MS. JINGWEN ZHANG (Orcid ID : 0000-0001-6979-6308)

DR. ADRIAN LOWE (Orcid ID : 0000-0002-4691-8162)

DR. CAROLINE LODGE (Orcid ID : 0000-0002-2342-3888)

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To the editor:

Cytokines play a key role as mediators in the immuno-pathogenesis of asthma.[1] Age at asthma onset and the presence of T-helper 2 mediated eosinophilic airway inflammation have been identified as two important and distinct factors for defining asthma phenotypes,[2] but little is known about longitudinal associations between systemic cytokine concentrations and asthma. In a previous investigation of serum cytokine concentrations among 44-year-old adults and asthma phenotypes, we found early-onset persistent asthma (from age 13 to 44 years) was associated with lower levels of interleukin (IL) -10, while asthma remission was associated with lower levels of IL-6 and TNF-α.[3] We hypothesised that, in middle-aged people with asthma serum cytokines might predict future asthma persistence beyond 44 years. Published studies regarding these potential associations are largely limited to childhood asthma, with only the Epidemiological study on the Genetics and Environment of Asthma (EGEA study) assessing cytokines profiles and asthma status longitudinally in adults, where they found patients with "high IL-1Ra and high IL-10" serum cytokine profiles had lower risks of worsening asthma control.[4]

Here, we examined whether serum cytokines were associated with asthma persistence during middle age, and if age-of-asthma onset modified any such association, using data from the Tasmania Longitudinal Health Study (TAHS). TAHS is a population-based cohort born in 1961, studied prospectively over 6 decades from ages 7 to 53 years.[5]

In the present analysis, participants with current asthma at ages 44 and/or 53 years were defined by self-reported asthma symptoms, or asthma-related healthcare utilization and/or asthma medication use

in the preceding 12 months.[6] Asthma persistence was defined as current asthma at both ages; asthma remission as having current asthma at age 44 years but no asthma at age 53 years; early-onset of asthma was defined as a parent reported history of asthma when participants were aged 7 and/or 13 years; and, late-onset asthma as no history of asthma at both these times, but positive self-report of current asthma in middle age.

Concentrations of serum cytokines, IL-4, IL-5, IL-6, IL-8, IL-10 and tumor necrosis factor (TNF)-α, were measured in peripheral blood samples collected from participants at age 44 years using a multiplex assay.[3] The cytokine concentrations were transformed on $\log_{10}$ scales and then categorized into binary variables (high (2^{nd} -5^{th} quintiles) versus low(1^{st} quintiles), Figure S1). Multivariable logistic regression was used to assess associations between each cytokine level and asthma persistence from age 44 years to 53 years, respectively, adjusting for sex, atopy, [3] smoking, season of assessment and BMI at baseline. Likelihood ratios were used to evaluate effect modification by early/late onset. Detailed definitions of the variables and statistical methods are explained in the supporting information.

Of 339 participants who had current asthma at age 44 years, 280 (83%) had serum cytokines measured and 201 (59%) also had asthma status documented at age 53 years (Figure S2). Of these, 57% (115/201) had persistent asthma while 43% had remitted asthma (Table 1). Atopy was more prevalent in early-onset asthma, while there were more females who had late-onset asthma, as found previously. [7] There were no other obvious differences between groups (Table1).

High levels of serum IL-4 (above the 1^{st} quintile) were associated with asthma persistence from age 44 to 53 years (adjusted odds ratio (aOR)= 2.65 [95% CI: 1.19− 5.90], p=0.017). High levels of serum IL-6 were marginally associated with asthma persistence (aOR=2.06 [95% CI: 0.94− 4.43], p=0.064) and age-of-asthma onset modified this association (p=0.015). For individuals with early-onset asthma, higher serum IL-6 levels were associated with asthma persistence (aOR=5.91 [95% CI: 1.64− 21.30], p=0.007), but this was not found for individuals with late-onset asthma. No associations were observed between serum levels of IL-5, IL-8, IL-10, TNF-α and the subsequent odds of asthma persistence during middle age, regardless of age-of asthma onset (Table 2).

