PEER REVIEW HISTORY

This paper was submitted to the THORAX but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Asthma and Risk of Non-Respiratory Tract Infection: A Population-Based Case Control Study
AUTHORS	Juhn, Young; Bang, Duk; Yang, Hyeon; Ryoo, Eell; Al-Hasan, Majdi; Lahr, Brian; Baddour, Larry; Yawn, Barbara

VERSION 1 - REVIEW

REVIEWER	Shearer, William Baylor College of Medicine, Immunology Allergy Rheumatology
REVIEW RETURNED	15-Jul-2013

GENERAL COMMENTS	Bang et al. describe the epidemiologic association between asthma and blood stream infections with community-acquired E.coli. This new association between atopic disease and systemic blood infection is quite a discovery that is important for both allergists and clinical immunologists. Although these discoveries by Dr. Young Juhn's research team do not prove causation, the growing number of such associations strongly suggests an immunologic mechanism of immunodysregulation as witnessed in patients with primary immunodeficiency that have asthma and atopic dermatits (e.g. Wiskott Aldrich syndrome) and secondary immunodeficiency (e.g. HIV-infected patients that have asthma and atopic dermatitis). 1. Regarding the analysis in this manuscript, what would the p values be in Table 3 if the Greenland entry criteria were much more restrictive at P<0.10? 2. Would path analysis be helpful in determining the total interactions of independent association with all variables?

REVIEWER	Johnston, Sebastian Imperial College London, Airway Disease Infection Section, National Heart and Lung Insitute
REVIEW RETURNED	17-Jul-2013

GENERAL COMMENTS	This manuscript reports a case control study investigating the hypothesis that persons with asthma may have an increased risk of bacterial infections outside the respiratory tract. The hypothesis is novel, the question is important, the study design is limited as the number of cases is only 259. However the authors have carried out a power calculation and the findings appear reasonably robust given

	the limitations in the study design. COMMENTS The authors can reference their work considerably better than they have done. For example line 97 should refer to the manuscripts by Talbot et al New England Journal, Klemets et al in Thorax and Pilishvili et al in Paediatrics. Line 99 This should refer to manuscripts reporting impaired innate interferon production in asthma, For example Wark et al Journal of Experimental Medicine 2005, Contoli et al Nature Medicine 2006 and those reporting impairment in induction of Th1-related cytokines namely Laza Stanca et al PLOS Pathogens 2011, Message et al PNAS 2008, Plummeridge et al Thorax 2000, Ho et al Chest 2002. The manuscripts by Contoli, Message and Ho are of particular relevance as these involved stimulation of cells with LPS and induction of Th1 cytokines in response to this gram negative stimulus. My major concerns with the data presented are summarised in table 2 where it is shown that there is no hint of a suggestion of increased risk of Ecoli infection with allergic rhinitis and atopic dermatitis which are both strongly linked with asthma. I would expect at least a trend in the same direction to be demonstrable, but there is none. The authors need to identify this as a weakness and discuss it accordingly. My other major concern is the extent of comorbid illness in this population. 7% had alcohol addiction, 12% had choric renal insufficiency, 7% had congestive heart failure, 6% had dementia, 20% coronary artery disease, 19% diabetes mellitus, 10% immunosuppressive therapy, 8% malignancy and 11% recurrent urinary tract infection. These are clearly not run of the mill asthmatics and this is further emphasised by their average age which is 61 years. The authors need to recognise this weakness in their data and discuss it appropriately. I would also like to see whether there is a trend for increased Ecoli in younger asthmatics without comorbid conditions. The authors should present analysis of this sub-group and discuss the robustness of their findings in the light of this sub-group analysis.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. “Bang et al. describe the epidemiologic association between asthma and blood stream infections with community-acquired E.coli. This new association between atopic disease and systemic blood infection is quite a discovery that is important for both allergists and clinical immunologists. Although these discoveries by Dr. Young Juhn's research team do not prove causation, the growing number of such associations strongly suggests an immunologic mechanism of immunodysregulation as witnessed in patients with primary immunodeficiency that have asthma and atopic dermatits (e.g. Wiskott Aldrich syndrome) and secondary immunodeficiency (e.g. HIV-infected patients that have asthma and atopic dermatitis).”

Response: We appreciate the reviewer’s comments.

2. “Regarding the analysis in this manuscript, what would the p values be in Table 3 if the Greenland entry criteria were much more restrictive at P<0.10?”

Response: Determining suitable entry criteria based on p-value in statistical modeling has been subject to a debate.(1, 2). Because we used a more conservative entry criteria (p=0.2) which allows a greater number of variables to be entered into our multivariate model than the suggested stringent criteria (p=0.1), the results are unlikely to be affected and statistical significance of the main association (or even effect size) can be rather enhanced. We hope this response is suitable in addressing this concern.

3. “Would path analysis be helpful in determining the total interactions of independent association with all variables?”

Response: We concur with the reviewer’s point on a more rigorous analysis such as path analysis to discern the interrelationship among significant variables.

