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ROTAVIRUS SYMPOSIUM

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Elevated Levels of Pre-existing Growth Factors and Chemokines are Associated with Better Rotavirus Vaccine Response in Malawian Children

The 14th International Rotavirus Symposium
Bali, Indonesia

Jere KC, Benedicto P, Chaguza C, Mandolo J, Chirombo J, Gordon M, Leslie A, Goodyear C, Cunliffe A and Iturriza-Gomara

University of Liverpool
Kamuzu University of Health Sciences
Malawi Liverpool Wellcome Clinical Research Programme

The Centre for Global Vaccine Research

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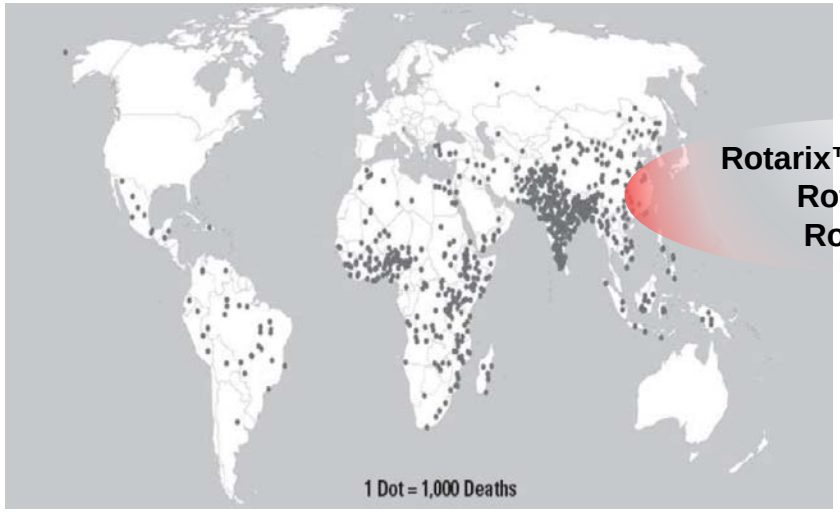
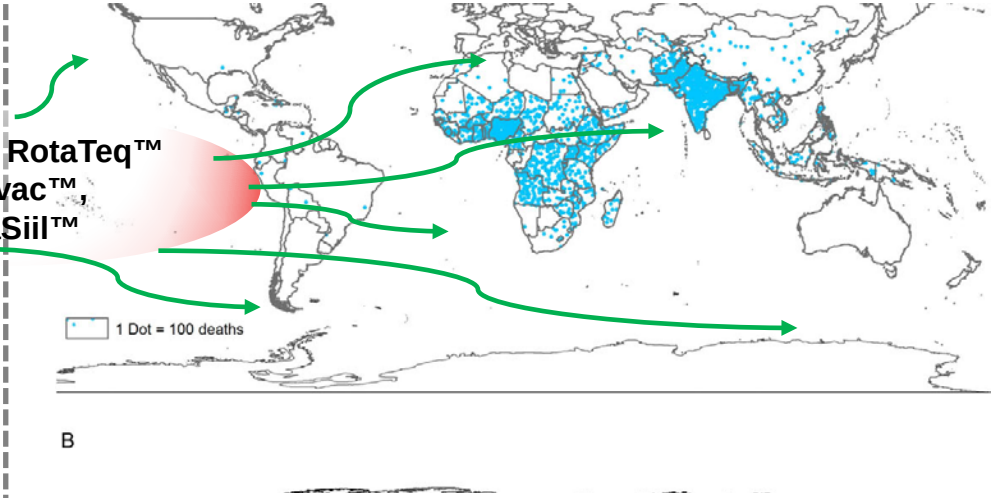
Presentation Outline

- **Background**
  - Rotavirus vaccine effectiveness in Malawi.
  - Study rationale.
- **Study design and methods**
- **Results and discussion**
  - Association between rotavirus vaccine response and pre-existing cytokine profiles.
  - Prediction of vaccine response based on pre-existing cytokine profiles.
- **Conclusion**

Global mortality cases caused by Rotavirus before and after vaccine introduction



Rotarix™, RotaTeq™
Rotavac™,
RotaSiil™



Pre-vaccine introduction
Responsible > 500,000 rotavirus-associated deaths

Post-vaccine introduction era
Responsible for 215,000 rotavirus - associated deaths

Glass and Parashar NIH, 2005; Tate et al. Clin Infect Dis, 2016

Rotavirus vaccine effectiveness in Malawi

- Rotavirus vaccination is highly cost-effective in Malawi.

