

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

Akhilesh Jha, Jake Dunning, Tanushree Tunstall, Ryan S. Thwaites, Long T. Hoang, The MOSAIC Investigators, Onn Min Kon, Maria C. Zambon, Trevor T. Hansel, Peter J. Openshaw

Supplemental Information

Table E1 | Multivariate logistic regression model of covariates associated with severe outcome

Table E2 | Serum cytokines and chemokines measured within 24 hours of admission

Table E3 | Nasal cytokines and chemokines measured within 24 hours of admission

Table E4 | Nasopharyngeal cytokines and chemokines measured within 24 hours of admission

Figure E1 | Serum level of CRP measured within 24 hours of admission

Figure E2 | Scatterplots of serum mediators differentiated by day of illness (DOI)

Figure E3 | Scatterplots of nasal mediators differentiated by day of illness (DOI)

Figure E4 | Scatterplots of nasopharyngeal mediators differentiated by day of illness (DOI)

Figure E5 | Expression of eosinophil associated genes in subjects with and without asthma

Table E1 | Multivariate logistic regression model of covariates associated with severe outcome

Outcome: Death or respiratory failure requiring invasive ventilation	P-value	Adjusted OR	95% CI
Asthma Present	0.0521	0.2947	0.0790 - 0.9537
Gender (female as reference)	0.2862	1.6550	0.6611 - 4.2674
Age (18-30 years as reference)			
Age 31-40	0.8939	1.0944	0.2847 - 4.1500
Age 41-50	0.6043	1.3669	0.4215 - 4.5728
Age >50	0.0818	3.1190	0.8890 - 11.7696
Admission >4 days	0.0001	5.9084	2.4540 - 15.4449
Seasonal flu vaccination	0.1342	0.4002	0.1120 - 1.2664
Inhaled corticosteroid use	0.2068	0.3573	0.0621 - 1.6321
Oral/I.V. corticosteroids	0.0154	3.4629	1.3036 - 9.9038
n=133			

Table E2 | Serum cytokines and chemokines measured within 24 hours of admission

Cytokine or chemokine	Median HC (n=36)	Median A (n=40)	Median NA (n=93)	Dunn's test with Bonferroni correction; <i>P</i> -value		
				A vs NA	A vs HC	NA vs HC
IFN- α 2 α	5.31	8.08	5.07	0.0099	0.0452	1.000
IFN- β	11.67	11.67	11.67	1.000	0.0042	0.0021
IFN- γ	0.32	1.38	2.32	0.1256	0.0806	0.0001
IFN - λ /IL-29	26.84	12.01	12.01	1.0000	0.3816	0.1327
TNF- α	5.17	4.67	6.64	0.0000	0.5235	0.0015
GMCSF	1.06	0.29	0.76	0.4591	0.8004	1.0000
IL-1 β	0.78	0.33	0.33	0.2631	1.0000	0.3032
IL-2	4.70	5.09	6.23	0.0906	1.0000	0.2109
IL-4	1.66	0.90	1.02	1.0000	0.0038	0.0011
IL-5	1.06	0.77	1.42	0.0010	0.2020	0.1791
IL-6	3.78	9.98	38.81	0.0005	0.0004	0.0000
IL-10	1.48	4.59	7.49	0.0411	0.0001	0.0000
IL-12p70	3.99	1.75	1.98	0.8897	0.0145	0.0177
IL-13	1.22	2.64	2.91	0.3131	0.0004	0.0000
IL-15	1.63	3.71	4.81	0.1809	0.0000	0.0000
IL-17	3.20	5.56	7.58	0.0197	0.0003	0.0000
CCL2/ MCP-1	415.82	435.19	595.56	0.0038	1.0000	0.0027
CCL3/ MIP-1 α	10.99	13.49	13.69	1.0000	0.0725	0.0166
CCL4/ MIP-1 β	165.28	125.66	133.52	0.7915	0.0179	0.0291
CCL11/ Eotaxin	1250.70	547.81	621.48	1.0000	0.0002	0.0000
CCL13/ MCP-4	640.96	357.80	394.07	0.8654	0.0017	0.0016
CCL17/ TARC	412.83	263.25	240.04	1.0000	0.0020	0.0007
CCL22/ MDC	4010.90	1381.39	1739.56	0.5969	0.0000	0.0000
CCL26/ Eotaxin-3	2.51	15.75	18.36	0.8089	0.0000	0.0000
CXCL8/ IL-8	7.61	10.99	25.78	0.0000	0.0643	0.0000
CXCL9/ MIG	44.64	68.72	114.22	0.0031	0.0016	0.0000
CXCL10/ IP-10	167.09	721.01	1409.26	0.0546	0.0000	0.0000
CXCL11/ ITAC	60.98	187.57	277.71	0.2949	0.0000	0.0000

