



Patterns of systemic and local inflammation in patients with asthma hospitalised with influenza

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Patients with asthma hospitalised with influenza are commonly female and lack classical type 2 nasal mucosal inflammation. They have good prognosis with enhanced type 1 interferon production and reduced systemic inflammation. <http://bit.ly/2Ovzz7O>

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ABSTRACT

Background: Patients with asthma are at risk of hospitalisation with influenza, but the reasons for this predisposition are unknown.

Study setting: A prospective observational study of adults with PCR-confirmed influenza in 11 UK hospitals, measuring nasal, nasopharyngeal and systemic immune mediators and whole-blood gene expression.

Results: Of 133 admissions, 40 (30%) had previous asthma; these were more often female (70% *versus* 38.7%, OR 3.69, 95% CI 1.67–8.18; $p=0.0012$), required less mechanical ventilation (15% *versus* 37.6%, Chi-squared 6.78; $p=0.0338$) and had shorter hospital stays (mean 8.3 *versus* 15.3 days, $p=0.0333$) than those without. In patients without asthma, severe outcomes were more frequent in those given corticosteroids (OR 2.63, 95% CI 1.02–6.96; $p=0.0466$) or presenting >4 days after disease onset (OR 5.49, 95% CI 2.28–14.03; $p=0.0002$). Influenza vaccination in at-risk groups (including asthma) were lower than intended by national policy and the early use of antiviral medications were less than optimal. Mucosal immune responses were equivalent between groups. Those with asthma had higher serum interferon (IFN)- α , but lower serum tumour necrosis factor, interleukin (IL)-5, IL-6, CXCL8, CXCL9, IL-10, IL-17 and CCL2 levels (all $p<0.05$); both groups had similar serum IL-13, total IgE, periostin and blood eosinophil gene expression levels. Asthma diagnosis was unrelated to viral load, IFN- α , IFN- γ , IL-5 or IL-13 levels.

Conclusions: Asthma is common in those hospitalised with influenza, but may not represent classical type 2-driven disease. Those admitted with influenza tend to be female with mild serum inflammatory responses, increased serum IFN- α levels and good clinical outcomes.

This study was approved by the NHS National Research Ethics Service, Outer West London REC (09/H0709/52, 09/MRE00/67) and is registered at Clinicaltrials.gov with trial number NCT00965354.

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Introduction

Influenza viruses cause a continuous threat to global health, mutating and spreading in both human and animal populations. The Influenza Clinical Information Network (FLU-CIN) reported that asthma was the commonest pre-existing risk factor for hospitalisation, being present in 25.3% of 1520 patients admitted with influenza A infection [1]. This apparent increased risk is reported in other studies, which show paradoxically that individuals with asthma experience less severe outcomes and are discharged earlier from hospital than those without asthma [2, 3]. There have been many studies of immune responses to influenza infection [4, 5], but none has focused on characterising the effect of asthma in the host responses to natural influenza.

The Mechanisms of Severe Acute Influenza Consortium (MOSAIC) recruited patients with clinical influenza presenting to hospitals in London and Liverpool (UK) during the winters of 2009/2010 and 2010/2011, periods of intense influenza activity. We previously reported enrichment for a host genetic variant of the interferon-inducible transmembrane protein 3 (*IFITM3*) allele single nucleotide polymorphism rs12252-C in hospitalised patients [6] and that circulating influenza viruses evolved and change in character over time [7] and the progression of whole-blood transcriptional signatures and mediator levels from interferon-induced to neutrophil-associated patterns in severe disease [8], but have not yet described the clinical details of the study population.

We now provide detailed clinicopathological analysis of the MOSAIC cohort, segregating patients with and without asthma and confirmed influenza and focusing on measures of nasal mucosal and systemic inflammation as potential causes of enhanced disease. Some of the results of this study have been reported previously in the form of an abstract [9].

Methods

Study design and cohort

Adult patients presenting with influenza-like symptoms were recruited between December 2009 and March 2011 from three hospitals in Liverpool and the Wirral (north-west England) and six hospitals in London. A detailed medical history, including the presence of a diagnosis of asthma, along with a record of prescribed medications on admission was obtained from case notes by specialised data collectors based on Department of Health guidelines and continued for the first 14 days of admission [10]. Three patients who were coded as having both asthma and chronic obstructive pulmonary disease (COPD) were allocated to the non-asthma group in order to avoid potential confounding (one patient had radiological evidence of emphysema; the second patient had clinical COPD and bronchiectasis secondary to crack-cocaine use and the third patient was clinically coded as having an exacerbation of COPD rather than asthma). The severity of respiratory illness was graded from 1 to 3 as described previously [8]. In addition, a panel of 36 healthy volunteers (with characteristics described elsewhere [8]) free of comorbidities or influenza-like symptoms were recruited to the study and nasal and blood samples were obtained at a single time point. The study was approved by the NHS National Research Ethics Service, Outer West London REC (09/H0709/52, 09/MRE00/67) and is registered at clinicaltrials.gov with trial number NCT00965354. Written informed consent was obtained from patients or their legally authorised representatives as well as healthy controls.

Sample collection

Nasopharyngeal aspirates (NPAs) and flocked nasopharyngeal swabs were taken as soon as possible, generally within 72 h of admission. Influenza virus infection status was assigned on the basis of results of influenza A/B reverse transcriptase (RT)-PCRs performed by laboratories serving the respective hospitals, with confirmation of these results by RT-PCR for influenza A, H1N1/2009 and influenza B at the West of Scotland Specialist Virology Centre. Antibody responses to A/Engl/195/2009(H1N1v) were detected using microneutralisation assays according to standard methods as previously described [11] at the Centre for Infections, Health Protection Agency (London, UK). Serum samples were tested at an initial dilution of 1:10 and a final dilution of 1:5120.

All other samples were collected within 24 h of admission to the hospital. Blood collection and NPA sampling was performed as described previously [8]. Nasosorption was used to sample mucosal lining fluid from the nose as detailed previously [12]. Additionally, whole blood was collected for microarray RNA profiling (Tempus blood RNA tube, Applied Biosystems/Ambion, Foster City, CA, USA). Research samples were collected within 24 h of admission and, where possible, at 48 h and in convalescence (≥ 4 weeks after presentation).

