Original Article

Increased Respiratory Viral Detection and Symptom Burden Among Patients with Primary Antibody Deficiency: Results from the BIPAD Study

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What is already known about this topic? Immunoglobulin replacement therapy is a central treatment for patients with primary antibody deficiency, with a major impact on lower airway infections; however, recurrent upper airway infections remain a major challenge despite individualized immunoglobulin replacement therapy dosing.

What does this article add to our knowledge? This is the largest upper airway infection study in primary antibody deficiency over 12 months with controls and comparative national sentinel sampling data. It identifies recurrent chronic upper respiratory infection and symptoms from a restricted pathogen subset, despite frequent use of prophylactic antibiotics and individualized immunoglobulin replacement therapy dosing.

How does this study impact current management guidelines? The study impacts current guidelines by the identification of the key pathogens, immunologic and social risk factors, the effect of prophylactic antibiotics, and the requirement for novel treatments to address this unmet clinical need.

BACKGROUND: Patients with primary antibody deficiency (PAD) are at increased risk of respiratory tract infections, but our understanding of their nature and consequences remains limited.
OBJECTIVE: To define the symptomatic and microbial burden of upper airway infection in adults with PAD relative to age-matched controls.
METHODS: Prospective 12-month observational study consisting of a daily upper and lower airway symptom score alongside fortnightly nasal swab with molecular detection of 19 pathogen targets.
RESULTS: A total of 44 patients and 42 controls (including 34 household pairs) were recruited, providing more than 22,500 days

of symptom scores and 1,496 nasal swabs. Swab and questionnaire compliance exceeded 70%. At enrollment, 64% of patients received prophylactic antibiotics, with a 34% prevalence of bronchiectasis. On average, patients with PAD experienced symptomatic respiratory exacerbations every 6 days compared with 6 weeks for controls, associated with significant impairment of respiratory-specific quality-of-life scores. Viral detections were associated with worsening of symptom scores from a participant's baseline. Patients with PAD had increased odds ratio (OR) for pathogen detection, particularly viral (OR, 2.73; 95% CI, 2.09-3.57), specifically human rhinovirus (OR, 3.60; 95% CI, 2.53-5.13) and parainfluenza (OR, 3.06; 95% CI, 1.25-7.50). *Haemophilus influenzae* and

Streptococcus pneumoniae were also more frequent in PAD. Young child exposure, IgM deficiency, and presence of bronchiectasis were independent risk factors for viral detection. Prophylactic antibiotic use was associated with a lower risk of bacterial detection by PCR.
CONCLUSIONS: Patients with PAD have a significant respiratory symptom burden associated with increased viral infection frequency despite immunoglobulin replacement and prophylactic antibiotic use. This highlights a clear need for future therapeutic trials in the population with PAD, and informs future study design. © 2020 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). (J Allergy Clin Immunol Pract 2021;9:735-44)

Key words: Immunodeficiency; Primary antibody deficiency; Hypogammaglobulinemia; Respiratory health; Quality of life; Bronchiectasis; Rhinovirus; COVID-19

INTRODUCTION

Patients with primary antibody deficiency (PAD) are at increased risk of sinopulmonary infections. Antibody replacement therapy decreases the risk of mortality and pneumonia, but recurrent minor infections remain common.[1-3] Decline in pulmonary function and progression to bronchiectasis in some have been reported despite immunoglobulin replacement therapy (IgRT).[4,5] Lung involvement in PAD is a major determinant of quality of life, and development of structural and functional lung impairment is predictive of mortality.[6] This has spurred optimization of our therapeutic approach, including individualization of IgG replacement dose and method to infection frequency[7] and long-term low-dose macrolide prophylaxis.[8] A number of studies have drawn attention to respiratory viral infections in the setting of PAD.[9-12] These have been limited by size,[10] retrospective design,[9] and lack of a control group,[9,11] and vary in approach to the management of PAD. The most detailed report to date includes 6210 days of data and 54 nasal swabs, profiling the UK winter season.[11] Thus, the nature, seasonality, and impact of these recurrent infections in a PAD cohort receiving optimized IgRT and high rates of macrolide prophylaxis remain poorly characterized. Greater understanding of the characteristics and pathogen spectrum of these infections experienced by antibody-deficient patients will help define the limitations of current treatments and develop improved therapies.

Study aims

We set out to characterize the burden of infection in people with PAD and age-matched contacts, by recording pathogens detected by fortnightly nasal swabbing and daily symptoms of respiratory tract infection, collected over 52 weeks. Primary aims were to establish the feasibility of this approach and define how patients with PAD differed from nonimmunodeficient controls in terms of symptom severity and duration, as well as frequency of pathogen detections. Secondary aims were to investigate possible relationships between symptoms, pathogen detections, and immunocompetence to support risk stratification of patients. Finally, we used sentinel screening data gathered over the same period to contextualize the pattern of microbial detections against pathogens circulating in the wider community.

METHODS

Participants

Patients were recruited from the Immunodeficiency Centre for Wales, Cardiff, if they had a diagnosis of PAD and had commenced immunoglobulin replacement for more than 3 months, with trough IgG level greater than or equal to 5 g/L. Healthy controls 18 years or older were recruited from household contacts of participants with PAD (where possible) or independently (including medical staff). Participants were excluded if they were younger than 18 years, were unable to provide informed consent, or secondary causes of hypogammaglobulinemia were identified.[13] All participants provided written informed consent. The study was approved by the Greater Manchester Research Ethics Committee (15/NW/0379).

Study design

For this observational prospective study, participants were asked to record symptoms of respiratory tract infection on a daily basis for a 52-week period. Participants also completed spirometry alongside the 22-item Sino-Nasal Outcome Test and St George's Respiratory Questionnaires at study entry, representing validated multidimensional measures of respiratory health status and quality of life concerning the upper and lower airways, respectively.[14,15] Data collection in the Burden of Infection in Primary Antibody Deficiency (BIPAD) study began in August 2015 and ended in January 2018.

Diary card

Participants were asked to record daily symptoms using a study-specific symptom score (BIPAD-Q) based on the Jackson Scale.[16] This includes upper and lower respiratory tract symptom components. A combined upper and lower airway symptom score of 0 (no symptoms) to 7 (multiple, severe symptoms) was generated by adding the scores for lower and upper respiratory tract symptoms (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Data on antibiotic use, serum immunoglobulin levels, medical history, and demographic characteristics were also extracted from clinical records of individuals with PAD.

