Letter to the Editor

Characterization of a high TNF-α phenotype in children with moderate-to-severe asthma

To the Editor:

Severe asthma is a heterogeneous disorder associated with multiple clinical phenotypes. Recognizing that the treatment of severe asthma might require targeted biologic approaches, we have read with interest reports of TNF-α antagonism in asthmatic adults.[1,2] TNF-α is a pleiotropic cytokine that binds to the type 1 TNF receptor and, on activation, induces canonical activation of nuclear factor κB (NF-κB). Although previous studies have demonstrated increased expression of both TNF-α and NF-κB in patients with severe asthma,[1,3,4] non–phenotype-directed antagonism of TNF-α in general populations of adults with moderate-to-severe asthma has failed to improve asthma outcomes.[2] However, smaller, non–placebo-controlled studies of adults with corticosteroid-refractory asthma and high TNF-α expression have demonstrated clinical improvements with TNF-α antagonism,[1,4] suggesting that treatment efficacy might be limited to selected phenotypes.

Given that children are not small adults and that children with asthma might require unique pharmacologic interventions,[5] the purpose of this study was to quantify the expression of TNF-α in children with moderate-to-severe persistent asthma and to further assess the associations between increased TNF-α expression and clinical features. Although children with moderate-to-severe asthma as a whole were characterized by increased TNF-α and NF-κB expression, we report that a subpopulation with high TNF-α expression (despite intensive corticosteroid therapy) is distinguished by the highest degree of symptoms and health care use. These findings highlight the multiple endotypes that likely exist within the population with severe asthma and suggest that phenotype-directed therapy might be warranted for these children.

Healthy nonasthmatic children aged 6 to 17 years and children with asthma confirmed by either 12% or greater reversibility in FEV_1 after albuterol administration or airway hyperresponsiveness to methacholine were enrolled from a severe asthma clinic in Atlanta, Georgia. Criteria for mild asthma included no treatment with asthma controller medications for at least 1 month and well-controlled asthma evidenced by an Asthma Control Questionnaire score of less than 0.75.[6] Children were classified as having moderate-to-severe asthma if they were receiving step 5 or step 6 therapy according to the National Asthma Education and Prevention Program asthma treatment guidelines (see Table E1 in this article's Online Repository at www.jacionline.org).[7] Children treated with step 4 therapy were also eligible provided they had poor asthma control evidenced by either an Asthma Control Questionnaire score of 1.25 or greater,[6] daytime asthma symptoms more than twice weekly, or nighttime asthma symptoms at least once per week. All children underwent phenotypic characterization, as described in the Methods section in this article's Online Repository at www.jacionline.org. A subset of children with moderate-to-severe asthma also underwent formal corticosteroid responsiveness testing with systemic triamcinolone acetonide.

PBMCs were isolated from whole blood through a density gradient. Plasma TNF-α concentrations were quantified by using a commercially available high-sensitivity kit with a detection limit of 0.106 pg/mL (R&D Systems, Minneapolis, Minn). PBMC mRNA gene expression was determined with a real-time PCR array (Human Antibacterial Response RT^2 Profiler; SABiosciences, Frederick, Md) after isolation of RNA with a commercial kit (RNeasy Mini; Qiagen, Valencia, Calif). Eight

TABLE I. Features of the participants

	Healthy control subjects (n = 15)	Mild well-controlled asthma (n = 8)	Moderate-to-severe asthma (n = 49)
Age (y)	10 (8-11)	12 (9-17)	12 (10-15)
Male sex	3 (20)	4 (50)	35 (71)§
Race			
White	1 (7)	0	4 (8)
Black	11 (73)	8 (100)	38 (78)
>1 Race	3 (20)	0	7 (14)
Body mass index percentile >95%	0	1 (13)	17 (35)§
Asthma duration (y)	—	6 (3-17)	11 (7-14)
Serum IgE (kU/L)*	46 (26-68)	34 (22-200)	204 (70-567)§‖
Exhaled nitric oxide (ppb)*	11 (8-12)	22 (13-56)§	28 (16-54)§
Pulmonary function			
FVC (% predicted)	100 (89-115)	106 (93-121)	103 (91-114)
FEV_1 (% predicted)	99 (93-110)	96 (84-114)	89 (76-102)§
FEV_1/FVC ratio	0.88 (0.84-0.94)	0.84 (0.74-0.90)	0.76 (0.70-0.83)§
FEF_{25-75} (% predicted)	98 (82-122)	89 (55-109)	67 (44-79)§
ACQ score†	—	0.14 (0-0.29)	1.29 (0.57-1.86)‖
AQLQ score‡	—	6.86 (6.58-6.98)	5.52 (4.76-6.32)‖

Data are shown as medians (interquartile ranges) or numbers (percentages).

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma Quality of Life Questionnaire.

*Data were logarithmically transformed before analyses.