HC=Healthy control; A=Asthma; NA=No Asthma

Table E3 | Nasal cytokines and chemokines measured within 24 hours of admission

Cytokine or chemokine	Median HC (n=36)	Median A (n=40)	Median NA (n=93)	Dunn's test with Bonferroni correction; <i>P</i> -value		
				A vs NA	A vs HC	NA vs HC
IFN- α 2a	1.31	1.31	1.31	1.0000	0.0000	0.0000
IFN- β	66.05	41.02	49.52	0.7356	0.0604	0.1247
IFN- γ	0.32	1.36	1.60	1.0000	0.0496	0.0495
IFN- λ (IL-29)	12.01	12.01	12.01	1.0000	0.0000	0.0000
TNF- α	1.31	1.83	3.86	0.0674	0.0312	0.0000
GMCSF	1.17	1.63	1.58	0.7172	0.1926	0.0208
IL-1 β	31.11	28.03	25.36	1.0000	0.9505	1.0000
IL-2	1.93	2.47	3.49	0.7123	1.0000	0.8696
IL-4	0.40	0.40	0.40	0.2432	0.0000	0.0000
IL-5	1.04	0.65	0.95	0.7025	0.2192	0.4762
IL-6	12.26	54.78	125.68	0.4013	0.0000	0.0000
IL-10	0.78	1.58	1.99	1.0000	0.0041	0.0001
IL-12p70	1.51	1.12	1.45	0.3472	0.3515	1.0000
IL-13	0.76	3.09	2.33	0.1510	0.0000	0.0002
IL-15	1.17	2.26	1.94	0.6601	0.0103	0.0235
IL-17	7.06	9.67	9.01	0.5367	0.2055	0.5915
CCL2/ MCP-1	49.16	160.36	161.93	0.7861	0.0014	0.0000
CCL3/ MIP-1 α	8.65	28.61	29.41	1.0000	0.0000	0.0000
CCL4/ MIP-1 β	23.33	92.86	135.37	0.6431	0.0005	0.0000
CCL11/ Eotaxin	138.53	253.14	229.45	0.3149	0.0000	0.0000
CCL13/ MCP-4	12.35	13.00	12.88	1.0000	0.4939	0.3527
CCL17/ TARC	4.96	17.67	14.37	0.3462	0.0543	0.2938
CCL22/ MDC	115.93	157.02	158.61	1.0000	0.3130	0.1788
CCL26/ Eotaxin-3	30.91	44.17	38.45	1.0000	0.4245	0.2513
CXCL8/ IL-8	1880.12	2177.42	2326.43	1.0000	1.0000	1.0000
CXCL9/ MIG	212.28	561.13	525.16	0.1771	0.0037	0.0625
CXCL10/ IP-10	773.80	5215.96	4619.65	0.2013	0.0000	0.0000
CXCL11/ ITAC	11.65	126.69	64.50	0.1026	0.0000	0.0010

