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Relationship between asthma status and antibody response pattern to 23-valent pneumococcal vaccination

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ABSTRACT

Objective: Asthma poses an increased risk for serious pneumococcal disease, but little is known about the influence of asthma status on the 23-valent serotype-specific pneumococcal antibody response. We examined differences in antibody titers between pre- and post-vaccination with 23-valent pneumococcal polysaccharide vaccine (PPSV-23) in relation to asthma status. **Methods:** Asthma status was retrospectively ascertained by the Predetermined Asthma Criteria in an existing vaccine cohort through comprehensive medical record review. Twenty-three serotype-specific pneumococcal antibody titers measured at baseline and 4–6 weeks post-vaccination were analyzed. Vaccine responses to PPSV-23 were calculated from pre- to post-vaccine titers for each of the serotypes. **Results:** Of the 64 eligible and enrolled subjects, 18 (28%) had asthma. Controls (i.e., subjects without asthma) demonstrated a statistically significant fold change response compared to their baseline for all serotypes, while those with asthma did not mount a significant response to serotypes 7F, 22F, and 23F. The overall vaccine response as measured by fold change over baseline was lower in subjects with asthma than controls. **Conclusions:** Poorer humoral immune responses to PPSV-23 as measured by fold change were more likely to be observed in subjects with asthma compared to controls. We recommend the consideration of asthma status when interpreting vaccine response for immune competence workup through larger studies. Further studies are warranted to replicate these findings.

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Introduction

Immune response to 23-valent pneumococcal polysaccharide vaccine (PPSV-23) is routinely employed to assess immune competence for evaluation of immune deficiencies [1]. While the guidelines exist regarding the administration and interpretation of the testing, there are many aspects to this assessment that make a comprehensive application to all populations difficult [1]. For example, the pattern of response to the various serotypes shows individual differences and may depend on multiple factors. Thus, a global overview of the immune response is challenging to ascertain. Additionally, asthma is associated with an increased risk of infections caused by *Streptococcus pneumoniae* infections [2]. For example, there is mounting evidence suggesting that subjects with asthma are at a

significantly increased risk of invasive pneumococcal disease, and the population-attributable risk percentage for asthma is 11% to 17% [2–4]. In this regard, the assessment of vaccine response in a background of asthma not only has implications related to interpretation, but it also helps clinicians have a better understanding of the mechanisms underlying the increased propensity to pneumococcal infections in subjects with asthma.

Studies evaluating pneumococcal titers have demonstrated the role of concurrent comorbidities in immune responses to pneumococcus. A recent study consisting of 16 subjects with asthma and 14 controls demonstrated that 44% of the subjects with asthma had 12 or more positive serotype-specific polysaccharide antibodies, whereas 86% of control subjects had

12 or more positive serotype-specific polysaccharide antibodies [5]. These results suggest that subjects with asthma may show pneumococcal antibody responses distinct from those of controls, although there are studies reporting otherwise [6]. Therefore, the consideration of the comorbid status may be a necessary caveat for interpretation of the results. However, limitations such as a cross-sectional design, lack of baseline antibody titers or reliance on *a priori* designated cutoffs (e.g. an adequate "protective" response to each pneumococcal serotype defined as a titer equal to or greater than 1.3 µg/mL antibody titers or as conversion of 70% of the serotypes tested with at least a 2-fold increase in the titers [1]) may hamper such conclusions to be made [5–7]. Additionally, dichotomizing continuous variables [e.g. >1.3 µg/mL vs. ≤1.3 µg/mL] facilitates analyses but may do so at the expense of loss of information such as pre-vaccination titer or magnitude of antibody responses [6,8].

In order to study the influence of asthma status on immune responses to PPSV-23 in adults, we conducted a wide supervised and unsupervised analysis in a vaccine cohort and determined differences in antibody titers between pre- and post-vaccination with 23-valent pneumococcal polysaccharide vaccine (PPSV-23) and fold change of each of the serotypes in relation to asthma status. The results of this *exploratory* study provide an insight into the relationship between asthma status and pneumococcal vaccine response.