Sample processing

Samples were processed using the ultrasensitive Meso Scale Discovery platform (Gaithersburg, MD, USA), based on electro-chemiluminescence quantitative patterned arrays to detect 28 cytokines and chemokines:

interferon (IFN)- α 2 α , IFN- β , IFN- γ , interleukin (IL)-29, tumour necrosis factor (TNF)- α , granulocyte-macrophage colony-stimulating factor, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-15, IL-17, CCL2, CCL3, CCL4, CCL11, CCL13, CCL17, CCL22, CCL26, CXCL8, CXCL9, CXCL10 and CXCL11. Microarray gene expression profiling was performed using HT12 V4 BeadChip arrays (Illumina, San Diego, CA, USA), containing >47 000 probes. Detailed methodology is available elsewhere [8].

Statistical analyses

Clinical features comparing populations with and without asthma were analysed using a combination of two-sided Fisher's exact test, Chi-squared test, unpaired t-test and two-tailed Mann-Whitney test, as indicated in figure legends. Mediators were compared using Kruskal-Wallis and Dunn's test with Bonferroni multiple correction. Logistic regression analysis was carried out to identify independent predictors of severe outcome and to investigate association of asthma in relation to severe outcome. All analyses were performed with Prism (version 7; GraphPad, La Jolla, CA, USA) and R package software (version 3.3.2) [13].

Results

Clinical features

40 (30.1%) out of 133 hospitalised adults had asthma (table 1). Among people with asthma, influenza A was responsible for 38 (95%) out of 40 cases (all H1), with influenza B causing two (5%) out of 40 cases. In individuals without asthma, 82 (88.2%) out of 93 cases were caused by influenza A (H1 n=77; H3 n=3; suspected H1 based on serology n=1; undetermined n=1), 10 out of 93 due to influenza B and 1 case with influenza A and B co-infection. Females were significantly overrepresented in those with asthma compared to those without (70% *versus* 38.7%, OR 3.69, 95% CI 1.67–8.18; p=0.0012). Patients with asthma had a lower mean \pm SEM length of stay (p=0.0333, 8.3 \pm 2.55 days *versus* 15.3 \pm 1.82 days), and were less likely to require intubation and ventilation at peak severity of disease (15% *versus* 37.6%, Chi-squared 6.78; p=0.0338). Relatively few patients with asthma died (5% *versus* 17.2%, p=0.0944), but were more likely to have received the seasonal influenza vaccine (37.5% *versus* 18.3%, p=0.0261) and inhaled corticosteroids (p<0.0001) prior to admission. Following admission, people with asthma were more likely to have received at least one dose of oral or intravenous corticosteroids during their inpatient stay (55% *versus* 25.8%, p=0.0016).

To determine predictors of a severe outcome (invasive ventilation or death), multiple regression modelling identified presentation to hospital >4 days after symptom onset and administration of in-hospital systemic steroids as factors associated with a worse outcome (supplementary table E1). When patients with and without a severe outcome were assessed separately, presentation to hospital >4 days after symptom onset (OR 5.49, 95% CI 2.28–14.03; p=0.0002) and administration of in-hospital systemic corticosteroids (OR 2.6250, 95% CI 1.02–6.96; p=0.0466) remained associated with a severe outcome in people without asthma, but not in those with asthma (data not shown). There was no significant difference between individuals with and without asthma in nasopharyngeal influenza viral load within 24 h of admission and kinetics based on day of sampling after self-reported onset of influenza symptoms (figure 1a). There was no difference in H1N1 geometric mean titres as measured by microneutralisation assay (figure 1b).

Systemic and mucosal immune response

All hospitalised patients with influenza had equivalent or greater systemic and mucosal inflammation relative to non-hospitalised healthy controls (figures 2 and 3, supplementary figure E1 and supplementary tables E2–E4). During the first 24 h of hospital admission, people with asthma had reduced systemic inflammation compared to those without as demonstrated by significantly lower serum levels of TNF- α (p<0.0001), IL-6 (p=0.0005), CXCL8 (p<0.0001), CXCL9 (p=0.0031), IL-10 (p=0.0411), IL-17 (p=0.0197) and CCL2 (p=0.0038). However, individuals with asthma had significantly higher levels of serum IFN- α 2a (p=0.0099; figure 1 and supplementary table E2). Interestingly, those with asthma had lower levels of serum IL-5 (p=0.0010), but comparable levels of IL-13 (p=0.3131). There were no significant differences in white blood cell count, neutrophils, lymphocytes or C-reactive protein between groups (supplementary figure E1).

There were no significant differences in 28 nasal or nasopharyngeal cytokine and chemokine levels measured within 24 h of admission between individuals with and without asthma (figure 2, supplementary tables E3 and E4). In particular, there was comparable mucosal induction of antiviral mediators IFN- α 2a, IFN- β , IFN- γ , IFN- λ and CXCL10 as well as the type 2 mediators IL-5 and IL-13.

Samples from subjects were collected within 24 h and again at 48–72 h after admission (if possible). To compare the kinetics of the host immune response to infection between patients with and without asthma, mediator levels were assessed based on day of illness, *i.e.* the interval between subject-reported onset of influenza-like symptoms and date of sampling (supplementary figures E2–E4). This analysis showed that those without asthma had evidence of greater systemic inflammation with significantly higher serum levels

TABLE 1 Clinical features of hospitalised influenza participants with and without asthma