Nasal swabbing

Following instruction, patients with PAD performed nasal swabbing every 2 weeks over a 12-month period (marked "routine swab"). Control participants enrolled during the first 6 months of the study were asked to submit additional "acute" nasal swabs only in the event of 2 or more new symptoms. A protocol amendment was passed in April 2016, standardizing all participants to the same routine collection schedule (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). Diaries and swabs were returned by post every fortnight. We have previously shown that

TABLE I. Study flowchart

Study components	Patients with PAD	Controls
Screened, n	68	42
Declined or excluded, n (% screened)	24 (of 68 = 35%)	0
Enrolled, n (% screened)	44 (of 68 = 65%)	42
Household pairs enrolled, n (% group)	34 (68 of 86 = 79%)	
Baseline questionnaire completion (% total expected)		
22-item Sino-Nasal Outcome Test	43 (of 44 = 98%)	39 (of 42 = 93%)
St George's Respiratory Questionnaires	43 (of 44 = 98%)	39 (of 42 = 93%)
Pulmonary function testing	42 (of 44 = 95%)	41 (of 42 = 98%)
Study dropout	7 (of 44 = 16%)	4 (of 42 = 10%)
Total swabs returned, n	870	626
Nasal swabs (2-weekly) compliance (% of total expected)	72% (of 1144 expected)	78% (of 745 expected)
Total daily symptom score data (d)	11,397	11,192
Daily questionnaire compliance (% of total expected)	71% (of 16,016 expected)	73% (of 15,288 expected)

Compliance is shown in parentheses, represented as a % of total expected. For nasal swabs received from controls, this was calculated following protocol amendment.

such methodology is suitable for reliable detection of respiratory viruses.[17]

Molecular analysis of upper respiratory swabs

Respiratory pathogen screening was performed using the NxTAG Respiratory Pathogen Panel (RPP; Luminex, Austin, Texas) targeting influenza A (typed to H1 and H3), influenza B, respiratory syncytial virus (A and B), parainfluenza types 1 to 4, adenovirus, human metapneumovirus, rhinovirus/enterovirus, *Mycoplasma pneumoniae*, and human coronaviruses (OC43, 229E, NL63, and HKU1). A laboratory- defined assay was then used to differentiate enteroviruses from rhinoviruses,[18] and a triplex assay performed targeting of *Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis.*[19] An endogenous control targeting human RNAseP was used to ensure sample integrity. Samples from the study and sentinel surveillance scheme were processed following identical protocols for viral detection testing. Full methodology is available in this article's Online Repository at www.jaci-inpractice.org. Strain-typing of human rhinovirus (HRV) was not performed.

Definitions

Symptomatic respiratory exacerbation (SRE) was defined by a symptom score of 2 or more occurring for 2 or more consecutive days as recorded by the patient, as previously described.[11] The SRE interval represents time elapsed until this score returned below 2. SREs were further defined as nasal swab positive or negative, and by the nature of pathogens detected within. To simplify analysis, pathogens were condensed into 12 types comprising 8 viruses (adenovirus, enterovirus, human metapneumovirus, influenza, parainfluenza, [nonpandemic seasonal] coronavirus, respiratory syncytial virus, and rhinovirus) and 4 bacteria. Child contact was defined as regular contact with children 10 years or younger for over 8 hours a day.

Data handling and statistical analysis

Diaries were transcribed and data curated in Microsoft Excel. Participant and swab data were analyzed in GraphPad Prism version 6.07 (GraphPad Software, San Diego, CA) with student *t* test, Mann-Whitney *U* test, or Fisher exact test as indicated. Symptom and swab data returned were analyzed until point of participant withdrawal. Public Health Wales data were collated in Microsoft Excel. Linear mixed-model fitting, multivariate linear regression, and Cox regression analysis were performed using R (version 3.4.0, R

Foundation, Vienna, Austria). Results were considered statistically significant at *P* less than .05.

RESULTS
Study population

A total of 44 patients with PAD and 42 healthy controls participated in the study, of which 34 pairs cohabited (Table I). PAD incorporated a range of diagnoses (Table II) including common variable immunodeficiency (CVID) (n = 30), specific antibody deficiency (n = 2), and hypogammaglobulinemia (n = 2) failing to fulfill diagnostic criteria for CVID.[20] Other diagnoses associated with humoral dysfunction were also represented including Good's syndrome (n = 2), and genetically defined primary immunodeficiency disease (including X-linked agammaglobulinemia (n = 2), signal transducer and activator of transcription 1-gain of function (n = 1), Wiskott-Aldrich syndrome (n = 1), and CD40 ligand deficiency (n = 1), and genetically undefined combined immunodeficiency (n = 3). Age, sex, and smoking status did not differ significantly between groups. Eleven patients and 15 control participants had regular daily exposure to children younger than 8 years. Unsurprisingly, patients with PAD clearly differed from controls in use of prophylactic antibiotics (56% vs 0%). Azithromycin was most frequently prescribed (16 of 25; see Table E1 in this article's Online Repository at www.jaci-inpractice.org), reflecting the emerging evidence for macrolide prophylaxis in PAD and non-PAD chronic infective respiratory disease.[8]

Feasibility

Ten patients with PAD declined to participate in the study at screening, whereas all healthy controls consented. Seven patients and 4 controls withdrew from the study after completing up to 6 months of study data (overall 13% study population), including 3 PAD and 4 control individuals who provided baseline questionnaire data but did not return swabs. Overall, we received 22,532 of a possible 31,304 days symptom score data (72%) (Table I). Compliance with routine nasal swab return was 72% in patients with PAD and 78% in controls (Table I).

SREs are more frequent in patients with PAD

As a group, patients with PAD clearly differed from controls in respiratory symptoms. We observed 219 SREs in patients

TABLE II. Participants' characteristics at enrollment

Characteristic	Patients with PAD	Controls	P value
Participants enrolled	44	42	—
Age (y), median age (range)	51.5 (21-78)	51.5 (28-77)	.785
Female patients, n (%)	21 (47.7)	25 (59.5)	.398
Bronchiectasis present on CT, n (%)	15 (34.1)	1 (2.38)	**.0002**
Smoking status			.872
Current smoker	3	2	
Ex-smoker	8	11	
Never smoker	33	29	
Prophylactic antibiotic use, n (%)	28 (63.6)	0 (0.0)	**<.0001**
Young child exposure >8 h/d	11 (25.0)	15 (35.7)	.280
Immunoglobulin replacement therapy	44	0	**<.0001**
IVIg	20		
SCIg	20		
fSCIg	4		
IgG trough level* (g/L) (range)	9.75 (6.18-15.3)		
IgA (g/L) (range)	0.06 (0.06-4.1)		
IgM (g/L) (range)	0.18 (0.05-5.1)		
FEV$_1$ (L), (Interquartile range)†	2.60 (1.81-3.17)	2.90 (2.34-3.43)	.07
FEV$_1$ predicted (%)‡ (Interquartile range)†	87.0 (69.8-99.5)	97.0 (88.5-108.5)	**.0005**
Quality of life			
22-item Sino-Nasal Outcome Test§ (interquartile range)	28 (16.0-43.0)	8 (2.0-29.0)	**.0003**
St George's Respiratory Questionnaires‖ (interquartile range)	29.3 (15.6-49.6)	7.89 (4.03-16.0)	**<.0001**