HC=Healthy control; A=Asthma; NA=No Asthma

Table E4 | Nasopharyngeal cytokines and chemokines measured within 24 hours of admission

Cytokine or chemokine	Median HC (n=36)	Median A (n=40)	Median NA (n=93)	Dunn's test with Bonferroni correction; <i>P</i> -value		
				A vs NA	A vs HC	NA vs HC
IFN-α2a	1.31	1.31	1.31	0.2611	0.0016	0.0000
IFN-β	11.67	11.67	11.67	1.0000	0.0394	0.0386
IFN-γ	0.32	1.05	0.32	0.2193	0.6190	0.8937
IFN-λ (IL-29)	12.01	12.01	12.01	0.5483	0.0000	0.0000
TNF-α	0.30	7.90	7.11	1.0000	0.0000	0.0000
GMCSF	0.29	2.01	2.42	1.0000	0.0000	0.0000
IL-1β	0.33	4.76	3.21	1.0000	0.0000	0.0000
IL-2	0.58	5.27	10.08	0.6876	0.0088	0.0001
IL-4	0.40	0.40	1.23	0.7800	1.0000	0.9828
IL-5	0.30	0.90	0.78	1.0000	0.6427	0.6659
IL-6	0.33	10.55	34.27	0.9277	0.0000	0.0000
IL-10	0.28	0.80	0.61	1.0000	0.0000	0.0000
IL-12p70	0.29	1.39	1.13	1.0000	0.2038	0.0796
IL-13	0.78	2.78	1.76	0.2548	0.0000	0.0000
IL-15	0.31	0.66	0.47	1.0000	1.0000	1.0000
IL-17	0.25	9.09	5.20	0.5273	0.0000	0.0000
CCL2/ MCP-1	1.70	66.62	123.56	1.0000	0.0000	0.0000
CCL3/ MIP-1α	1.37	28.04	26.42	1.0000	0.0000	0.0000
CCL4/ MIP-1β	5.25	279.03	135.08	0.8527	0.0076	0.0001
CCL11/ Eotaxin	14.98	850.31	536.97	1.0000	0.0000	0.0000
CCL13/ MCP-4	2.94	38.24	38.26	1.0000	0.0000	0.0000
CCL17/ TARC	4.96	47.84	29.73	0.5485	0.0000	0.0000
CCL22/ MDC	54.39	435.31	375.91	0.8612	0.0000	0.0000
CCL26/ Eotaxin-3	2.51	26.86	17.95	1.0000	0.0000	0.0000
CXCL8/ IL-8	10.40	2173.69	888.60	1.0000	0.0000	0.0000
CXCL9/ MIG	2.52	360.53	216.47	0.9507	0.0000	0.0000
CXCL10/ IP-10	11.98	9004.99	3389.03	0.7205	0.0000	0.0000
CXCL11/ ITAC	0.81	134.15	135.70	0.7444	0.0000	0.0000

