

Original Research

Received 06 July 2026

Revised 07 Aug 2026

Accepted 20 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (17s): 1491–1503

KEYWORDS:

Bronchial asthma; children; undifferentiated connective tissue dysplasia; zinc deficiency; magnesium deficiency; component-resolved diagnostics.

Undifferentiated Connective Tissue Dysplasia and Microelement Imbalance as Determinants of Allergic Phenotype in Children with Bronchial Asthma: A Prospective Cross-Sectional Study

Turdikul A. Bobomurodov¹, Diloram T. Abdullaeva¹, Maktuba Kh. Mirrakhimova¹, Dilnoza T. Bobomurodova¹, Adiba M. Saidkhanova¹, Mohigul K. Ismatova¹, Sarvarbek S. Sobirov¹, Nigora T. Akramova^{2*}

¹Department of Paediatric Allergology, International Allergy Center, 1st Clinic, Tashkent State Medical University, Tashkent, Uzbekistan

²Department of Faculty and Hospital Therapy, Rheumatology and Occupational Diseases No. 1, Tashkent State Medical University, Tashkent 100109, Uzbekistan

*Correspondence: Nigora T. Akramova, MD, PhD

E-mail: akramovanigoraturdikulovna@gmail.com

ORCID iDs: T.A. Bobomurodov

0000-0002-9021-4576

D.T. Abdullaeva

0000-0002-2749-9222

M.Kh. Mirrakhimova

0000-0003-2802-4357

D.T. Bobomurodova

0000-0003-3862-1753

A.M. Saidkhanova

0000-0002-1970-1451

M.K. Ismatova

0009-0001-0992-4790

S.S. Sobirov

0009-0005-2487-3935

N.T. Akramova

0009-0006-9572-3835

ABSTRACT: Bronchial asthma (BA) remains poorly controlled in 30–40% of children despite guideline-based therapy, and undifferentiated connective tissue dysplasia (NDCT) and zinc/magnesium (Zn/Mg) deficiency may jointly amplify airway inflammation and allergen sensitisation. This study characterised molecular sensitisation, airway function and inflammatory phenotype in children with BA and comorbid NDCT, and assessed the relation of Zn and Mg depletion to disease severity. In a prospective cross-sectional study reported per STROBE, 98 children aged 7–18 years were enrolled at the International Allergy Centre, Tashkent State Medical University: BA+NDCT (n=32), BA–NDCT (n=34) and healthy controls (n=32). NDCT was graded with the modified Kadurina scale; component-resolved diagnostics used the ALEX2 multiplex array (>300 allergen

molecules); spirometry (ATS/ERS 2019), FeNO (ATS 2011) and serum Zn/Mg were measured at enrolment. Children with BA+NDCT showed greater airway obstruction (FEV_1 $61.2 \pm 11.8\%$ vs $74.8 \pm 13.2\%$ predicted), higher FeNO (52.3 ± 18.7 vs 34.6 ± 15.2 ppb) and lower serum Zn (7.96 ± 1.35 vs 10.08 ± 1.57 $\mu\text{mol/L}$) and Mg (0.52 ± 0.10 vs 0.74 ± 0.10 mmol/L; all $p < 0.001$). Polysensitisation was found in 84.4% vs 44.1% (OR 7.0; 95% CI 2.4–20.6; $p < 0.001$). In multivariable regression, NDCT (adjusted OR 4.82; 95% CI 1.91–12.15) and Zn depletion (aOR 1.58 per $\mu\text{mol/L}$ decrease; 95% CI 1.21–2.07) were independently associated with moderate-to-severe BA, and the combined NDCT+Zn+Mg model yielded AUC 0.88 (95% CI 0.81–0.95). NDCT combined with Zn/Mg depletion was associated with a distinct paediatric BA phenotype marked by polysensitisation, eosinophilic airway inflammation and greater airflow obstruction. These cross-sectional, hypothesis-generating findings warrant prospective testing of routine NDCT phenotyping and micronutrient profiling.

1. INTRODUCTION

Bronchial asthma remains the commonest chronic inflammatory airway disease of childhood, with the ISAAC Phase III survey putting its global prevalence at roughly 14% [1]. Inhaled corticosteroids (ICS) and, more recently, biologics directed at IgE and type-2 cytokines have improved outcomes, yet a sizeable minority of children, somewhere between 30% and 40%, still fail to achieve good control [2, 3]. That this gap has proved so durable suggests that mechanisms beyond the classical Th2 paradigm are at work in at least some patients.

One candidate is undifferentiated connective tissue dysplasia (NDCT), a genetically and metabolically determined condition in which collagen architecture, the extracellular matrix and basement-membrane integrity are all subtly disturbed [4]. Some degree of NDCT is reported in a quarter to a third of children. Where it affects the airway, the resulting epithelial weakness is thought to let allergens cross the mucosa more readily and to predispose to polysensitisation, a pattern that recurs in difficult, severe asthma [5, 6]. This fits the broader epithelial barrier hypothesis, in which loss of epithelial integrity drives allergic sensitisation and persistent type-2 inflammation [7]; NDCT can be viewed as a constitutional, structurally rooted version of that barrier failure. Connective tissue dysplasia has likewise been tied to bronchial obstruction and airway remodelling in paediatric series [5, 8].