CT, Computed tomography; *fSCIg*, facilitated subcutaneous immunoglobulin; *IVIg*, intravenous immunoglobulin; *SCIg*, subcutaneous immunoglobulin.
Bold indicates statistical significance ($P < .05$).
*Serum IgG level measured immediately before next *IVIg* infusion.
†Unpaired *t* test.
‡Based on the European respiratory guidelines of 1993.
§Sino-Nasal Outcome Test: a validated measure of burden of sinusitis, with 22 items scored between 0 and 5, with total score of 0 (best) and 110 (worst).
‖St George Respiratory Questionnaire: a validated measure of respiratory-related quality of life, scored between 0 (best) and 100 (worst). Median values are shown unless stated;
P values were calculated using the Mann-Whitney test for continuous variables and Fisher exact test for categorical variables unless otherwise stated.

with PAD, compared with 79 SREs reported by controls over 11397- and 11192-day follow-up, respectively. Patients with PAD reported greater daily symptom scores and lower weekly well-being (Figure 1, *A* and *B*). The median duration of a symptom-free period in patients with PAD was only 6 days (95% CI, 4-8), compared with 42 days (95% CI, 25-63) reported by controls (Figure 1, *C*). Length of SREs was comparable between groups (Figure 1, *D*), with median duration in patients with PAD of 4 days (95% CI, 4-6) and of 3 days in controls (95% CI, 3-4). Specific patient subgroups showed a trend toward longer median SRE duration, notably those with CVID: 5 days (95% CI, 4-7 days) and bronchiectasis: 6 days (95% CI, 5-12 days). Some patients experienced prolonged respiratory symptoms, including 2 patients reporting more than 180 days (both CVID, 1 with bronchiectasis). Average 22-item Sino-Nasal Outcome Test and St George's Respiratory Questionnaires scores were 20.5 and 23 points higher than those of controls, respectively, Minimum clinically significant differences have been reported in the context of chronic rhinosinusitis as 9 for the 22-item Sino-Nasal Outcome Test[15] and 4 to 8 for St George's Respiratory Questionnaires in chronic obstructive pulmonary disease.[14] Thus, these 2 validated multidimensional questionnaires appear consistent with a repeated respiratory symptom burden leading to marked impairment of quality of life. Together this indicates a significant and chronic respiratory burden in patients with PAD featuring recurrent symptom episodes, despite medical therapy with prophylactic antibiotics and IgRT.

Pathogen detections within BIPAD

We observed 436 pathogen detections in patients with PAD and 198 in control nasal samples, representing 50% and 32% of total swabs submitted by these groups, respectively. The effect of introducing routine nasal swab sampling for healthy controls was to increase the number of negative swabs (data not shown). Specific pathogen detection rates and odds ratios are summarized in Table III. HRV dominated, with parainfluenza also detectable at a greater rate among patients with PAD. Viral codetection with bacterial species was found at increased frequency in patients with PAD relative to controls, with *H influenzae* accounting for most. Conversely, *M catarrhalis* detection was more common in control participants.

To contextualize seasonality and significance of viral detection within BIPAD, we analyzed patterns of circulating respiratory viruses based on results of swabs sent to the Public Health Wales reference laboratory during the BIPAD study period (Figure 2; see Figure E3 in this article's Online Repository at www.jaci-inpractice.org). Briefly, this encompasses swabs provided by sentinel general practices alert for seasonal influenza illness in the community or tested following respiratory symptoms during inpatient admissions at ward-based or higher-care levels. HRV appears infrequently in BIPAD controls sampled at equivalent intensity to patients with PAD, whereas both HRV and parainfluenza virus are detectable year-round among symptomatic adults tested for respiratory symptoms.
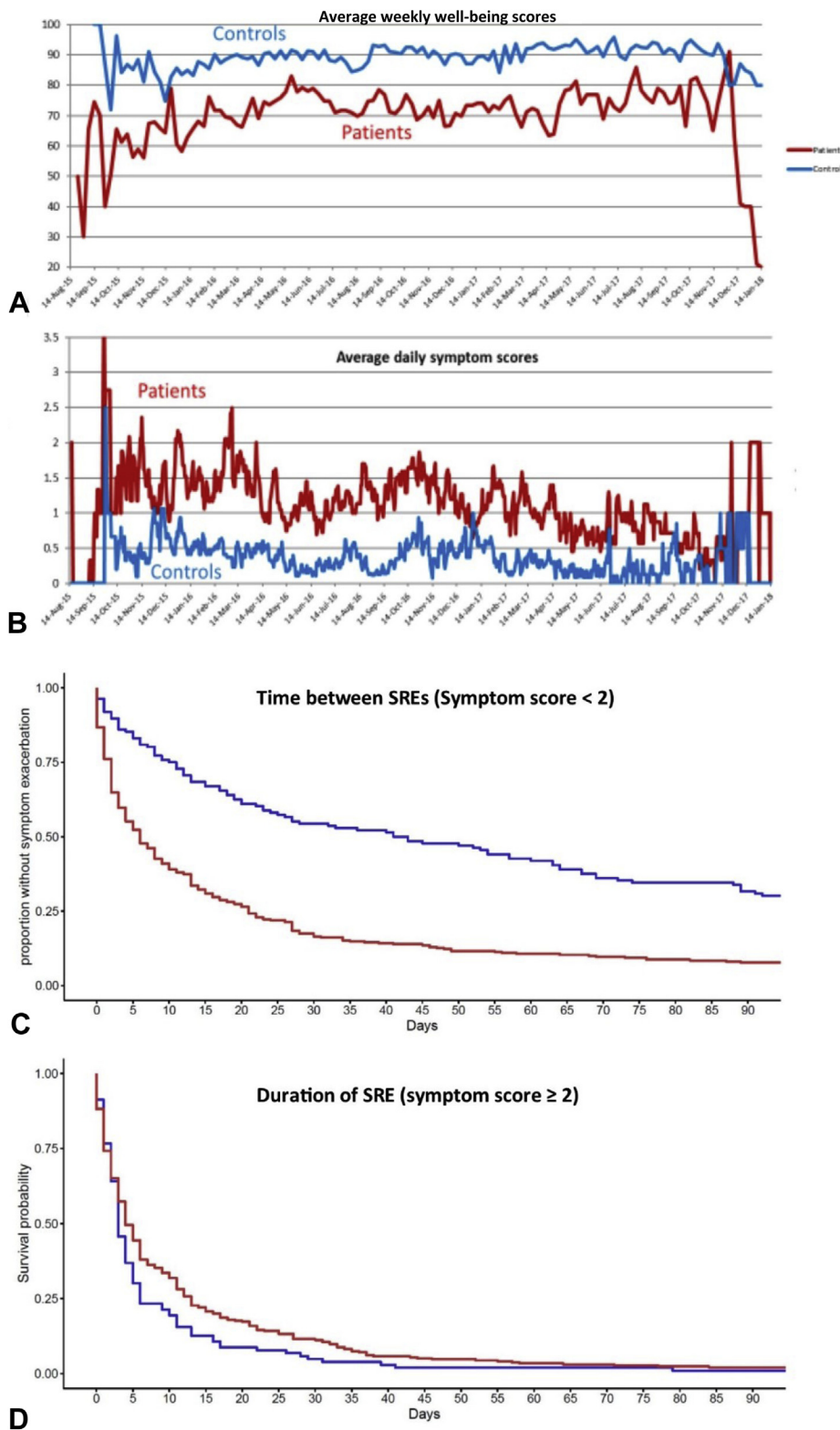
FIGURE 1. Participant-reported symptom scores. Participants self-reported (**A**) overall well-being on a weekly basis using the visual analog scale score (score 0-100, with 100 being best possible) and (**B**) daily respiratory symptom score (BIPAD-Q; a score of 7 denotes multiple and maximal symptoms). (**C**) Duration in days for which participants were without significant respiratory symptoms, approximating to time spent "well." (**D**) Length of time for which each symptomatic exacerbation persists. Patients with PAD in red and controls in blue.