†Scores on the Asthma Control Questionnaire range from 0 to 6, with lower scores indicating better asthma control and 0.5 as the minimal important difference.

‡Scores on the Asthma Quality of Life Questionnaire range from 1 to 7, with higher scores indicating better quality of life and 0.5 as the minimal important difference.

§$P < .05$ versus control subjects.

‖$P < .05$ versus patients with mild asthma.

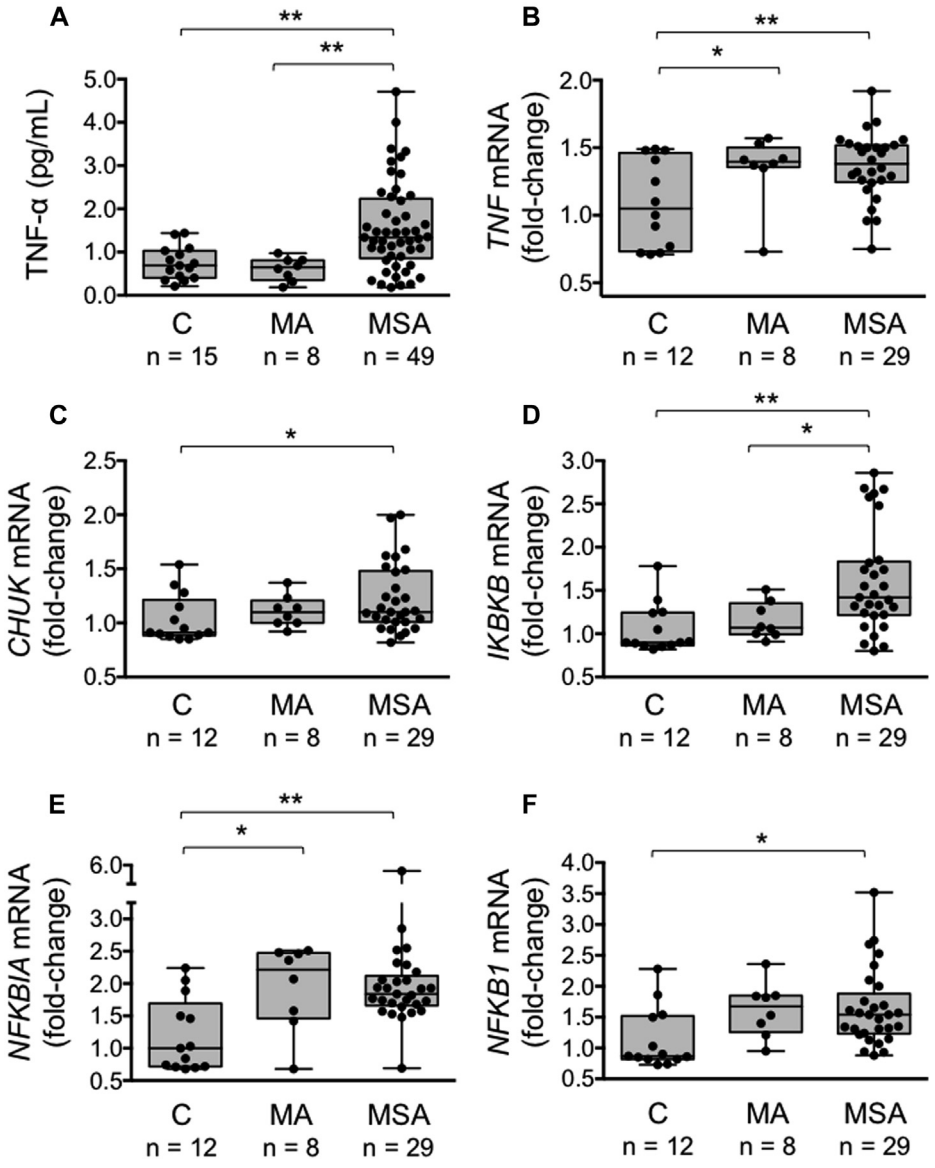

FIG 1. Plasma concentrations of TNF-α **(A)** and mRNA gene expression of *TNF* **(B)**, *CHUK* **(C)**, *IKBKB* **(D)**, *NFKBIA* **(E)**, and *NFKB1* **(F)** in healthy control subjects *(C)*, children with mild asthma *(MA)*, and children with moderate-to-severe asthma *(MSA)*. *Horizontal lines* represent the median. $*P < .05$ and $**P < .01$.

microliters of the first-strand cDNA synthesis reaction was preamplified (RT^2 PreAMP PCR Master Mix and RT^2 PreAMP Human Antibacterial Response Primer Mix, SABiosciences), and the equivalent of 0.8 μL of cDNA was added to each well of the array plate. Genes of interest included *CHUK, IKBKB*, *NFKB1*, *NFKBIA*, and *TNF* (see Table E2 in this article's Online Repository at www.jacionline.org). The cycle threshold values of the target cDNAs for each subject were normalized to the average cycle threshold of 5 housekeeping genes (*GAPDH*, *ACTB*, *RPL13A*, *B2M*, and *HPRT1*).