Molecular allergology has, in parallel, changed how sensitisation is assessed. The ALEX2 multiplex platform measures specific IgE against more than 300 individual allergen molecules in a single run, separating genuine primary sensitisation from cross-reactivity and grouping patients by clinically meaningful clusters such as PR-10 proteins, profilins, lipid transfer proteins (LTPs) and storage proteins [9–11]. When the epithelial barrier is compromised, as in NDCT, these same molecules take on added importance, since they mediate much of the cross-reactivity between pollen and plant-food allergens [12].

Zinc and magnesium sit at the intersection of all of these processes. Both are required for immune regulation, collagen synthesis and antioxidant defence. Zinc shapes the Th1/Th2 balance, supports regulatory T-cell function and underpins vitamin D and glucocorticoid receptor signalling [13, 14]; magnesium stabilises mast-cell membranes, dampens bronchial hyperreactivity and is a cofactor for glutathione synthetase [15, 16]. Deficiency of either is common in chronically inflamed children and has been linked to weaker ICS responses and stronger allergen reactivity [13, 15]. It is therefore plausible that, when NDCT and micronutrient depletion occur together, structural vulnerability and immune dysregulation reinforce one another and push disease towards greater severity.

What has been missing is a study that examines these strands together. No adequately powered paediatric cohort has, to our knowledge, looked simultaneously at NDCT, molecular sensitisation, lung function, airway eosinophilia (FeNO) and trace-element status in the same children. Defining such a phenotype would have practical consequences for diagnosis, risk stratification and the case for micronutrient supplementation as adjunctive treatment.

Objective. To describe the molecular sensitisation, lung function (spirometry and FeNO) and inflammatory phenotype of children whose bronchial asthma co-occurs with NDCT, and to assess how far zinc and magnesium depletion relates to disease severity.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

A prospective cross-sectional study was conducted at the International Allergy Centre of the 1st Clinic, Tashkent State Medical University, Department of Paediatric Allergology (Tashkent, Republic of Uzbekistan) between January 2023 and December 2024. The study was approved by the Institutional Ethics Committee (Protocol No. 7, 15 January 2023) and conducted in accordance with the Declaration of Helsinki (2013). Written informed consent was obtained from all parents/guardians; assent was obtained from patients aged ≥ 12 years. The study is reported in compliance with the STROBE statement for cross-sectional studies [17]; the participant flow is shown in **Figure 1**.

2.2 Sample Size Calculation

Based on pilot data ($n=22$), the mean difference in serum Zn between BA+NDCT and BA-NDCT groups was $2.1 \mu\text{mol/L}$ ($\text{SD}=1.4$), yielding a Cohen's d of 1.50. Using a two-sided $\alpha=0.05$ and power $(1-\beta)=0.80$, a minimum of 22 patients per group was required (G*Power 3.1). To ensure 80% power for secondary endpoints with smaller effect sizes ($d \approx 0.80$ for FeNO and FEV_1), a minimum of 26 participants per group was necessary. We recruited 32–34 per group (total $N=98$) to accommodate $\sim 15\%$ attrition and provide power for subgroup, regression, and ROC analyses.

2.3 Participants

Of 117 children screened, 98 met eligibility criteria and were stratified into: BA+NDCT ($n=32$), BA-NDCT ($n=34$), and healthy age-/sex-matched controls ($n=32$). Inclusion criteria: age 7–18 years; confirmed BA per GINA 2023 [18]; ability to perform spirometry; absence of acute infection within 4 weeks; complete clinical, laboratory, and spirometric data. Exclusion criteria: severe systemic comorbidities (congenital heart disease, immunodeficiency); differentiated connective tissue disorders (Marfan, Ehlers–Danlos); systemic corticosteroid therapy at enrolment; or incomplete data.

2.4 NDCT Assessment

NDCT severity was graded using the modified Kadurina phenotypic scoring scale [4], systematising external and visceral markers across musculoskeletal, dermal, cardiovascular, ophthalmological, and dental domains. Total score: 0–6 = absent/minimal; 7–12 = mild; 13–18 = moderate; ≥ 19 = severe. Two independent paediatricians scored each participant; inter-rater reliability was excellent (Cohen's $\kappa=0.83$).

2.5 Spirometry, Lung Volumes and FeNO

Spirometry (FEV_1 , FVC, FEV_1/FVC , PEF, FEF_{25-75}) was performed on a calibrated spirometer with an integrated body plethysmograph (MasterScreen Body, Vyaire Medical, Germany) following the ATS/ERS 2019 technical standards for the standardisation of spirometry [19]. At least three acceptable and two reproducible manoeuvres (FEV_1 and FVC within 150 mL) were obtained; predicted values and lower limits of normal were derived from the GLI-2012 all-age reference equations [20], and results were interpreted according to the ERS/ATS 2022 interpretive strategy standard [21].