Bar-Zeev et al. Clin Infect Dis 2016, Tate et al. NEJM, 2018

- Rotarix vaccination is effective in preventing severe rotavirus diarrhoea episodes among infants (>60%), with population incidence of rotavirus hospitalisations reduced by 43%.

Bar-Zeev et al. Lancet, 2015; Bar-Zeev & Jere et al. CID, 2016; Bennet et al Vaccine, 2018

- Rotavirus vaccination reduced infant diarrhoea deaths by a third in rural Malawi (34%).

Bar-Zeev et al Lancet Global Health, 2018

- Vaccine protects against some unusual strains.

Jere et al. J Virol, 2018; Jere et al. Emerg Infect Dis 2019

Unanswered questions?

- **Why rotavirus vaccines underperform in low-middle income settings** (<60% VE) compared to high income countries (>90% VE)?
- Why **certain children respond better** to rotavirus vaccine than others?
- What are the rotavirus-related **innate** and **adaptive immune responses in humans**?

Rotavirus immunology: innate and adaptive immune responses?

- IgA used as proxy of vaccine take and response to natural infection.
- B and T cell not fully understood.
- Cytokine response described in natural rotavirus infections ***but not exclusive in vaccinated individuals***.

Patel et al. J Infect Dis, 2009; Estes & Greenberg Fields Virology, 2013; Parker et al. Nat Commun 2021

Role of cytokines in rotavirus infection

- **Responses to rotavirus pathogenesis and immunity:** IFN-y (Th1 response), IL-2, IL-12 (Th2 response), IL-4, IL-6 and IL-10 (Th3 response) cytokines.
- **Stimulation of the immune response, inhibiting rotavirus binding and/or replication** (Th1 and Th2 groups of cytokines).
- **Plays immunomodulatory function to reduce rotavirus-associated diarrhoea and enhances immune responses in experimental rotavirus infection** (Th1/Th2 cytokine responses).
- ***Understanding of cytokine-induced immune responses to viral infection and vaccination is required***.

Azim et al J Med Virol 2003; Ghandhi et al Cytokines, 2013.

Study objectives

- Define the cytokine profile in rotavirus vaccine responders and non-responders.
- Identify cytokines that could predict response to rotavirus vaccination.
- Identify cytokines that could potentially be used as potential vaccine adjuvants.

Materials and Methods

Study Design

Timelines for immunisation and sample collection at Chilomoni Health Centre (CHC), Blantyre

	Birth	Enrolment	Dose 1			Dose 2			Final Draw
Week of life	Wk0	Wk1	Wk6	Day 4 post dose 1	Day 6 Post dose 1	Wk10	Day 4 Post dose 2	Day 6 post dose 2	Wk14
Blood			X	X					X
Stool			X	X	X	X	X	X	X
Place	CHC	CHC	CHC	CHC	Home visit	CHC	Home visit	Home visit	CHC

X

Breast milk
Maternal blood

Laboratory assays and Data analysis

- VP6 and Rotarix-specific NSP2 RT-PCR assays were used to detect exposure to wildtype rotavirus and Rotarix vaccine shedding, respectively.
- ELISA was used to quantify rotavirus specific IgA and IgG.
- Luminex was used to quantify cytokine levels (Milliplex Human Cytokine/Chemokine Magnetic Bead Premixed 38- Plex Kit, Merck Life Sciences, UK).