Methods

Study design

This was a *retrospective* analysis of vaccine response in a convenience sample of subjects who were enrolled in a previous prospective vaccine cohort study for the evaluation of pneumococcal vaccine response. The vaccine cohort study was approved by the institutional review boards at the Mayo Clinic, MN and Olmsted Medical Center, MN.

Subjects

Vaccine response data were obtained from residents of Olmsted County, Minnesota, who were recruited for a vaccine cohort study. These subjects were individuals who were self-defined as 'healthy' and met the following inclusion/exclusion criteria. Subjects were excluded if they: (1) had previous or current diagnosis of an immunodeficiency (primary and secondary); (2) had previous or current diagnosis of a

rheumatological disorder (Rheumatoid arthritis, Lupus, Sjögren, and vasculitis), cancer (chronic lymphocytic leukemia, non-Hodgkin lymphoma, and B-cell malignancy), diabetes, active infection (pneumonia, otitis media, HIV, and EBV), renal disease (nephrotic syndrome and protein losing enteropathy), or other chronic diseases (multiple sclerosis, etc); (3) had current or previous use (within the last 6 months) of systemic/inhaled corticosteroids, captopril, fenclofenac, sulfasalazine, or other immunosuppressive agents (cyclosporin, methotrexate, and mycophenolic acid), anti-convulsants (phenytoin and carbamazepine), gold, d-penicillamine, or anti-malarials (quinine, chloroquine, and hydroxychloroquine); (4) had previous pneumococcal vaccination; or (5) were pregnant. Enrollment was delayed two weeks if subjects had signs and/or symptoms suggestive of upper respiratory infections or viral illness.

Plasma 23 serotype-specific pneumococcal antibody measurement

Twenty-three serotype-specific pneumococcal antibody titers (IgG) were measured using a fluorescent, multiplex bead-based immunoassay at Mayo Clinic Clinical Immunology Laboratory at baseline (before administration of PPSV-23) and 4–6 weeks post-vaccination [9,10]. Changes in the post-vaccination titers over pre-vaccine levels were calculated as fold-changes for each of the serotypes.

Ascertainment of asthma

We retrospectively applied asthma ascertainment criteria on the enrolled subjects. Those subjects who fulfilled the Predetermined Asthma Criteria (PAC) in Table 1 were considered to have asthma, and those who did not meet the criteria were categorized as control subjects. These criteria for asthma have been widely used in asthma research in the past [3,5,11–15] and were found to have high reliability [16,17]. For example, both epidemiological [3,14,15,18] and laboratory studies [19–21] using the PAC have consistently demonstrated the associations of asthma status (as either exposure or outcome) with environmental factors, genetic factors, and/or the risk of serious infections (i.e., pneumococcal diseases). Apart from the advantages basing asthma status on the PAC as an asthma criterion (e.g., 1) minimizing ascertainment bias for asthma status by using International Classification of Diseases [ICD] codes or self-report and 2) availability of index date of asthma better defining temporality), our greatest rationale for using the PAC in the present study is its consistency and

Table 1. Ascertainment criteria used to define asthma in the cohort.

Patients were considered to have *definite* asthma if a physician made a diagnosis of asthma and/or if each of the following 3 conditions were present.

Patients were considered to have *probable* asthma if the first 2 of the following 3 conditions were present:

1. History of cough with dyspnea and/or wheezing, OR history of cough and/or dyspnea plus wheezing on examination
2. Substantial variability in symptoms from time to time or periods of weeks or more when symptoms were absent
3. Two or more of the following:
 - Sleep disturbance caused by nocturnal cough and wheeze,
 - Nonsmoker (14 y or older)
 - Nasal polyps
 - Blood eosinophilia higher than 300/ μ L
 - Positive wheal and flare skin tests OR elevated serum IgE level
 - History of hay fever or infantile eczema OR cough, dyspnea, and wheezing regularly on exposure to antigen
 - Pulmonary function tests showing one FEV₁ or FVC < 70% predicted and another with at least 20% improvement to an FEV₁ of higher than 70% predicted OR a methacholine challenge test showing $\geq$ 20% decline in FEV₁
 - Favorable clinical response to bronchodilator

Patients were excluded from the study if any of these conditions were present:

- Pulmonary function tests that showed FEV₁ to be consistently below 50% predicted or diminished diffusion capacity
- Tracheobronchial foreign body at or about the incidence date
- Hypogammaglobulinemia (IgG less than 2.0 mg/mL) or other immunodeficiency disorder
- Wheezing occurring only in response to anesthesia or medications
- Bullous emphysema or pulmonary fibrosis on chest radiograph
- PiZZ α_1 -antitrypsin
- Cystic fibrosis
- Other major chest disease, such as juvenile kyphoscoliosis or bronchiectasis

FVC, forced vital capacity; and FEV₁, forced expiratory volume in one second.

coherence of our earlier work based on the PAC which showed increased risk of pneumococcal diseases and impaired humoral immune responses associated with asthma status defined by the PAC [3,5,11,12,18–21]. We have included both definite and probable asthma according to the criteria because most cases of probable asthma (85%) become definite over time [17,22]. For the laboratory studies such as this, we included those who had asthma diagnosis by clinicians in addition to meeting the PAC in order to make sure they have true asthma [19–21].

Bipartite network visualization

The continuous data on baseline titers for each of pneumococcal serotypes was used for the generation of the network graph. This is one of only a few suitable unsupervised agnostic data analyses taking into account baseline titers, individual serotypes, and individual fold changes of antibody responses. Data were scaled using a min-max algorithm (feature scaling) using the formula,

$$x' = \frac{x - \min(x)}{\max(x) - \min(x)}$$

where x' is the scaled value for pneumococcal antibody titers, x is the original value, $\min(x)$ is the minimum value for that variable across all subjects, and $\max(x)$ is the maximum value for that variable across all subjects. This standardizes the vaccine response of each serotype and preserves the proportionality of response for each serotype from 0 to 1. The data were then converted into a format suitable for use in Gephi (www.Gephi.org), an open-source software for graph

visualization and network analysis [23,24]. A bipartite graph was generated, following which force directed algorithm was applied to reveal inherent patterns in data since these algorithms allow for strongly connected nodes to come closer together and weakly connected nodes to move away from each other in the graph based on mathematical relationships.

The results of bipartite network (the term bipartite refers to a network with two nodes, i.e., subjects and serotype) were expressed in a graphical format consisting of three main network basics: nodes, edges (i.e. lines connecting between nodes), and measures of location such as centrality (which pertain to the relative spatial properties of the network). The network in our study is formed by a set of nodes connected in pairs by edges and edges can connect only nodes from different sets of nodes (i.e., edges connect only subjects and serotype-specific titers; edges neither connect subjects to subjects nor serotypes to serotypes). The edges reflect the relation between subjects and their serotype-specific titers at baseline (the shorter the edges, the stronger relation between subjects and serotype-specific titers) and also graphically result in clusters of related entities with the resultant measure of centrality. We used weighted degree centrality (WDC) as a centrality measure since it is a relative value derived from the total number and magnitude of connections to a node. Using baseline titers in a continuous variable format as a basis for graphical analysis, we modeled fold increase response in each subject across all serotypes in relation to asthma. Cumulative fold-changes for all serotypes and subjects

were calculated and scaled by a factor of 0.1 and 0.5, respectively for graphical overlay in the network. Node size in our analysis represents fold-change from pre-vaccination to post-vaccination values with larger nodes depicting higher fold-changes and vice versa. Asthma status was then overlaid on the subject's nodes to assess location in the graph.

Statistical analysis

Characteristics of subjects with and without asthma were compared using the Wilcoxon rank-sum test to examine statistical differences for continuous variables between groups and the Chi-square/Fisher's exact tests were used for categorical variables where appropriate. For each serotype, the Wilcoxon signed-rank test was used for paired analysis of pre and post vaccination. The Benjamini-Hochberg method was used for multiple testing corrections. All statistical tests were two-sided and statistical significance was determined at a p values <0.05 . Analyses were performed by using JMP 10 (SAS Institute Inc., Cary, NC).

Results

Characteristics of study subjects

The study enrolled 64 eligible subjects; of whom, 31 (48%) were male, 58 (91%) Caucasian, the mean age ($\pm$ SD) 43.1 years ($\pm$ 13.8), and 18 (28%) had a history of asthma. The demographic and clinical characteristics of the subjects with and without asthma are presented in Table 2. There were no differences in the clinical variables except that the incidence of other atopic diseases was higher in the subjects with asthma than in control subjects.