Static lung volumes were measured by whole-body plethysmography according to the ATS/ERS standardisation of lung-volume measurement [22]. Functional residual capacity ($\text{FRC}_{\text{pleth}}$) was determined from panting manoeuvres against a closed shutter at a frequency of 0.5–1.0 Hz; linked expiratory-reserve-volume and slow inspiratory-vital-capacity manoeuvres were then used to derive residual volume (RV) and total lung capacity (TLC). The mean of at least three technically acceptable $\text{FRC}_{\text{pleth}}$ measurements agreeing within 5% was used, with RV and TLC expressed as a percentage of GLI-predicted values. Air trapping was defined a priori as an RV/TLC ratio exceeding 120% of the predicted value, consistent with lung hyperinflation. Bronchodilator reversibility was tested 15 min after 400 μg inhaled salbutamol (metered-dose inhaler with spacer); a positive response was defined as an FEV_1 increase $\geq 12\%$ and $\geq 200 \text{ mL}$.

FeNO was measured before spirometry (NObreath, Bedfont Scientific, UK) at a constant expiratory flow of 50 mL/s, per the ATS 2011 clinical practice guideline [23]; the mean of three readings agreeing within 10% was recorded.

2.6 Allergy Evaluation

Evaluation included: standardised allergy history and examination; skin prick tests; total serum IgE by immunonephelometry; specific IgE by ImmunoCAP (Thermo Fisher Scientific, Sweden); and CRD by ALEX2 multiplex array (Macro Array Diagnostik, Austria), detecting specific IgE to >300 allergen molecules. The ALEX2 platform has been validated against ImmunoCAP ISAC in independent studies and includes a CCD inhibitor to suppress clinically irrelevant cross-reactive carbohydrate determinants [11, 24]. CRD results differentiated true sensitisation from cross-reactivity and classified profiles by molecular cluster, per the EAACI Molecular Allergology User's Guide 2.0 [9].

2.7 Microelement Determination

Fasting venous blood was collected in metal-free tubes. Serum Zn was measured by atomic absorption spectrophotometry (Perkin Elmer AAnalyst 800; CV<5%). Serum Mg was quantified by the xylydyl blue colorimetric method (Beckman Coulter AU680; CV<3%). Reference intervals (paediatric): Zn 11.5–18.5 $\mu\text{mol/L}$; Mg 0.65–1.05 mmol/L. All measurements were performed in duplicate.

2.8 Asthma Phenotyping

Inflammatory BA phenotypes were classified using induced sputum (where feasible), peripheral blood eosinophilia, FeNO, total IgE, and atopic status, following current paediatric severe-asthma endotyping frameworks [25–27]: (1) atopic eosinophilic; (2) non-atopic eosinophilic; (3) neutrophilic; (4) paucigranulocytic; and (5) obesity-associated.

2.9 Statistical Analysis

SPSS Statistics v.26.0 and GraphPad Prism v.9 were used. Continuous variables are presented as $M \pm SD$ or median (IQR) depending on normality (Shapiro–Wilk). Between-group comparisons: one-way ANOVA with Tukey post-hoc (parametric) or Kruskal–Wallis with Mann–Whitney U (non-parametric). Correlation: Spearman's rank coefficient (r_s). Categorical variables: χ^2 or Fisher exact test, with odds ratios and 95% CIs. A multivariable binary logistic regression model identified independent predictors of moderate-to-severe BA, adjusting for age, sex, NDCT status, serum Zn, serum Mg, polysensitisation, and FeNO; model fit was assessed by the Hosmer–Lemeshow test and Nagelkerke R^2 . ROC analysis determined diagnostic accuracy of predictors. Significance: $p < 0.05$ (two-tailed); Bonferroni correction for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1 Participant Flow and Baseline Characteristics

Of 117 screened patients, 98 were enrolled (exclusions: 8 incomplete data, 6 differentiated CTD, 5 acute infection; **Figure 1**). Mean age was 11.6 ± 3.3 years; 59 (60.2%) were male. Groups were well-matched for age, sex, and BMI (all $p > 0.05$; Table 1). Moderate or severe BA was significantly more prevalent in BA+NDCT (81.3% vs 44.1%; $p = 0.001$). Baseline IgE, blood eosinophils, FeNO, and NDCT scores differed significantly across groups (all $p < 0.001$).