	Asthma	No asthma	p-value	Chi-squared	OR (95% CI)
Total participants	40 (30)	93 (70)			
Demographics					
Female	28 (70)	36 (38.7)	0.0012		3.69 (1.67–8.18)
Age years			0.9699	0.06	NA
18–30 [#]	11 (27.5)	26 (30.0)			
31–45 [#]	14 (35)	32 (34.4)			
46–60 [#]	11 (27.5)	23 (24.7)			
>60 [#]	4 (10)	12 (12.9)			
Ethnicity			0.5810	1.96	NA
White [#]	27 (67.5)	62 (66.7)			
Asian/Asian British [#]	1 (2.5)	8 (8.6)			
Black/Black British [#]	8 (20)	14 (15.1)			
Chinese [#]	4 (10)	9 (9.7)			
Comorbidities and co-factors					
Any comorbidities other than asthma	25 (62.5)	62 (66.7)	0.6930		0.83 (0.71–1.24)
Chronic pulmonary conditions excluding asthma/COPD	3 (7.5)	11 (11.8)	0.5519		0.60 (0.16–2.30)
Chronic neurological disorders	2 (5)	6 (6.5)	1.0000		0.76 (0.15–3.96)
Chronic renal disease	3 (7.5)	5 (5.4)	0.6965		1.43 (0.32–6.28)
Chronic hepatic disease	1 (2.5)	4 (4.3)	1.0000		0.57 (0.06–5.27)
Chronic cardiovascular disease	10 (25)	19 (20.4)	0.6478		1.30 (0.54–3.12)
Diabetes	5 (12.5)	9 (9.7)	0.7587		1.33 (0.42–4.26)
Chronic obesity	10 (25)	17 (18.3)	0.4809		1.49 (0.61–3.62)
HIV positivity	7 (17.5)	8 (8.6)	0.1466		2.25 (0.76–6.71)
Immunodeficiency (excluding HIV)	9 (22.5)	15 (16.1)	0.4615		1.51 (0.60–3.81)
Current or active cancer	3 (7.5)	19 (20.4)	0.0778		0.32 (0.09–1.14)
Haematological disorder	0	5 (5.4)	0.3217		0.20 (0.01–3.68)
Current or ex-smoker	17/34 (50)	37/69 (53.6)	0.8344		0.86 (0.38–1.97)
Current smoker	13/34 (38.2)	22/69 (31.9)	0.5106		1.38 (0.59–3.24)
Pregnancy (in women of childbearing age)	3/23 (13)	10/30 (33.3)	0.1151		0.30 (0.07–1.26)
Seasonal vaccine	15 (37.5)	17 (18.3)	0.0261		2.68 (1.17–6.14)
H1N1 vaccine	8 (20)	7 (7.5)	0.0684		3.07 (1.03–9.16)
Pre-admission inhaled corticosteroids	16 (40)	6 (6.5)	<0.0001		9.67 (3.41–27.4)
Admission					
Oral/ <i>i.v.</i> corticosteroids on day 1 of admission	15 (37.5)	13 (14)	0.0046		3.69 (1.55–8.80)
In-hospital oral/ <i>i.v.</i> corticosteroid use (≥1 dose)	22 (55)	24 (25.8)	0.0016		3.51 (1.62–7.29)
Admission ≤2 days after symptom onset	15 (37.5)	32 (34.4)	0.8435		1.14 (0.53–2.47)
Admission ≤4 days after symptom onset	24 (60)	49 (52.7)	0.4547		1.35 (0.63–2.86)
Days between onset of symptoms and admission [¶]	3 (2–7)	4 (2–8)	0.3941		
Oxygen saturation on admission ≤92%	6/33 (18.2)	23/71 (32.4)	0.2389		0.48 (0.18–1.37)
Oxygen administered	8/26 (30.1)	28/63 (44.4)	0.3423		0.56 (0.21–1.47)
Pneumonia on chest radiography	3/20 (15)	14/47 (29.8)	0.2382		0.42 (0.11–1.65)
Antibiotic use at admission	28/33 (84.8)	69/73 (94.5)	0.1329		0.33 (0.08–1.30)
Peak respiratory score			0.0338	6.78	NA
Peak respiratory score 1 [#]	15 (37.5)	27 (29.0)			
Peak respiratory score 2 [#]	19 (47.5)	31 (33.3)			
Peak respiratory score 3 [#]	6 (15)	35 (37.6)			
Length of stay days [*]	8.3±2.55	15.3±1.82	0.0333		
Influenza type			0.4492	1.60	
Influenza A positive [#]	38 (95)	82 (88.2)			
Influenza B positive [#]	2 (5)	10 (10.8)			
Influenza A+B co-infection [#]	0	1 (1.1)			
Influenza subtype			0.4903	2.42	
Influenza H1 positive [#]	38/38 (100)	77/82 (94.0)			
Influenza H3 positive [#]	0	3/82 (3.7)			
Undetermined [#]	0	1/82 (1.2)			
Suspected influenza H1 based on serology [#]	0	1/82 (1.2)			
Rhinovirus positive	1/36 (2.8)	9/83 (10.84)	0.2788		0.23 (0.03–1.93)
Pre-hospital antiviral use	2/33 (6)	1/77 (1.3)	0.2137		4.90 (0.43–56.1)
In-hospital antiviral use (≥1 dose)	37/39 (94.9)	85/89 (95.5)	0.9999		0.87 (0.20–4.74)
Antiviral use ≤48 h from onset of symptoms	14/39 (35.9)	26/89 (29.2)	0.5351		1.36 (0.62–3.05)
Antiviral use on day 1 of admission	28/35 (80)	62/73 (84.9)	0.58		0.71 (0.27–1.87)

Continued

TABLE 1 Continued

	Asthma	No asthma	p-value	Chi-squared	OR (95% CI)
Positive for any bacteria 0–4 days post-onset of symptoms (PCR or culture)	10 (25)	20/92 (21.7)	0.6595		1.20 (0.50–2.87)
Positive for any bacteria 5–10 days post-onset of symptoms (PCR or culture)	11 (27.5)	25/92 (27.2)	1.000		1.02 (0.44–2.34)
Positive for any bacteria ≥11 days post-onset of symptoms (PCR or culture)	13 (32.5)	19/92 (20.7)	0.1849		1.85 (0.80–4.25)
Death	2 (5)	16 (17.2)	0.0944		0.25 (0.06–1.16)

Data are presented as n (%), median (interquartile range) or mean±SEM, unless otherwise stated. Six patients without asthma on pre-admission inhaled corticosteroids had comorbidities including chronic obstructive pulmonary disease (n=4) and chronic lung disease (n=2). Where there is incomplete data about medication and comorbidities available from medical records, a denominator value is shown. All statistical analyses were performed using Fisher’s exact test, unless indicated otherwise. #: Chi-squared test; †: Mann–Whitney test; ‡: unpaired t-test.

of TNF-α (days 3–12), IL-6 (days 4–11), IL-5 (days 11–19), IL-17 (days 4–7), IL-10 (days 4–8), CCL2 (days 3–5), CXCL8 (days 2–13), CXCL9 (days 5–12) and CXL10 (days 5–11). In the nasal mucosa, people without asthma had higher TNF-α levels (days 8–10) while all other mediators demonstrated similar kinetics.

Total IgE (a marker of atopy) and periostin (a marker of IL-4/-13 activation) were measured in serum, with no significant differences seen between those with and without asthma (figure 4). The use of pre-admission inhaled corticosteroids in patients with asthma (figure 5) and administration of systemic corticosteroids within the first 24 h of admission in those with and without asthma (figures 6 and 7) did not significantly affect serum or nasal mucosal levels of IL-5, IL-13 or periostin.

