# Blood cytokine profiles – potential biomarkers for asthma persistence into adulthood?

# Authors:

Wei Chern Gavin Fong, MSc 1,2, Latha Kadalayil, PhD 1,2, Laurie Lau, PhD 2, Ramesh J Kurukulaaratchy, DM 1,2, S Hasan Arshad, DM 1,2.

# Institutions

1The David Hide Asthma and Allergy Research Centre, Isle of Wight, United Kingdom.
2Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.

# Conflict of interest statementThe authors declare that they do not have any relevant conflicts of interest.

# Corresponding author

**Professor Hasan Arshad**
The David Hide Asthma and Allergy Research Centre, Isle of Wight, United Kingdom.
& School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, United Kingdom.
Level F, South Academic Block, Southampton General Hospital
Southampton SO16 6YD, United Kingdom
Telephone: +44 (0)23 81203366
Email: S.H.Arshad@soton.ac.uk

# Funding

This work was funded by the National Institutes of Health [grant numbers R01 AI061471, R01HL082925, R01 AI091905, and R01 HL132321], Asthma UK for 10 years assessment (Grant no. 364. PI: S.H. Arshad), and the David Hide Asthma and Allergy Research Trust for 26 years assessment (PI: S.H. Arshad). The funders did not contribute to study design, data collection, analysis, interpretation of data or in the preparation of the manuscript

**Keywords:** asthma; cohort studies; asthma persistence; longitudinal studies; respiratory hypersensitivity; cytokines; biomarker; predictor

Word count text: around 1000 words
Tables/ Figures: 3

To the Editor,

The persistence of asthma into adulthood is thought to occur in 35-65% of children with asthma and is associated with poorer outcomes including impaired lung function (1). Predicting asthma persistence in an outpatient setting using biomarkers could help personalize care with a view to improving clinical outcomes. Cytokines may be viable candidate biomarkers as they can be measured in the peripheral blood and are fundamental to the immunobiology of asthma. While there have been cross-sectional evaluations of cytokines in asthma (2), there have been limited longitudinal assessments. This study aimed to characterize the relationship between serum cytokine levels in adolescence (10 years) and in young adulthood (18 years) with subsequent asthma persistence, using longitudinal data from the Isle of Wight birth cohort.

Briefly, children (N=1456) were enrolled at birth and followed up at ages 1 (94.4%), 2 (84.5%), 4 (83.6%), 10 (94.3%), 18 years (90.2%), and 26 years (70.9%) (3). Additionally, at age 10 (N=282) and age 18 (N=398), serum samples of participants were analysed for measurement of cytokine concentrations: Interleukin-5 (IL-5) and Interferon-gamma (IFN-g). IL-5/IFN-g ratio was also examined as a surrogate marker of Th1/ Th2 skewing. Relevant clinical characteristics of the study participants with cytokine data are in Table 1. Cytokines were log-transformed for analysis. Asthma was defined as having a physician diagnosis of asthma and either the presence of wheezing or requirement for asthma medications in the last 12 months. Case definition for persistent asthma was; asthma present at 10 to 18, 18 to 26, or 10 to 26 years of age. Controls were defined as no asthma or non-persistent asthma; (asthma at one time point but not at the following time point, eg: 10 but not at 18 or 18 but not 26 years). Atopy was defined as having a positive skin prick test. Detailed study methodology is described elsewhere (3). Multivariable logistic regression modelling was performed to assess the relationship between log-transformed cytokines and asthma, adjusting for Sex (dichotomous), Maternal smoking (dichotomous, questionnaire at birth), Family (maternal, paternal or sibling) history of atopic disease (dichotomous, questionnaire at birth) and Cord IgE levels (>0.5kU/L, dichotomous). Subgroup analyses were also conducted using the subjects’ atopic state at age 10 and 18 for stratification. Statistical analyses were conducted using Stata (version 15, College Station, TX). Logistic regression results were presented as adjusted odds ratios (aOR) and 95% confidence intervals (CI). P-values <0.05 were regarded as statistically significant.

At age 10, 18 (6.4%) of 282 individuals with cytokine data had asthma, of whom 61.1% (n=11) had persistent asthma to age 18 while 6 (30%) had persistent asthma up to age 26. In multivariable analysis, a higher serum IL-5/IFN-g ratio at age 10 was associated with asthma persistence between ages 10 to 18 (Table 1) [aOR (95% CI): 4.06 (1.24, 13.25), P= 0.020] and with asthma persistence from age 10 to age 26 (Table 2) [aOR (95% CI): 3.62 (1.10, 11.94), P=0.034]. No other statistically significant associations were observed.