HC=Healthy control; A=Asthma; NA=No Asthma

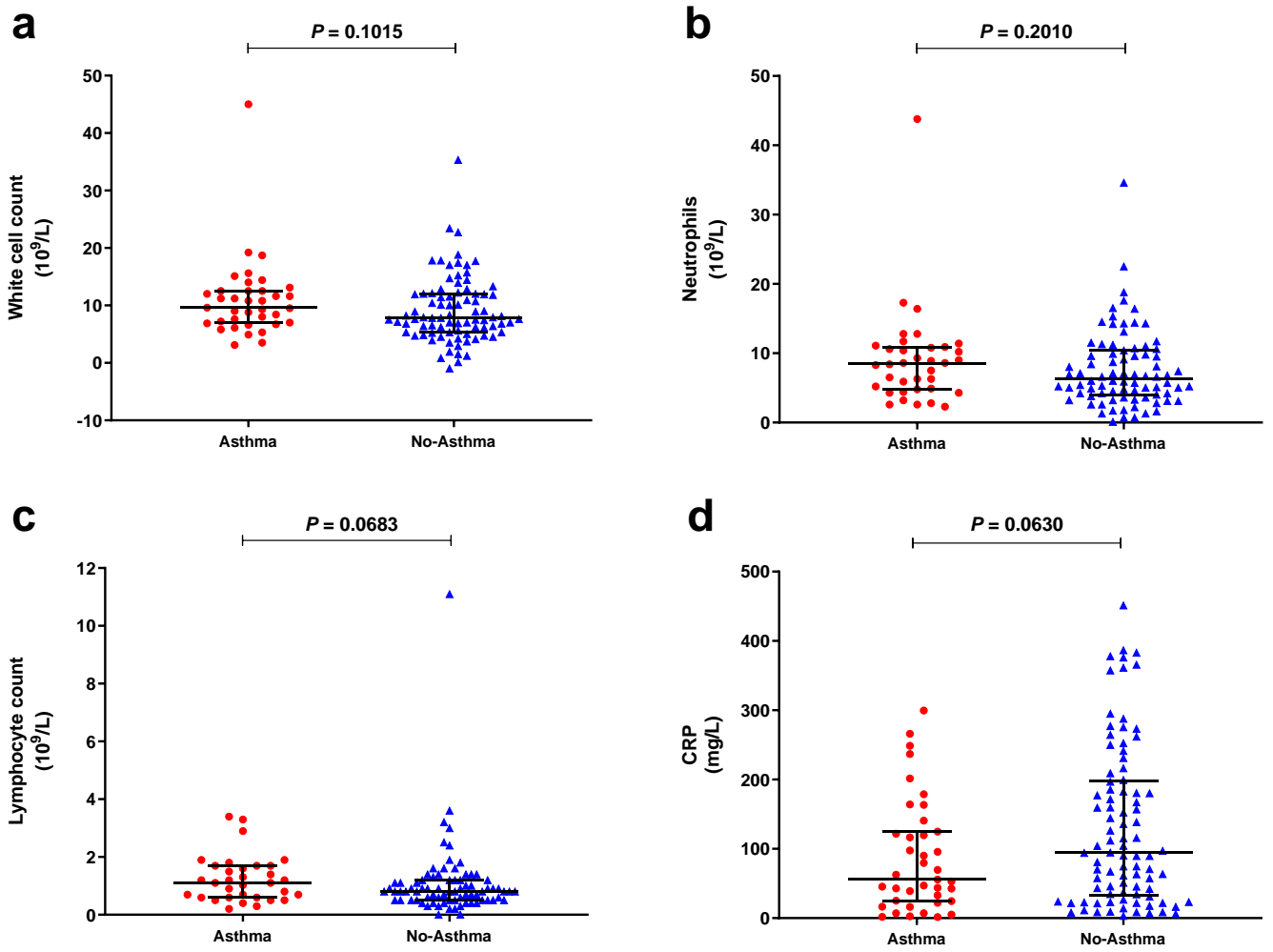


Figure E1 | Serum white cell count differential and CRP levels in subjects with and without asthma
 Measured within 24 hours of admission in individual subjects: (a) white cell count (asthma $n=37$, no asthma $n=84$), (b) neutrophils (asthma $n=36$, no asthma $n=82$), (c) lymphocytes (asthma $n=33$, no asthma $n=79$) and CRP (asthma $n=37$, no asthma $n=90$). Horizontal bar represents the median and error bars the interquartile range. Statistical analysis performed using Mann-Whitney test.