TABLE III. Pathogen detections within BIPAD

Swab number and pathogen detection	Patients with PAD (n = 41)	Controls (n = 38)	Odds ratio (95% CI)	P value (Fisher exact test)
Total number of swabs	870	626	—	—
Total number of pathogen detections per group, positive swab fraction (%)	436 (50.1)	198 ((31.6)	2.17 (1.75-2.69)	<.0001
Viral detection	266 (30.6)	87 (13.9)	2.73 (2.09-3.57)	<.0001
Bacterial detection	279 (32.1)	141 (22.5)	1.62 (1.28-2.06)	<.0001
Dual positive virus and bacteria	109 (12.5)	30 (4.8)	2.85 (1.87-4.32)	<.0001
HRV	179 (20.6)	42 (6.7)	3.60 (2.53-5.13)	<.0001
Coronavirus	27 (3.1)	12 (1.9)	1.64 (0.824-3.26)	.188
Parainfluenza	25 (2.9)	6 (1.0)	3.06 (1.25-7.50)	**.0098**
Human metapneumovirus	16 (1.8)	13 (2.1)	0.883 (0.422-1.85)	.850
Influenza	13 (1.5)	9 (1.4)	1.04 (0.442-2.45)	>.999
Enterovirus	10 (1.1)	6 (1.0)	1.20 (0.434-3.32)	.803
Respiratory syncytial virus	7 (0.8)	3 (0.3)	1.68 (0.434-6.54)	.535
Adenovirus	7 (0.8)	2 (0.3)	2.53 (0.524-12.2)	.319
Hemophilus influenzae	182 (20.9)	28 (4.5)	5.65 (3.74-8.54)	<.0001
Streptococcus pneumoniae	44 (5.1)	16 (2.6)	2.03 (1.14-3.63)	**.0159**
Mycoplasma pneumoniae	0	0	—	—
Moraxella catarrhalis	115 (13.2)	110 (17.6)	0.715 (0.538-0.950)	**.0229**

Bold indicates statistical significance (*P* < .05).

Upper airway pathogen detection is associated with increase in acute symptom score

To define the association between a positive pathogen detection and respiratory symptoms, we first quantified the average daily symptom score for the week of swab sampling, modeling an acute symptom episode as previously described.[21] To account for interindividual variation and the presence of chronically reported symptoms, any change was compared with the individual's 12-month average symptom score. Both patients with PAD and controls showed an increase in symptoms in the 7-day period around a viral detection from their baseline (Figure 3, *A* and *B*). Pathogen-negative swabs were associated with a symptom-free status in controls, and net improvement in symptom scores in patients with PAD (Figure 3, *C*). To compare long-term symptom burdens experienced by patients and controls, we fitted a regression model interacting study groups with positive viral or bacterial detections. To account for unobserved shared exposures and the resulting lack of independence, we included a random effect for each patient-control pair (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). In the absence of pathogen detections, patients had a higher background level of symptoms, comparable to those of control patients during a viral detection. During viral detection, both patients and controls experienced a similar rise in symptom burden. Considering bacterial detections, patients again showed a higher background symptom burden, but little increase in symptom scores when bacteria were detected. Controls showed a small increase when bacteria were detected, but not elevating them to the background level of the patients.

Identification of risk factors for viral and bacterial detection

We next performed multiple linear regression, investigating the frequency of detection of pathogens to identify whether immune or clinical characteristics could help risk-stratify patients, focusing on viral detections given their greater association with respiratory symptoms. In univariate analysis, lower trough level of IgG was associated with increased viral or pathogen-positive detection rates. However, because clinical adjustment of IgG dosing was performed on the basis of infection frequency reported during the protocol, this was excluded from subsequent multivariate analysis (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). Regular contact with young children emerged as the single strongest risk factor for viral detection (adjusted odds ratio, 2.16; 95% CI, 1.45-3.22). This association was robust to inclusion and exclusion of trough IgG level. Interestingly, the presence of bronchiectasis and lower levels of IgM in serum were also independently associated with a greater rate of viral detection. Serum IgA appeared protective in univariate but not multivariate analysis, whereas participant age had no significant impact. Prophylactic antibiotic use was associated with markedly decreased bacterial detection rates in univariate analysis (odds ratio, 0.261; 95% CI, 0.190-0.359), with the effect of prophylactic antibiotics on bacterial detections shown in Figure E4 in this article's Online Repository at www.jaci-inpractice.org.

DISCUSSION

This study provides the most detailed characterization of the symptomatology and microbial diversity within patients with PAD and controls to date. Through quantification of the rate and effect of upper respiratory tract pathogen detections relative to controls, we reveal the gulf in symptom-related quality of life, despite evidence-based therapies including prophylactic antibiotics[8] and IgRT.[7]