At age 18, 14 (10.3%) of 398 participants with cytokine data had asthma of whom 58.5% (24) had persistent asthma to age 26. A higher serum IL-5 at age 18 was independently associated with asthma persistence between 18-26 [aOR (95% CI): 2.29 (1.11, 4.72), P= 0.024] in multivariable analysis. No other significant associations were observed.

Subgroup analyses at age 18 found that the association between higher IL-5 levels at age 18 with asthma persistence from age 18-26 was only seen in atopic individuals [aOR (95% CI): 4.72 (1.32, 16.95), P=0.017], but not in non-atopic participants (Table 3). Conversely, subgroup analyses at 10 found no association between cytokines and asthma persistence from age 10-18, and ages 10-26 in atopic and non-atopic participants (Table 3).

Sensitivity, specificity, false positives (FP) and false negatives (FN) were estimated at different concentrations of IL-5 at 18 years as cut-offs to design a preliminary IL-5 test to predict asthma persistence. The best IL-5 cut-off was selected based on the diagnostic odds ratio (DOR) which incorporates sensitivity, specificity, FP and FN into a single metric to evaluate the test performance (4). In the 80 individuals analysed for persistence from 18 to 26 years (range of IL-5 at age 18: 0.05 to 8.86 pg/mL, median: 0.34 pg/mL), a IL-5 cut-off of 0.45 pg/mL had the highest DOR (Sensitivity: 69%, Specificity: 69%, FP: 31%, FN: 31%, DOR: 4.93) and therefore is the best cut-off to predict asthma persistence based on our data.To our knowledge, this is the first report to describe the longitudinal relationship between serum cytokines and asthma during adolescence and adulthood. Our findings highlight the potential utility of peripheral blood IL-5, IFN-g and the IL-5/IFN-g ratio as biomarkers to predict asthma persistence in adolescents and young adults. One of the key findings is that a higher IL-5/IFN-g ratio is seen in adolescents who have persistent asthma into adulthood. This observation is in keeping with our current understanding that skewing towards T2 inflammation is central in asthma, especially asthma in adolescence which is predominantly allergic (5). The role of T2 inflammation is also highlighted by our subgroup analyses, which showed that the associations between cytokines and disease persistence were only present in our atopic subjects from 18 to 26 years. Indeed, it has previously been shown that in atopic asthmatics, a lower Th1/Th2 ratio, as measured in IFN-g/IL-4, corresponded with disease severity (6).

Our other key finding was that higher serum IL-5 levels at age 18 were associated with increased odds of persistent asthma between 18-26. Indeed, we showed that serum IL-5 levels above 0.45 pg/mL had clinically meaningful sensitivity and specificity in predicting asthma persistence between these ages. These findings corroborate current understanding, whereby IL-5 is a known key mediator of asthma, particularly T2 asthma (7). IL-5 is central in enabling eosinophil survival, differentiation, maturation, migration and proliferation, and thereby is likely to be implicated in asthma persistence. One question arising from our findings is whether early treatment with anti-IL-5 / anti-IL-5R monoclonal antibody therapies could therefore facilitate subsequent disease remission (8). The significance surrounding IL-5 in our data and asthma persistence contrasted with the findings of a recent report by the Tasmanian health study, which found IL-4, but not IL-5, to be associated with asthma persistence. However, that group focused on asthma in middle-age, and its persistence further into adulthood, which may explain the different findings (9).

Our study’s strength lies in its prospective, longitudinal nature, multiple follow-ups, high retention, and that we used standard, physician diagnosis for asthma, reducing bias. The study is limited by the relatively small number of subjects with cytokine measures, especially at 10 years of age, which limits the statistical power of this study and may have led to possible false-negative results. However, key, consistent messages emerged which merit further research to determine the utility of serum cytokines as biomarkers for asthma persistence.

In conclusion, peripheral blood IL-5 and IL-5/IFN-g ratio may be useful biomarkers to predict the persistence of asthma into adulthood. However, larger prospective studies are needed to further elucidate the utility of cytokines for such purposes.

# Authors:

Wei Chern Gavin Fong, MSc 1,2, Latha Kadalayil, PhD 1,2, Laurie Lau, PhD 2, Ramesh J Kurukulaaratchy, DM 1,2, S Hasan Arshad, DM 1,2.

# Institutions

1The David Hide Asthma and Allergy Research Centre, Isle of Wight, United Kingdom.
2Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.