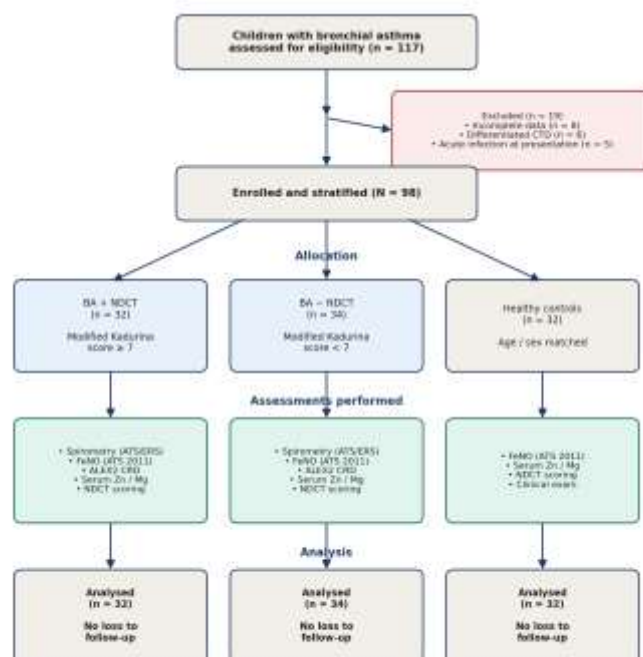


Figure 1. STROBE participant flow diagram

STROBE — Strengthening the Reporting of Observational Studies in Epidemiology; BA — bronchial asthma; NDCT — undifferentiated connective tissue dysplasia; CTD — connective tissue disorder; CRD — component-resolved diagnostics.

Table 1. Demographic and clinical characteristics of study groups

Parameter	BA+NDCT (n=32)	BA-NDCT (n=34)	Control (n=32)	p-value
Age, years (M±SD)	11.3±3.1	11.9±3.6	11.5±2.9	0.87
Male sex, n (%)	21 (65.6)	20 (58.8)	18 (56.3)	0.71
BMI, kg/m ² (M±SD)	19.1±3.2	18.7±2.9	19.3±2.8	0.69
Moderate/severe BA, n (%)	26 (81.3)	15 (44.1)	—	0.001*
Total IgE, kU/L (median, IQR)	512 (328–764)	316 (194–462)	38 (20–72)	<0.001*
Blood eosinophils ×10 ⁹ /L (M±SD)	0.72±0.23	0.46±0.19	0.17±0.06	<0.001*
FeNO, ppb (M±SD)	52.3±18.7	34.6±15.2	11.8±4.9	<0.001*
NDCT score (M±SD)	16.4±4.2	3.2±1.8	1.1±0.9	<0.001*
ICS use, n (%)	32 (100)	28 (82.4)	—	0.011*
Comorbid AR, n (%)	28 (87.5)	21 (61.8)	0	<0.001*

Parameter	BA+NDCT (n=32)	BA-NDCT (n=34)	Control (n=32)	p-value
Comorbid AD, n (%)	18 (56.3)	10 (29.4)	0	0.018*

AR — allergic rhinitis; AD — atopic dermatitis; ICS — inhaled corticosteroids; FeNO — fractional exhaled nitric oxide.

* $p < 0.05$ vs comparator (Bonferroni-corrected).

3.2 Pulmonary Function and FeNO

Spirometric indices were significantly impaired in BA+NDCT relative to BA-NDCT across all parameters (Table 2). Mean FEV₁ was $61.2 \pm 11.8\%$ predicted in BA+NDCT vs $74.8 \pm 13.2\%$ in BA-NDCT ($\Delta = 13.6\%$; $p < 0.001$). Post-bronchodilator FEV₁ reversibility was greater in BA+NDCT ($22.4 \pm 8.1\%$ vs $15.6 \pm 6.8\%$; $p < 0.001$), and air trapping (RV/TLC $> 120\%$) was present in 56.3% vs 26.5% ($p = 0.01$), suggesting small airway involvement. FeNO was markedly elevated in BA+NDCT (52.3 ± 18.7 ppb) vs BA-NDCT (34.6 ± 15.2 ppb; $p < 0.001$) and controls (11.8 ± 4.9 ppb; $p < 0.001$), indicating greater eosinophilic airway inflammation.

Table 2. Spirometric parameters and FeNO by study group

Spirometric Parameter	BA+NDCT (n=32)	BA-NDCT (n=34)	p-value	MCID
FEV ₁ , % predicted (M $\pm$ SD)	61.2 ± 11.8	74.8 ± 13.2	$< 0.001^*$	$\geq 12\%$
FVC, % predicted (M $\pm$ SD)	74.5 ± 9.3	83.2 ± 8.7	$< 0.001^*$	$\geq 10\%$
FEV ₁ /FVC ratio (M $\pm$ SD)	0.64 ± 0.07	0.72 ± 0.06	$< 0.001^*$	—
PEF, % predicted (M $\pm$ SD)	58.7 ± 14.1	72.3 ± 12.6	$< 0.001^*$	—
Post-BD FEV ₁ reversibility, % (M $\pm$ SD)	22.4 ± 8.1	15.6 ± 6.8	$< 0.001^*$	$\geq 12\%$
Positive BD response, n (%)	29 (90.6)	22 (64.7)	0.01*	—
FEF _{25–75} , % predicted (M $\pm$ SD)	48.3 ± 17.6	62.9 ± 16.4	$< 0.001^*$	—
Air trapping (RV/TLC $> 120\%$), n (%)	18 (56.3)	9 (26.5)	0.01*	—