The baseline (pre-vaccine) and post-vaccine pneumococcal antibody titers and asthma status

Subjects with asthma tended to demonstrate higher baseline titers in certain serotypes (Table 3). However, none of these serotype comparisons met the significance threshold ($p \leq 0.05$) after adjustment for multiple comparisons (Benjamini-Hochberg). The numbers of serotype titers greater than the threshold of 1.3 $\mu\text{g/mL}$ were not significantly different between subjects with and without asthma at baseline (17.5 [12–20.5] vs. 14.5 [8–20]). For post-vaccination titers, the subjects with asthma had a trend of higher median values in certain serotypes (Table 4). Again, none of the serotype comparisons met the significance threshold after adjustment for multiple comparisons (Benjamini-Hochberg).

Table 2. Characteristics of the participants.

Variable	Subjects with asthma ($n = 18$, 28%)	Subjects without asthma ($n = 46$, 72%)	p value*
Age at baseline, Median (IQR**)	41.5 (28.8–51.0)	43.5 (32.5–57.0)	0.43
Sex			0.69
Female, n (%)	10/18 (56)	23/46 (50)	
Race/Ethnicity			1.00
White, n (%)	16/18 (89)	42/46 (91)	
Non-White, n (%)	2/18 (11)	4/46 (9)	
Years of Education***			0.80
Median (IQR)	16 (13.5–16.5)	16 (14.0–17.0)	
Any atopic condition (atopic dermatitis and/or allergic rhinitis), n (%)	16/18 (89)	21/44 (48)	<0.01

* p values was calculated by χ^2 test for categorical variables or Wilcoxon Rank Sum test for continuous variables between groups.

**IQR, Inter-quartile range between 25th and 75th percentile.

***Missing education information for two subjects without asthma.

Table 3. Comparison of serotype specific pre-vaccine (baseline) titers between subjects with and without asthma.

Serotype	Subjects with asthma		Subjects without asthma		p value ^a
	Median ($\mu\text{g/mL}$)	IQR	Median ($\mu\text{g/mL}$)	IQR	
1	2.65	1.07–9.20	1.25	0.15–3.40	0.029
2	2.25	0.90–5.40	1.15	0.30–2.70	0.052
3	2.60	0.50–5.20	1.20	0.70–2.10	0.067
4	0.35	0.17–1.12	0.20	0.05–0.75	0.223
5	4.65	3.50–11.10	3.75	1.67–9.50	0.167
6B	5.95	1.30–17.07	2.80	1.40–5.47	0.162
7F	9.65	5.52–24.15	4.95	1.47–11.47	0.013
8	5.60	2.50–13.77	1.45	0.77–4.45	0.004
9	5.80	2.75–14.07	1.90	0.80–6.00	0.008
9V	2.65	1.17–7.42	2.00	0.67–6.00	0.160
10A	8.20	4.52–24.52	2.90	1.05–11.02	0.021
11A	1.25	0.45–2.82	0.90	0.50–2.32	0.488
12F	0.85	0.20–4.37	0.30	0.10–1.02	0.037
14	3.20	1.37–12.22	2.00	0.60–7.20	0.422
15B	1.35	0.70–2.92	0.90	0.30–2.15	0.159
17F	8.15	4.15–34.50	7.05	1.50–14.15	0.180
18C	0.65	0.17–1.85	1.10	0.30–1.82	0.418
19A	6.35	1.47–16.15	3.65	1.40–9.67	0.234
19F	3.15	1.65–12.07	3.75	1.55–11.17	0.916
20	2.30	1.07–5.85	1.30	0.47–3.70	0.053
22F	19.10	8.87–56.87	6.95	2.90–23.17	0.021
23F	12.85	4.97–37.52	6.80	2.00–15.60	0.105
33F	2.10	0.87–12.82	1.25	0.60–2.90	0.066

^aUnadjusted.

IQR: Inter-quartile range between 25th and 75th percentile.