# Conflict of interest statementThe authors declare that they do not have any relevant conflicts of interest.

# Corresponding author

**Professor Hasan Arshad**
The David Hide Asthma and Allergy Research Centre, Isle of Wight, United Kingdom.
& School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, United Kingdom.
Level F, South Academic Block, Southampton General Hospital
Southampton SO16 6YD, United Kingdom
Telephone: +44 (0)23 81203366
Email: S.H.Arshad@soton.ac.uk

# Author contributions

Wei Chern Gavin Fong: Formal Analysis (equal), Methodology (equal), Writing – Original Draft Preparation (lead), Writing – review and editing (equal), Visualization (equal). Latha Kadalayil: Formal Analysis (equal), Visualization (equal), Methodology (equal), Writing – review and editing (equal). Laurie Lau: Writing – review and editing (equal), Investigation (lead). Ramesh Kurukulaaratchy: Supervision (supporting), Methodology (supporting), Writing – review and editing (equal). Hasan Arshad: Conceptualization (lead), Formal Analysis (supporting), Funding Acquisition (lead), Supervision (lead), Writing – review and editing (equal).

# References

1. Arshad SH, Raza A, Lau L, Bawakid K, Karmaus W, Zhang H, et al. Pathophysiological characterization of asthma transitions across adolescence. Respiratory Research. 2014 Nov 29;15(1):1–11.

2. Waserman S, Nair P, Snider D, Conway M, Jayaram L, McCleary LM, et al. Local and systemic immunological parameters associated with remission of asthma symptoms in children. All Asth Clin Immun. 2012 Oct 8;8(1):16.

3. Arshad SH, Holloway JW, Karmaus W, Zhang H, Ewart S, Mansfield L, et al. Cohort Profile: The Isle Of Wight Whole Population Birth Cohort (IOWBC). International Journal of Epidemiology. 2018 Aug 1;47(4):1043–1044i.

4. Glas AS, Lijmer, JG, Prins MH, Bonsel GJ, Bossuyt MMP, The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiology. 2003, 56:1129-1135.

5. Kurukulaaratchy RJ, Matthews S, Arshad SH. Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease. Allergy. 2005;60(10):1280–6.

6. Leonard C, Tormey V, Burke C, Poulter LW. Allergen-induced cytokine production in atopic disease and its relationship to disease severity. Am J Respir Cell Mol Biol. 1997 Sep;17(3):368–75.

7. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. Immunity. 2019 Apr 16;50(4):975–91.

8. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti‐IL5 therapies for asthma. Cochrane Database of Systematic Reviews [Internet]. 2017 [cited 2021 May 17];(9). Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD010834.pub3/full

9. Zhang J, Walters EH, Tang MLK, Lowe AJ, Lodge CJ, Bui D, et al. Serum cytokine concentrations and asthma persistence to middle age. Allergy. 2020;75(11):2985–8.

# Tables

|  |  |
| --- | --- |
| **Clinical characteristics** | **Percentage (%), frequency (n)** |
| **Asthma (yes)** |
| Age 10 | 6.4% (18) |
| Age 18 | 10.3% (41) |
| **Persistent asthma (yes)** |  |
| Age 10 to 18 | 3.9% (11) |
| Age 18 to 26 | 6.2% (24) |
| Age 10 to 26 | 2.1% (6) |
| **Atopic (yes)** |
| Age 10 | 13% (36) |
| Age 18 | 26.2% (102) |
| **Sex, Female** |
| Age 10 | 53.5% (151) |
| Age 18  | 51.8% (206) |
| **Maternal smoking, Yes** |
| Age 10 | 17.6% (49/) |
| Age 18 | 19.1% (75/) |
| **Cord IgE, high (≥0.5 kU/L)** |
| Age 10 | 10.9% (25) |
| Age 18 | 10.6% (33) |
| **Family history of atopic disease** **(asthma, eczema, rhinitis, food allergy)** |
| Age 10 | 60.7% (170) |
| Age 18 | 62.7% (247) |

**Table 1. Clinical characteristics of the study participants with cytokine data.**

|  |  |  |  |
| --- | --- | --- | --- |
| Cytokines  | Cases/ subjects | aOR (95% CI) | P-value |
| **Asthma persistence** |
| **Age 10-18** |
| IFN-g at 10 years | 7/216 | 0.27 (0.04, 1.82) | 0.201 |
| IL-5 at 10 years | 8/218 | 2.01 (0.85, 4.80) | 0.114 |
| IL-5/IFN-g ratio at 10 years | 7/216 | 4.06 (1.24, 13.25) | **0.020** |
| **Age 18-26** |
| IFN-g at 18 years | 16/232 | 1.41 (0.50, 4.00) | 0.518 |
| IL-5 at 18 years | 16/237 | 2.29 (1.11, 4.72) | **0.024** |
| IL-5/IFN-g ratio at 18 years | 16/228 | 1.90 (0.85, 4.26) | 0.115 |
| **Age 10-26** |
| IFN-g at 18 years | 6/232 | 0.70 (0.13, 3.81) | 0.680 |
| IL-5 at 18 years | 6/235 | 2.41 (0.99, 5.89) | 0.052 |
| IL-5/IFN-g ratio at 18 years | 6/232 | 3.62 (1.10, 11.94) | **0.034** |