To our knowledge, this is the first study to evaluate the longitudinal relationship between serum cytokines and asthma persistence in adults. It provides supporting evidence that elevated IL-4 concentrations are related to subsequent asthma persistence over the subsequent 9 years. This could be explained by the physiological actions of IL4 which have longer-term consequences, as IL-4 has been shown to inhibit apoptosis of T cells to maintain the allergic responses in persistent and refractory asthma.[2,8] Interestingly, we found that elevated serum IL-6 was associated with subsequent asthma persistence, but only in the subgroup with early-onset disease. We did not find any associations between serum IL-10 levels and asthma persistence. These were unexpected as

previously we found that lower serum IL-6 concentrations were related to asthma remission only in the subgroup with late-onset disease, and that people with early-onset current asthma had lower serum IL-10 concentrations.[3] Another study also reported that higher IL-6 was related to worse asthma outcomes, regardless of onset-age-of asthma.[9] The EGEA study found high IL-10/IL-1Ra profile was associated with reduced subsequent risks of worsening asthma control,[4] however this study cannot be directly compared. The EGEA study defined asthma control (worsening, stable, or improving) by changes of self-reported asthma symptoms severity over two time points. Another explanation is that asthma persistence is associated with alteration of immune responses, skewing Th1/Treg-dominated response towards Th2/Th17-dominated responses;[10] therefore the combinations of different cytokines may better predict the persistence of asthma. One study found increased IL-10/IFN-γ ratios at age 1 year were associated with developing asthma by age 4 years.[11] Unfortunately, neither IFN-γ nor IL-1Ra were measured in the TAHS cohort.

The limitations of our study are small sample size due in part to attrition. Furthermore, participants included in the analysis had higher proportions of current smokers, ex-smokers and atopy, compared with those lost-to-follow-ups (Table S1). Nevertheless, this is a population-based cohort that was asthma enriched to maximize the power to detect associations. The cytokines were only measured once at age 44 years. As skewness from Th1 to Th2 subsets and Th17 mediated immune reactions are important pathways in the pathogenesis of asthma, T helper (Th) 1-related cytokines (interferon (IFN)-γ, IL-12) and Th-17 related cytokines (IL-17, IL-21, IL-22) would also be promising biomarkers for asthma (Figure S3).[1] But they were not measured in the current study.

In summary, our findings suggest that serum concentrations of IL-4 may provide a prognostic marker for the persistence of asthma, while IL-6 may be a prognostic marker for the persistence of early-onset asthma in middle-age. This analysis supports a rationale to evaluate anti-IL4R therapies in clinical trials, but not for those relating to IL-10. Further studies with larger sample sizes and measuring a more comprehensive panel of cytokines at multiple time points, will help to further advance understanding of the roles of cytokines in asthma natural history and phenotyping.

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Authors: Jingwen Zhang [a.], Eugene H. Walters [a.], Mimi LK. Tang [b, c.], Adrian J. Lowe [a.], Caroline J. Lodge [a.], Dinh Bui [a.], Rangi Kandane-Rathnayake [d.], Bircan Erbas [e.] Garun S. Hamilton [d, f.], Bruce R. Thompson [g.], Michael J. Abramson [h.], Graham G. Giles [i.], Jennifer L. Perret [a, j.] *, Shyamali C. Dharmage [a.]*

Affiliation:

[a] Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, VIC Australia

[b] Allergy and Immune Disorders, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, VIC Australia

[c] Department of Pediatrics, University of Melbourne, VIC Australia

[d] School of Clinical Sciences, Monash University, Clayton, VIC Australia

[e] School of Psychology and Public Health, La Trobe University, VIC Australia

[f] Monash Lung and Sleep, Monash Health, Clayton, VIC Australia

[g] Faculty of Health, Arts and Design, Swinburne University of Technology, Hawthorn, VIC Australia

[h] School of Public Health & Preventive Medicine, Monash University, Melbourne, VIC, Australia