The most notable finding is the increased rate of detection for common circulating upper respiratory viral pathogens (notably HRV) among individuals with PAD, despite current therapies. Other viral pathogens including adenovirus, respiratory syncytial virus, and nonpandemic seasonal coronavirus showed similar trends toward increased detection frequency in patients with PAD. This pattern is reminiscent of specific immune defects associated with recurrent viral susceptibility such as
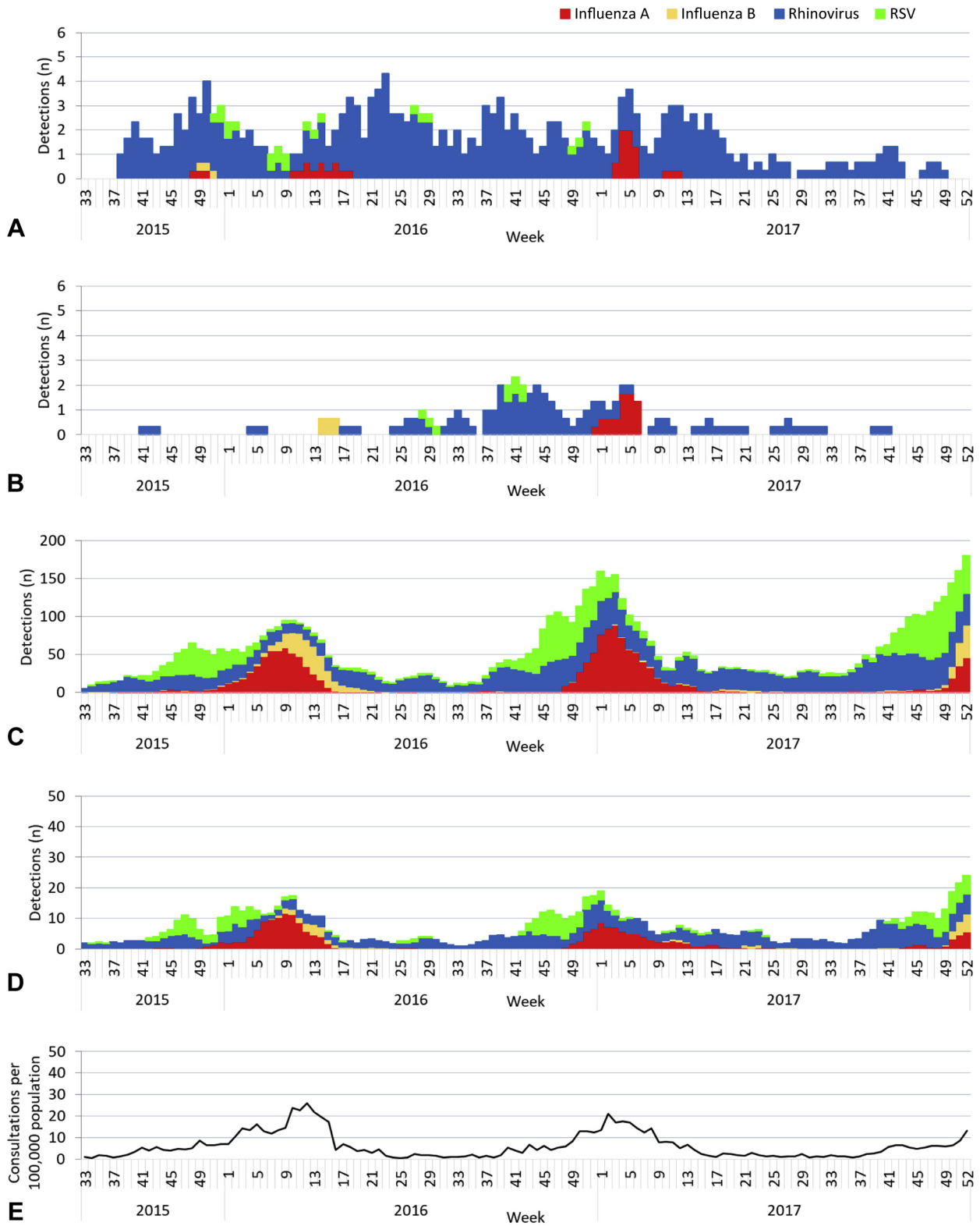
FIGURE 2. BIPAD and PHW detections of selected pathogens. Weekly numbers of confirmed cases of influenza A, influenza B, rhinovirus, and RSV in (**A**) BIPAD patients and (**B**) BIPAD controls compared with (**C**) confirmed cases in Wales* and (**D**) cases confirmed in intensive care units in Wales. Case numbers are presented as rolling 3-week averages. (**E**) The Welsh sentinel GP ILI weekly consultation rate is also provided. *Data predominantly (>95%) from hospital patients. *ICU*, Intensive care unit; *GP*, general practice; *ILI*, influenza-like illness; *RSV*, respiratory syncytial virus.
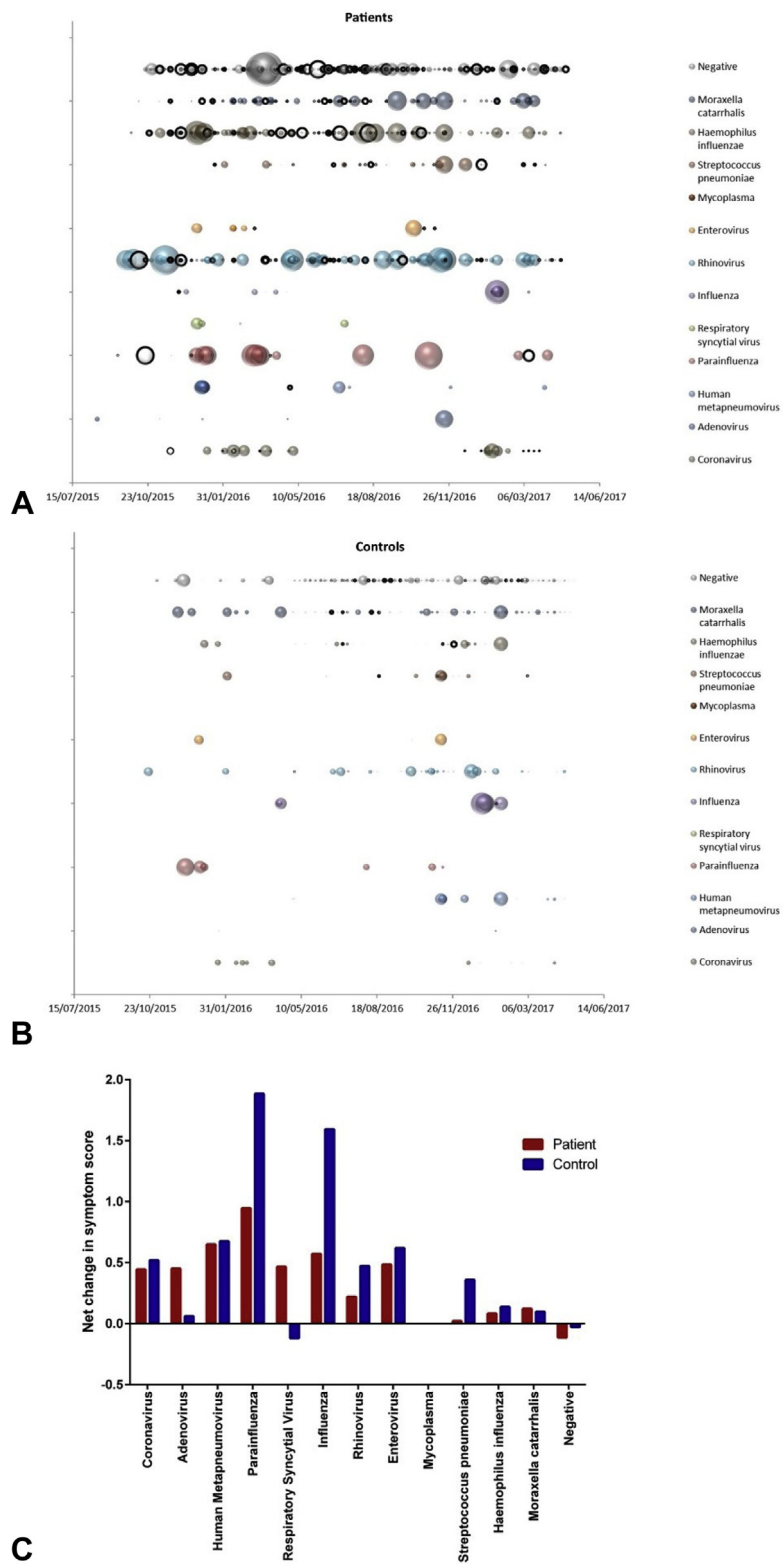
FIGURE 3. Pathogen detection and acute change from participant's baseline score. Bubble charts depicting dynamic change from (**A**) patients or (**B**) controls mean symptom score, during the 7-day period around each swab received. Solid bubbles, worsening of symptoms; hollow, improvement. (**C**) Symptom scores by pathogen type and participant group.

