)	5.80 (2.36–6.12)	<0.001

Table 3 Independent association between urticaria activity score (UAS) and mean platelet volume (MPV): summary of multivariate analyses. Two general linear mixed models (each child contributed paired UAS and MPV values) were fitted, one with UAS as a dependent variable and the other one with MPV as a dependent variable[†]. Effects are coefficients (95% CI)

	Model for UAS		Model for MPV	
	Coeff. (95%CI)	P	Coeff. (95%CI)	P
MPV (in the analysis of UAS; by 1 fL)	3.38 (2.04, 5.07)	<0.001	–	–
UAS (in the analysis of MPV; by 1 point)	–	–	0.04 (0.03–0.05)	<0.001
ASST positive (<i>vs</i> negative)	7.41 (3.35, 11.5)	<0.001	–0.19 (–0.82, 0.45)	0.557
ASST*MPV interaction (analysis of UAS)	–1.30 (–4.40, 1.82)	0.407	–	–
MPV at ASST negative (by 1 fL)	4.03 (1.58, 6.48)	–	–	–
MPV at ASST positive (by 1 fL)	2.73 (0.61, 4.86)	–	–	–
ASST*UAS interaction (analysis of MPV)	–	–	0.01 (–0.01, 0.03)	0.533
UAS at ASST negative (by 1 point)	–	–	0.05 (0.02, 0.05)	–
UAS at ASST positive (by 1 point)	–	–	0.04 (0.02, 0.06)	–

[†]Each model had two default independents – one of the primary interest (MPV or UAS), and the result of the autologous serum skin test (ASST) and their interaction to test whether the MPV↔UAS association was conditional on the ASST status. Adjustments in both models were for: age, baseline C-reactive protein, platelet count, whether anti-allergic treatment was employed and whether stool was positive for *Candida*; and were selected from demographic, serology and laboratory results through a backward selection procedure.

larger, more reactive) platelets in CSU (triggering coagulation, favouring histamine release), but larger platelets might also be a simple consequence of bone marrow stimulation by increased systemic inflammation.⁸ Present data and reports in adults suggest that platelets/MPV in CSU deserve further evaluation as potential prognostic markers or therapeutic targets.

AUTHORS' CONTRIBUTION

Anamarija Čavčić conceived the study, performed allergological tests and drafted the manuscript. Irena Ivković Jureković contributed to data collection and interpretation of the results. Vladimir Trkulja contributed to study design, analysed the data and drafted the manuscript. All authors critically revised the article, read and approved the final manuscript.

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spontaneous urticaria. *Allergol. Immunopathol. (Madr)* 2015; 43: 10–3.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Data S1. Supplemental methods.

Figure S1. Mean platelet volume in children with chronic

spontaneous urticaria (CSU, cases), healthy controls and non-CSU control children.

Figure S2. Association between chronic spontaneous urticaria (CSU) and mean platelet volume (MPV) as determined 4 weeks after initial presentation of the CSU patients (i.e., at reduced disease activity assessed by the means of the urticaria activity score).

Table S1. Children with chronic spontaneous urticaria: main diagnostic work-up results and commenced treatments.

Supplemental Material to Research Letter

Higher mean platelet volume is associated with pediatric chronic spontaneous urticaria and with a more severe disease in the affected children

Supplemental Methods

Supplemental Figure S1 - Mean platelet volume in children with chronic spontaneous urticaria (CSU, cases), healthy controls and non-CSU control children.

Supplemental Table S1 – Children with chronic spontaneous urticaria: main diagnostic work-up results and commenced treatments.

Supplemental Figure S2 - Association between chronic spontaneous urticaria (CSU) and mean platelet volume (MPV) as determined four weeks after initial presentation of the CSU patients (i.e., at reduced disease activity assessed by the means of the urticaria activity score).