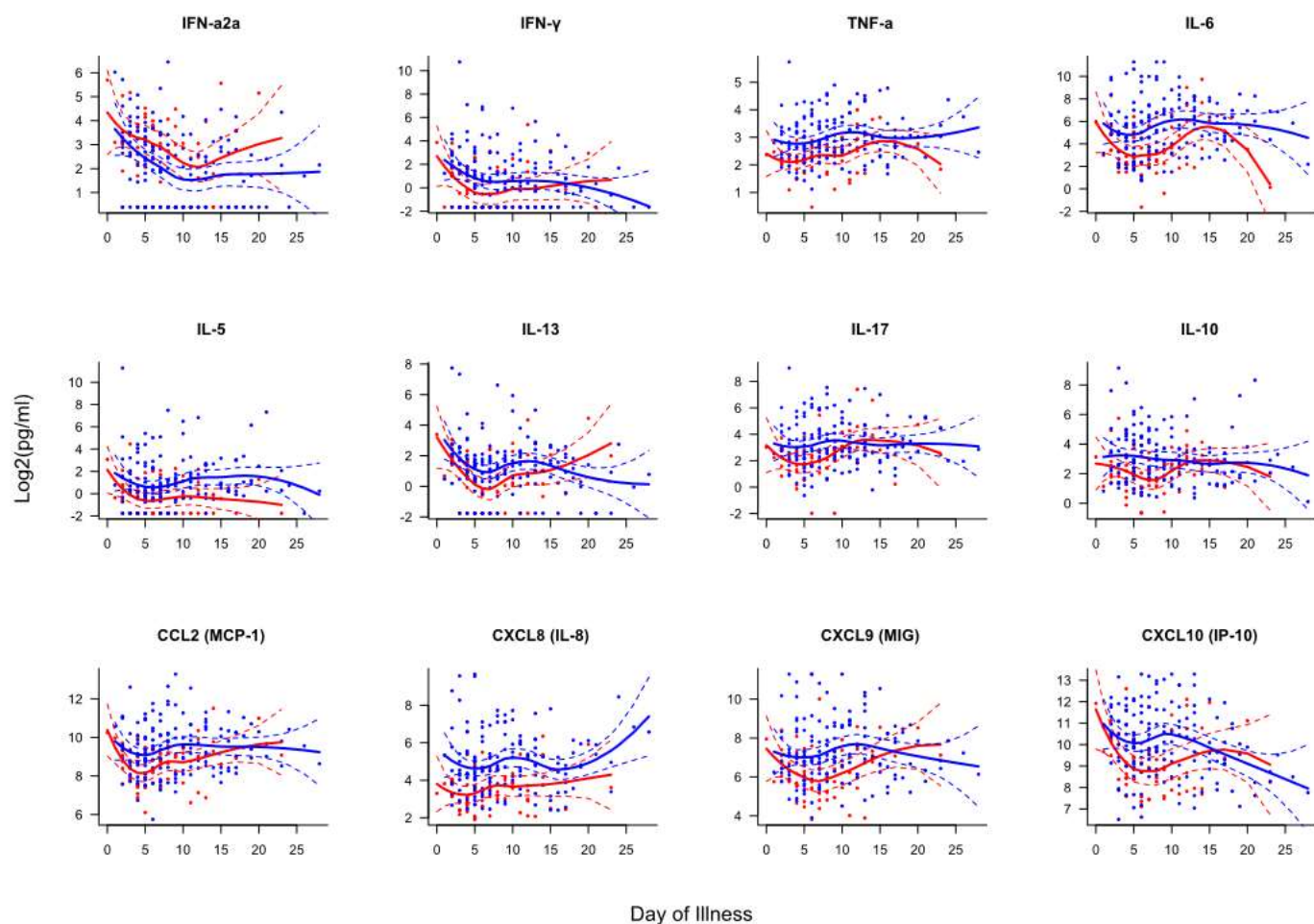


Figure E2 | Scatterplots of serum mediators differentiated by day of illness (DOI)

Dots represent individual samples up to two samples collected per subject. Red dots represent asthma (n=64), blue dots represent no asthma (n=155). First sample collected within 24 hours of admission and second sample between 48-72 hours of admission. DOI represents number of days elapsed between subject-reported symptom onset and time of sampling. 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 the 95% CI.