Seventy-two children, including 15 healthy nonasthmatic children, 8 children with mild, well-controlled asthma, and 49 children with moderate-to-severe asthma, were included in the final analyses (see Fig E1 in this article's Online Repository at www.jacionline.org). Features of the groups are shown in Table I. Children with moderate-to-severe asthma were more likely to be obese males and had increased serum IgE and exhaled nitric oxide concentrations, greater airflow obstruction, poorer asthma control, and decreased asthma-related quality of life, despite a median dose of 1000 μg/d inhaled fluticasone (mean ± SD, 818 ± 287 μg/d). Children with moderate-to-severe asthma also had higher plasma TNF-α concentrations and increased mRNA expression of TNF-α and other genes downstream of TNF-α activation and binding, including *NFKB1* (Fig 1).

Given the heterogeneity in TNF-α expression levels observed in children with moderate-to-severe asthma and the overlap in TNF-α values between groups, a TNF-α cut point of 1.25 pg/mL was selected for further stratification. This cut point had a sensitivity and specificity of 59% and 91%, respectively, for detection of moderate-to-severe asthma compared with the other groups (see Fig E2 in this article's Online Repository at

www.jacionline.org). The demographic features of the resulting low TNF-α expression and high TNF-α expression groups were not statistically different (see Table E3 in this article's Online Repository at www.jacionline.org). However, children with moderate-to-severe asthma with high TNF-α concentrations were characterized by increased health care use in the previous year, poorer symptom control, and greater airflow obstruction than children with low TNF-α concentrations (see Table E4 in this article's Online Repository at www.jacionline.org). In the subset of children who underwent treatment with systemic triamcinolone (see Table E5 in this article's Online Repository at www.jacionline.org), TNF-α concentrations remained stable in most children, although a few outliers were noted (see Fig E3 in this article's Online Repository at www.jacionline.org). Similar results were also noted when a TNF-α cut point of 1.05 pg/mL was used, although some significance was lost (see Table E6 in this article's Online Repository at www.jacionline.org).

These preliminary observations suggest that, similar to studies in adults,[1,4] there is a subpopulation of children with moderate-to-severe asthma with persistently high TNF-α expression and poor asthma control despite aggressive corticosteroid treatment who might benefit from anti–TNF-α therapy. Although our focus on systemic TNF-α expression might be criticized, we[8] and others[9] have previously described systemic inflammatory alterations in patients with severe asthma that are similar to those in the airways. The use of PMBCs also permitted the inclusion of healthy children and children with mild asthma. Nonetheless, these preliminary findings highlight a potential endotype or mechanism within the larger heterogeneous population with severe asthma that might contribute to poor asthma control. However, additional studies on TNF-α polymorphisms, TNF-α expression in other resident cells, and the longitudinal stability of TNF-α expression in relation to key asthma clinical outcomes, such as exacerbations, might be useful.

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REFERENCES

1. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, et al. Evidence of a role of tumor necrosis factor alpha in refractory asthma. N Engl J Med 2006; 354:697-708.
2. Holgate ST, Noonan M, Chanez P, Busse W, Dupont L, Pavord I, et al. Efficacy and safety of etanercept in moderate-to-severe asthma: a randomised, controlled trial. Eur Respir J 2011;37:1352-9.
3. Gagliardo R, Chanez P, Profita M, Bonanno A, Albano GD, Montalbano AM, et al. IkappaB kinase-driven nuclear factor-kappaB activation in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol 2011;128: 635-45, e1-2.
4. Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, et al. Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax 2005;60:1012-8.
5. Szefler SJ, Chmiel JF, Fitzpatrick AM, Giacoia G, Green TP, Jackson DJ, et al. Asthma across the ages: knowledge gaps in childhood asthma. J Allergy Clin Immunol 2014;133:3-14.
6. Juniper EF, Bousquet J, Abetz L, Bateman ED, Committee G. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med 2006;100:616-21.
7. National Asthma E, Prevention P. Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma—summary report 2007. J Allergy Clin Immunol 2007;120(Suppl):S94-138.
8. Fitzpatrick AM, Stephenson ST, Hadley GR, Burwell L, Penugonda M, Simon DM, et al. Thiol redox disturbances in children with severe asthma are associated with posttranslational modification of the transcription factor nuclear factor (erythroid-derived 2)-like 2. J Allergy Clin Immunol 2011;127:1604-11.
9. Goleva E, Jackson LP, Gleason M, Leung DY. Usefulness of PBMCs to predict clinical response to corticosteroids in asthmatic patients. J Allergy Clin Immunol 2012;129:687-93.e1.