[i] Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, VIC Australia

[j] Institute for Breathing and Sleep, Melbourne, VIC Australia

*Perret JL and Dharmage SD should be considered joint senior author

Corresponding author: Shyamali Dharmage, Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, 207, Bouverie Street, Carlton, VIC 3052, Australia; Phone: +61 3 83440737; Fax:+61 3 9349 5815; Email: s.dharmage@unimelb.edu.au

Author Contributions:

Study concept and design: SCD, EHW, MJA, DPJ, GGG, JLP

Acquisition of data: SCD, EHW, MJA, RKR, MLKT, GH, BRT

Analysis and interpretation of data: JZ, JLP, SCD, MJA, EHW, CJL, AJL, DB, RKR, MLKT, BE, AJL, CJL

Drafting of the manuscript: JZ, JLP, SCD, MLKT

Critical revision of the manuscript for important intellectual content: All authors

Statistical analysis: JZ, JLP, SCD, AJL

Obtained funding: SCD, EHW, MJA

Administrative, technical and material support: SCD

Study supervision: SCD, EHW, MJA, MLKT

Conflict of Interest:

Michael Abramson holds investigator-initiated grants for unrelated research from Pfizer and Boehringer-Ingelheim; undertaken an unrelated consultancy and received assistance with conference attendance from Sanofi; and received a speaker's fee from GSK. JLP has received a travel grant from Boehringer-Ingelheim.

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Table 1. Baseline characteristics of participants at age 44 years stratified by age-of asthma onset

Characteristics	Overall	Late-onset[†]	Early-onset[†]	P[‡]
	N=201	N=73	N=120	
Asthma Persistence	115 (57%)	31 (42%)	78 (65%)	0.002
Male Gender	143 (42%)	22 (30%)	63 (53%)	0.002
Atopy[†]	152 (76%)	49 (67%)	98 (83%)	0.011
Smoking Status				0.254
Non-smoker	98 (49%)	31 (43%)	64 (53%)	
Ex-smoker	54 (27%)	20 (27%)	31 (26%)	
Current smoker	49 (24%)	22 (30%)	25 (21%)	
Season of attendance in the laboratory study				0.444
Winter	84 (42%)	26 (49%)	46 (38%)	
Summer	17 (9%)	5 (7%)	11 (9%)	
Autumn	49 (24%)	15 (21%)	34 (29%)	
Spring	51 (25%)	17 (23%)	29 (24%)	
BMI[†]				0.593
Underweight (BMI <18.5kg/m^2)	1 (0.5%)	1 (1%)	0	
Normal (18.5kg/m^2 ≤ BMI < 25kg/m^2)	54 (27%)	20 (28%)	33 (28%)	
Overweight (25kg/m^2 ≤ BMI < 30kg/m^2)	80 (40%)	29 (40%)	48 (40%)	
Obese I&II (30kg/m^2 ≤ BMI < 40kg/m^2)	59 (29.5)	21 (29%)	34 (28%)	
Obese III (BMI ≥ 40kg/m^2)	6 (3%)	1 (2%)	5 (4%)	
Asthma severity[†¶]				0.132
Asymptomatic, intermittent	55 (36%)	15 (27%)	39 (43%)	
Mild persistent	45 (29%)	20 (35%)	23 (25%)	
Moderate-severe persistent	54 (35%)	21 (38%)	29 (32%)	
Medication[†§]				
Regular OCS/ICS/LABA use in the past 12 months	53 (30%)	16 (24%)	34 (33%)	0.216
Regular OCS use in the past 12 months	3 (2%)	1 (2%)	2 (2%)	0.752
ICS use in the past 1 month	45 (27%)	13 (21%)	30 (31%)	0.156
ICS use in the past 12 months	50 (30%)	15 (24%)	32 (33%)	0.213
OCS use for asthma attacks/flare-ups in the past 12 months	3 (2%)	1 (2%)	2 (2%)	0.752
CRP concentration (mean ± SD in µg/ml) [†§]	4.47 ± 8.16	4.87 ± 6.15	4.44 ± 6.15	0.243
FVC pre-bronchodilator (mean ± SD in L) [§†]	4.31 ± 0.92	4.33 ± 0.92	4.29 ± 0.88	0.425
FEV$_1$/FVC ratio pre-bronchodilator (mean ± SD %) [§†]	74 ± 7.06	73 ± 7.15	75 ± 7.14	0.249

