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SUPPORTING INFORMATION

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Plasma adenosine is linked to disease activity and response to treatment in patients with chronic spontaneous urticaria

To the Editor,

Urticaria is a common, mast cell-driven disease presenting with wheals or angioedema or both.¹ Chronic spontaneous urticaria (CSU), the most common phenotype of urticaria, is clinically defined by the spontaneous appearance of wheals and/or angioedema for more than 6 weeks.² Some of the mediators involved in the pathogenic mechanisms of CSU, including CRP, ESR, D-dimer, and several interleukins, have been identified as potential biomarkers useful for the activity and response to therapies, but further studies are required.^{3,4} Adenosine (ADO) is an endogenous nucleoside found throughout human cells that are involved in dilating blood vessels and increasing blood flow. More importantly, ADO has been known to be a major modulator of mast cell function for more than 40 years and is thought to play a role in the pathogenesis of asthma by its actions on mast cells.^{5,6} Adenosine potentiates anti-IgE-induced degranulation of human umbilical cord blood-derived mast cells (hUCBMCs), and the mast cell activation is amplified in the presence of IL-4 by amplifying the pro-inflammatory effect of adenosine.⁵ Nonselective activation adenosine receptors can directly stimulate degranulation of mouse bone marrow-derived mast cells and human LAD2 mast cells.⁶ Because mast cells are the main effector cells in the pathogenesis of CSU,^{1,3} we hypothesized that ADO levels may be different between CSU patients and healthy population. Also, we aimed to investigate the correlation between plasma ADO levels and clinical characteristics of CSU patients.

Adenosine levels in plasma from both CSU patients and healthy subjects were measured by high-performance liquid chromatography (HPLC),⁷ and the potential of ADO as a biomarker for predicting disease activity and treatment outcomes was evaluated in patients with CSU. (More detail can be found in Appendix S1). CSU patients were enrolled from the ongoing cohort study, Cohort for Adult CSU in China. Diagnosis of CSU was made by specialists according to the EAACI/GA²LEN/EDF/WAO Guideline of Urticaria 2018.² All enrolled CSU patients were uniformly treated with nonsedating H1-antihistamine monotherapy at licensed doses. CSU patients were

categorized into two groups based on their modified urticaria activity score in 7 days (UAS7) scores, according to Hawe et al⁸ (UAS7 0-15, mild and moderate; 16-42, severe) both at baseline and after antihistamine monotherapy for 4 weeks. The dermatology life quality activity (DLQI) of urticaria was used to assess the quality of life for the patients. According to our previous research,⁹ the responders were designated as those with UAS7 scores achieving 50% or more significant improvement as compared with the baseline before treatment. Nonresponders were defined as those patients who had a decrease in UAS7 scores below 50%. This study was approved by the ethics committee boards of Xiangya Hospital, Central South University, China. All participants signed informed consent (ethical approval number: 201904112).

The ADO levels between healthy controls and CSU patients were analyzed by *t* test. Receiver operating characteristics (ROC) curve analysis was used to determine the optimal ADO cutoff to distinguish the responder group from the nonresponder group. Linear regression models were used to investigate the association between clinical data and the ADO level. Data were analyzed using the GraphPad Prism 8.0 and SPSS 18.0 statistical software.

This study firstly included 24 healthy controls and 48 CSU patients. (Their characteristics are listed in Table S1 in Appendix S1). Among the 48 patients, there were 24 with mild and moderate CSU and 24 with severe CSU. The mean plasma ADO level in the patients was significantly higher than that in the healthy control (386.6 ± 200.1 vs 112.3 ± 37.9 , $P \leq .001$) (Figure 1A). Secondly, 220 patients (their demographic and clinical characteristics are listed in Table S2) were enrolled to investigate the association between the ADO level and clinical characteristics of CSU patients. Based on this cohort, we found that plasma ADO levels in patients with severe disease ($n = 177$) were significantly higher than those in patients with mild and moderate disease ($n = 43$) (483.3 ± 250.6 vs 185.8 ± 113.3 , $P \leq .001$) (Figure 1B). Furthermore, ADO levels were lower in the responder group ($n = 132$) than those in the nonresponder group ($n = 45$) in 177 severe CSU patients (450.8 ± 248.0 vs 578.8 ± 250.6 , $P < .01$) (Figure 1C). We then performed correlation analysis and found that the ADO level in plasma of CSU patients was positively