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Characterization of a “high” TNF-~ phenotype in moderate-to-severe asthmatic children

ONLINE REPOSITORY

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Detailed Methods

Subjects. Healthy non-asthmatic children age 6-17 years and children with asthma confirmed by either ≥ 12% reversibility in the forced expiratory volume in one second **(FEV_1)** after albuterol administration or airway hyperresponsiveness to methacholine were enrolled. Exclusion criteria for all children included premature birth before 35 weeks' gestation, current smoking, or other co-morbid disorders such as immunodeficiency, aspiration or vocal cord dysfunction. This study was conducted under approval from the Emory University Institutional Review Board. Written informed consent was obtained from the parents or legal guardians. Written assent was also obtained from children 12 to 17 years of age, whereas verbal assent was obtained from children 6 to 11 years of age.

Asthma severity classification. Criteria for mild asthma included no treatment with asthma controller medications for at least one month and well-controlled asthma evidenced by an Asthma Control Questionnaire Score **(ACQ)** < 0.75.[1] Children were classified as having moderate-to-severe s asthma if they were receiving Step 5 or Step 6 therapy according to the National Asthma Education and Prevention Program asthma treatment guidelines **(Table E1)**.[2] Children treated with Step 4 therapy were also eligible provided they had poor asthma control evidenced by either an ACQ score ≥ 1.25[1], daytime asthma symptoms more than twice weekly, or nighttime asthma symptoms at least once per week.

Characterization procedures. Children were evaluated during a research-only outpatient visit that was rescheduled if the following were reported within the preceding 2 weeks: 1) upper respiratory viral symptoms such as rhinorrhea, 2) an acute worsening of asthma symptoms, 3) antibiotic use, or 4) systemic glucocorticoid use. Subjects completed questionnaires about their medical history and asthma symptoms over the previous 2 weeks.

Spirometry was performed with a portable spirometer (KoKo Legend; Ferraris, Louisville, Colorado) at baseline and after four inhalations of albuterol sulfate (90 µg/actuation) delivered through a non-electrostatic valved holding chamber with a mouthpiece. The best of three forced vital capacity **(FVC)** maneuvers was interpreted.[3] Population reference equations were used to calculate percent predicted values for FVC, the forced expiratory volume in one second **(FEV_1)**, and the mid-expiratory flow rate at 25-75% of vital capacity **(FEF_{25-75})**.[4] Fractional nitric oxide in the exhaled breath was quantified using online methods (NIOX MINO; Aerocrine, Inc, New Providence, New Jersey) with a fixed flow rate of 0.05 L/s and exhalation duration of 6 seconds.[5] Whole blood (30-40 mL) was collected into serum separation tubes and heparinized tubes containing a density gradient for PBMC isolation (Vacutainer® CPT™, Becton, Dickinson and Company, Franklin Lakes, New Jersey). Asthma control and quality of life were quantified with the Asthma Control Questionnaire[6] and the Pediatric Asthma Quality of Life Questionnaire,[7] respectively. Serum immunoglobulin E **(IgE)** concentrations were determined using a commercially-available assay kit according to the manufacturer's instructions (Calbiotech, Spring Valley, California). A subset of children with moderate-to-severe asthma also underwent formal corticosteroid responsiveness testing with systemic triamcinolone acetonide (1 mg/kg injection, 60 mg maximum dose). These children returned to the clinic in 2 weeks and the characterization procedures were repeated.

Laboratory methods. Plasma TNF-α concentrations were quantified using a commercially available, high-sensitivity kit with a detection limit of 0.106 pg/mL (R&D Systems, Minneapolis, Minnesota). mRNA gene expression was assessed in isolated PBMCs with a real-time polymerase chain reaction array (Human Antibacterial Response RT^2 *Profiler*,™ SABiosciences, Frederick, MD) after isolation of RNA using a commercial kit (RNeasy® Mini,

Qiagen, Valencia, California). 8 µl of the first-strand cDNA synthesis reaction was pre-amplified (RT^2PreAMP PCR Master Mix and RT^2 PreAMP Human Antibacterial Response Primer Mix, SA Biosciences) and the equivalent of 0.8 µl cDNA was added to each well of the array plate. Genes of interest included *CHUK*, *IKBKB*, *NFKB1*, *NFKBIA*, and *TNF* **(Table E2)**. The cycle threshold **(CT)** values of the target cDNAs for each subject were normalized to the average CT of 5 housekeeping genes (*GAPDH, ACTB, RPL13A, B2M* and *HPRT1*).

Statistical analyses. Statistical analyses were performed with IBM® SPSS® Statistics (Version 21, SPSS Inc, Chicago, Illinois). Data that were not normally distributed were logarithmically transformed prior to analysis. mRNA gene expression was analyzed relative to the healthy control group and was expressed as fold change. Group differences were assessed using chi-square tests and analysis of variance, with Fisher's Least Significant Difference post-hoc tests for comparison of group means. Receiver Operating Characteristic curve analysis was used to select cut-points of plasma TNF-α for further stratification. Logistic regression analyses were performed to determine the association between plasma TNF-α concentrations and asthma characteristics. Where indicated, models were adjusted for sex, race, and body max index to minimize potential confounding on the selected variables of interest. Significance was defined as $\alpha < 0.05$ using two-tailed tests.