FVC post-bronchodilator (mean ± SD in L) †	4.32 ± 0.91	4.30 ± 0.92	4.34 ± 0.85	0.337
FEV_1/FVC ratio pre-bronchodilator (mean ± SD %) †	77 ± 6.79	75 ± 6.93	79 ± 6.47	0.650

† Missing data in 8 participants for onset-age of asthma, 2 participants for atopy, 8 participants for age-of-asthma onset, 1 participant for BMI, 47 participants for asthma severity, 22 participants for regular OCS/ICS/LABA use in the past 12 months, 55participants for OCS use in the past 12 months, 34 participants for ICS use in the past 1 month, 37 participants for ICS use in the past 12 months, 55 participants for OCS use for asthma attacks/flare-ups in the past 12 months, 142 for CRP, 132 for pre-bronchodilator FVC and FEV1, 133 for post-bronchodilator FVC and FEV1.

‡ P-values from Pearson tests for categorical variables and t tests for numerical variables.

§ OCS = oral corticosteroids; ICS = inhaled corticosteroids; LABA = long acting $beta_2$ agonist; CRP= C reactive protein; FVC= forced vital capacity; FEV1= forced expiratory volume in one second.

¶ Asthma severity defined by frequency of asthma symptoms and attacks/flare ups: Asymptomatic, intermittent (no symptoms in the last month, less than 3 attacks in the past 12 months); mild persistent (symptoms less than once a week, attacks less than once a month); moderate-severe persistent (weekly to daily symptoms, monthly to persistent attacks

Table 2. Associations between serum cytokines concentrations and asthma persistence from age 44 years to 53 years

Cytokines†	Asthma Persistence from age 44 years to 53 years								
	Unadjusted OR (95%CI)	P	Adjusted OR‡ (95%CI)	P	Stratified Analysis				P for interaction§
					Early-onset aOR‡ (95% CI)	P	Late-onset aOR‡ (95% CI)	P	
	N=201		N=197		N=118		N=70		N=189
IL-4	2.19 (1.03, 4.63)	0.041	2.65 (1.19, 5.90)	0.017	4.01 (1.24, 13.0)	0.020	2.30 (0.27, 9.36)	0.245	0.567
IL-5	1.52 (0.71, 3.28)	0.282	1.66 (0.73, 3.77)	0.224	1.58 (0.54, 4.63)	0.409	2.16 (0.40, 11.6)	0.369	0.638
IL-6	2.00 (0.97, 4.11)	0.060	2.06 (0.96 ,4.43)	0.064	5.91 (1.64, 21.3)	0.007	0.77 (0.24, 2.50)	0.669	0.015
IL-8	1.18 (0.59, 2.39)	0.630	1.25 (0.58, 2.68)	0.572	1.06 (0.35, 3.25)	0.919	2.33 (0.60, 9.11)	0.223	0.584
IL-10	1.05 (0.55,2.03)	0.877	1.04 (0.52, 2.08)	0.902	1.48 (0.59, 3.76)	0.406	0.92 (0.23, 3.78)	0.913	0.447
TNF-α	1.00 (0.52, 1.92)	0.991	0.98 (0.50, 1.93)	0.952	1.21 (0.50, 2.92)	0.672	0.97 (0.26, 3.56)	0.960	0.806

† Serum cytokine concentrations, high levels (2^{nd} to 5^{th} quintiles) versus low levels (1^{st} quintile)

‡ Adjusted for atopy, BMI, season, sex, smoking measured at age 44 years

§ P values from likelihood ratio tests, comparing models with and without age-of asthma onset as the effect modifier