In whole-blood RNA, there were no differentially expressed genes found between individuals with and without asthma after correction for multiple testing (data not depicted). Blood eosinophil counts were unavailable, but gene transcripts that serve as markers of eosinophilia (CLC, CEBPE, DACH1, EMR1, EMR4P and LGALS12 [12]) were measured. CEBPE, DACH1 and LGALS12 were not significantly different between groups while CLC, EMR1 and EMR4P were higher in people without asthma, indicating that those with asthma in this study were unlikely to have raised eosinophil levels (supplementary figure E5).

Discussion

In this study, asthma was the major identifiable predisposing condition in patients hospitalised with influenza. Influenza cases with asthma tended to be female (70%), have a shorter period of hospitalisation

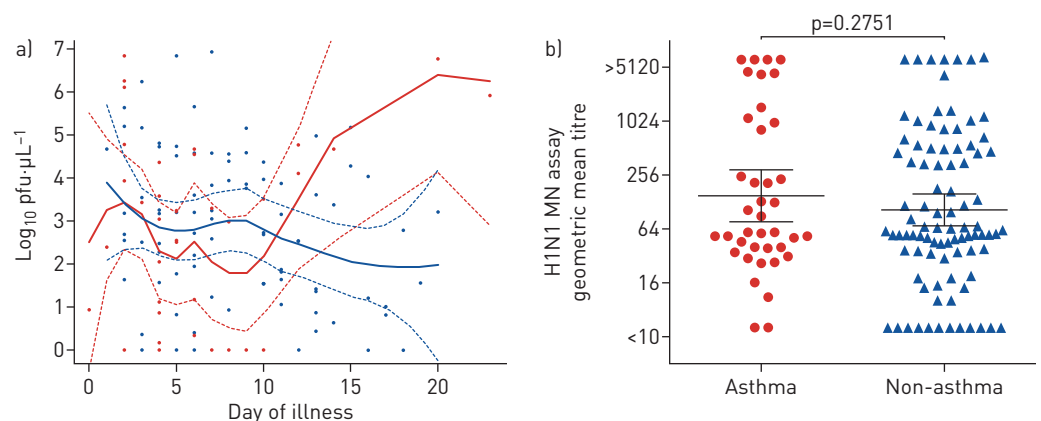


FIGURE 1 Nasopharyngeal influenza viral load and serological response to H1N1 in subjects with and without asthma. a) Viral load kinetics based on number of days elapsed between subject-reported symptom onset and time of sampling. Dots represent individual samples with up to two samples collected per subject; red dots represent asthma (n=37), blue dots represent no asthma (n=88). First sample collected within 24 h of admission and second sample between 48 and 72 h of admission to a Mechanisms of Severe Acute Influenza Consortium (MOSAIC) hospital. Locally weighted scatterplot smoothing (LOESS) fits plotted in red for asthma samples and blue for non-asthma samples. Solid line represent LOESS fit and dashed lines 95% CI. b) H1N1 geometric mean titre as measured by microneutralisation (MN) assay. Horizontal line represents the geometric mean and error bars the 95% CI. Statistical analysis performed using unpaired t-test.

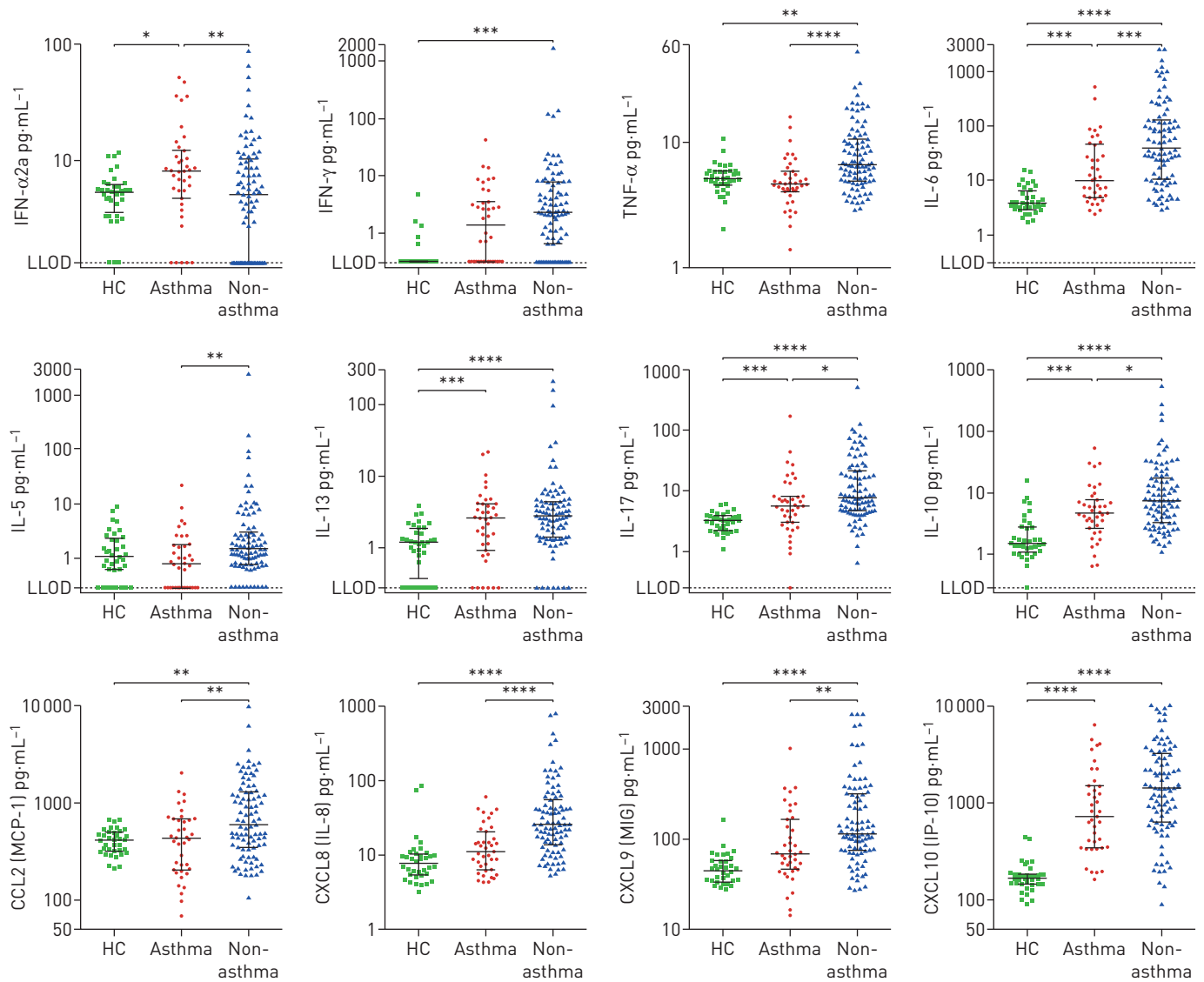


FIGURE 2 Serum mediators measured within 24 h of admission to hospital in individuals with and without asthma. Dots represent individual patients (healthy control (HC) n=36, asthma n=39, non-asthma n=91) and error bars the median and interquartile range. Statistical analysis performed using Kruskal–Wallis and Dunn’s test with Bonferroni multiple correction. IFN: interferon; TNF: tumour necrosis factor; IL: interleukin; MCP: monocyte chemoattractant protein; MIG: monokine induced by γ -IFN; IP: IFN- γ -induced protein; LLOD: lower limit of detection. *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.