Table 2. Longitudinal evaluation of the associations between IL-5, IFN-g and IL-5/ IFN-g ratio ratio with asthma persistence. Cytokines measurements were log transformed for analysis. . Case definition for persistent asthma was; asthma present at 10 to 18, 18 to 26 years or 10-18-26 years of age. Controls were defined as no asthma or non-persistent asthma; (asthma at one time point but not at the following time point, eg: 10 but not at 18 or 18 but not at 26 years). Models for persistence from age 10-18 and age 18-26 were adjusted for sex (male/female), maternal smoking (yes/no), family history of atopic disease (yes/no) and cord IgE levels (binary, ≥0.5kU/L). Models for persistence from age 10-26 were only adjusted for sex (male/female) and family history of atopic disease (yes/no). aOR: Adjusted odds ratio. 95% CI: 95% confidence intervals. IFN-g: Interferon-gamma. IL-5: Interleukin 5.

|  |  |  |  |
| --- | --- | --- | --- |
| Cytokines  | Cases/ subjects | aOR (95% CI) | P-value |
| **Asthma persistence, age 10-18**  |
| **Atopic individuals** |
| IFN-g at 10 years | 4/32 | 1.49 (0.06, 34.30) | 0.803 |
| IL-5 at 10 years | 4/32 | 2.45 (0.32, 19.11) | 0.391 |
| IL-5/IFN-g ratio at 10 years | 4/32 | 1.71 (0.29, 10.06) | 0.552 |
| **Non-atopic individuals** |
| IFN-g at 10 years | 6/228 | 1.21 (0.21, 7.00) | 0.832 |
| IL-5 at 10 years | 7/231 | 2.13 (0.83, 5.49) | 0.117 |
| IL-5/IFN-g ratio at 10 years | 6/228 | 2.30 (0.61, 8.72) | 0.219 |
| **Asthma persistence, age 18-26**  |
| **Atopic individuals** |
| IFN-g at 18 years | 13/77 | 2.34 (0.63, 8.69) | 0.206 |
| IL-5 at 18 years | 13/80 | 4.72 (1.32, 16.95) | **0.017** |
| IL-5/IFN-g ratio at 18 years | 13/76 | 1.73 (0.59, 5.10) | 0.322 |
| **Non-atopic individuals** |
| IFN-g at 18 years | 6/221 | 0.48 (0.09, 2.65) | 0.396 |
| IL-5 at 18 years | 6/225 | 1.26 (0.39, 4.05) | 0.697 |
| IL-5/IFN-g ratio at 18 years | 6/228 | 1.75 (0.56, 5.49) | 0.338 |
| **Asthma persistence, age 10-26**  |
| **Atopic individuals** |
| IFN-g at 10 years | 3/30 | 0.70 (0.03, 17.55) | 0.827 |
| IL-5 at 10 years | 3/30 | 10.84 (0.68, 73.73) | 0.092 |
| IL-5/IFN-g ratio at 10 years | 3/30 | 8.41 (0.54, 129.68) | 0.127 |
| **Non-atopic individuals** |
| IFN-g at 10 years | 3/200 | 1.43 (0.15, 13.97) | 0.756 |
| IL-5 at 10 years | 3/202 | 2.24 (0.72, 7.02) | 0.165 |
| IL-5/IFN-g ratio at 10 years | 3/200 | 2.57 (0.49, 13.45) | 0.262 |

Table 3. Subgroup analyses of the longitudinal associations between IL-5, IFN-g and IL-5/ IFN-g ratio with asthma persistence, stratified by atopic status. Cytokines measurements were log-transformed for analysis. Case definition for persistent asthma was; asthma present at 10 to 18, 18 to 26 years or 10-18-26 years of age. Controls were defined as no asthma or non-persistent asthma; (asthma at one time point but not at the following time point, eg: 10 but not at 18 or 18 but not 26 years). Atopy was defined as having a positive skin prick test. All models were adjusted for sex (male/female) and family history of atopy (yes/no). aOR: Adjusted odds ratio. 95% CI: 95% confidence intervals. IFN-g: Interferon-gamma. IL-5: Interleukin 5.