Fold-change response to pneumococcal antibody titers and asthma status

The change in the titers of pneumococcal serotypes expressed as a ratio of post-vaccination titers over pre-vaccination titers was calculated (Table 5). Fold change response to serotype 9 and 23F was higher in control subjects than those with asthma, but comparisons did not meet the significance threshold after adjustment for multiple comparisons (Benjamini-Hochberg). Median fold-change was lower in the subjects with asthma compared to control subjects in 18 of 23 serotypes and the subjects with asthma did not

Table 4. Comparison of serotype specific post-vaccine titers between subjects with and without asthma.

Serotype	Subjects with asthma		Subjects without asthma		<i>p</i> value ^a
	Median (μg/mL)	IQR	Median (μg/mL)	IQR	
1	9.65	3.77–47.85	6.30	2.90–31.42	0.496
2	7.15	4.00–24.55	2.70	1.50–13.15	0.064
3	5.20	2.97–10.75	5.85	2.07–9.95	0.708
4	2.70	1.10–7.20	1.55	0.50–4.42	0.193
5	13.75	8.00–28.15	6.45	2.97–23.05	0.103
6B	23.50	3.77–69.90	9.60	3.10–28.22	0.122
7F	11.50	5.82–26.70	5.15	3.42–16.25	0.031
8	14.85	6.72–35.80	7.00	3.75–20.05	0.052
9	15.05	5.87–25.25	13.65	5.15–26.22	0.697
9V	7.85	2.87–17.10	4.95	2.22–19.67	0.627
10A	17.15	5.20–80.92	7.45	2.07–24.77	0.019
11A	4.10	2.67–15.90	5.05	1.60–18.32	0.952
12F	1.45	0.77–10.25	0.95	0.30–2.85	0.113
14	13.6	4.07–49.65	14.50	2.97–47.70	0.904
15B	7.30	3.65–20.12	11.10	3.97–32.50	0.455
17F	23.90	10.07–67.0	9.75	4.72–29.97	0.070
18C	6.30	2.12–20.20	8.40	1.80–19.27	0.805
19A	24.55	5.42–176.35	19.45	4.17–76.52	0.420
19F	11.0	3.92–27.62	12.85	4.05–30.27	0.799
20	6.85	2.9–17.77	4.40	1.65–19.87	0.317
22F	21.8	10.42–68.20	11.10	3.65–31.35	0.041
23F	12.1	4.5–61.7	10.80	2.87–32.25	0.390
33F	9.35	3.27–24.22	7.20	1.95–20.82	0.535

^aUnadjusted.

IQR: Inter-quartile range between 25th and 75th percentile.

Table 5. Comparison of fold changes in response to PPV-23 vaccine between asthmatics and non-asthmatics.

Serotype	Subjects with asthma		Subjects without asthma		<i>p</i> value ^a
	Median	IQR	Median	IQR	
1	4.48***	1.75–9.67	5.81***	3.22–20.65	0.147
2	2.15**	1.14–25.98	4.41***	1.76–7.62	0.335
3	2.03***	1.45–4.69	3.90***	1.65–10.75	0.153
4	7.71***	2.70–13.43	6.50***	2.00–50.93	0.748
5	1.87**	1.09–3.99	1.64***	1.17–3.47	0.822
6B	3.82***	1.20–6.18	2.39***	1.23–7.30	0.632
7F	1.08	0.98–1.32	1.25***	0.97–1.85	0.201
8	1.76***	1.40–4.44	4.25***	1.39–9.36	0.167
9	1.58**	1.16–3.17	4.10***	1.46–9.34	0.016
9V	2.02***	1.29–3.02	2.95***	1.44–6.40	0.169
10A	1.24**	0.99–2.72	1.26***	1.08–3.23	0.725
11A	3.93***	1.62–8.39	4.45***	2.66–10.72	0.463
12F	1.43**	1.00–3.79	2.12***	1.15–4.75	0.353
14	2.51***	1.35–7.83	2.57***	1.10–10.71	0.681
15B	6.12***	2.52–12.99	10.06***	3.50–32.59	0.143
17F	2.10*	1.15–3.48	2.05***	1.31–6.62	0.464
18C	10.82***	6.17–17.93	8.85***	3.75–20.20	0.424
19A	3.21***	1.18–14.4	4.46***	1.59–14.18	0.560
19F	1.81***	1.39–8.17	2.47***	1.31–7.72	1.0
20	2.53**	1.23–4.74	3.05***	1.16–11.06	0.259
22F	1.07	1.0–1.32	1.16*	0.98–1.63	0.424
23F	1.0	0.94–1.17	1.21**	0.99–2.51	0.023
33F	2.03***	1.01–8.16	4.13***	1.97–11.42	0.067