and a reduced requirement for invasive ventilation than those without asthma. Extensive monitoring of mucosal and systemic inflammation over time showed relatively low levels of systemic inflammation but equal mucosal inflammation in subjects with asthma, compared to those without.

The sex bias noted in this study is reflected in a larger UK-based cohort in which 61% of asthma patients were female, compared to only 39% of those without asthma [1] (Puja Miles, Medicines and Healthcare Products Regulatory Agency, UK; personal communication). It is well described that middle-aged females have a propensity to present with non-atopic asthma [14], highlighting that non-allergic triggers (such as infection) are likely to be key determinants of asthma exacerbations in this subgroup. A range of subtypes of asthma have been extensively characterised [15]. Many individuals with asthma have increased type 2 inflammation associated with mast cell and eosinophil infiltration of the airways [16]. Such patients with allergic asthma typically have elevated plasma IL-5 and IL-13, identifying them as suitable for biologic therapies that target these cytokines [17, 18]. In addition, IL-5 and IL-13 are elevated in nasosorption samples from individuals with allergic asthma (even when stable before exacerbation) compared with healthy non-atopic controls [19], as well as nasal periostin, IgE and IL-13 in severe asthma [20]. In this study, there was a remarkable lack of elevation in serum or nasal type 2 mediators (IL-5 and IL-13) and no significant

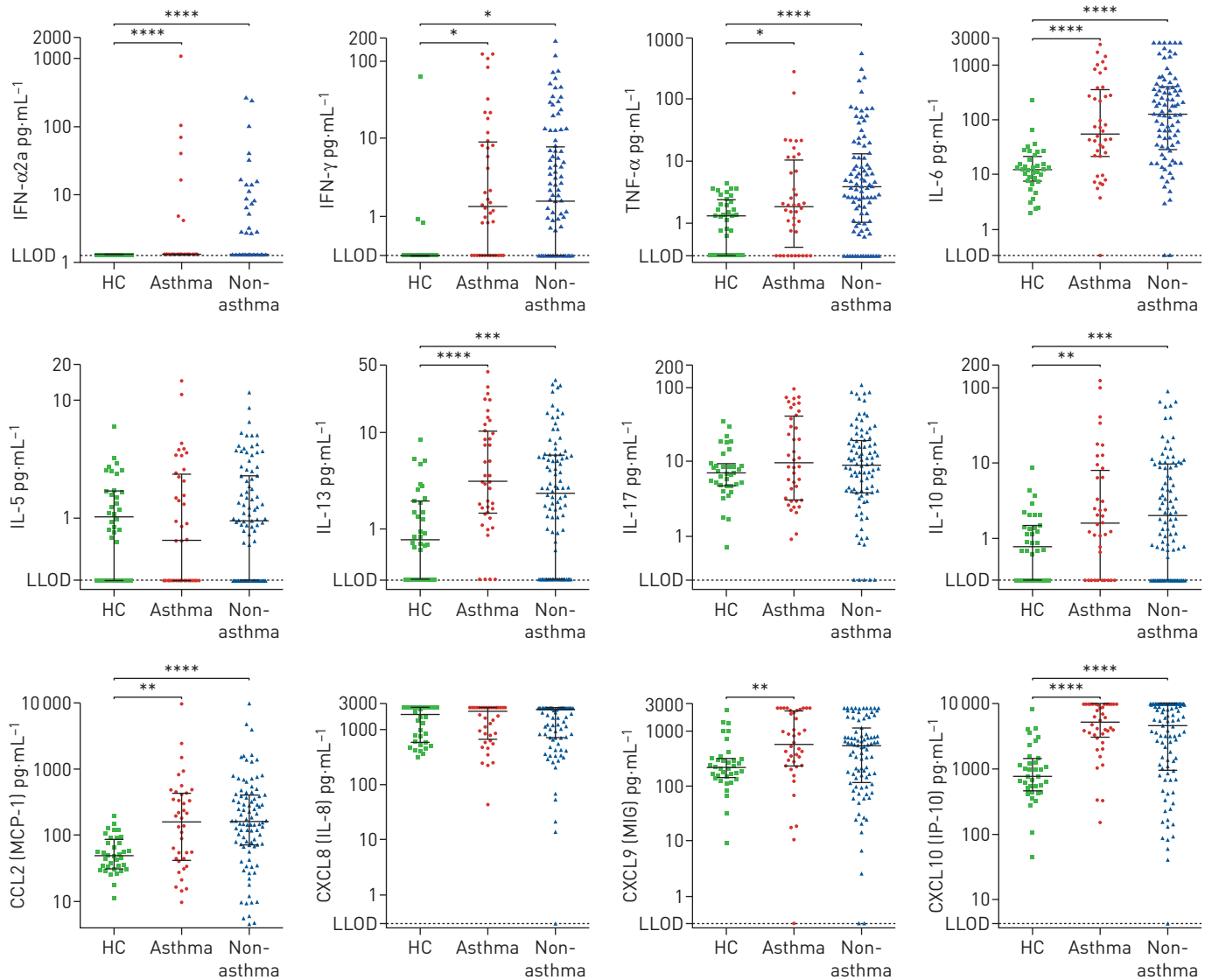


FIGURE 3 Nasal mediators measured within 24 h of admission to hospital in individuals with and without asthma. Dots represent individual patients (healthy control (HC) n=36, asthma n=40, non-asthma n=92) and error bars the median and interquartile range. Statistical analysis performed using Kruskal–Wallis and Dunn’s test with Bonferroni multiple correction. IFN: interferon; TNF: tumour necrosis factor; IL: interleukin; MCP: monocyte chemoattractant protein; MIG: monokine induced by γ -IFN; IP: IFN- γ -induced protein; LLOD: lower limit of detection. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