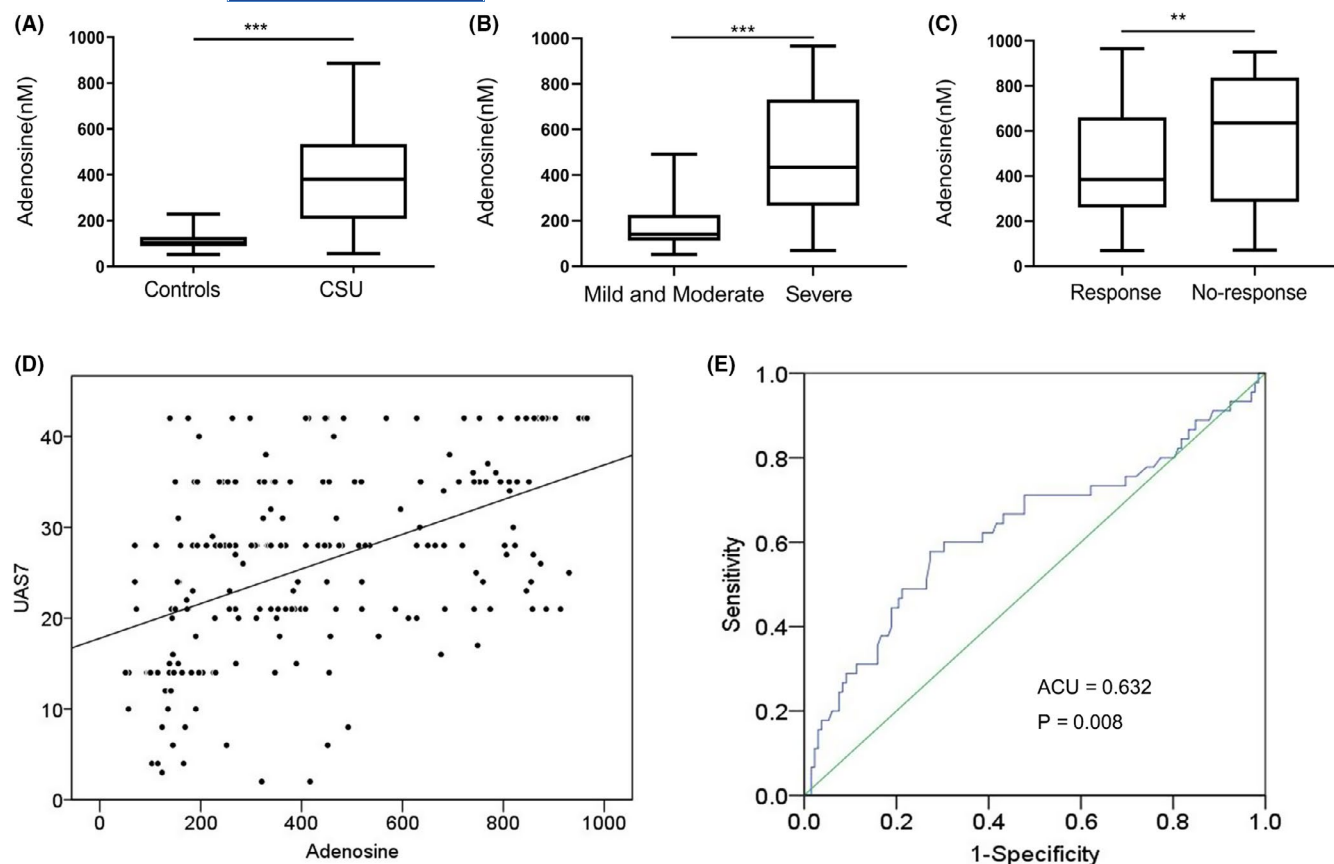


FIGURE 1 The ADO levels between CSU patients and healthy controls (A). The mean plasma ADO level (mean $\pm$ SD) of the CSU patients ($n = 48$) was significantly higher than that in the healthy control ($n = 24$) (386.6 ± 200.1 vs 112.3 ± 37.9 ; $***P \leq .001$, t test). Differences in ADO levels between mild and moderate group and severe group of CSU patients ($n = 220$) (B). The mean plasma ADO level of the severe group ($n = 177$) was significantly higher than that of the mild and moderate group ($n = 43$) (483.3 ± 250.6 vs 185.7 ± 113.3 ; $***P \leq .001$, t test). Differences in ADO levels between responder group ($n = 132$) and nonresponder group ($n = 45$) in 177 severe CSU patients (C). The ADO level was significantly lower in the responder group than that in the nonresponder group (450.8 ± 248.0 vs 578.8 ± 250.6 ; $**P < .01$, t test). Correlation analysis between the ADO level and UAS7 scores (D). The ADO level in plasma of CSU patients was positively correlated with the UAS7 scores ($R = 0.50$, $P < .01$; Spearman's test). ROC curve analysis of ADO levels from severe CSU patients as a cutoff to define responders vs nonresponders (E). The cutoff value of ADO was 603 nmol/L for predicting the patients with no response to nonsedating H1-antihistamine monotherapy after 4 wk of treatment

correlated with UAS7 ($R = 0.50$, $P < .01$) (Figure 1D). However, the ADO level has no correlation with DLQI scores, age, disease duration, level of CRP, ESR, and 25-OH-VD, smoking history, and drinking history (Table S2). ROC curve analysis showed that the ADO level can well predict nonresponders among severe CSU patients ($AUC = 0.632$, $P = .008$, Figure 1E). The 177 severe CSU patients were classified into two subgroups with high and low ADO levels using the optimal cutoff value of 603 nmol/L. The high ADO group