^aUnadjusted.Superscript asterisk denotes statistical significance of fold change (i.e. pre-vaccine to post vaccine with a group using paired Wilcoxon Signed-Rank Test). **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

IQR: Inter-quartile range between 25th and 75th percentile.

p values in the last column is comparison between groups (non-asthmatics vs. asthmatics).

mount a significant response to serotypes 7F, 22F, or 23F.

A global unsupervised overview of baseline titers and fold-changes by network visualization

This bipartite network graph is a mathematical representation of a global overview of patterns among individual patients, serotypes, and fold-changes of pneumococcal antibody responses taking into account the baseline data. The topology of the network demonstrated a core-periphery relationship of the nodes. The nodes in the center depict the strongly connected nodes. For example, the nodes representing the subjects in the center were those with higher baseline titers compared to those in the periphery, which were weakly connected nodes, and thus had lower baseline titers. The size of nodes which depicts fold-changes shows that serotype nodes located in the center are smaller (e.g. 7F or 10A), thus portraying smaller fold-changes, compared to the nodes located in the periphery, portraying higher fold changes (e.g. 15B or 19A). Asthma status was then assigned to subjects in the network using conditional overlay (black nodes = fulfilled asthma ascertainment criteria [i.e. asthma], and white nodes = did not fulfill asthma ascertainment criteria, Figure 1). The majority of the control subjects' nodes (white) were larger and located in the periphery. Overall, maximum fold change (post/prevaccine) was observed for serotypes nodes depicted

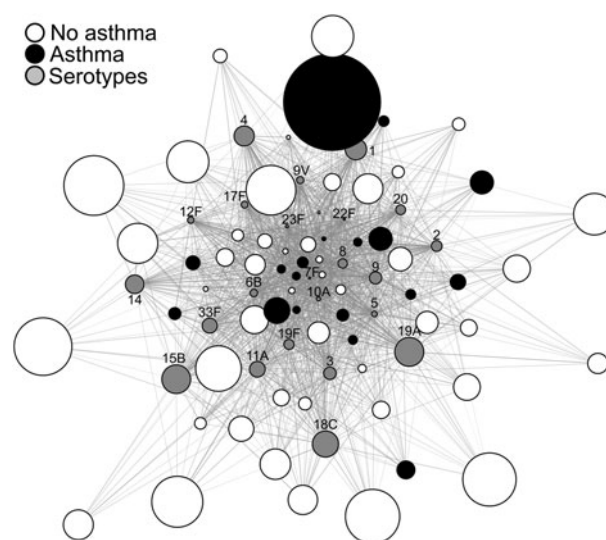


Figure 1. Network graph depicting simultaneously the relationships between basal titers of all 23 serotypes (grey nodes) for all subjects, the vaccine response as fold change (node sizes) for summative patterns of pneumococcal antibody responses in control subjects (white) to those with asthma (black).

by the greatest node sizes (15B, 18C, and 19A), which were closer to the white nodes (control subjects). Thus, this method allowed us to simultaneously examine the relationship of pre-vaccination titers of all 23 serotypes for all 64 subjects, including the vaccine response as fold changes (thus incorporating the post-vaccine response as well) and their asthma status in the network for integrated patterns of pneumococcal antibody responses.

In addition to the visual inspection, we further wished to confirm the network pattern using traditional analytic approaches. Central position in the network is defined by high WDC reflecting pre-vaccine titers. In the highest quartile of WDC, there were proportionally more subjects with asthma (43.7%, 7/16); thus, confirming their position in the center of the network. Meanwhile, the lowest quartile (Q1) of WDC included 12.5% of the subjects with asthma (2/16) compared to controls, thereby confirming their peripheral position. Additionally, the median node size (reflecting the cumulative fold change) in control subjects was 90.7 (interquartile range [IQR] 53.2 to 147.1) compared to those with asthma (50.4 [IQR 36.6 to 85.8], $p = 0.03$), corroborating the larger node sizes (i.e., fold change) in the control subjects taking into account the WDC or pre-vaccine titers.