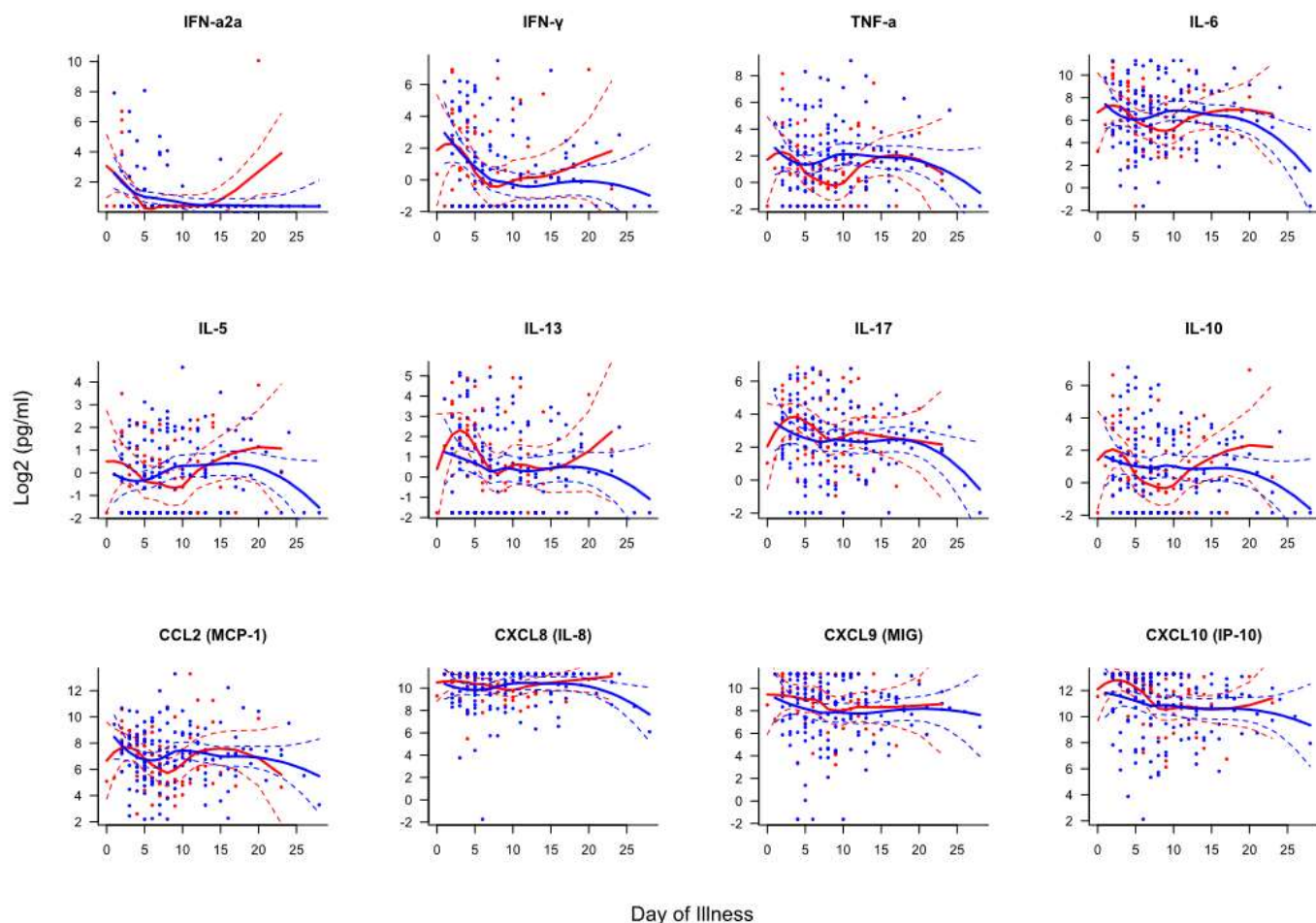


Figure E3 | Scatterplots of nasal mediators differentiated by day of illness (DOI)

