



Temporal relationships of cytokines, chemokines and cellular biomarkers during Human Rhinovirus (HRV) infection in asthmatics

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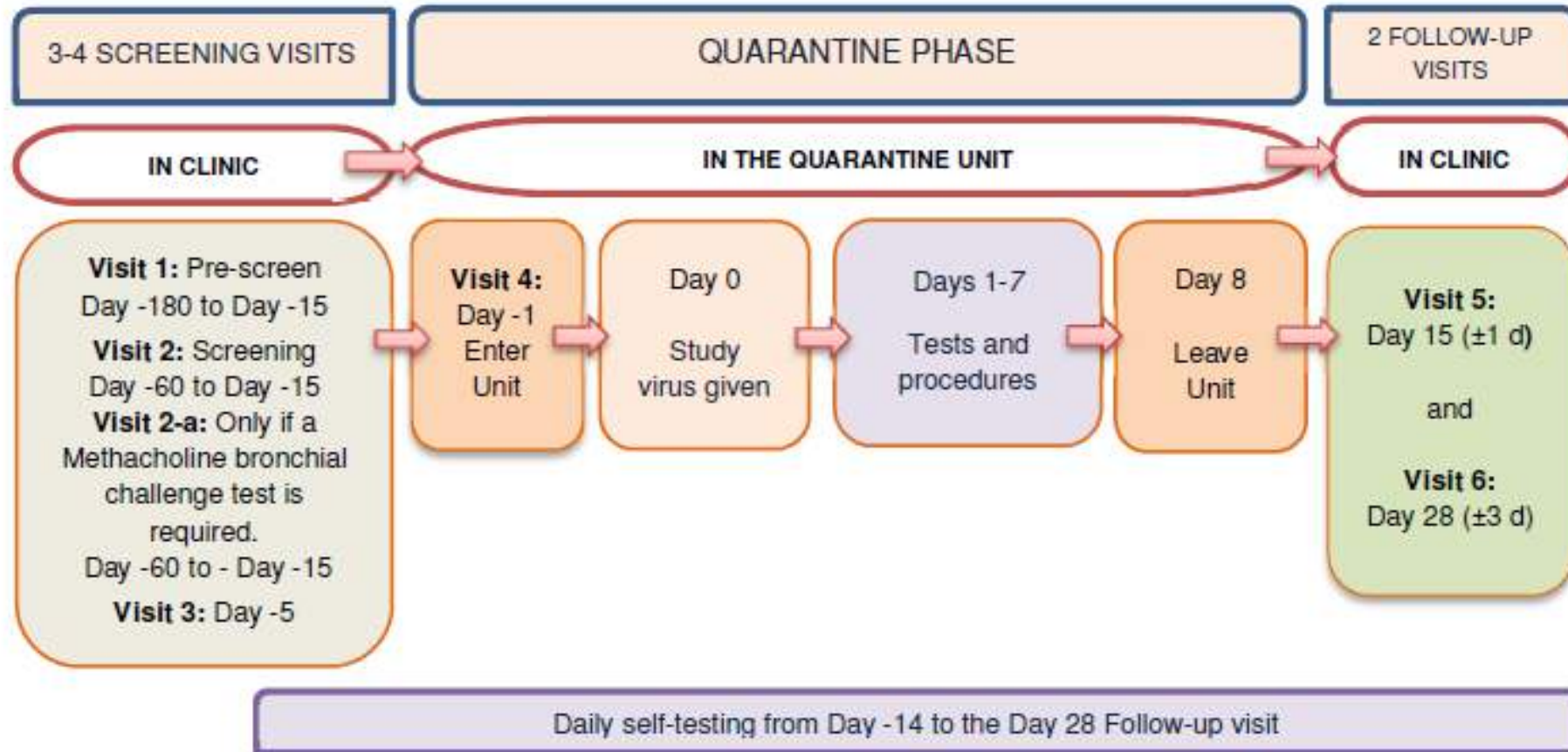
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Aims



- Explore the clinical response to HRV infection in a population of mild intermittent Asthma
- Identify baseline immunity markers to HRV induced Asthma worsening during infection
- Investigate the relationship between blood cells, NELF and blood biomarkers/cytokines to clinical responses during infection
- Interpretation of combined results