Supplemental references

Supplemental methods

General design and ethics

This case-control study aimed to evaluate the relationship between mean platelet volume (MPV) and chronic spontaneous urticaria (CSU) in children: a) by comparing MPV in children with manifest CSU to that in healthy children (healthy controls) and children suffering from recurrent non-CSU allergic, infectious or non-allergic, non-infectious diseases (non-CSU controls). The latter control group was included since MPV might be related to inflammatory/allergic conditions other than CSU;¹⁻⁵ b) by evaluating the ability of MPV to discriminate between CSU and health/non-CSU disease; c) by evaluating the relationship between severity of CSU (urticaria activity score, UAS) and MPV. No diagnostic procedure beyond the routine ones standard in the management of children with respective diseases was undertaken. All parents/guardians were informed about and consented to the implemented procedures. Institutional Ethics committee approved the study.

Patients and procedures

Cases were children (<18 years of age) with verified CSU in line with the European Academy of Allergy and Clinical Immunology (EAACI) criteria.⁶ They were included in the present analysis if at least 3 months had elapsed since the last corticosteroid use and at least 7 days had elapsed since the last antihistamine use. At the beginning of the diagnostic work-up, while the urticarial rash was active, they underwent the autologous serum skin test (ASST) to identify chronic autoimmune urticaria (CAU), a form of CSU mediated by IgE. A serum-induced wheal ≥ 1.5 mm in diameter as compared to a saline-induced wheal at 30 minutes post-injection was considered as a positive test.⁷ Diagnostic work-up and subsequent treatment followed the EAACI guidelines.⁶ Clinical severity of the disease (UAS)⁶ and mean platelet volume (MPV) were determined at presentation and 4 weeks after the initial evaluation (with treatment in-between). They were age (± 6 months) and sex-matched to healthy controls and non-CSU controls (1:1:1) within a three-month window. Healthy controls were recruited among children visiting the hospital for regular control check-ups after mild non-recurrent diseases, typically minor injuries and a few cases of infective gastro-enterocolitis, all with confirmed absence of a disease (i.e., with regained healthy condition). Standard hematology tests included in the present analysis were obtained at the final visit confirming the absence of a disease. Control children with recurrent disease unrelated to CSU were recruited among children known to suffer from either an atopic disease (asthma, allergic rhinoconjunctivitis, n=20) or recurrent infectious conditions (most commonly respiratory or urinary tract infections, n=17), or non-allergic, non-infectious conditions (gastro-esophageal reflux disease, n=13). Standard laboratory tests were obtained at the start of their diagnostic work-up and were included in the

present analysis if ≥ 3 months / ≥ 7 days had elapsed since the last corticosteroid or antihistamine dose, respectively. Neither cases nor controls were included if suffering from conditions known to potentially affect platelet function (obesity, diabetes mellitus, hypertension, any heart disease or pulmonary embolism), or if treated with antiplatelets or anticoagulants.

Peripheral venous blood samples were collected using Greiner Vacuette System (Greiner Bio-One GmbH, Kemsminster, Austria) with EDTA and were analyzed using an automated blood counter (Beckman Coulter LH 750, Miami, USA) within < 2 hours at room temperature to prevent platelet swelling.⁸ Over the years, standard routine quality control checks at the central institutional laboratory consistently showed $< 5\%$ intra- and inter-assay variability for platelet parameters.

Planned analyses

Considering the study design (cross-sectional case-control), analyses were planned in “two directions”, i.e., a variable was to be considered as a dependent in one analysis, and as an independent in the “reverse” analysis. We aimed: a) to estimate adjusted differences in MPV between children with CSU and controls; in reverse, to estimate the ability of MPV to discriminate between CSU and health/non-CSU; b) to estimate association between UAS and MPV considering UAS as a dependent variable and MPV as an independent, and *vice-versa*.