Iturriza-Gomara et al. J Virol 2002; Estes & Greenberg Fields Virology, 2013

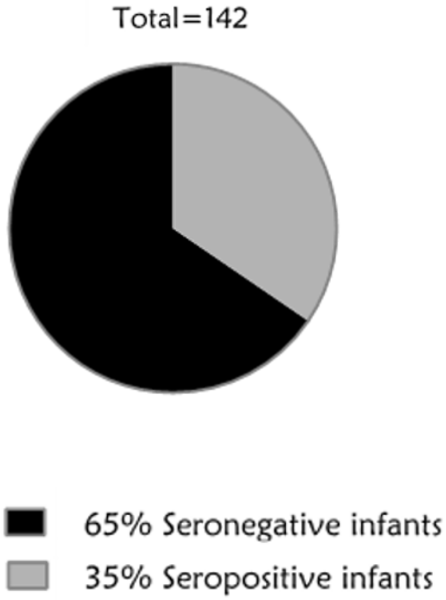
Cytokine groups

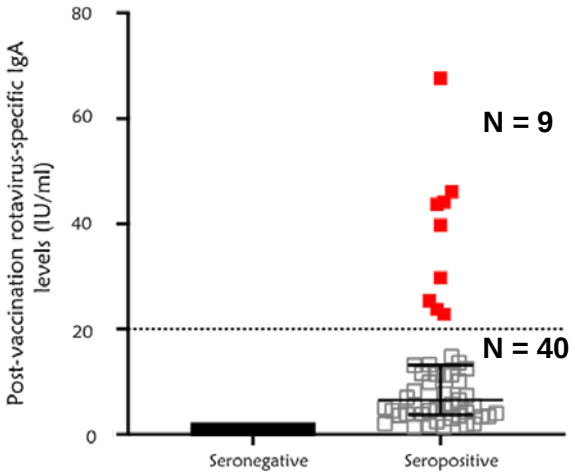
Growth Factors	Chemokine	Adaptive	Anti-Inflammatory	Pro-Inflammatory	Others
• EGF • FGF-2 • TGF-A • GCSF • GM CSF • IL3 • IL7 • VEGF	• Eotaxin • Fractakine • CRO-alpha • IL-8 • IP-10 • MCP-1 • MCP-3 • MDC • MIP-1A • MIP-1B	• IFN-A2 • IFN-gamma • IL-2 • IL-4 • IL-5 • IL-9 • IL-12P40 • IL-12P70 • IL-13 • IL-15 • IL-17A	• IL-IRA • IL-10	• IL-1A • IL-1B • IL-6 • TNF-A	• sCD40L

Findings and Discussion

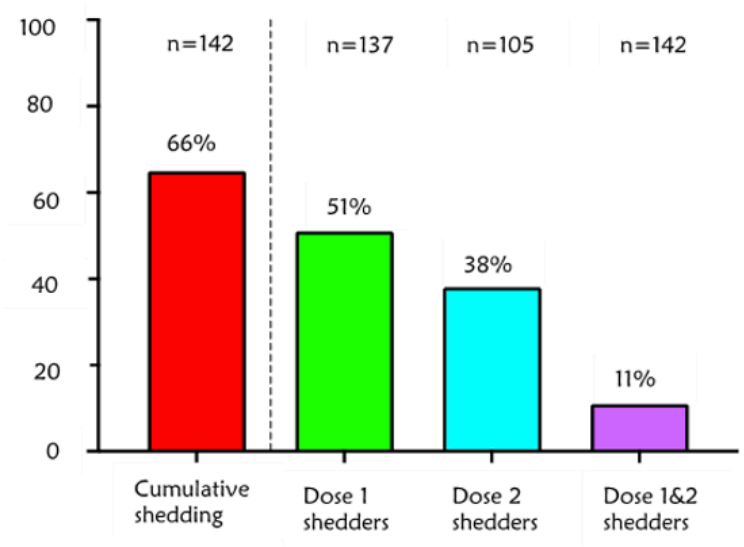
Low seroconversion and low Rotarix (RV1) vaccine virus shedding

Proportion of seropositive infants following Rotarix vaccination





Rotarix vaccine shedding post dose 1 and 2



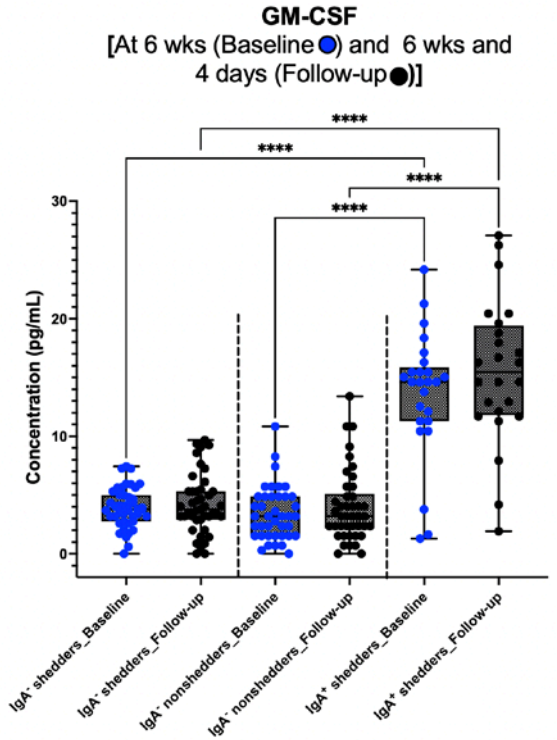
Clinical Characteristics of the study participants