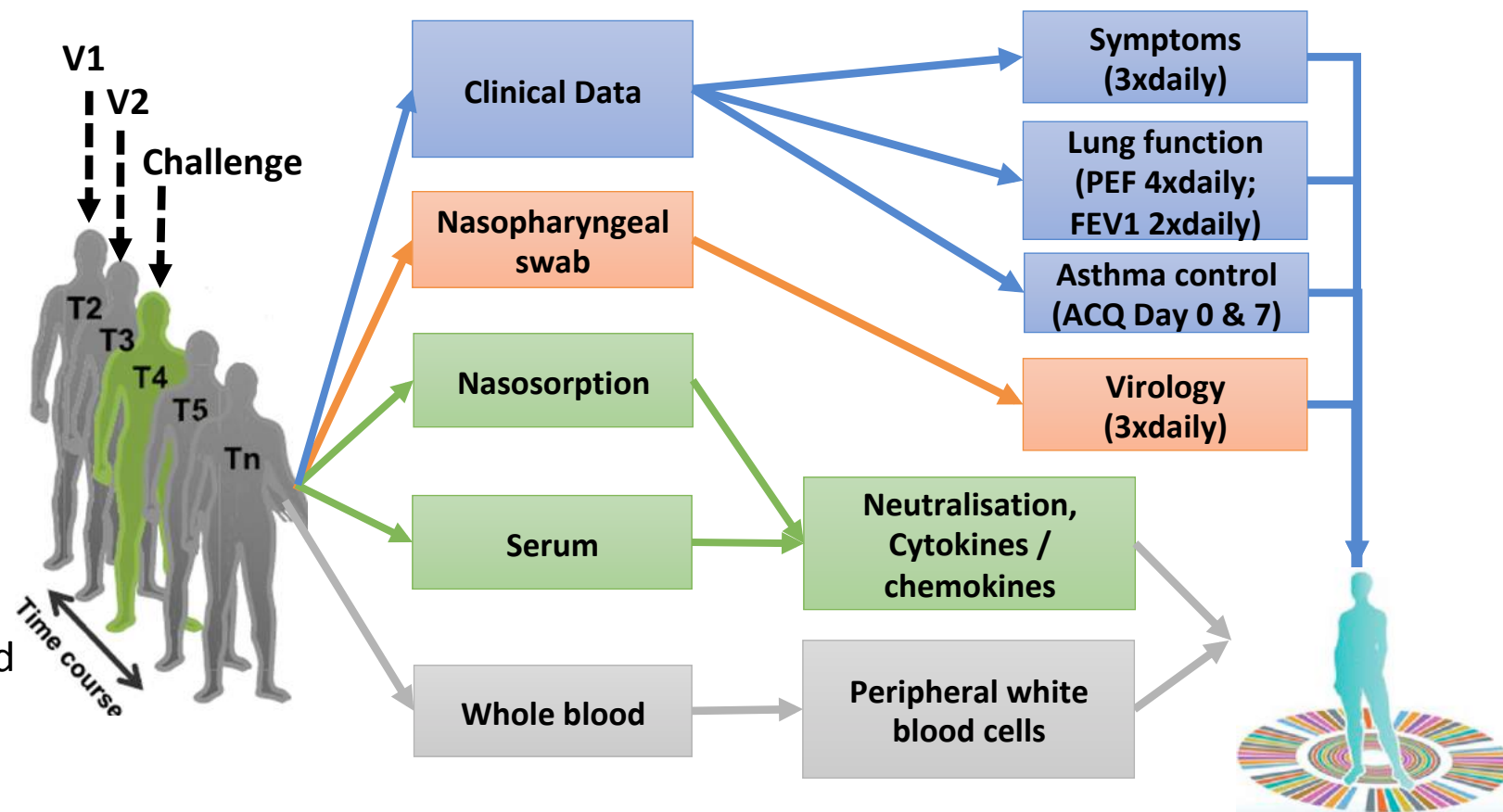
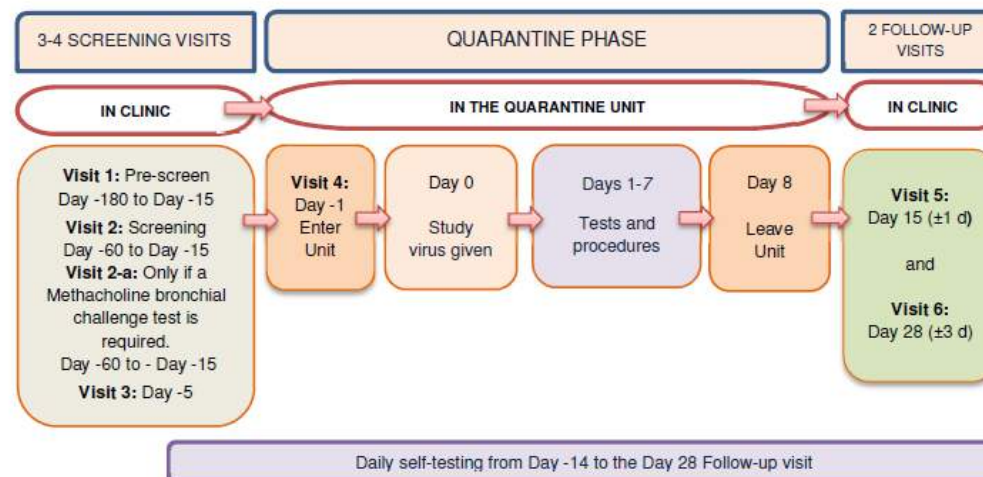
Study design



20 mild asthmatic subjects (GINA 1):

- 13 given HRV and 11 became infected
- 7 given diluent

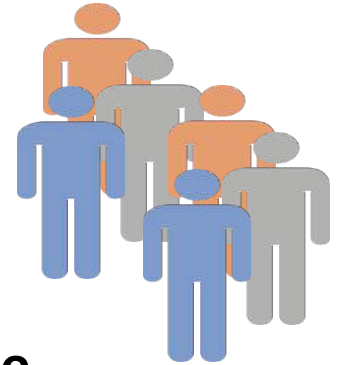
Study design



20 mild asthmatic subjects (GINA 1):

- 13 given HRV and 11 became infected
- 7 given diluent
- 2 non-evaluable

Demographics and Baseline characteristics



Baseline characteristics	Asthma Infected (n=11)	Asthma Uninfected (n=7)
Age	26 ± 4.7	27 ± 9.0
Sex (% male)	9 (82)	6 (86)
Ethnicity		
White	10 (91)	7 (100)
African/Caribbean	1 (9)	0 (0)
GINA 1	11 (100)	7 (100)
Lung function - baseline		
PEF % predicted	93.7 ± 13.6	91.9 ± 16.9
FEV1 % predicted	91.0 ± 9.4	91.9 ± 11.8
Asthma control - Day 0		
ACQ5	0.49 ± 0.23	0.31 ± 0.27
ACQ6	0.47 ± 0.21	0.3 ± 0.22
ACQ7	0.58 ± 0.2	0.43 ± 0.31
Blood eosinophils – Day 0		
percentage	4.6 ± 2.3	5 ± 2.7
count (10 ⁹ /L)	0.28 ± 0.15	0.3 ± 0.18
FeNO ppb - Day 0	48 ± 26	31 ± 11
Serum total IgE - Day 0		
Skin prick test +ve	100%	100%
Max wheal size (mm)	17 ± 15	18 ± 12
Number of +ve skin prick tests	2.3 ± 0.79	1.9 ± 0.83
Maximum wheal SI	2.2 ± 0.36	1.7 ± 0.40