FEV₁ — forced expiratory volume in 1 s; FVC — forced vital capacity; PEF — peak expiratory flow; FEF_{25–75} — forced expiratory flow at 25–75% FVC; BD — bronchodilator; RV — residual volume; TLC — total lung capacity; MCID — minimal clinically important difference. * $p < 0.001$ (ANOVA, Tukey post-hoc, Bonferroni-corrected).

3.3 Microelement Status

Significant differences in serum microelements were observed across all groups (Table 3). BA+NDCT showed the most severe depletion: mean Zn 7.96 ± 1.35 $\mu\text{mol/L}$ (38% below the lower reference limit) and Mg 0.52 ± 0.10 mmol/L (20% below). Both were significantly lower than in BA-NDCT (Zn 10.08 ± 1.57 $\mu\text{mol/L}$, $p < 0.001$; Mg 0.74 ± 0.10 mmol/L, $p < 0.001$), which in turn differed from controls. NDCT severity correlated inversely with serum Zn ($r_s = -0.61$; $p < 0.001$) and Mg ($r_s = -0.55$; $p < 0.001$), serum Zn with total IgE ($r_s = -0.52$; $p < 0.001$), and serum Mg with FeNO ($r_s = -0.47$; $p < 0.001$).

Table 3. Serum zinc and magnesium concentrations by study group

Group	n	Zn, $\mu\text{mol/L}$ [ref 11.5–18.5]	p vs ctrl	Mg, mmol/L [ref 0.65–1.05]	p vs ctrl
BA+NDCT	32	7.96 ± 1.35	$<0.001^{*†}$	0.52 ± 0.10	$<0.001^{*}$ †
BA–NDCT	34	10.08 ± 1.57	$<0.001^{*}$	0.74 ± 0.10	$<0.001^{*}$
Control	32	12.84 ± 1.21	—	0.86 ± 0.09	—

* $p < 0.001$ vs Control; † $p < 0.001$ vs BA–NDCT. Reference intervals: Zn 11.5–18.5 $\mu\text{mol/L}$; Mg 0.65–1.05 mmol/L (paediatric).

3.4 Molecular Sensitisation Profiles

CRD revealed substantially different molecular profiles between groups (Table 4). Polysensitisation (≥ 3 components) was detected in 84.4% of BA+NDCT vs 44.1% of BA–NDCT (OR 7.0; 95% CI 2.4–20.6; $p < 0.001$). Cross-reactive patterns dominated in BA+NDCT: profilins (Bet v 2 homologues) in 68.8%, LTPs (Pru p 3 homologues) in 53.1%, PR-10 proteins (Bet v 1 homologues) in 81.3%. The mean number of sensitised components was 6.8 ± 2.4 vs 3.2 ± 1.9 ($p < 0.001$).

Table 4. Molecular sensitisation profiles by component-resolved diagnostics (CRD)

Molecular Component	BA+NDCT (n=32)	BA–NDCT (n=34)	OR (95% CI)	p-value
Polysensitisation (≥ 3 components), n (%)	27 (84.4)	15 (44.1)	7.0 (2.4–20.6)	$<0.001^{*}$
PR-10 proteins (Bet v 1 group)	26 (81.3)	17 (50.0)	4.4 (1.5–13.1)	0.006*
Profilins (Bet v 2 group)	22 (68.8)	9 (26.5)	6.0 (2.1–17.2)	0.001*
Lipid transfer proteins (Pru p 3 group)	17 (53.1)	7 (20.6)	4.4 (1.5–13.1)	0.006*
Storage proteins (Ara h 2 group)	10 (31.3)	9 (26.5)	1.3 (0.4–3.8)	0.65
Major HDM allergens (Der p 1/2)	32 (100)	28 (82.4)	—	0.011*
Mean no. of sensitised components	6.8 ± 2.4	3.2 ± 1.9	—	$<0.001^{*}$

OR — odds ratio; CI — confidence interval; HDM — house dust mite. * $p < 0.05$ (Fisher exact test, Bonferroni-corrected). Polysensitisation defined as specific IgE ≥ 0.35 kU/L to ≥ 3 distinct allergen molecule groups.

3.5 NDCT Severity and Asthma Severity

Among the 66 children with BA, a statistically significant gradient between NDCT severity grade and BA severity was confirmed (Table 5). The proportion of patients with any degree of NDCT rose from 11.1% in intermittent BA to 83.3% in severe persistent BA. Of 12 patients with severe persistent BA, 10 (83.3%) had at least mild NDCT and 4 (33.3%) had severe NDCT. Spearman correlation between NDCT score and BA severity grade was $r_s = 0.68$ ($p < 0.001$). Note that allergic rhinitis and atopic dermatitis were comorbidities (reported in Table 1) rather than separate enrolment strata; Table 5 reports unique BA patients only, avoiding double-counting across diagnoses.