References

1. Juniper EF, Bousquet J, Abetz L, Bateman ED, Committee G. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med 2006; 100:616-21.

2. National Asthma E, Prevention P. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol 2007; 120:S94-138.

3. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005; 26:319-38.

4. Wang X, Dockery DW, Wypij D, Fay ME, Ferris BG, Jr. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol 1993; 15:75-88.

5. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011; 184:602-15.

6. Juniper EF, Gruffydd-Jones K, Ward S, Svensson K. Asthma Control Questionnaire in children: validation, measurement properties, interpretation. Eur Respir J 2010; 36:1410-6.

7. Juniper EF, Guyatt GH, Feeny DH, Griffith LE, Ferrie PJ. Minimum skills required by children to complete health-related quality of life instruments for asthma: comparison of measurement properties. Eur Respir J 1997; 10:2285-94.

Table E1. Stepwise approach for the management of asthma in children and adolescents, as recommended by the National Asthma Education and Prevention Program's Expert Panel Report-3.[2]

Step	Recommended therapy
Step 1	As-needed short-acting bronchodilators
Step 2	Daily low-dose ICS, or daily controller such as a leukotriene receptor antagonist
Step 3	Daily low-dose ICS plus a second controller medication, or daily medium-dose ICS
Step 4	Daily medium-dose ICS plus a second controller medication
Step 5	Daily high-dose ICS plus a second controller medication
Step 6	Daily high-dose ICS plus a second controller medication plus systemic corticosteroids

ICS = inhaled corticosteroid

Table E2. Genes of interest for mRNA gene expression analyses of the TNF-α/NF-κB signaling pathway. Genes were selected from the Human Antibacterial Response RT^2 *Profiler*^{TM} array, SABiosciences, Frederick, MD.

Gene symbol	Gene name	Common name for encoded protein
TNF	Tumor necrosis factor	TNFα
CHUK	Conserved helix-loop-helix ubiquitous kinase	I kappa B kinase alpha (IKKα)
IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	I kappa B kinase beta (IKKβ)
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	I kappa B alpha
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	Nuclear factor kappa B p50 subunit (p50)

Table E3. Features of children with moderate-to-severe asthma stratified by "low" (<1.25 pg/mL) versus "high" (≥1.25 pg/mL) baseline plasma TNF-α concentrations. Data are shown as the median (interquartile range) or the number (percent). Groups are not statistically different.

	TNF-α "low" moderate-to-severe asthma N = 20	TNF-α "high" moderate-to-severe asthma N = 29
Age (years)	11 (8 – 15)	12 (11 – 16)
Males	12 (60)	23 (79)
Race		
White	1 (5)	3 (10)
Black	18 (90)	20 (69)
More than one race	1 (5)	6 (21)
Body mass index percentile >95%	6 (30)	11 (38)
Asthma duration (years)	9 (6 – 12)	11 (7 – 15)
Daily asthma medications		
ICS without LABA	3 (15)	3 (10)
ICS with LABA	17 (85)	26 (90)
Montelukast	15 (75)	18 (62)
Omalizumab	1 (5)	1 (3)
Oral corticosteroids	2 (10)	1 (3)
Serum IgE (kU/L)[1]	147 (23 – 622)	238 (136 – 564)
Exhaled nitric oxide (ppb)[1]	24 (17 – 54)	30 (16 – 56)

ICS = inhaled corticosteroid, LABA = long-acting beta agonist

[1] Data were logarithmically transformed prior to analyses.

Table E4. Associations between "high" (≥ 1.25 pg/mL) versus "low" (< 1.25 pg/mL) baseline plasma TNF-α concentrations and asthma characteristics in children with moderate-to-severe asthma.

Baseline Asthma Characteristic	Adjusted OR[1]	95% CI	p-value
Healthcare Utilization			
Emergency room visit (previous year)	3.64	(1.11, 11.87)	0.033
≥3 oral corticosteroid bursts (previous year)	0.89	(0.27, 2.94)	0.850
Hospitalization (previous year)	0.75	(0.18, 3.18)	0.691
Intubation (ever)	1.73	(0.51, 5.82)	0.376
Asthma Control			
Asthma Control Questionnaire (ACQ) score ≥1.25[2]	3.65	(1.06, 12.59)	0.040
Daytime symptoms daily[3]	7.31	(1.92, 27.86)	0.004
Daytime symptoms more than twice weekly[3]	4.32	(1.16, 16.08)	0.029
Nighttime symptoms one time per week or more[3]	4.30	(1.18, 15.59)	0.027
Airflow obstruction[4]	3.61	(1.01, 12.97)	0.050

[1] Adjusted for sex, race (white versus non-white) and body mass index percentile (≥95% versus <95%).