Dots represent individual samples with up to two samples collected per subject. Red dots represent asthma (n=65), blue dots represent no asthma (n=153). First sample collected within 24 hours of admission and second sample between 48-72 hours of admission. DOI represents number of days elapsed between subject-reported symptom onset and time of sampling. 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 the 95% CI

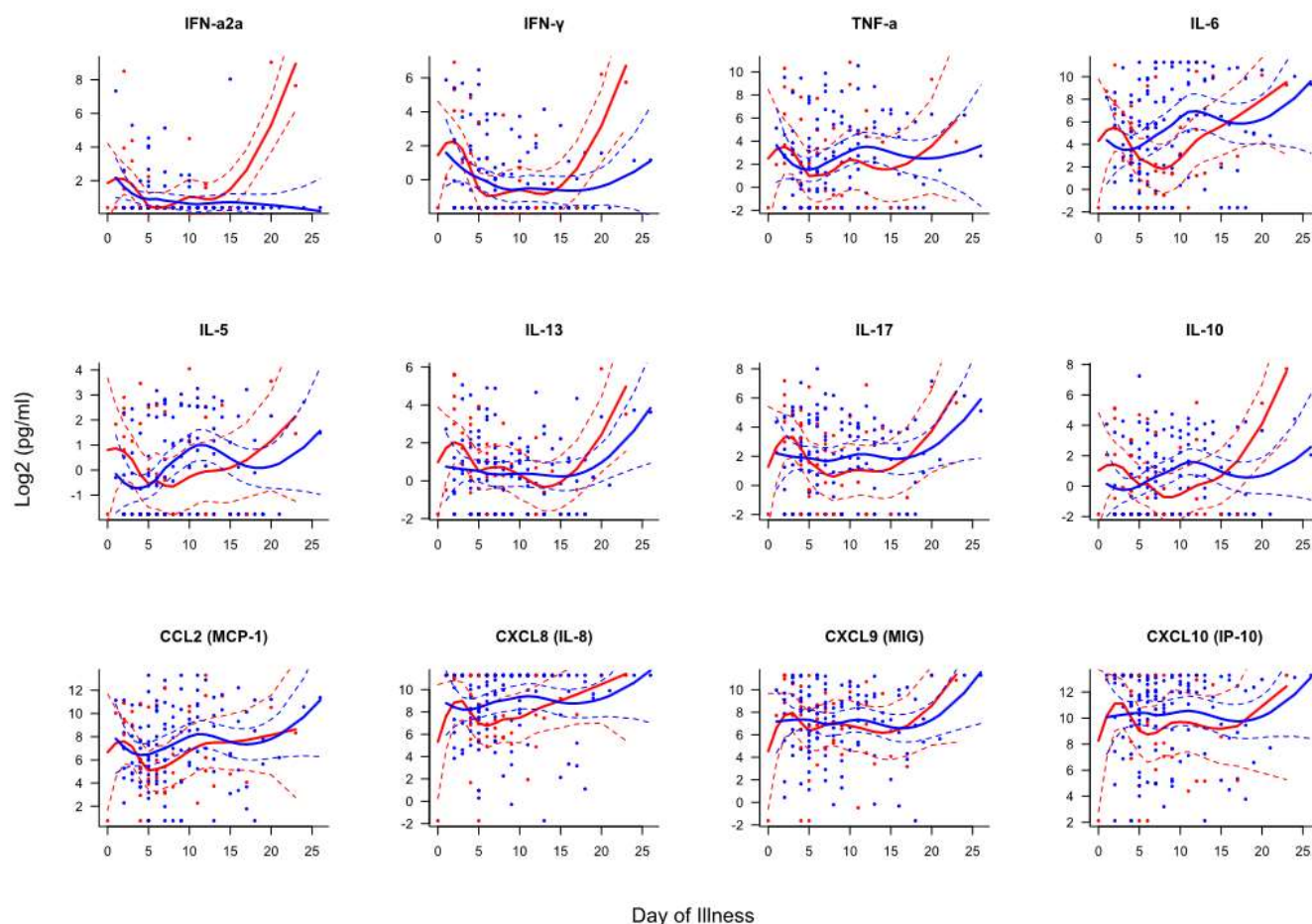


Figure E4 | Scatterplots of nasopharyngeal mediators differentiated by day of illness (DOI)

Dots represent individual samples up to two samples collected per subject. Red dots represent asthma (n=49), blue dots represent no asthma (n=123). First sample collected within 