has both higher UAS7 and DLQI scores compared with the low ADO group (Table 1), which suggested that patients with high ADO levels may be more likely to have more severe CSU disease.

This study demonstrated that ADO levels were elevated in patients with CSU. In particular, they were higher in patients with severe CSU than those in patients with mild and moderate CSU. Additionally, high ADO levels were significantly correlated with the UAS7 scores and the therapeutic efficacy of nonsedating

	Plasma ADO ≤ 603 nmol/L ($n = 115$)	Plasma ADO > 603 nmol/L ($n = 62$)	Total ($n = 177$)	P
Age, years	39.41 ± 14.50	40.08 ± 14.02	39.65 ± 14.34	.76
Female sex, n (%)	73 (63.48%)	45 (72.58%)	118 (73.25%)	.22
Duration, months	38.67 ± 57.79	21.49 ± 32.33	29.04 ± 50.50	.09
UAS7	31.89 ± 6.89	28.11 ± 8.23	29.47 ± 7.57	.001
DLQI	5.50 ± 3.06	4.41 ± 3.92	4.83 ± 3.41	.04

TABLE 1 Difference in clinical characteristics between high and low ADO subgroups in 177 severe CSU patients

H1-antihistamines (especially those with ADO value ≥ 630 nmol/L). These results indicated that ADO may be a new candidate biomarker for predicting disease activity and the treatment outcome of patients with CSU.

This study has several limitations. Firstly, a bias existed in sample collection at enrollment because we only collected data from patients in one central, not in multicentral. Secondly, the sample size for ROC analysis was small. Thirdly, the follow-up period was relatively short (1 month).

In conclusion, we investigated plasma ADO level in CSU patients, and its potential use as a biomarker for predicting disease activity and response to the conventional dose of H1-antihistamine treatment. However, the role of ADO as a potential biomarker needs further study with a larger population and longer follow-up.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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