Age – 18-55

Gender – 5 Female/15 Male

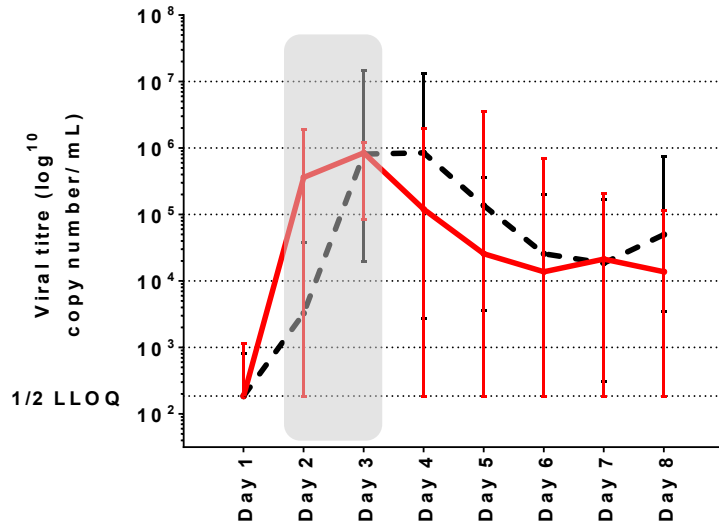
Atopic - skin prick test positive

Sensitivity – Reversible + PC₂₀<16 mg/ml

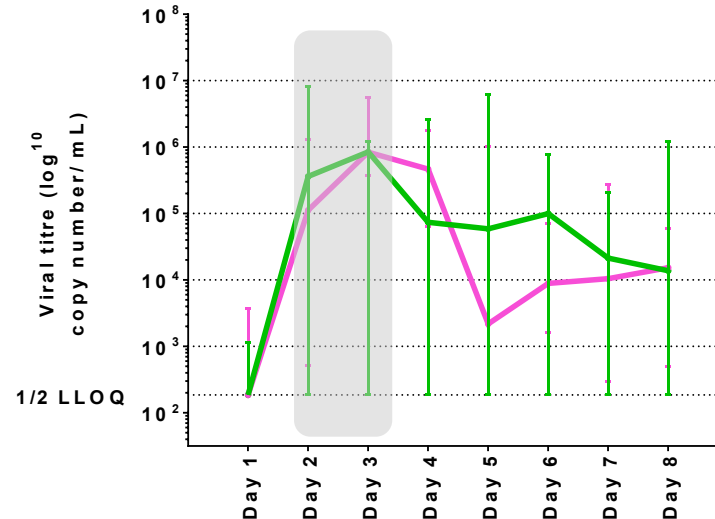
Asthma Medication – SABA PRN

Smoking history – non-smoker/< 10 pack yrs

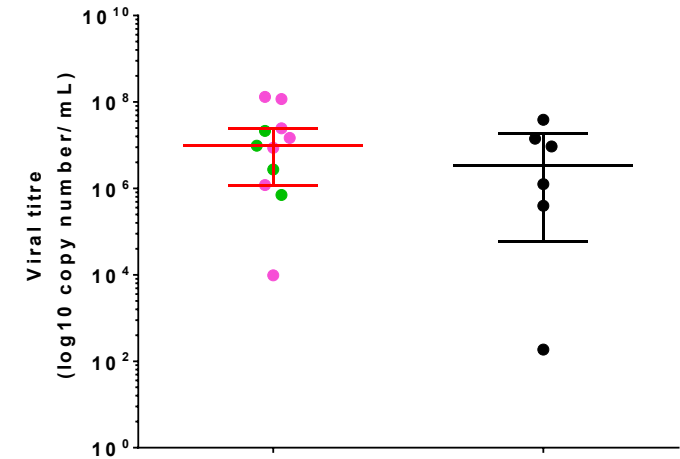
Clinical Endpoint: Virology



Infected with asthma (red, n=11)
Infected healthy (black, n=6)



Infected with asthma and reduced control (green, n=4), Infected with asthma and no reduced control (pink, n=7)



Infected with asthma and reduced control (green, n=4), Infected with asthma and no reduced control (pink, n=7)

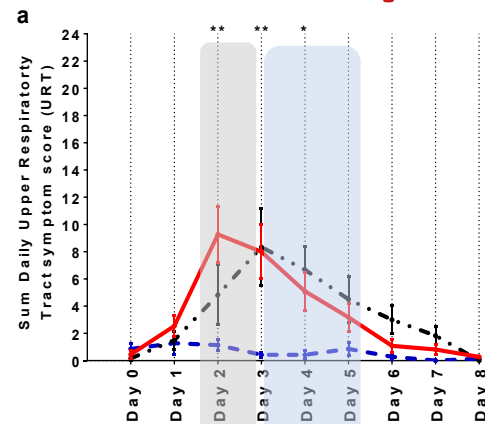
Healthy infected subjects have similar magnitude and profile of virus shedding to infected subjects with asthma

Infected subjects with clinically significant asthma worsening (ACQ rise) have similar viral curves to those with no asthma worsening

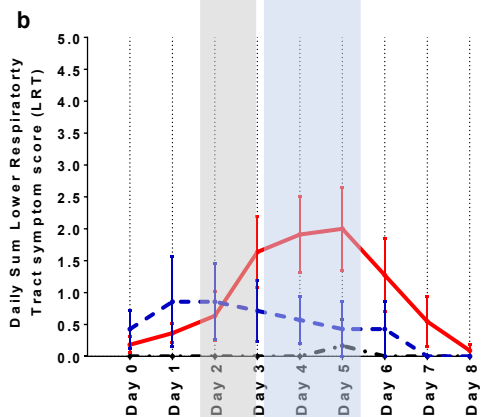
Clinical endpoints: ACQ change and intercorrelations