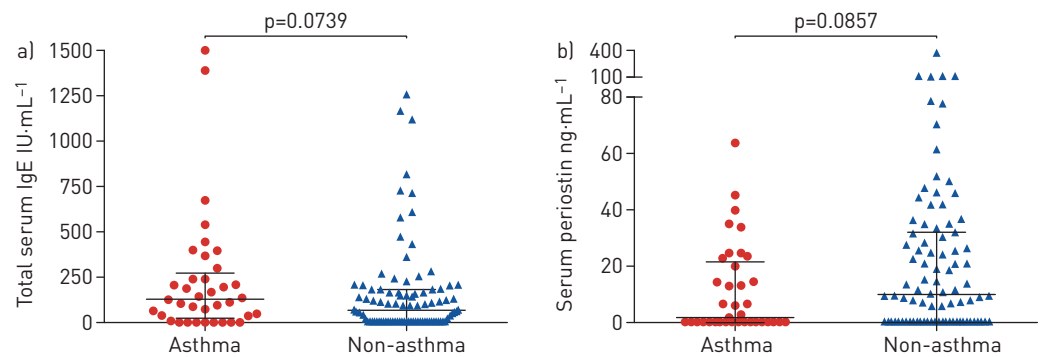


FIGURE 4 Serum levels of total IgE and periostin in subjects with and without asthma. a) Total serum IgE and b) serum periostin levels measured within 24 h of admission in individual subjects (asthma n=37, non-asthma n=92). Horizontal bar represents the median and error bars the interquartile range. Statistical analysis performed using Mann–Whitney test.

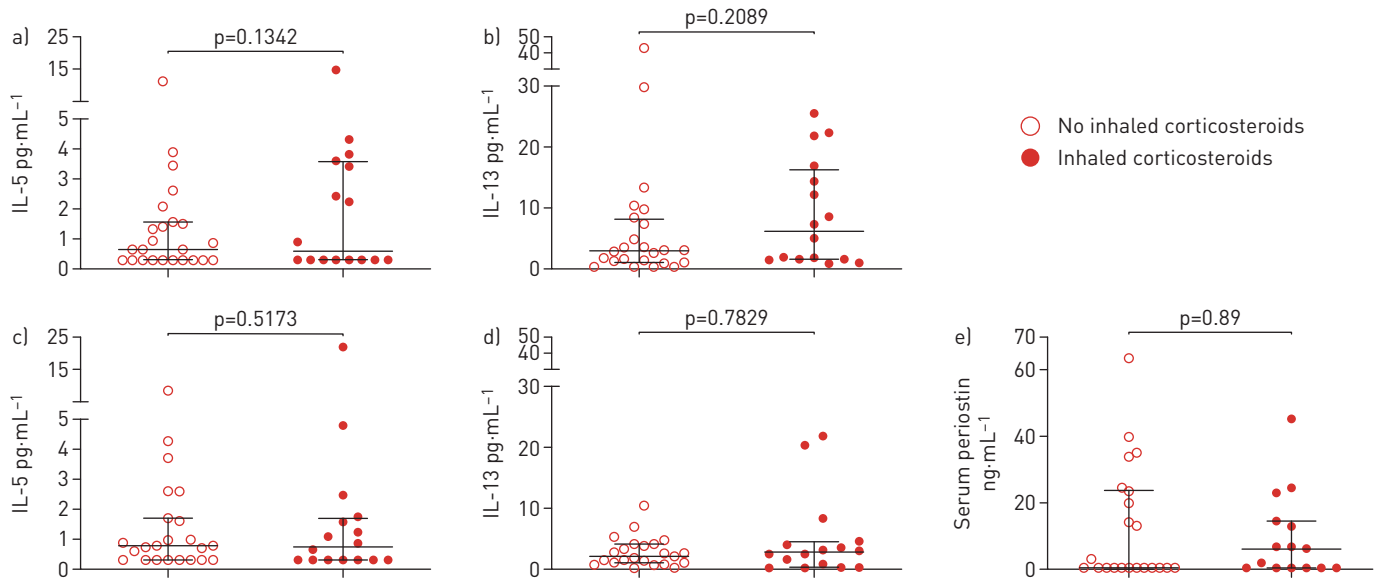


FIGURE 5 Type 2 inflammatory response in subjects with asthma based on presence or absence of pre-admission inhaled corticosteroids. Nasal a) interleukin (IL-5 and b) IL-13 levels measured within 24 h of admission; subjects on pre-admission inhaled corticosteroids (n=16), subjects not on pre-admission inhaled corticosteroids (n=24). Serum c) IL-5 and d) IL-13 levels measured within 24 h of admission; subjects on pre-admission inhaled corticosteroids (n=16), subjects not on pre-admission inhaled corticosteroids (n=23). e) Serum periostin levels measured within 24 h of admission; subjects on pre-admission inhaled corticosteroids (n=22), subjects not on pre-admission inhaled corticosteroids (n=22). Horizontal bar represents the median and error bars the interquartile range. Statistical comparison performed using Mann-Whitney test.

rise in total serum IgE, periostin and blood eosinophil-specific gene expression in individuals with asthma relative to those without asthma. These findings raise the possibility that individuals with asthma who are at risk of severe influenza may be of a specific disease endotype: females with minimal type 2 inflammation and a lack of raised total IgE. This endophenotype is sometimes described as “intrinsic” asthma, a classification ascribed to RACKEMANN [21], who noted that intrinsic asthma generally affects older individuals with frequent virally-induced exacerbations [22]. However, important caveats to this interpretation are that