Sample size

We intended to enroll at least 40 children with CSU (cases) and 40 of each of the two types of controls. This sample size would have provided 85% power to detect a difference in MPV of 1.7 fL (one third of the range of normal values) between cases and any of the control groups at two-sided alpha level 0.05 assuming that standard deviation in each group is up to 2.5 (half of the normal range of values). This sample size would have also provided 87% power to distinguish an area under the receiver operating characteristics curve (ROC curve) of ≥ 0.85 from an area of 0.70 at alpha 0.05, i.e., to distinguish between “good to very good” discriminative properties of MPV and “fair to poor” discriminative properties regarding presence of CSU vs. either health or non-CSU recurrent disease; and 83% power to identify an odds ratio ≥ 2.0 or ≤ 0.5 for the outcome “CSU”, associated with 1 SD increase in MPV (with 1:1 cases and controls ratio) at two-sided alpha 0.05.

Statistics

General linear models were fitted to MPV data to determine adjusted differences between cases and controls. Generalized linear (logit) models with ROC analysis were used to test the ability of MPV to discriminate between cases and controls. General linear mixed models were used to evaluate independent association between UAS and MPV based on repeated data. We used SAS 9.4 for Windows (SAS Inc., Cary, USA) software.

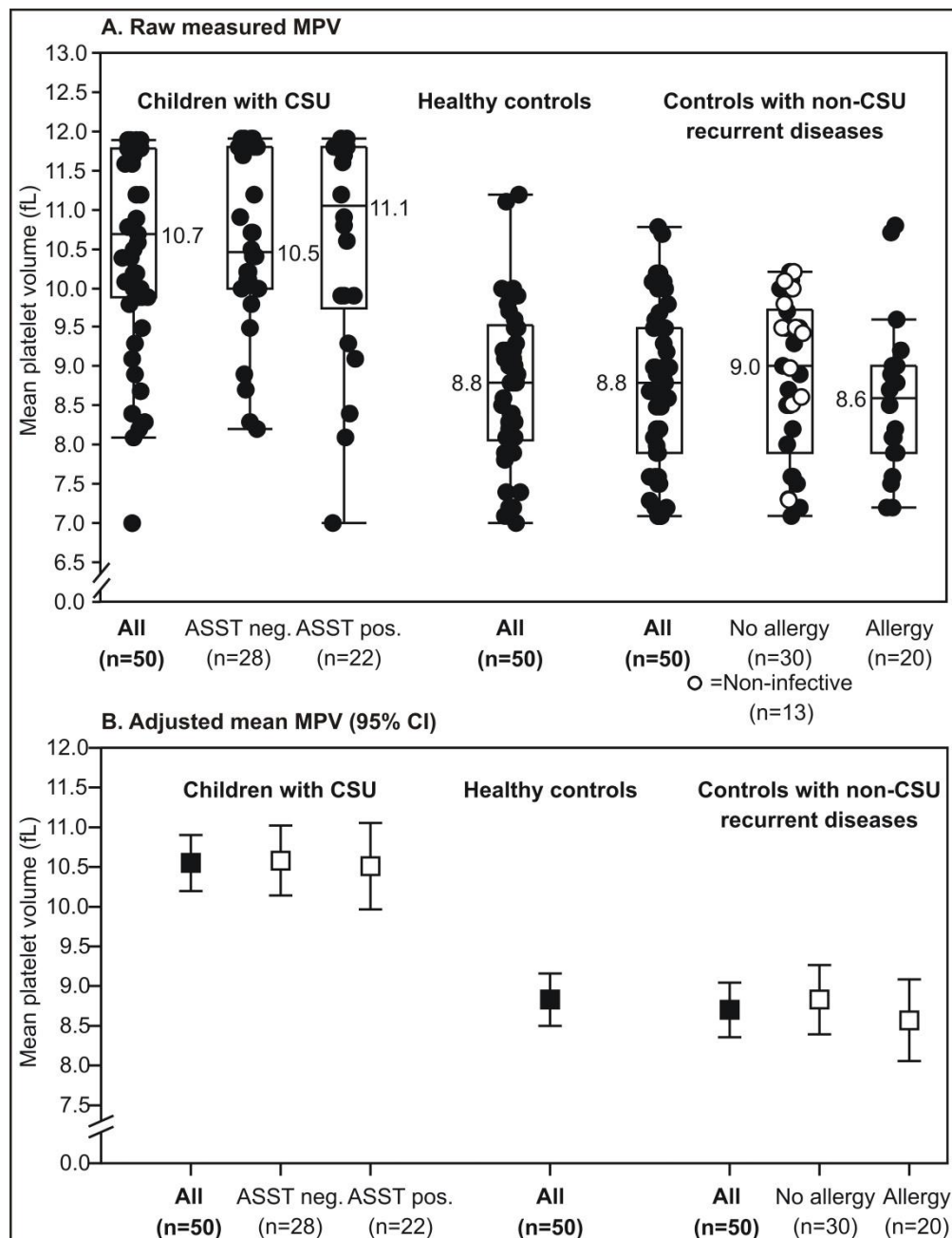