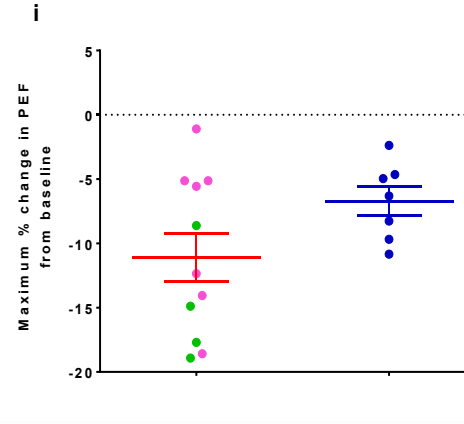
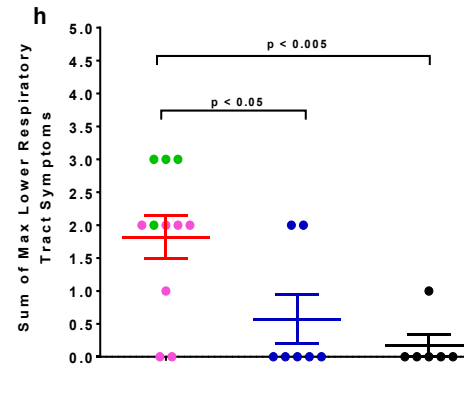
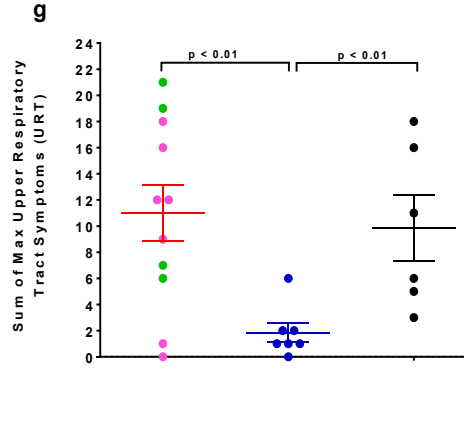
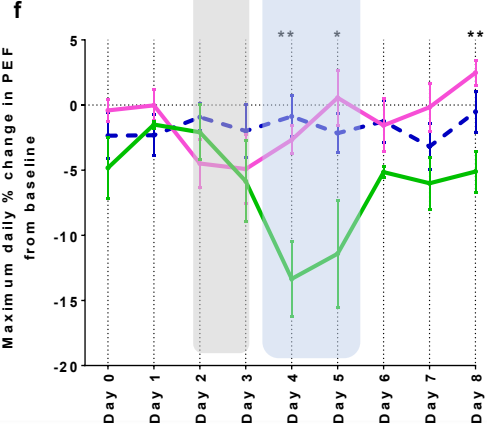
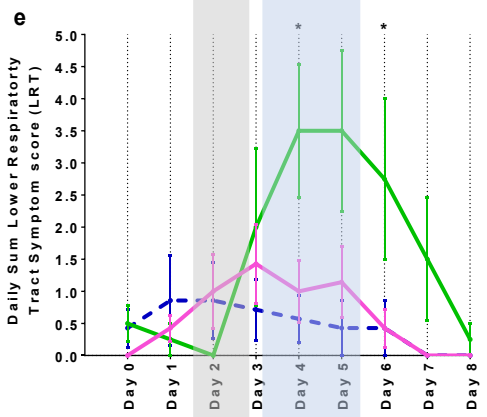
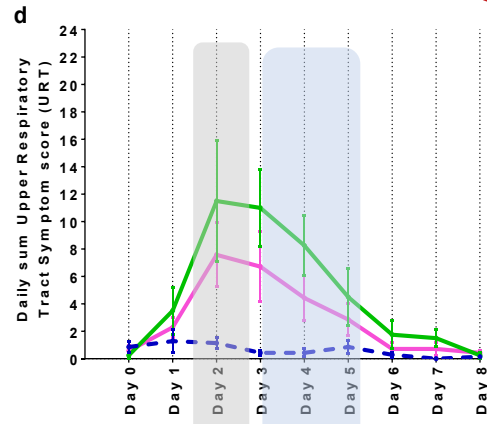
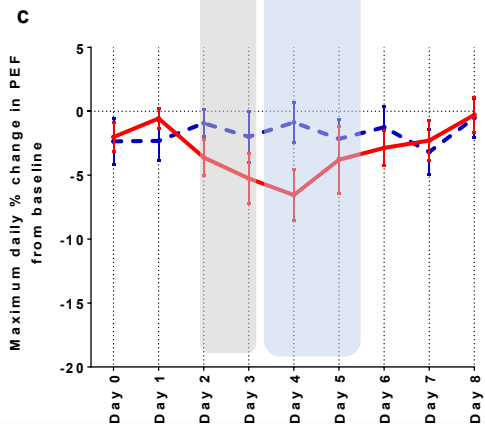
URT



LRT



PEF



Infected subjects with asthma have:

- an early onset of URT symptoms (Day 2,3)
- Delayed LRT symptoms and PEF fall (Day 3,4,5)

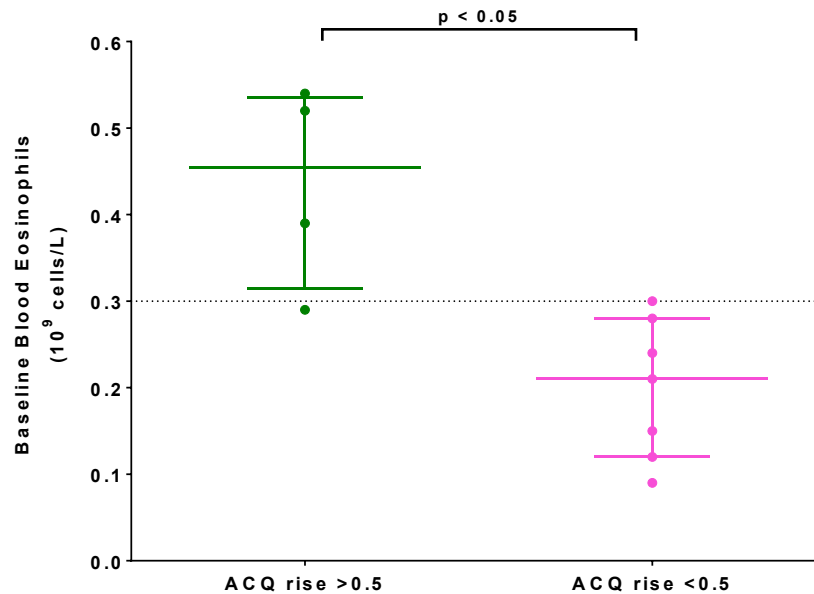
Infected subjects with reduced asthma control have:

- Higher URT
- Higher LRT symptoms (Day 4,5,6)
- Greater PEF falls (Day 4,5)

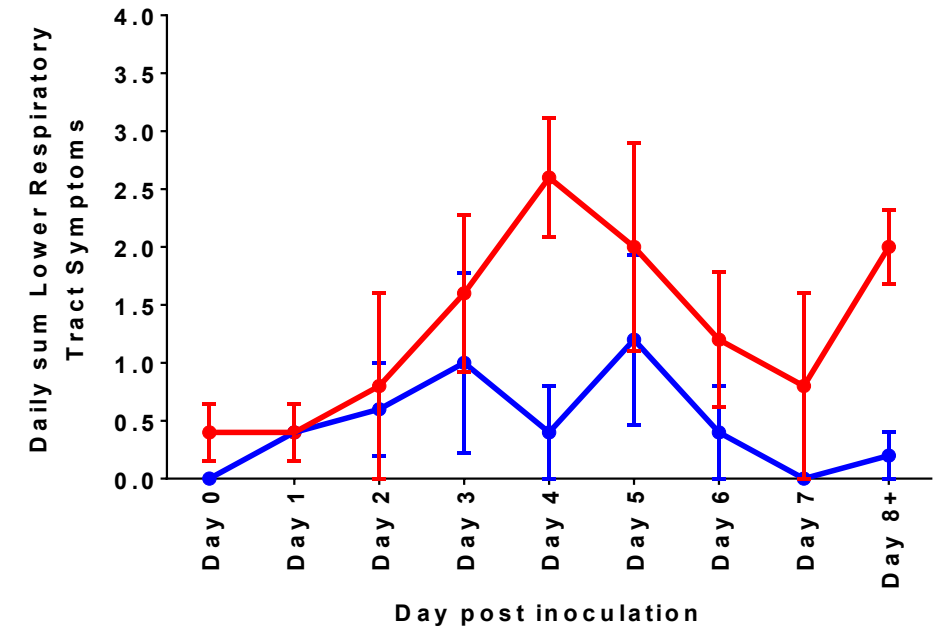
Immune Biomarkers: Baseline Susceptibility



Higher BL blood eosinophils associated with greater increases in ACQ (i.e. greater loss of asthma control)



LRTS for infected subjects with high (red) and low (blue) baseline NELF IL-5

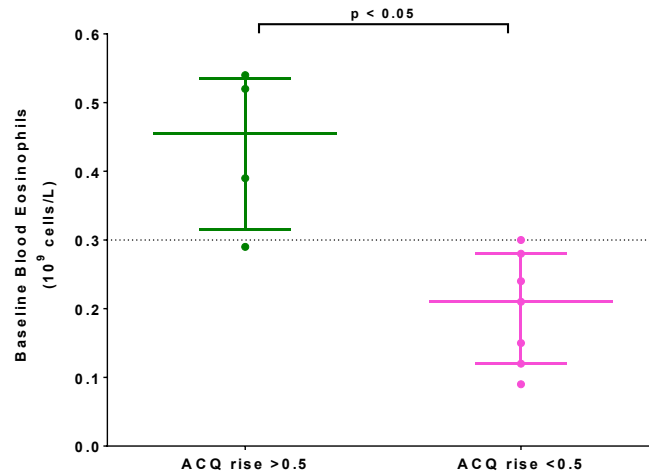


Type 2
immunity
(e.g.
Eosinophils,
IL-5)

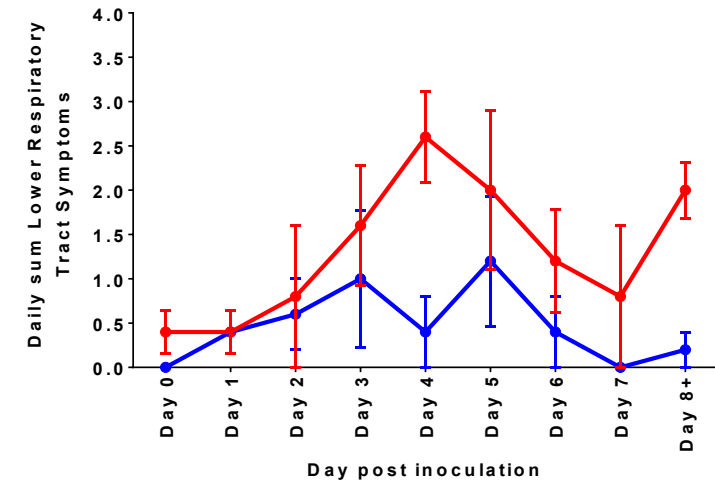
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Type 2
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Asthma control:

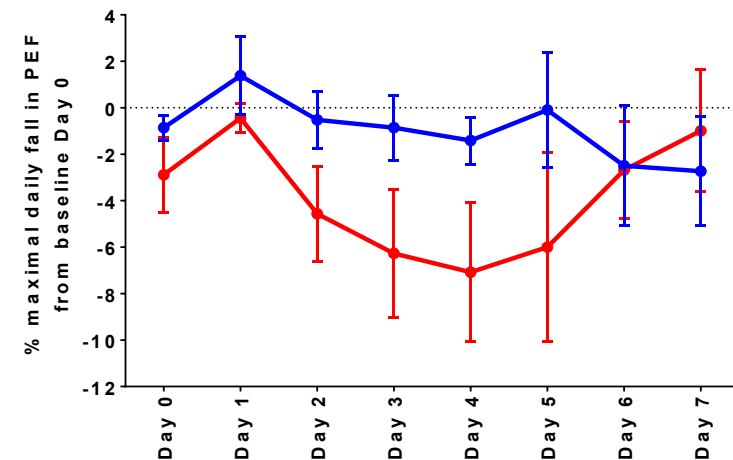
- Lymphocyte count correlated with ACQ-7 change ($r = 0.75$ $p < 0.05$)

Lung function:

- NELF TNF-alpha and CXCL10 correlated with PEF fall ($r = 0.59$, $p < 0.01$ and $r = 0.64$, $p < 0.05$ respectively).