Table 5. Distribution of NDCT severity grades by bronchial asthma severity (BA patients only, n=66)

BA severity	n	No NDCT	Mild NDCT	Moderate NDCT	Severe NDCT	% with NDCT
Intermittent	9	8	1	0	0	11.1%

BA severity	n	No NDCT	Mild NDCT	Moderate NDCT	Severe NDCT	% with NDCT
Mild persistent	21	16	4	1	0	23.8%
Moderate persistent	24	8	3	11	2	66.7%
Severe persistent	12	2	0	6	4	83.3%
Total (BA patients)	66	34	8	18	6	48.5%

Row totals equal the number of unique BA patients in each severity category; column totals equal the number of patients in each NDCT grade. By definition, all 34 BA–NDCT patients fall in the “No NDCT” column (Kadurina score <7). Fisher exact test for NDCT grade vs BA severity: $p < 0.001$.

3.6 Factors Independently Associated with Moderate-to-Severe Asthma

In the multivariable logistic regression model (Table 6), NDCT presence (adjusted OR 4.82; 95% CI 1.91–12.15; $p = 0.001$), serum Zn depletion (aOR 1.58 per 1 $\mu\text{mol/L}$ decrease; 95% CI 1.21–2.07; $p < 0.001$), serum Mg depletion (aOR 1.41 per 0.1 mmol/L decrease; $p = 0.009$), polysensitisation (aOR 3.24; $p = 0.010$), and elevated FeNO (aOR 1.49 per 10 ppb; $p = 0.011$) were independently associated with moderate-to-severe BA after adjustment for age and sex. The model demonstrated good fit (Hosmer–Lemeshow $p = 0.41$; Nagelkerke $R^2 = 0.62$). These associations are cross-sectional and do not imply causation.

Table 6. Multivariable logistic regression: independent predictors of moderate-to-severe bronchial asthma

Predictor variable	Adjusted OR	95% CI	p-value
NDCT present (vs absent)	4.82	1.91 – 12.15	0.001*
Serum Zn (per 1 $\mu\text{mol/L}$ decrease)	1.58	1.21 – 2.07	<0.001*
Serum Mg (per 0.1 mmol/L decrease)	1.41	1.09 – 1.83	0.009*
Polysensitisation (≥ 3 components)	3.24	1.32 – 7.95	0.010*
FeNO (per 10 ppb increase)	1.49	1.10 – 2.02	0.011*
Age (per year)	1.05	0.92 – 1.20	0.47
Male sex	1.31	0.59 – 2.91	0.51

Adjusted OR — odds ratio adjusted for all variables listed in the model. Dependent variable: moderate-to-severe BA vs intermittent/mild. Model fit: Hosmer–Lemeshow $p = 0.41$; Nagelkerke $R^2 = 0.62$; $n = 66$. * $p < 0.05$.

3.7 ROC Analysis

ROC analysis used a single, consistent outcome — moderate-to-severe BA (41 of 66 BA patients) — for the structural and micronutrient predictors, while FeNO was evaluated against the atopic eosinophilic phenotype (Table 7). The combined NDCT+Zn+Mg model showed the highest discrimination for moderate-to-severe BA (AUC 0.88; 95% CI 0.81–0.95; sensitivity 87.8%, specificity 84.0%). Individual predictors showed moderate discrimination: serum Zn $< 9.5 \mu\text{mol/L}$ (AUC 0.79), NDCT score ≥ 10 points (AUC 0.78), and serum Mg $< 0.62 \text{ mmol/L}$ (AUC 0.74). FeNO $> 38 \text{ ppb}$ discriminated the atopic eosinophilic phenotype (AUC 0.82). These cut-offs were derived from a single-centre sample at the Youden-optimal point and require external validation before clinical use.

Table 7. ROC analysis: diagnostic accuracy of predictors for severe bronchial asthma

Predictor → outcome	AUC (95% CI)	Sens. (%)	Spec. (%)	Cut-off
NDCT score → mod./severe BA	0.78 (0.69–0.87)	80.5	76.0	≥10 pts
Serum Zn → mod./severe BA	0.79 (0.70–0.88)	80.5	72.0	<9.5 µmol/L
Serum Mg → mod./severe BA	0.74 (0.64–0.84)	73.2	68.0	<0.62 mmol/L
FeNO → atopic eosinophilic phenotype	0.82 (0.73–0.91)	87.5	76.5	>38 ppb
Combined (NDCT+Zn+Mg) → mod./severe BA	0.88 (0.81–0.95)	87.8	84.0	—

AUC — area under the ROC curve; Sens. — sensitivity; Spec. — specificity. Outcome for structural/micronutrient predictors: moderate-to-severe BA (n=41/66); FeNO evaluated against the atopic eosinophilic phenotype. Combined model = logistic regression of NDCT score + serum Zn + serum Mg. Cut-offs at the Youden-optimal point; all AUC $p < 0.001$; 95% CI by DeLong method.