Figure S1. Mean platelet volume (MPV) in children with chronic spontaneous urticaria (CSU) (cases – overall and by autologous serum skin test, ASST), healthy controls and non-CSU control children. **A.** Individual measured values (circles), medians (horizontal lines with a numerical value), quartiles (boxes) and inner fences (bars). Circles outside fences are outliers. **B.** Adjusted mean MPV values with 95% confidence intervals. A general linear model was fitted to MPV with independents: group (cases, healthy controls or non-CSU controls), age, sex, blood cell counts, hemoglobin, hematocrit, C-reactive protein, condition is immunologic or not (ASST positive or not for cases, allergic disease or not for non-CSU controls) group*immunologic condition interaction.

Table S1. Children with chronic spontaneous urticaria: main diagnostic work-up results and administered treatments, overall and by the autologous serum skin test (ASST) results. Data are count (percent) or median (minimum-maximum) if not stated otherwise.

	All	ASST negative	ASST positive
N	50	28	22
<i>Candida spp.</i> in stool	19 (38.0)	12 (42.9)	7 (31.8)
<i>S. pyogenes</i> in nasopharyngeal swab	2 (4.0)	0	2 (9.1)
Antistreptolysin O titer >200 IU/mL	8 (16.0)	3 (10.7)	5 (22.7)
Serology indicates actual infection ^a	11 (22.0)	3 (10.7)	8 (33.4)
Subsequent treatments			
Antihistamines	30 (60.0)	17 (60.7)	13 (59.1)
Montelukast	2 (4.0)	0	2 (9.1)
Systemic corticosteroids	1 (2.0)	0	1 (4.5)
Systemic azoles	13 (26.0)	8 (28.6)	5 (22.7)
Azithromycin	1 (2.0)	1 (3.6)	0
Mebendazole	1 (2.0)	0	1 (4.5)
Dietary measures	2 (4.0)	2 (7.2)	0

^aAntibody profiles indicated Epstein-Barr Virus infection (different stages) in 4 children, acute immune response to *Mycoplasma pneumonia* in 4 children, to *Chlamydia spp.* in 3 children, *Helicobacter pylori* in one child and to *Toxocora canis* in one child (in 3 children, more than one infection was apparently on-going).