Our study may be limited to perform path analysis since it may be difficult to take into account individual comorbid conditions, which are important variables in interpreting the results but have a small sample size and have different biological background. In addition, we believe that at the current conceptual stage, the first step would be to determine the association between asthma status and risk of non-respiratory tract infection such as community acquired E coli blood stream infection (BSI). As a next step, we will attempt to identify the immunologic mechanisms underlying the association between asthma and risk of E coli BSI and discern the interrelationship among other risk factors through path analysis based on a future study with a larger sample size. We appreciate the reviewer’s excellent comments.

Reviewer: 2

1. ‘This manuscript reports a case control study investigating the hypothesis that persons with asthma may have an increased risk of bacterial infections outside the respiratory tract. The hypothesis is novel, the question is important, the study design is limited as the number of cases is only 259. However the authors have carried out a power calculation and the findings appear reasonably robust given the limitations in the study design.”

Response: We appreciate the reviewer’s comments.

2. “The authors can reference their work considerably better than they have done. For example line 97 should refer to the manuscripts by Talbot et al New England Journal, Klemets et al in Thorax and Pilishvili et al in Paediatrics--- This should refer to manuscripts reporting impaired innate interferon production in asthma, For example Wark et al Journal of Experimental Medicine 2005, Contoli et al Nature Medicine 2006 and those reporting impairment in induction of Th1-related cytokines namely Laza Stanca et al PLOS Pathogens 2011, Message et al PNAS 2008, Plummeridge et al Thorax 2000, Ho et al Chest 2002. The manuscripts by Contoli, Message and Ho are of particular relevance as these involved stimulation of cells with LPS and induction of Th1 cytokines in response to this gram negative stimulus.”

Response: We incorporated all references into the revised manuscript.

3. “My major concerns with the data presented are summarized in table 2 where it is shown that there is no hint of a suggestion of increased risk of E coli infection with allergic rhinitis and atopic dermatitis which are both strongly linked with asthma. I would expect at least a trend in the same direction to be

demonstrable, but there is none. The authors need to identify this as a weakness and discuss it accordingly."

Response: The reviewer raises an important concern. We were also puzzled by the study findings on the lack of associations of allergic rhinitis or atopic dermatitis with risk of E coli BSI. We can interpret this finding in two ways.

First, this lack of associations of allergic rhinitis or atopic dermatitis with risk of E coli BSI might be stemming from biological differences from asthma because allergic rhinitis and atopic dermatitis share similar biological background with asthma but have not necessarily shown the same epidemiologic features as asthma.(3-9) These differential epidemiologic features among different atopic conditions might be due to complex interaction (effect modification) by genetic and environmental factors.(10)

Alternatively, we suspect that this lack of association of allergic rhinitis and atopic dermatitis with risk of BSI can be due to misclassification bias since ascertainment of allergic rhinitis and atopic dermatitis by a physician diagnosis can be less specific and objective than that for asthma, potentially resulting in significant errors leading to a null hypothesis. However, food allergy is relatively more specific and objective than allergic rhinitis and atopic dermatitis since it frequently relies on lab test (food sensitization test). Our study results show food allergy was, indeed, associated with risk of E coli BSI in univariate analysis.

We included a brief summary of the above response in the manuscript and hope our response addresses the reviewer's concern.

4. "My other major concern is the extent of comorbid illness in this population. 7% had alcohol addiction, 12% had choric renal insufficiency, 7% had congestive heart failure, 6% had dementia, 20% coronary artery disease, 19% diabetes mellitus, 10% immunosuppressive therapy, 8% malignancy and 11% recurrent urinary tract infection. These are clearly not run of the mill asthmatics and this is further emphasized by their average age, which is 61 years. The authors need to recognize this weakness in their data and discuss it appropriately."

Response: We acknowledge the reviewer's concern. We discussed this limitation in the Discussion section of the revised manuscript. Briefly, the prevalence of comorbid conditions in our study subjects are relatively high due to relatively an older age group of study subjects as the reviewer pointed out. As discussed in the Discussion section (the first paragraph of page 15), the prevalence of common chronic conditions (e.g., coronary heart disease: 15%) in our study was similar to the national average (7.1-19.8% depending on age).

In addition, because of the concern that the reviewer raised, we adjusted the main results on the association between asthma and risk of E coli BSI for all significant comorbid conditions individually instead of collectively (i.e., comorbid conditions as a binary variable: any vs. none).

However, we do acknowledge and caution readers for careful interpretation.

5. "I would also like to see whether there is a trend for increased Ecoli in younger asthmatics without comorbid conditions. The authors should present analysis of this sub-group and discuss the robustness of their findings in the light of this sub-group analysis."

Response: We acknowledge the reviewer's comments. We assessed the interaction term between asthma and age in relation to risk of E coli BSI. The p-values for testing a significant interaction between asthma and categorized age were as follows: p=0.285 for age cutoff of 65 years (i.e., ≥65 vs. <65 years), p=0.958 for age cutoff of 40 years (i.e., ≥40 vs. <40 years), and p=0.417 for age cutoffs of

40 years and 65 years (i.e., <40, 40-65, vs. >65 years). As a result, we have no evidence of a differential asthma effect on risk of E coli BSI across age strata. We summarized the results in the revised manuscript in the Results and Discussion section.

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