[2] Scores on the ACQ range from 0 to 6, with lower scores indicating better asthma control.

[3] Symptoms were assessed over the previous 2 weeks.

[4] Airflow obstruction was determined by either FEV_1 < 80% predicted or FEV_1/FVC < 0.85.

Table E5. Features of children with moderate-to-severe asthma who received triamcinolone. Data are stratified by "low" (<1.25 pg/mL) versus "high" (≥1.25 pg/mL) baseline plasma TNF-α concentrations. Data are shown as the median (interquartile range) or the number (percent). Groups are not statistically different.

	TNF-α "low" N = 11	TNF-α "high" N = 17
Age (years)	11 (9 – 14)	12 (10 – 15)
Males	8 (73)	13 (77)
Race		
White	1 (9)	1 (6)
Black	9 (82)	11 (65)
More than one race	1 (9)	5 (29)
Body mass index percentile >95%	4 (36)	8 (47)
Asthma duration (years)	9 (6 – 13)	11 (7 – 14)
Daily asthma medications		
ICS without LABA	2 (18)	1 (6)
ICS with LABA	9 (82)	16 (94)
Montelukast	9 (82)	12 (71)
Omalizumab	1 (9)	1 (6)
Oral corticosteroid	2 (18)	0
Serum IgE (kU/L)[1]	492 (266 – 1712)	237 (147 – 478)
Exhaled nitric oxide (ppb)[1]	24 (21 – 60)	21 (13 – 36)
Pulmonary function		
FVC (% predicted)	97 (83 – 107)	105 (99 – 114)
FEV_1 (% predicted)	87 (73 – 104)	94 (84 – 103)
FEV_1/FVC	0.82 (0.66 – 0.90)	0.76 (0.69 – 0.82)
FEF_{25-75} (% predicted)	68 (42 – 102)	64 (57 – 81)

[1] Data were logarithmically transformed prior to analysis

Table E6. Associations between "high" versus "low" baseline plasma TNF-α concentrations and asthma characteristics in children with moderate-to-severe asthma, with a TNF-α cut-point of 1.05 pg/mL.

Baseline Asthma Characteristic	Adjusted OR[1]	95% CI	p-value
Healthcare Utilization			
Emergency room visit (previous year)	4.52	(1.31, 15.58)	0.017
≥3 oral corticosteroid bursts (previous year)	0.89	(0.24, 3.01)	0.806
Hospitalization (previous year)	2.52	(0.45, 14.26)	0.295
Intubation (ever)	3.07	(0.79, 11.97)	0.105
Asthma Control			
Asthma Control Questionnaire (ACQ) score ≥1.25[2]	2.99	(0.82, 10.89)	0.098
Daytime symptoms daily[3]	8.87	(1.91, 41.22)	0.005
Daytime symptoms more than twice weekly[3]	5.56	(1.38, 22.45)	0.016
Nighttime symptoms one time per week or more[3]	5.46	(1.24, 24.01)	0.025
Airflow obstruction[3]	1.99	(0.57, 6.94)	0.281

[1] Adjusted for sex, race (white versus non-white) and body mass index percentile (≥95% versus <95%).

[2] Scores on the ACQ range from 0 to 6, with lower scores indicating better asthma control.

[3] Symptoms were assessed over the previous 2 weeks.

[4] Airflow obstruction was determined by either FEV_1 < 80% predicted or FEV_1/FVC < 0.85.

Figure Legends

Figure E1.