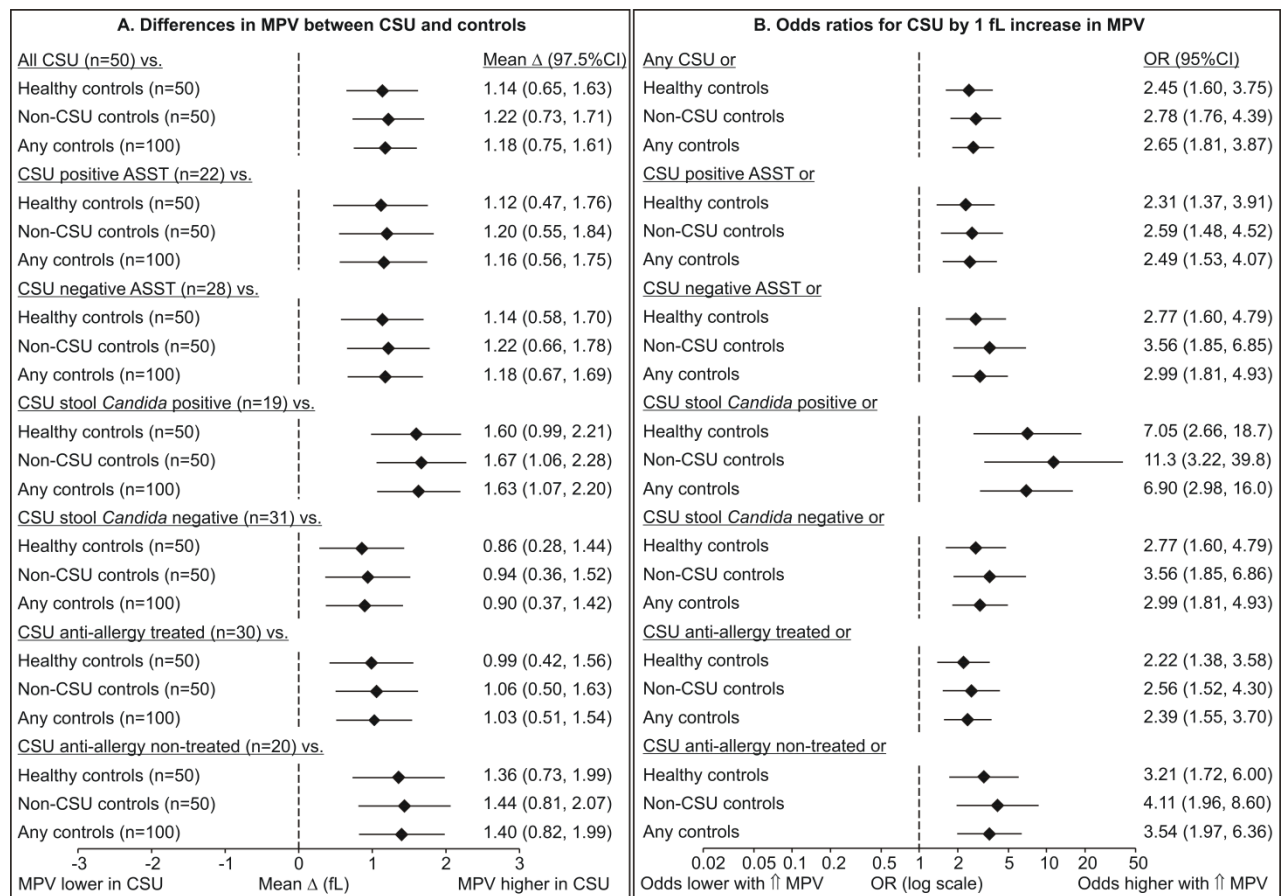


Figure S2. Association between chronic spontaneous urticaria (CSU) and mean platelet volume (MPV) as determined four weeks after the initial presentation of the CSU patients (i.e., at reduced disease activity assessed by the means of the urticaria activity score). **A.** Adjusted (age, sex) mean differences between CSU patients (overall and by subsets) and different control children (healthy or non-CSU disease). For each comparison (different subsets of CSU children based on the autologous serum skin test [ASST], stool colonization with *Candida*, or anti-allergy treatment received or not since the initial presentation.) with controls, a separate general linear model was fitted to MPV. Between-group (“cases” vs. “controls”) differences are given with 97.5% CI to adjust for multiple comparisons (two control groups). **B.** Adjusted (age, sex) odds ratios of CSU associated with 1 fL increase in MPV. A separate logistic model was fitted to the binary outcomes “CSU or control”, were CSU referred to all CSU patients, or specific subsets based on ASST, *Candida* colonization or received anti-allergy treatment.

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