interferon induced with helicase C domain 1 which encodes melanoma differentiation-associated protein 5[22,23] and interferon regulatory factor 7 deficiency.[24] Here, we identify major risk factors including young child contact, a likely surrogate of viral pathogen exposure,[25] and defects in circulating immunoglobulin isotypes relevant for protection at the mucosal barrier. Although exceeding any previous work, it is likely that sample size limited our ability to detect differential infection rates for pathogens circulating with lower prevalence in the adult community. It is also possible our pathogen detection rates represent an underestimate of true infective burden, given the adherence to a degree of social distancing already practiced by patients with PAD and their households (reported informally by participants).

Strengths of our study include a high compliance rate, despite the intensity and duration of the surveillance protocol. Participant dropout over the study period was 13%, which compares favorably to prospective longitudinal studies in chronic obstructive pulmonary disease.[26] Consistent with infection, viral detections were associated with increased symptom score and independently associated with risk factors such as presence of bronchiectasis in multivariate analysis. Through use of a multiplex PCR approach, we show that HRV and *H influenzae* detections frequently co-occur. Although limited by lack of paired sputum cultures, this association has been robustly described in studies examining chronic obstructive pulmonary disease exacerbations, where coinfection is associated with greater impairment of lung function and prolonged inpatient stays.[27,28] HRV has similarly been reported to increase *H influenzae* and *S pneumoniae* detection rates and exacerbation risk in children with asthma.[21] A range of studies suggest dynamic relationships between HRV, *H influenzae,* and the respiratory epithelium, where coinfection favors pathogen persistence and potentiates inflammation.[29-31] In secondary immunodeficiency related to hematopoietic stem cell transplantation, pretransplant rhinovirus infection increases mortality risk.[32] It highlights the need for targeted strategies to prevent or treat respiratory tract infection in PAD.[33,34] Our findings link deficiency of mucosally active immunoglobulin isotypes to viral susceptibility, providing further support for development of nebulized therapies to help restore a humoral immune barrier.[35] We have also generated an extensive biobank amenable for next-generation sequencing and bioinformatic approaches to illuminate intra-household transmission dynamics.[36]

We believe that BIPAD defines an important baseline before the global emergence of coronavirus disease 2019 (COVID-19). At time of writing, evidence for which immunodeficiencies are associated with increased susceptibility, severity, or duration of COVID-19 among immunodeficient individuals is not yet clear but will be informed by ongoing national and international studies. However, kinetic analysis of COVID-19 infection within the immunocompetent patient strongly implicates the need for a coordinated humoral immune response,[37] mirroring the immunologic signature of successful vaccination.[38] Our finding of increased susceptibility to a range of viral pathogens in PAD, despite current therapy and independent of age, is convergent with failure of this immune response. This supports the advice for social distancing of immunodeficient patients and potentially consideration of limiting young child exposure may also be considered.[39] Finally, prolonged symptom and viral detection reported in immunocompromised individuals here, and elsewhere,[12] may prove relevant when considering infection control to halt ongoing transmission chains.

CONCLUSIONS

BIPAD highlights frequently circulating viruses such as HRV and parainfluenza as dominant pathogens in patients with PAD and reveals wider susceptibility patterns. Recurrent viral infection likely contributes significantly to the recurrent respiratory burden observed despite current therapies. BIPAD highlights a clear need for future therapeutic trials in the PAD population. By extension, it supports social distancing including consideration of limiting young child contacts for this vulnerable patient group during the COVID-19 pandemic.

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ONLINE REPOSITORY

METHODS
Swab processing and multiplex PCR analysis

Once received in the laboratory, the dry flocked swab was processed as previously described.[E1] Briefly, the swab was broken into 0.9 mL of a guanidinium thiocyanate—based lysis buffer (BioMérieux, Basingstoke, UK), vortexed, and left to stand for 10 minutes. Nucleic acid purification was performed using the automated extraction platform NucliSens EasyMag (BioMérieux, Basingstoke, UK) on 200 μL of the preprocessed lysis buffer into an elution volume of 110 μL. The remaining lysis buffer containing the swab was stored at −80°C. In addition to the exogenous internal control (bacteriophage MS2) added to the sample before nucleic acid purification following the NxTAG RPP protocol, an endogenous control was used targeting human RNAseP to ensure sample integrity and quality. A sample was reported as poor quality if the RNAseP threshold crossing value (ct) was greater than 37. Samples from the study and sentinel surveillance scheme were processed following identical protocols.