3.8 Inflammatory Phenotypes

The atopic eosinophilic phenotype predominated in BA+NDCT (68.8%), consistent with markedly elevated FeNO (>38 ppb) and high blood eosinophilia ($0.72 \pm 0.23 \times 10^9/L$). Neutrophilic and paucigranulocytic phenotypes predominated in BA–NDCT (55.9%), with lower FeNO and IgE. A non-atopic eosinophilic phenotype was found in 12.5% of BA+NDCT cases, representing a potentially distinct endotype with elevated eosinophils but absent environmental sensitisation.

3.9 Discussion

The principal finding of this study is that children whose asthma coincided with connective tissue dysplasia and low zinc and magnesium formed a recognisable clinical group, set apart by broader molecular polysensitisation, higher FeNO and poorer spirometry. To our knowledge it is the first adequately powered look at this combination in a Central Asian paediatric population. The design is cross-sectional, so we cannot speak to cause or sequence; the mechanisms we sketch below are offered as interpretation and as a starting point for longitudinal and interventional work.

The functional findings invite a structural reading. FEV₁ was some 14 percentage points lower in the BA+NDCT group, and bronchodilator reversibility was greater, which is at least compatible with collagen weakness in the airway wall adding to the obstruction produced by eosinophilic inflammation. We cannot show this directly, but the idea is biologically reasonable: collagen XVII and the laminins that are disturbed in NDCT normally help keep allergens away from subepithelial antigen-presenting cells, and the epithelium itself acts as a sentinel for allergic inflammation [5, 28]. If that barrier leaks, more intact allergen reaches the immune system, the threshold for sensitisation falls, and the sensitisation profile widens. That is exactly the pattern we saw, with PR-10 and profilin sensitisation (pan-allergens that depend on mucosal penetration) dominating the BA+NDCT group [9, 12]. Earlier paediatric series had already linked connective tissue dysplasia to bronchial obstruction [5]; our spirometric and plethysmographic data add objective lung-function and air-trapping measurements to that picture.

FeNO reinforced the same impression. At a mean of 52 ppb the BA+NDCT group sat above the high cut-off the ATS applies in children, pointing to brisk eosinophilic inflammation [23]. Stubbornly high FeNO during ICS treatment has been tied both to genuinely poorer steroid response and to imperfect adherence, and FeNO-guided strategies have been trialled to cut exacerbations in children [23, 29]. A high FeNO may therefore flag a less ICS-responsive subgroup, though a cross-sectional snapshot cannot establish that.

The zinc data deserve particular comment. Mean serum zinc in the BA+NDCT group ran about 38% below the lower reference limit. Paediatric meta-analysis shows a mixed overall picture for zinc in asthma [13], so the depth of depletion specifically in our dysplasia subgroup hints at a connective-tissue-linked mechanism rather than a generic asthma effect. Zinc is a cofactor for the matrix metalloproteinases that remodel collagen and a broad regulator of immunity, so a shortfall could plausibly hamper

collagen synthesis, in keeping with worsening dysplasia, while also relaxing regulatory T-cell control and tilting the balance towards Th2 [14, 30, 31]. Zinc additionally enables glucocorticoid receptor translocation to the nucleus, which offers one testable route to the ICS resistance often suspected in these children [14].

Magnesium may contribute along a parallel track. Low magnesium destabilises mast-cell membranes and favours histamine release and smooth-muscle contraction [15, 32], and systematic reviews credit magnesium with bronchodilatory and anti-inflammatory effects, with oral supplementation modestly improving control and lung function in stable asthma [15, 16]. As a cofactor for glutathione synthetase, magnesium also props up antioxidant defence; its loss could aggravate the oxidative stress already present in NDCT and, with it, epithelial damage and allergen permeability [16, 32].

Reassuringly, the multivariable model pointed in the same direction as the bivariate comparisons. NDCT, low zinc, low magnesium, polysensitisation and FeNO each remained associated with moderate-to-severe asthma after adjustment, whereas age and sex did not. This is consistent with a real pathophysiological contribution that demographic confounding does not explain, although a regression cannot prove it. The composite of dysplasia score plus zinc and magnesium discriminated moderate-to-severe disease better than any single marker (AUC 0.88) and may be worth developing as a stratification tool, but it needs prospective external validation before any clinical use.

Finally, the inverse correlations between dysplasia severity and both zinc ($rs=-0.61$) and magnesium ($rs=-0.55$) are themselves of interest. Correlation cannot separate shared cause from coincidence, but there is a plausible candidate: collagen-rich connective tissue is the body's largest zinc store, so the architectural disruption of NDCT might reduce zinc retention and disturb its homeostasis. That possibility deserves a study of its own [4, 14].