Type 1
immunity
(TNF α ,
IFN γ)
&
Innate
immunity
(CXCL10)

Max daily % fall in PEF for infected subjects with high (red) and low (blue) baseline NELF CXCL10



Nasosorption biomarkers: post inoculation changes



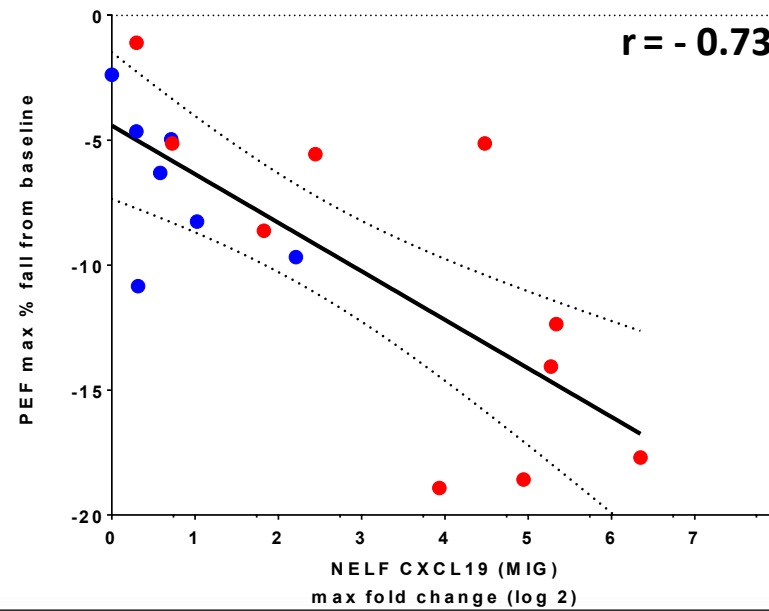
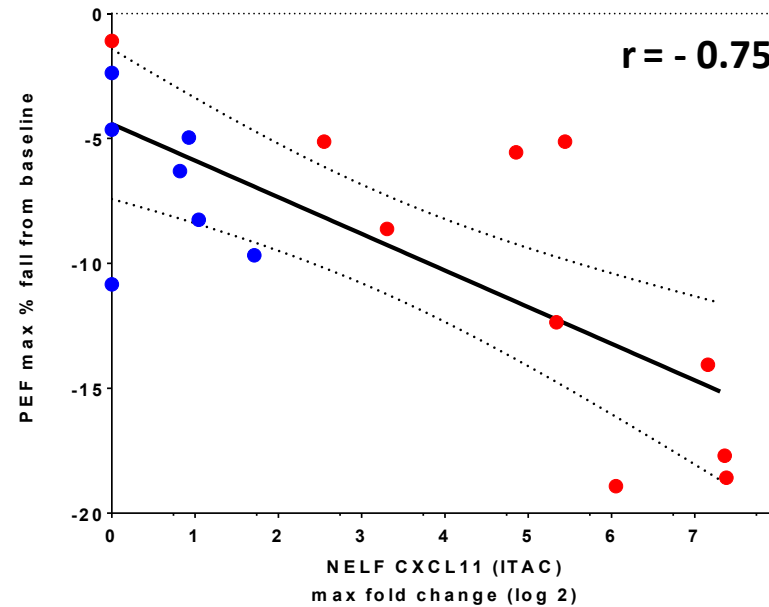
Chemokines for IL-2 activated T cells & NK cells

PEF:

- CXCL19 (MIG), $r = -0.75$
- CXCL11 (ITAC), $r = -0.73$
- CXCL10 (IP-10), $r = -0.68$

LRT:

- CXCL10 (IP-10), $r = 0.63$
- CXCL11 (ITAC), $r = 0.62$



Recruitment of immune cells and inflammation, & allergic response

PEF:

- CCL13 (MCP-4), $r = -0.69$
- CCL5 (RANTES), $r = -0.65$

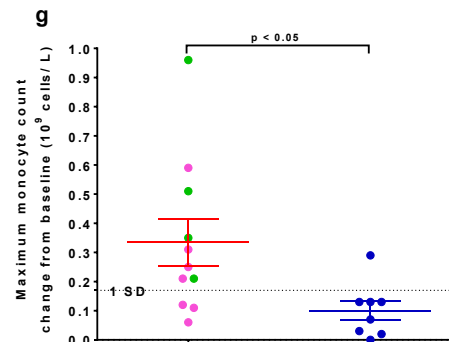
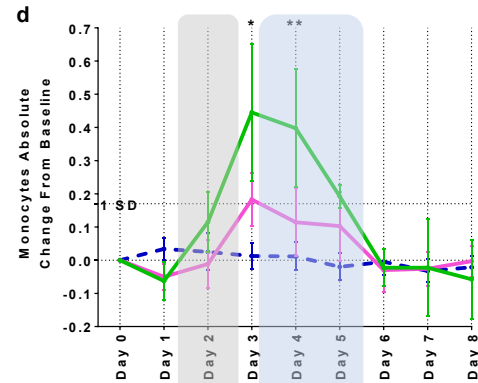
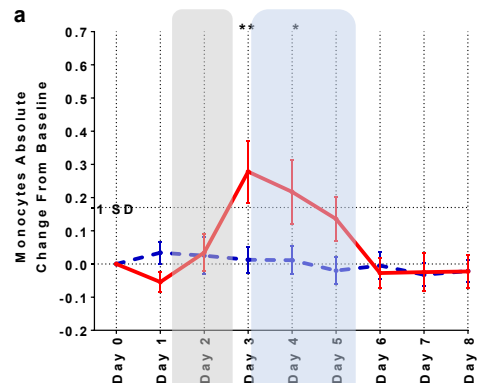
LRT:

- CCL8 (MCP-2), $r = 0.63$

Blood Cell Differentials: Post Inoculation relationships



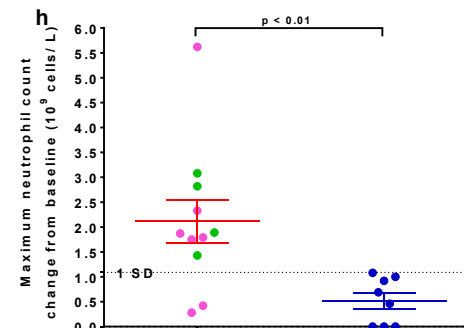
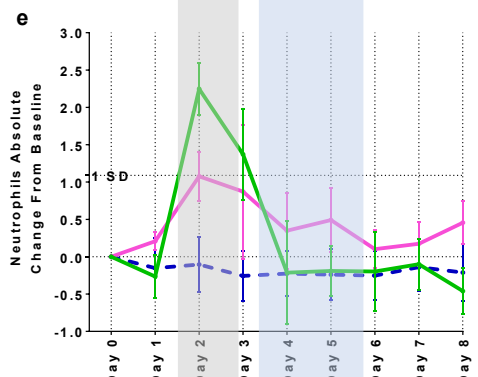
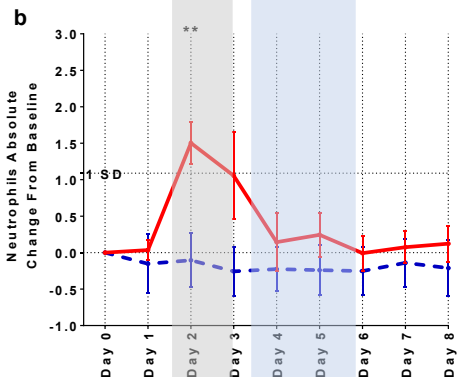
Monocytes



Infected subjects had:

- Greater change in monocytes,
- Greater change in neutrophils
- Lower L%:M% ratio

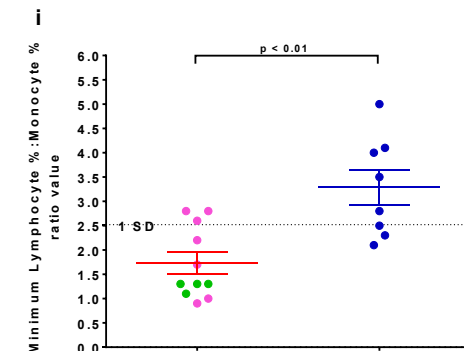
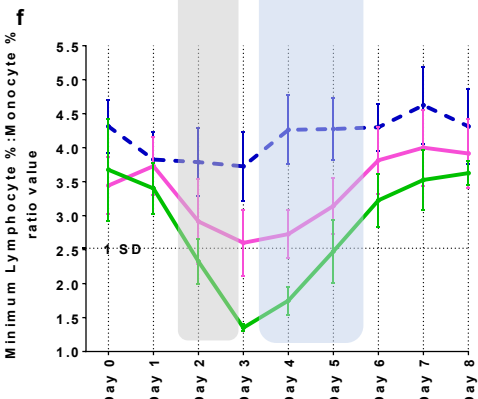
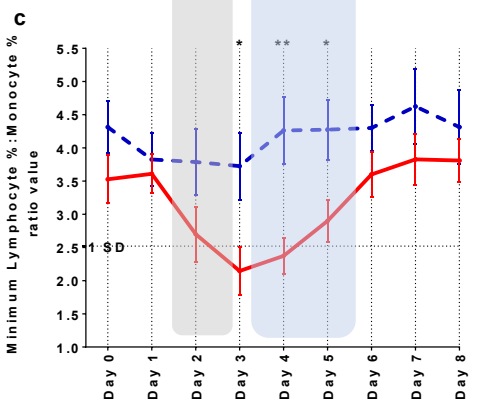
Neutrophils



Those with reduced in control had:

- Greater change in monocytes
- Greater change in neutrophils
- Lower L%:M% ratio

L%:M%



PEF and LRTS correlated with cells:

- Monocytes:
 - PEF ($r=-0.70$) & LRTS ($r=-0.75$)
- Neutrophils: PEF ($r=-0.75$)
- L%:M%: PEF ($r=0.63$)

Clinical, Cellular, and Biomarker Response to Infection



Recruitment of immune cells
and inflammation, & allergic
response

Chemokines for IL-2
activated T cells & NK cells

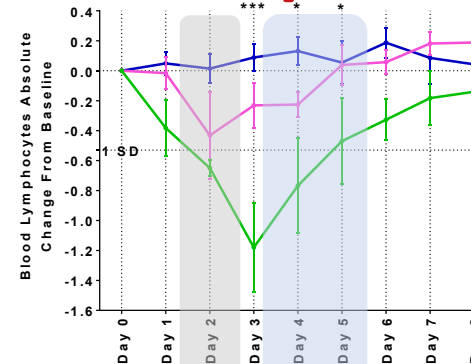
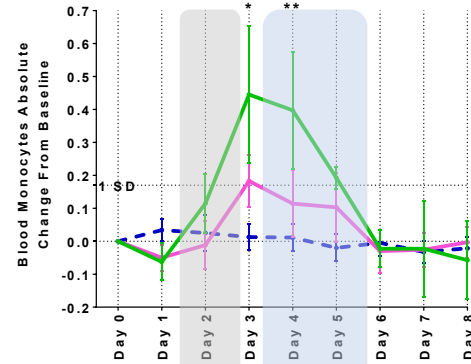
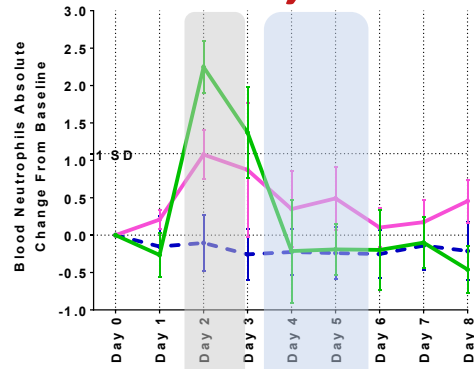
PEF, LRT, and blood cell changes correlated with NELF chemokines/ cytokines			
	Mono	Neut	L:M
CCL8	X	X	
CCL13	X	X	
CCL5		X	
CXCL10	X	X	X
CXCL9	X	X	X
CXCL11	X	X	X
IFN γ		X	
IFN α	X	X	
IL-10	X	X	X
IL-6			X