Discussion

The aim of this exploratory study was to determine the vaccine response patterns to PPSV-23 in relation to asthma status among an existing adult vaccine cohort. We found that while individual serotype titers did not differ by asthma status; on a continuous scale, cumulatively poor responses to PPSV-23 vaccine as measured by fold change from baseline were likely to be observed in the subjects with asthma compared to control subjects after taking into account prevaccine titers.

The small fold change in postvaccine titers in the subjects with asthma may reflect higher baseline antibody titers to pneumococcus, which, on their own, were not significant to discriminate the subjects with asthma from the control subjects. The effect of baseline titers on pneumococcal vaccine response has been previously documented [25]. Also, high colonization rates of pneumococcus have been reported in subjects with asthma [26] and may subsequently influence the baseline titers. In the network analysis of the current study subjects, more subjects with asthma were centrally distributed, supporting the finding of higher baseline antibody titers to pneumococcus in those

with asthma. The conceptual and analytic framework for bipartite networks has been described in the literature [27–30]. The basis for network graph is that networks in biological or social domains are not mathematically random, but follow organizing rules based on data and are reflected in their topological patterns that distinguish them from randomly linked networks [28]. In the presented network, the more centrally located nodes indicate that they have higher WDC and thus have higher baseline antibody titers. In our network analysis, this is how we took into account the effect of baseline titers (i.e., titers before vaccination) in assessing the relationship between pneumococcal antibody response patterns and asthma status. The edges (i.e., lines) represent closeness or relatedness between two entities of interest which are mathematically similar to Euclidean distance measuring a distance between two physical points (i.e., the shorter the edges, the closer the points or entities). Taken together, our global network analyses results suggest that poor responders to PPSV-23 vaccine as measured by fold change from baseline titers were more likely to be observed in the subjects with asthma compared to control subjects taking into account prevaccine titers.

Previous studies have examined pneumococcal responses in children with asthma [31] and atopic children [32]. For example, Lee et al [31] reported that children with asthma showed suboptimal antibody responses to PPSV-23, compared to controls. Furthermore, Arkwright et al [32] demonstrated that patients with atopic dermatitis had a poorer antibody response to pneumococcal vaccine, compared to those without such condition. These data potentially suggest that suboptimal responses might be an unrecognized characteristic of asthma (i.e., potential endotypes) in a subgroup of subjects with asthma. Corresponding with previous studies, our analysis in adults also indicates poor immune responses to pneumococcal vaccine in the subjects with asthma, but in the context of elevated baseline titers.

Differential baseline titers and supposed colonization patterns between subjects with and without asthma might suggest differential susceptibility and immune functions between the two groups with regard to pneumococci. Immune mechanisms underlying suboptimal antibody response to PPSV-23 in the subjects with asthma are unknown. Pneumococcal polysaccharide is a T-cell – independent type II (TI-2) antigen. Recently, it has been demonstrated that neutrophils in the marginal zone lymphoid follicles produce cytokines that drive isotype class-switching of

marginal zone B cells to produce IgG antibodies against polysaccharide antigens without T cell help [33]. Despite the fact that antibody responses to TI-2 antigens are regarded as T-cell independent, T-B cell interactions may still be required to produce optimal responses to pneumococcal polysaccharide antigens [34,35]. In this respect, asthma has a Th2-predominant immune environment, which has been reported to suppress humoral immune responses to pneumococci in humans and mice [11,36]. The literature and our study findings invite a question as to the possibility of interaction of asthma status, pneumococcal infection or colonization, humoral immune responses, and their clinical implications. Future large-scale prospective studies are needed to address these questions.