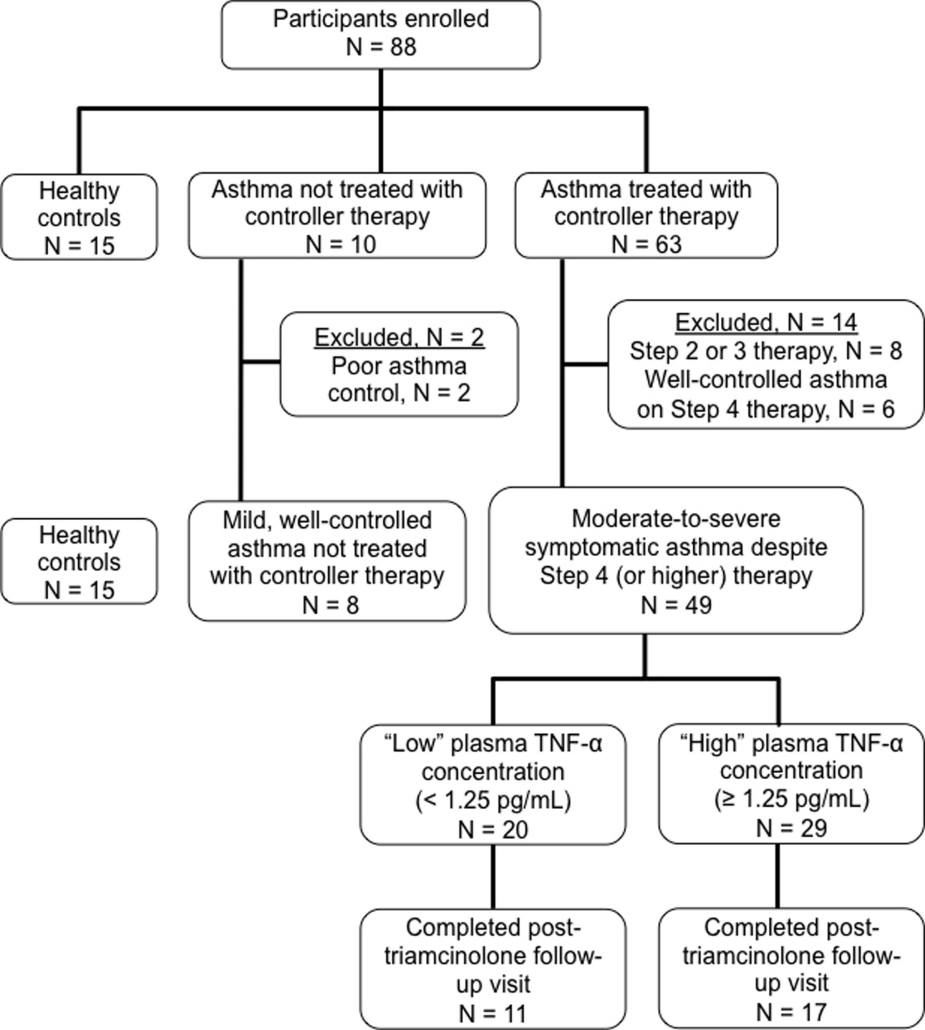
Flowchart depicting participant enrollment in the study.

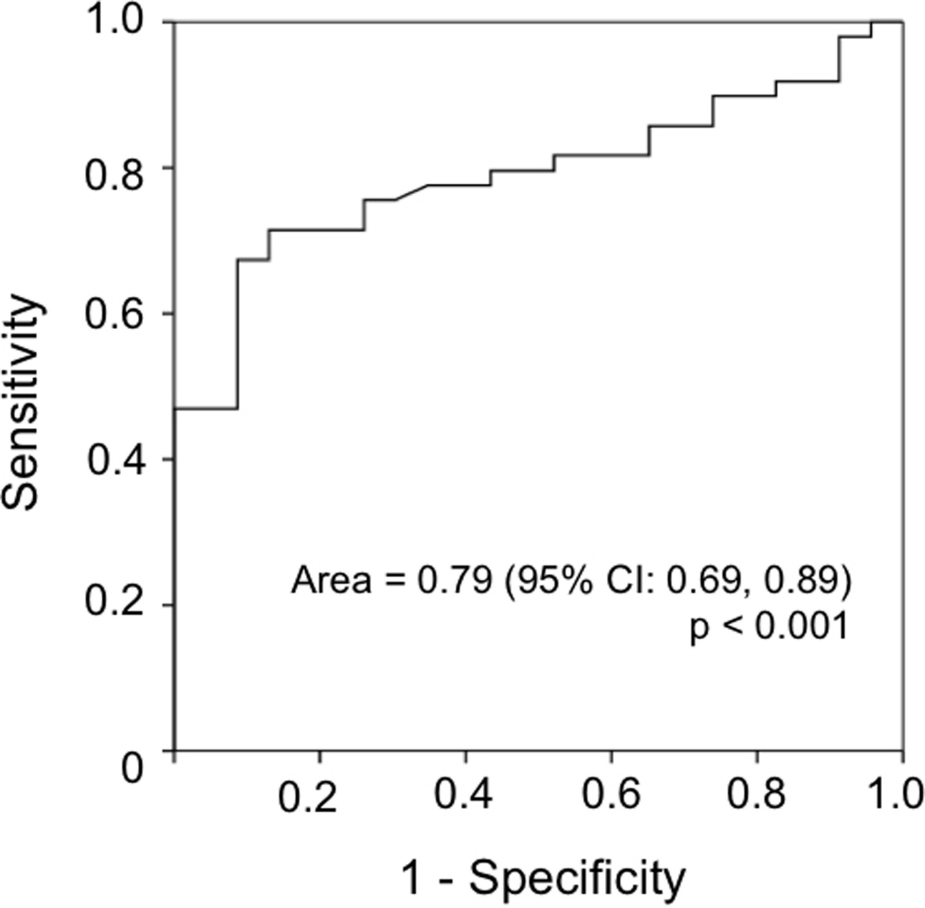
Figure E2.

Receiver Operating Characteristic (ROC) curve analysis depicting the sensitivity and specificity of plasma TNF-α cut-points for detection of moderate-to-severe asthma (as compared to mild asthma or no asthma).