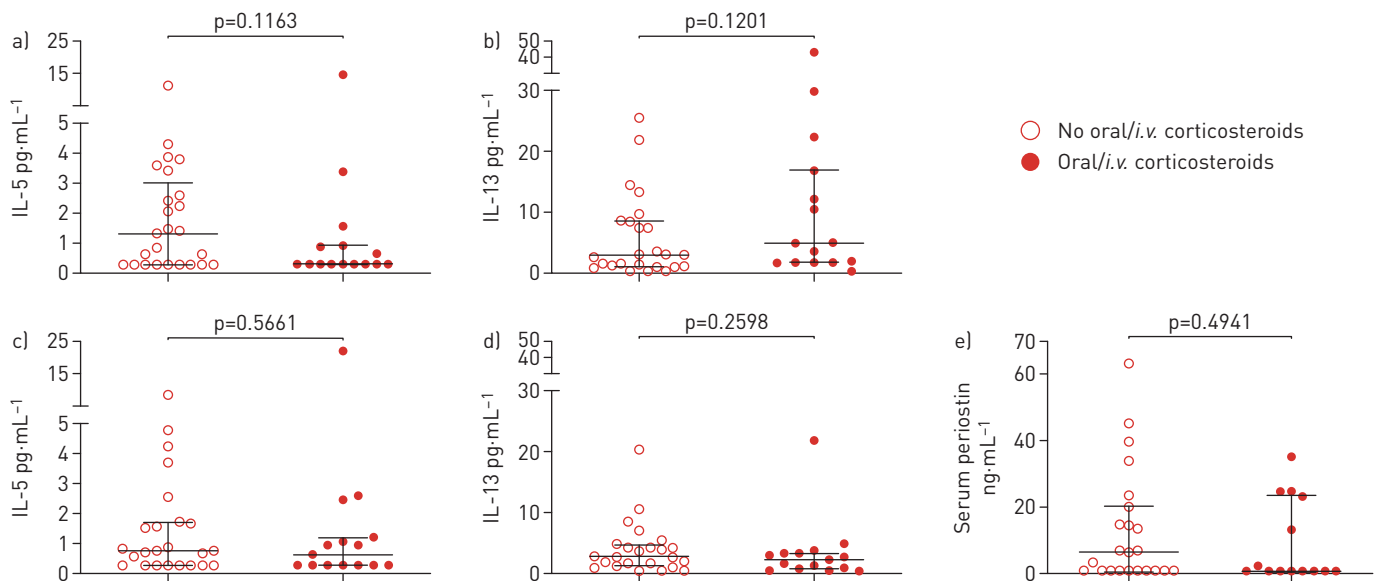


FIGURE 6 Type 2 inflammatory response in subjects with asthma based on administration of oral/intravenous corticosteroids within 24 h of admission. Nasal a) interleukin (IL)-5 and b) IL-13 levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=15), subjects not on oral/i.v. corticosteroids (n=25). Serum c) IL-5 and d) serum IL-13 levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=15), subjects not on oral/i.v. corticosteroids (n=24). e) Serum periostin levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=14), subjects not on oral/i.v. corticosteroids (n=23). Horizontal bar represents the median and error bars the interquartile range. Statistical comparison performed using Mann-Whitney test.

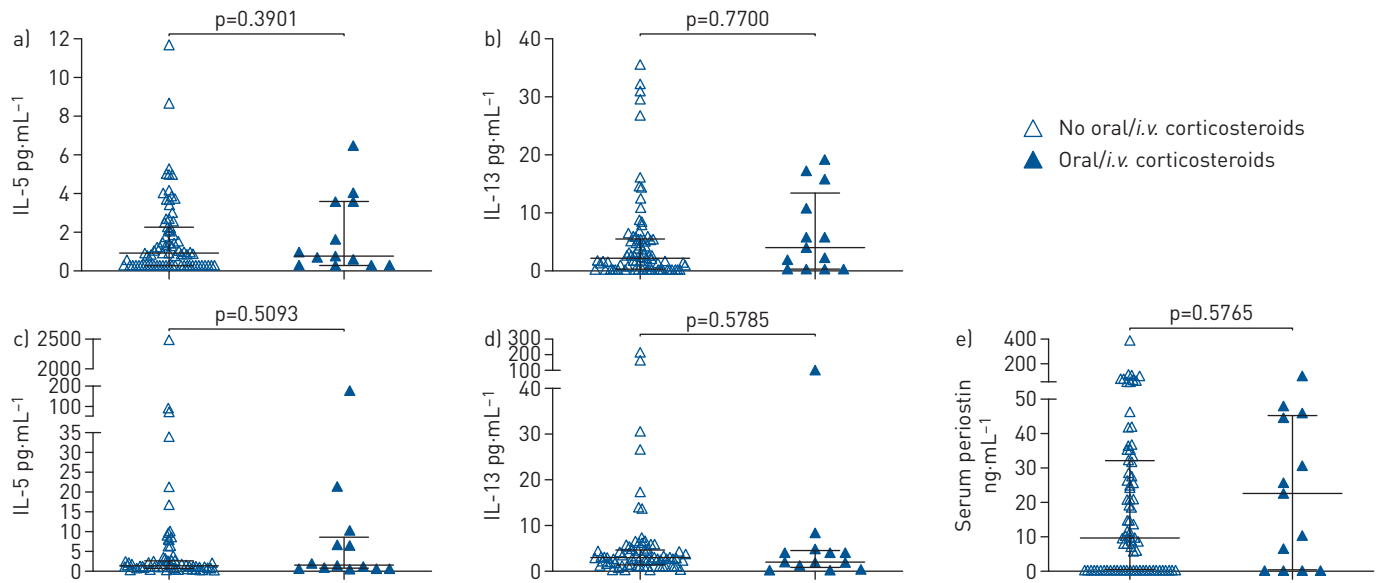


FIGURE 7 Type 2 inflammatory response in subjects without asthma based on administration of oral/intravenous corticosteroids within 24 h of admission. Nasal a) interleukin (IL)-5 and b) IL-13 levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=13), subjects not on oral/i.v. corticosteroids (n=79). Serum c) IL-5 and d) IL-13 levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=13), subjects not on oral/i.v. corticosteroids (n=78). e) Serum periostin levels measured within 24 h of admission; subjects on oral/i.v. corticosteroids (n=13), subjects not on oral/i.v. corticosteroids (n=77). Horizontal bar represents the median and error bars the interquartile range. Statistical comparison performed using Mann-Whitney test.

we do not know about other features that predispose to atopy, namely the presence of allergic rhinitis or atopic dermatitis symptoms and skin-prick or specific IgE responses to allergens. Additionally, the pre-existing or convalescent status of type 2 inflammatory mediators in both groups are unknown and could therefore have been confounded by the presence of influenza infection.

Serosurveys show that the first wave of the 2009 pandemic influenza A H1N1 infected one in three children in the UK [11], with only a small minority suffering severe disease. The role of type 2 cytokines in the pathogenesis of influenza remains unclear. Although T-helper 2 cells are major producers of IL-5 and IL-13 [23], other cells may also contribute. For example, infection of airway epithelial cells can induce the secretion of epithelial-derived cytokines such as IL-25, thymic stromal lymphopoietin (TSLP) and IL-33, which interact with innate lymphoid cells (ILCs) to induce the release of type 2 cytokines [24, 25]. TSLP induces type 2 cytokine production, even in naive CD4⁺ cells and indirectly affects dendritic cell function; it may have protective antiviral effects in mice [26]. Influenza infection can enhance production of alveolar macrophage derived IL-33, resulting in increased IL-13 release by ILCs and airway hyperreactivity in mice [27]. IL-33 may also have an important role in epithelial injury repair after influenza infection [28, 29].