Greatest changes (i.e., antibody responses) across all the subjects were observed for serotype 19A, 15B, 18C, 4, and 1, which represent the top quartile of serotypes for cumulative fold changes. On the other hand, the fold change was least for 23F, 22F, and 7 serotypes, suggesting that there exists a reduced responsiveness to these serotypes in our study population. Additionally, only the subjects with asthma did *not* mount a statistically significant response to serotypes 7F, 22F, and 23F, likely due to their high baseline titers while all control subjects did. While this finding may suggest the prevailing colonization and herd immunity, the nature of this finding needs to be studied by addressing the following questions: whether the differential antibody responses among various serotypes suggest intrinsic differential immunogenicity of serotypes [37]; and whether asthma status or pneumococcal epidemiology influences these questions, given the large proportion of subjects affected by asthma in the United States.

This *unsupervised* part of the study is also an attempt to analyze the pneumococcal vaccine response data without *a priori* assigned *arbitrary cutoffs* (2-fold, 4-fold, 1.3 $\mu\text{g/mL}$, 70% or more of pneumococcal serotypes, etc.) as the current clinical recommendations are not designed to incorporate multiple aspects of this complex assay at the same time (e.g. number of serotypes, baseline values, cutoffs for positivity for individual serotypes, cutoffs for protectivity, and fold changes for optimal response) [1,7,25,38–40]. This analysis simultaneously takes into account many of these variables in an integrated manner which utilizes a high level overview as a supplement to the current analysis method of pneumococcal vaccine response. This analytic approach might be a useful tool in the field of asthma and vaccine research in the future.

Nevertheless, the present study has limitations. First, the study may lack sufficient power to differentiate individual serotype differences between subjects with and without asthma due to a relatively small number of subjects; therefore, a reliable conclusion may be difficult to be made. Recognizing the lack of sufficient power, the differences were visualized in the global network. We speculate that these findings need to be replicated in a prospective study with a large sample size. Second, we used a convenience sample of subjects enrolled in a previous prospective vaccine cohort study; therefore, the current study may not represent the general population. Although we speculate that our study subjects may be reasonable for the aim of this exploratory study, the study findings need to be replicated in future large general population-based studies with random sampling method. Third, information on pulmonary function was not available on all subjects since the asthma ascertainment was made retrospectively based on the predefined criteria as shown in Table 1. Fourth, even though a fold change was the robust discriminating factor of asthma status in this analysis, this in itself may not inform of underlying mechanisms since it was likely dependent on collective factors influencing the baseline values of vaccine titers and immune responses. Fifth, no data on immunological markers (e.g., B cell functions or Th1 and Th2 cytokines) or colonization patterns were measured. Finally, we excluded those who had used systemic or inhaled corticosteroids, thus limiting the asthma severity to mild or intermittent. How these findings would relate to those with severe asthma warrants further studies.

This study has the following strengths. First, serotype-specific antibody responses to PPSV-23 vaccines were measured before and after vaccination in a US cohort in order to ascertain fold increases in each serotype-specific antibody. Second, we used conventional analytical approaches and supplemented with a global network approach based on the raw vaccine response data (without arbitrary cutoffs or *a priori* categorization) in order to explore the relationship between fold changes in vaccine response and asthma status. Networks have been increasingly used to analyze global patterns in data including cytokines, drugs-targets, symptoms, microbiome data, etc. and to offer a parallel view of the experimental data which supplements conventional analysis [41–45]. Third, for the *unsupervised* analysis, we did not use *a priori* cutoff points [1,5,7,11,25,38,40] and based the current analysis on quantitative data alone in order to generate the network with the final overlay of asthma

status. Thus, the present findings have a few important clinical implications: (1) clinicians should be aware that the subjects with asthma may not demonstrate a robust response to pneumococcal vaccine, (2) such inability to mount a robust PPSV-23 vaccine response might be an unrecognized feature of asthma, and (3) these findings provide evidence that the inclusion of asthma status might affect interpretation of immune competence assessment utilizing PPSV-23 vaccine.

Conclusion/key findings

Poorer responses to PPSV-23 as measured by a fold increase were more likely to be observed in the subjects with asthma. Our study results deserve further investigation with a large sample size to identify the clinical and immunological characteristics of subjects with asthma with poor immune responses to pneumococcal or other vaccines.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. Part of this analysis was presented at the ERS meeting in 2015.

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