Daily Symptom Score "BIPAD-Q"
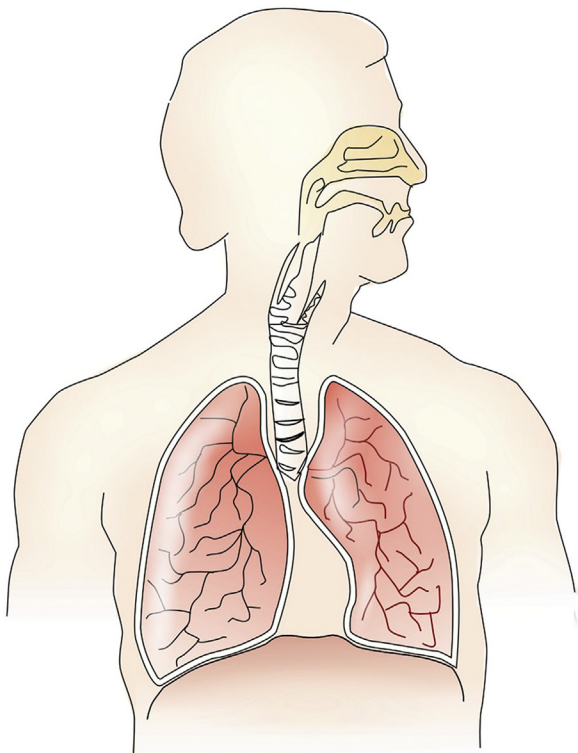
Upper respiratory tract symptoms

0: No symptoms
1: Mild stuffy or runny nose
2: Moderate stuffy or runny nose
3: Severe, unable to breathe through nose, unable to sleep due to symptoms

Lower respiratory tract symptoms

1: Cough
2: Increased sputum volume
3: Change in sputum colour
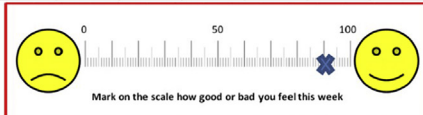4: New shortness of breath
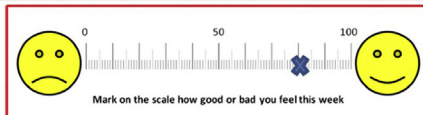
7: Total

FIGURE E1. BIPAD-Q and example weekly scoring questionnaire. Summary of the BIPAD-Q daily upper and lower airway scoring alongside a representative participant diary card covering a 2-week period that would accompany the posted 2 weekly nasal swab. *VAS*, Visual analog scale.
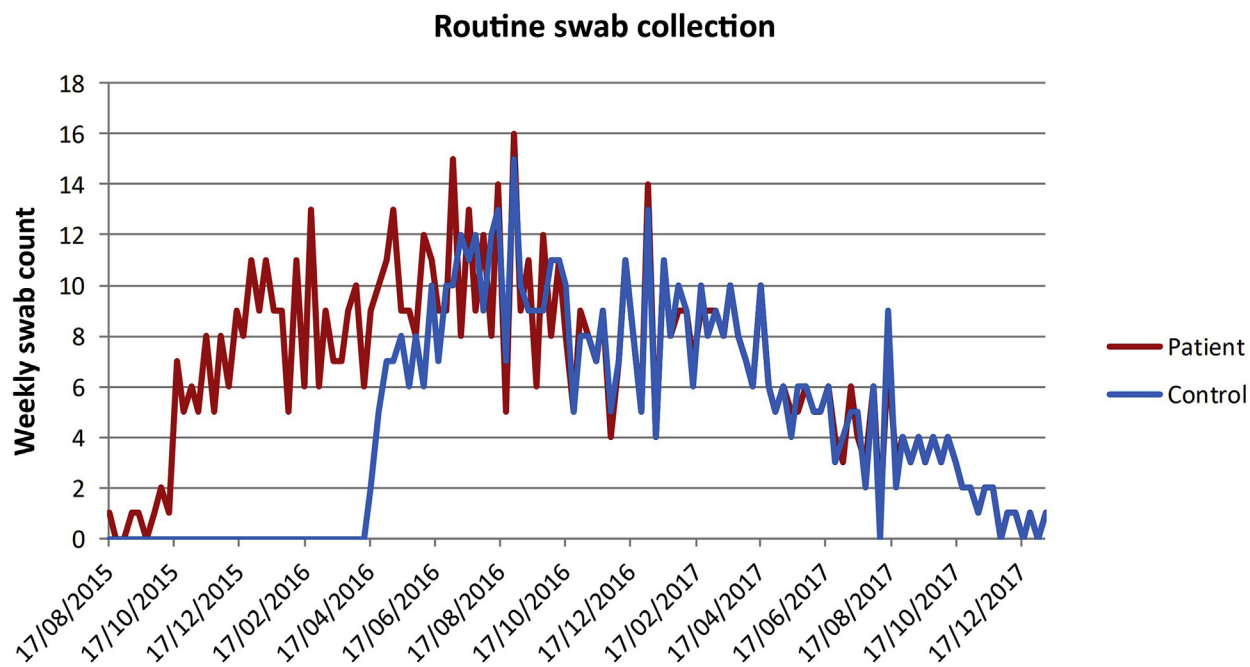
Routine swab collection