3.10 Clinical Implications

If these associations hold up prospectively, the practical message is straightforward. A child with moderate-to-severe asthma who also carries the external stigmata of connective tissue dysplasia, or whose disease behaves more stubbornly than expected, is a reasonable candidate for Kadurina scoring, a check of serum zinc and magnesium, and component-resolved testing where it is available. These are inexpensive, widely accessible steps that together could flag the subgroup described here and, in time, identify children in whom correcting a micronutrient deficit might add something to inhaled therapy.

3.11 Limitations

Several limitations temper these conclusions. The cross-sectional design is the most important: it shows associations but cannot fix their direction, and only longitudinal work will tell whether dysplasia and micronutrient loss precede severe disease or accompany it. Induced sputum, the reference method for inflammatory phenotyping, could not be obtained in every younger child, so we relied on FeNO as a surrogate. We did not sequence collagen genes, and so cannot tie the phenotype to specific molecular variants. Dietary intake of zinc and magnesium was not formally recorded, leaving nutrition as an unmeasured contributor to the serum values. The single tertiary-centre setting may also have skewed recruitment towards more severe cases. Finally, although the study was powered for its main comparisons, the smallest subgroups, severe persistent asthma in particular ($n=12$), are modest and should be read cautiously.

4. CONCLUSION

In this paediatric cohort, asthma accompanied by connective tissue dysplasia and low zinc and magnesium marked out a distinct clinical phenotype: more molecular polysensitisation, higher FeNO, poorer spirometry, an atopic eosinophilic profile and a more severe course. A reasonable interpretation is that a weakened epithelial barrier in NDCT lets allergens through more easily and broadens sensitisation towards cross-reactive components, while zinc and magnesium depletion helps sustain Th2 dominance and mast-cell reactivity, the two together feeding a cycle of allergic inflammation. That cycle remains a hypothesis built on cross-sectional associations, and confirming it will require longitudinal and interventional studies.

Pending confirmation in larger prospective studies, the following directions are proposed:

- Systematic NDCT phenotypic assessment using the modified Kadurina scale may be considered in the evaluation of children with moderate-to-severe allergic disease.
- Serum Zn and Mg quantification may add value to the workup of children with BA, particularly with NDCT stigmata or refractory disease, pending validation of clinical utility.

- ALEX2-based component-resolved diagnostics is a useful tool for sensitisation profiling in NDCT-associated BA and may inform allergen-specific immunotherapy decisions.
- Prospective randomised controlled trials are warranted to test whether Zn and Mg supplementation provides adjunctive benefit in NDCT-associated BA, targeting the composite biomarker cluster identified here.

ACKNOWLEDGEMENTS

The authors thank the nursing and laboratory staff of the International Allergy Centre, 1st Clinic, Tashkent State Medical University, for their assistance with sample collection and lung-function testing, and the participating children and their families for their cooperation.

AUTHOR CONTRIBUTIONS

Turdikul A. Bobomurodov: Writing—Review & Editing.

Diloram T. Abdullaeva: Conceptualization, Writing—Original Draft, Writing—Review & Editing, Supervision.

Maktuba Kh. Mirrakhimova: Methodology, Writing—Review & Editing.

Dilnoza T. Bobomurodova: Writing—Review & Editing.

Adiba M. Saidkhanova: Methodology, Formal Analysis, Writing—Review & Editing.

Mohigul K. Ismatova: Writing—Review & Editing.

Sarvarbek S. Sobirov: Conceptualization, Investigation, Data Curation, Formal Analysis, Writing—Original Draft, Writing—Review & Editing.

Nigora T. Akramova: Writing—Review & Editing.

All authors have read and agreed to the published version of the manuscript.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The study was conducted within the research programme of the Department of Paediatric Allergology, Tashkent State Medical University.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The ALEX2 platform was used under standard clinical service conditions; no compensation or support was received from any manufacturer.

ETHICS STATEMENT

Approved by the Institutional Ethics Committee of Tashkent State Medical University (Protocol No. 7, 15 January 2023). All procedures complied with the Declaration of Helsinki (2013). Written informed consent/assent was obtained from all participants.

DATA AVAILABILITY STATEMENT

The anonymised dataset supporting the findings is available from the corresponding author upon reasonable request, subject to local ethics committee approval for data sharing.

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; ATS, American Thoracic Society; AUC, area under the curve; BA, bronchial asthma; BD, bronchodilator; CRD, component-resolved diagnostics; ERS, European Respiratory Society; FEV₁, forced expiratory volume in 1 s; FeNO, fractional exhaled nitric oxide; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; IgE, immunoglobulin E; LTP, lipid transfer protein; Mg, magnesium; NDCT, undifferentiated connective tissue dysplasia; OR, odds ratio; RV, residual volume; TLC, total lung capacity; Zn, zinc.

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