24 hours of admission and second sample between 48-72 hours of admission. Day of illness represents number of days elapsed between subject-reported symptom onset and time of sampling. 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 the 95% CI

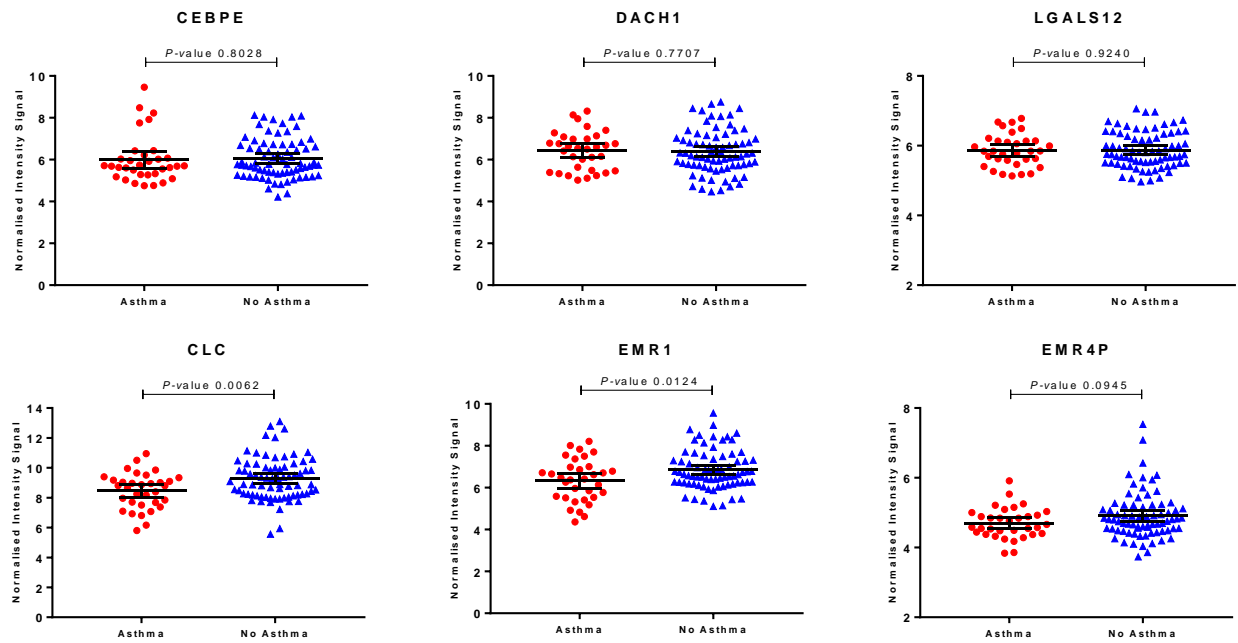


Figure E5 | Expression of eosinophil associated genes in subjects with and without asthma Whole blood samples were collected within 24 hours of admission for gene expression. RNA was extracted and analysed using Illumina microarray and the data normalized. Dots represent individual patients (asthma=33, no asthma=74) with horizontal bar representing the mean and error bars the 95% CI. Statistical analysis performed using unpaired t-test