FIGURE E2. Effect of protocol amendment on nasal swab submission. Initially only patients with PAD provided 2 weekly swabs; however, following a protocol amendment in April 2016, controls also provided 2 weekly swabs mirroring the patients.
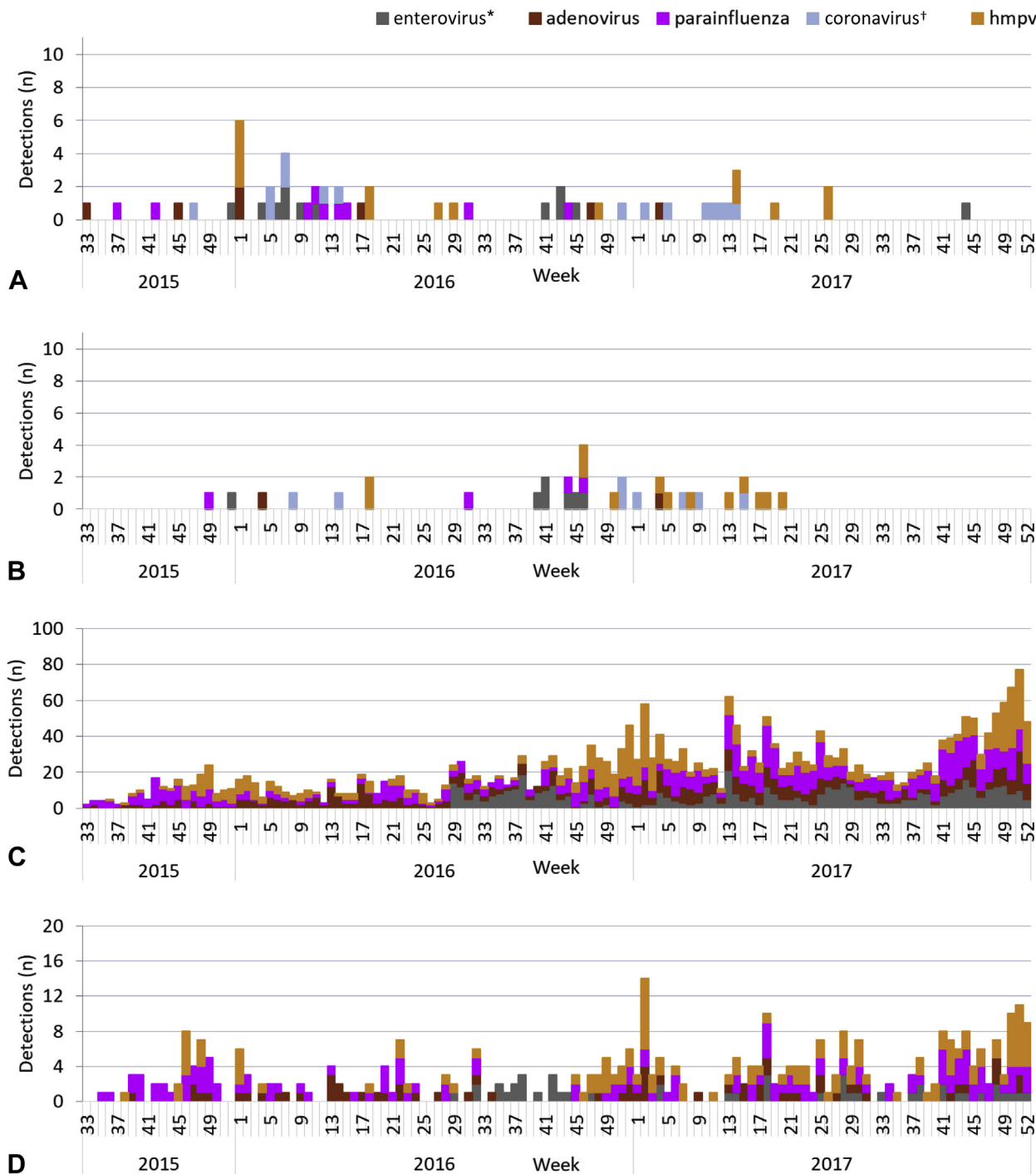
FIGURE E3. Extended viral detection patterns in BIPAD and Public Health Wales Surveillance. Weekly numbers of confirmed cases of enterovirus, adenovirus, parainfluenza, coronavirus, and human metapneumovirus in (**A**) BIPAD patients and (**B**) BIPAD controls compared with (**C**) confirmed cases in Wales (data predominantly [>95%] from hospitalized patients) and (**D**) cases confirmed in intensive care units in Wales. *hmpv*, Human metapneumovirus; *ICU*, intensive care unit. *Enterovirus testing was not routinely carried out in standard respiratory screen tests until W29 2016. †Samples undergoing routine respiratory screen are not routinely tested for coronaviruses.
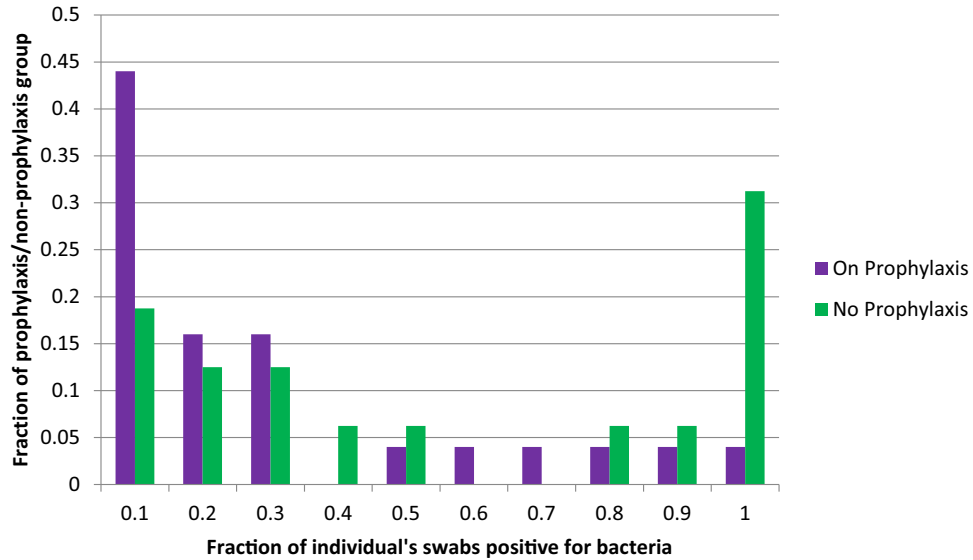
FIGURE E4. Effect of antibiotic prophylaxis on bacterial detections. The effect of antibiotic prophylaxis on bacterial detections represented as the fraction of an individual's swabs positive for bacteria (purple, on prophylaxis; green, no prophylaxis).

TABLE E1. Antibiotic prophylaxis in use by patients with PAD

Antibiotic prophylaxis	No. of patients
Antibiotic prophylaxis in use	28
Amoxicillin	2
Azithromycin	15
Cefuroxime	1
Clarithromycin	1
Coamoxiclav	5
Colomycin (nebulized) and cotrimoxazole (oral)	1
Doxycycline	2
Trimethoprim	1
No prophylaxis	16

TABLE E2. Linear mixed-model fit examining interaction between patient group and infection type on symptom burden

Variable	Coefficient	95% CI_lower bound	95% CI_upper bound	P value
Intercept	0.380	0.173	0.588	.000648
Patients with PAD	0.711	0.579	0.844	7.23×10^{-25}
Virus-positive swab	0.768	0.517	1.02	2.49×10^{-09}
Patients: Virus-positive	−0.238	−0.537	0.0621	.120
Intercept	0.417	0.205	0.631	.000281
Patients with PAD	0.852	0.706	0.999	8.76×10^{-29}
Bacteria-positive swab	0.363	0.121	0.606	.00340
Patients: Bacteria-positive	−0.380	−0.684	−0.0755	.0147

TABLE E3. Identification of viral susceptibility risk factors

Variable considered	Univariate				Multivariate			
	OR	95% CI lower bound	95% CI upper bound	Unadjusted P value	OR	95% CI lower bound	95% CI upper bound	Adjusted P value
Age	0.991	0.981	1.00	.0907	0.727	0.982	1.01	.388
IgM*	0.626	0.397	0.937	**.0325**	0.612	0.394	0.900	**.019**
IgA*	0.733	0.585	0.893	**.0038**	0.854	0.680	1.05	.152
Regular child contact	2.04	1.446	2.89	**.0000486**	2.16	1.45	3.22	**.000158**
On antibiotic prophylaxis	0.813	0.603	1.10	.174	0.676	0.451	1.01	.0558
CD3$^+$CD4$^+$ count <400	1.08	0.783	1.48	.645	0.852	0.581	1.24	.408
Bronchiectasis	1.46	1.052	2.03	**.023**	1.76	1.20	2.59	**.00384**

OR, Odds ratio.

IgG was excluded from analysis because clinical practice allows adjustment of trough dose on the basis of clinical symptoms.

Bold indicates statistical significance ($P < .05$).

*Continuous variables.

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E1. Moore C, Corden S, Sinha J, Jones R. Dry cotton or flocked respiratory swabs as a simple collection technique for the molecular detection of respiratory viruses using real-time NASBA. J Virol Methods 2008;153:84-9.