IFN deficiency has been described in cultured cells from asthma volunteers following *ex vivo* human rhinovirus infection [30–32], whereas robust IFN- γ and IFN- λ responses have been found *in vivo* during exacerbations in children with asthma [33, 34]. A recent study of the effects of fluticasone on bronchial biopsy explants from patients with and without asthma infected *ex vivo* with influenza did not demonstrate any difference in epithelial cell infection rates between groups. While there was a reduction in the secretion of innate immune mediators (including IFN- γ , CXCL10, monocyte chemoattractant protein-1 and IL-6), type 1 IFNs were undetectable [35]. Our current study had the advantages of *in vivo* direct sampling of the blood and airway mucosa, showing increased serum levels of IFN- α 2a in patients with asthma, but comparable levels of interferons (α , β , γ , λ) and related chemokines (CXCL10 and CXCL11) in mucosal fluids. Indeed, of all the serum proteins measured the only mediator that was increased in patients with asthma compared to those without asthma was IFN- α 2a. This suggests that patients with asthma do not have impaired antiviral immunity in response to pandemic H1N1 influenza and the combination of enhanced serum IFN- α 2a with a relative lack of proinflammatory mediators may have played a protective role among those with asthma, leading to a milder course of disease.

We found that the use of in-hospital systemic corticosteroids was associated with poor outcome in those without asthma, but not in those with asthma. A meta-analysis of unselected patients with influenza-related complications found that systemic corticosteroid use is associated with worse outcomes [36].

More recently, a placebo-controlled randomised clinical trial in primary care confirmed the lack of any beneficial effect of oral corticosteroids for acute lower respiratory tract symptoms in patients without asthma [37]. We found that the use of in-hospital systemic corticosteroids was associated with poor outcome in those without asthma, but not in those with asthma. However, the fact that corticosteroid use is more prevalent in patients who have increased influenza-related complications may have contributed to this association.

Multivariate logistic regression analysis indicated that presentation to hospital ≥ 4 days after symptom onset was associated with worse outcome among patients without asthma. Individuals with asthma have an abnormal respiratory epithelium with airway hyperreactivity and may present to hospital primarily due to enhanced mucosal responses and bronchospasm. We speculate that the increased number of asthma patients being hospitalised with exacerbations may partly be due to early onset of respiratory symptoms caused by inflammation in the conducting airways. By contrast, people without asthma may present with respiratory failure caused by inflammation in the distal gas-exchanging parts of the lung.

A slower decline in viral load has been associated with increased severity of influenza infection and immunodysregulation [38]. We found that viral load measured within 24 h of admission, subsequent clearance of virus, or serological responses to H1N1 was not significantly different between patients with and without asthma. This is consistent with our conclusions that different systemic immune responses between clinical groups are not due to an inability to control viral replication, but rather to differences in host response. Antiviral medication (oseltamivir and zanamivir) is recommended for those with risk factors for influenza complications, including asthma [39]. Antivirals were very rarely given prior to hospital admission in our patients (asthma $n=2$, non-asthma $n=1$), so cannot explain differences in immune responses measured on admission. They were not administered in 20% of asthma patients in the first 24 h of admission for whom prescribing data was available, but most did receive at least one dose during the course of their admission. Other studies show that administration of antivirals early during illness and prior to admission significantly reduces hospital admission rates [40], and that antivirals within 48 h of symptom onset reduces mortality in hospitalised patients [41]. Our study found that 14 (35.9%) out of 39 asthma patients and 26 (29.2%) out of 89 patients without asthma received antivirals within 48 h of symptom onset. This may have been due to patients presenting later in their illness or a lack of awareness or resources available in primary care. While more individuals with asthma received the seasonal influenza vaccine compared to those without asthma, vaccination rates were low compared to national norms. Immunisation with H1N1 vaccine was especially rare, but it is notable that some of our cases did appear to have been appropriately vaccinated and yet developed severe influenza.

Influenza vaccination coverage in European countries varies widely and use of antivirals is patchy, highlighting the need for more effective education programmes for both the general population and healthcare providers in early case recognition, and consideration of early empirical antiviral therapy to reduce influenza-attributable healthcare utilisation among patients with asthma [42]. The development of more internationally coordinated registries that accurately collate vaccination status along with medical and sociodemographic details to assess the impact of public health interventions over time may also be warranted [42].

Our study has important limitations. It was designed to investigate all patients admitted with influenza rather than to address the issue of the effects of pre-existing asthma on the course of influenza. Asthma diagnosis was based on a review of clinical notes and patient-reported diagnosis of asthma and we lack spirometric data or information about baseline severity and nature of symptoms (*e.g.* persistent *versus* intermittent asthma). We do not have blood eosinophil counts, exhaled breath nitric oxide or sputum cell counts. Only 40% of asthma patients were on inhaled corticosteroids. However, national guidance at the time of recruitment did not require its use in those with mild asthma [43]. Additionally, clinical studies recruiting those with confirmed asthma commonly have only half of participants taking inhaled corticosteroids [19]. In future studies, prospective endophenotyping of asthma patients, coupled with the measurement of mucosal and systemic immune parameters might further enhance understanding of mechanisms of disease during viral exacerbations [44]. Large cohort studies with well-characterised subjects such as the Severe Asthma Research Programme (SARP) [45] and Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) [46] have the potential to identify how underlying endophenotypes influence susceptibility to infection and predict treatment response. Despite its limitations, we wish to report our findings in relation to asthma because of the unique nature of the MOSAIC study and the likelihood that an opportunity to study a pandemic in this way may not arise again.

In summary, our study suggests that patients with asthma hospitalised with influenza are predominantly female and have a good prognosis, with reduced systemic inflammation but comparable mucosal responses to individuals without asthma. Notably, serum total IgE levels and nasal IL-5 and IL-13 levels were not

statistically different between patients with and without asthma. Our study highlights the value of assessing the airway mucosal and systemic host immune response to influenza, suggesting investigation of prospective targeted therapeutic and preventative strategies in well-characterised asthma patients with intrinsic disease.

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