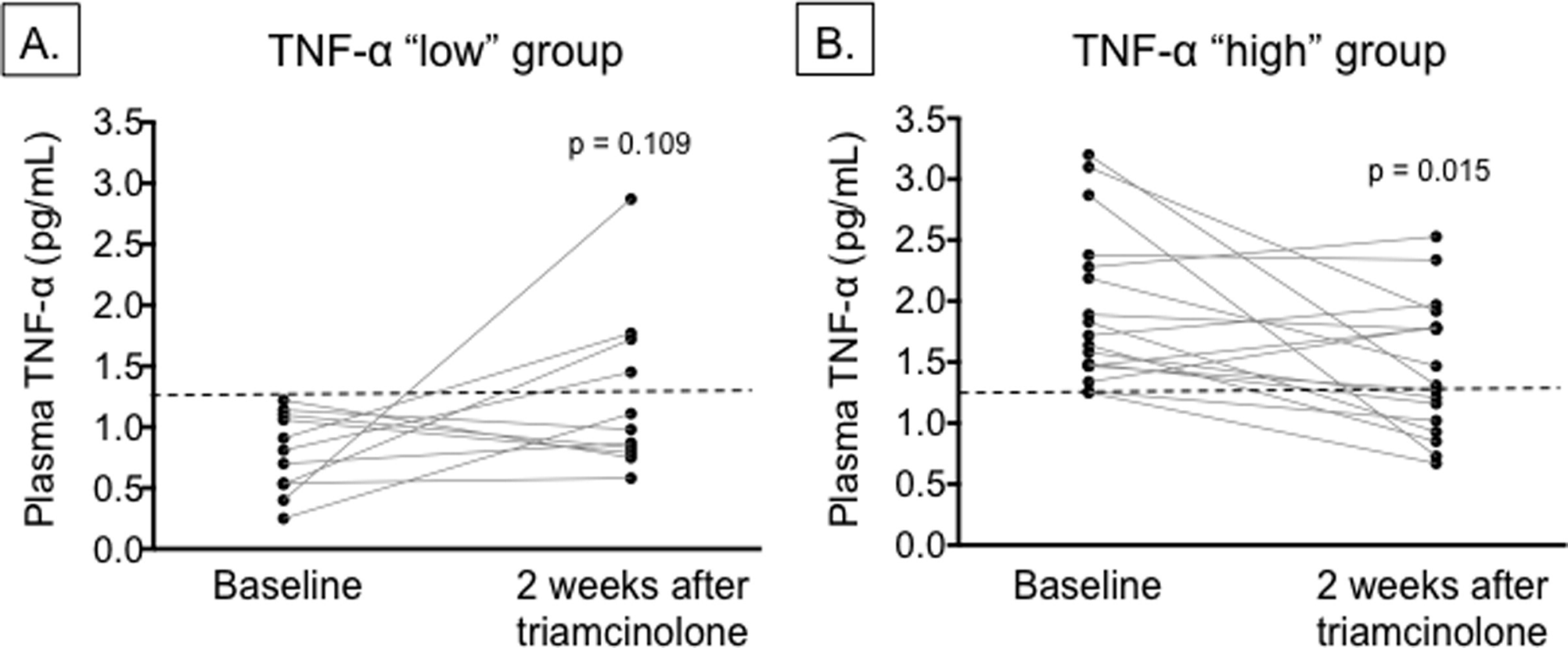
Figure E3.

Plasma TNF-α concentrations before and after systemic triamcinolone acetonide administration, stratified by (A) “low” versus (B) “high” baseline TNF-α values. The dashed line represents the TNF-α cut-point of 1.25 pg/mL that was used for stratification.


Participants enrolled
N = 88
Healthy controls
N = 15
Asthma not treated with controller therapy
N = 10
Asthma treated with controller therapy
N = 63
Excluded, N = 2
Poor asthma control, N = 2
Excluded, N = 14
Step 2 or 3 therapy, N = 8
Well-controlled asthma on Step 4 therapy, N = 6
Healthy controls
N = 15
Mild, well-controlled asthma not treated with controller therapy
N = 8
Moderate-to-severe symptomatic asthma despite Step 4 (or higher) therapy
N = 49
“Low” plasma TNF-α concentration
(< 1.25 pg/mL)
N = 20
“High” plasma TNF-α concentration
(≥ 1.25 pg/mL)
N = 29
Completed post-triamcinolone follow-up visit
N = 11
Completed post-triamcinolone follow-up visit
N = 17



Cut-point	Sensitivity	1 - Specificity
0.25	0.95	0.91
0.50	0.85	0.65
0.75	0.78	0.40
1.00	0.71	0.17
1.25	0.57	0.09
1.50	0.38	0


A.
TNF-α "low" group
Plasma TNF-α (pg/mL)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
p = 0.109
Baseline
2 weeks after triamcinolone
B.
TNF-α "high" group
Plasma TNF-α (pg/mL)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
p = 0.015
Baseline
2 weeks after triamcinolone