Interferon, anti-inflammatory

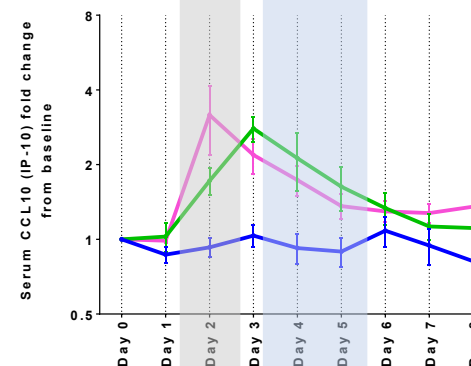
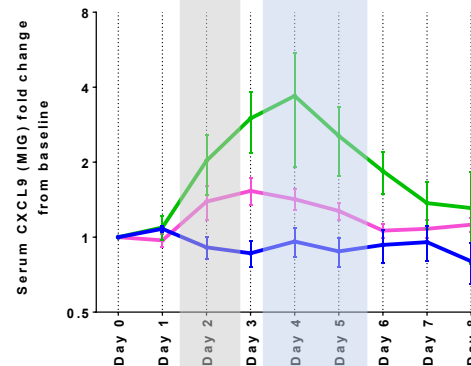
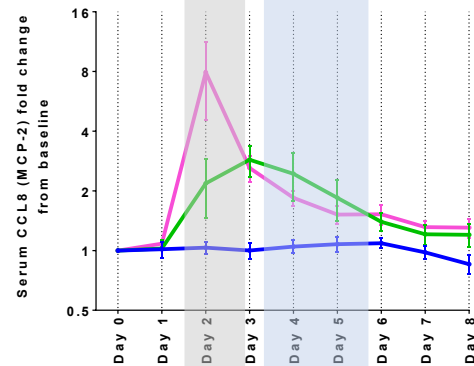
Clinical, Cellular, and Biomarker Response to Infection



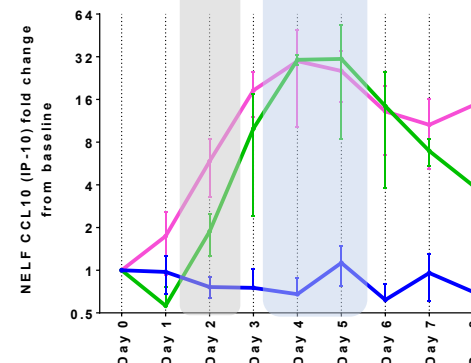
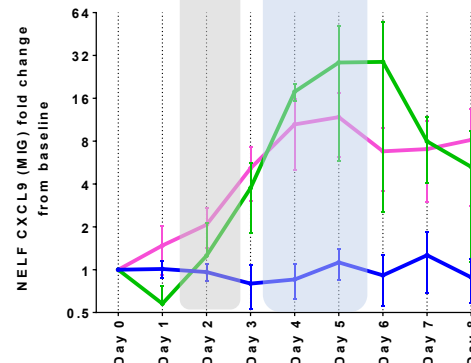
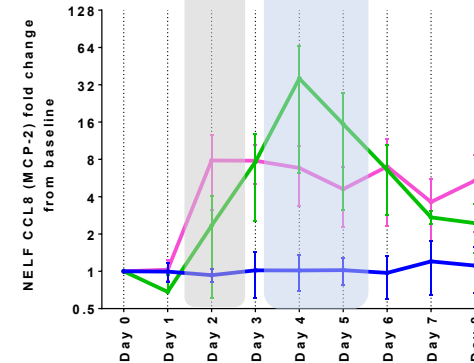
CELLS



BLOOD



NELF



CCL8 (MCP-2)

CXCL9 (MIG)

CXCL10 (IP-10)

Time course of chemokines and cells in relation to clinical response:

Early phase (Day 2,3)

- Viral and URT peaks
- Changes in blood cells
- Rise in chemokine markers

Later phase (Day 3,4,5,6)

- LRT and PEF changes
- Migration of immune cells to the respiratory tract
- Rises in chemokine markers in NELF



Summary of findings

- Hypothesis generating challenge study using HRV-16 rhinovirus mild intermittent asthma
- HRV infection induced asthma worsening (ACQ, LRT, PEF)
- Asthma worsening was associated with:
 - Raised baseline type 2 immune pathway markers
 - Post-inoculation changes in
 - blood cells
 - nasal chemokines/cytokines
 - however not type 2



Clinical relevance and future direction

- Within a well studied phenotype different clinical responses to infection associates with baseline characteristics and the subsequent immune response
- Impacting on T2 pathways prior to viral infection may reduce exacerbation frequency, severity and duration
- Further studies to better understand phenotypes/endotypes and the interaction of immune pathways in viral infection (Innate, T1, T2, chemokines) is needed

Acknowledgements



*Your trusted and experienced
Human challenge CRO partner!*

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- Pawel Rucki
- Olesya Rusyn
- Laura Krizman



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