Characteristics	IgA+ shedders, N = 30 (1)	IgA- shedders, N = 37 (1)	IgA- non-shedders, N = 31 (1)	p-value (2)
Birth weight (Kgs)	3.00 (2.70-4.30)	3.00 (2.70-4.60)	2.80 (2.60-4.90)	0.4752
Gender				>0.9999
Female	13/30 (43%)	20/37 (54%)	18/31 (58%)	
Male	17/30 (57%)	17/37 (46%)	13/31 (42%)	
Mother HIV status				>0.9999
HIV uninfected	28/30 (93%)	28/37 (76%)	20/31 (65%)	
HIV infected	2/30(7%)	9/37 (24%)	11/31 (35%)	
Weight (Kgs)	5.00 (4.48-6.50)	4.70(4.48-6.00)	4.70(4.20-6.90)	0.4515
Breast feeding (Yes)				>0.9999
Yes	29/30 (97%)	37/37 (100%)	31/31 (100%)	
No	1/30(3%)	0	0	
Mother on medication				>0.9999
Yes	0	0	0	
No	30/30 (100%)	37/37 (100%)	31/31 (100%)	
Shedding				0.5
Post-Dose 1	18/30 (60%)	25/37 (68%)	0	
Post-Dose 2	12/30 (40%)	12/37 (32%)	0	
IgA GMC	9.56 (6.60-13.84)	0	0	<0.0001
Pre-existing IgG GMC	223.60 (140.10-357.00)	347.30. (219.80-548.90)	245.8 (155.70-388.00)	0.4321

[1]Median (Range); n / N (%); GMC (95% CI)

[2]Wilcoxon rank sum test; Pearson's Chi-squared test

Vaccine responders had significantly elevated **levels of growth factors and most chemokines prior to vaccination**

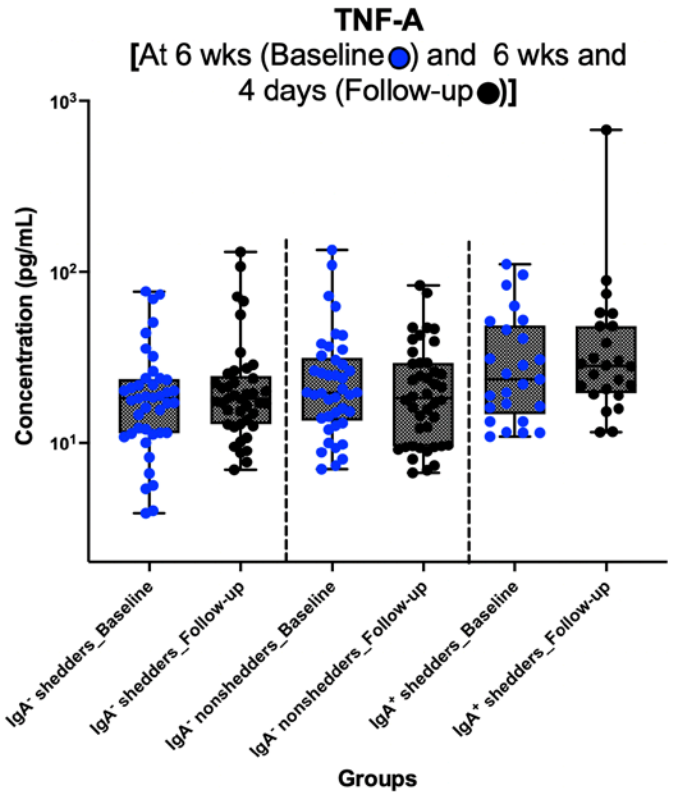


	Growth Factors (Median, pg/mL)						
	IgA⁻ Shedders		IgA⁻ Nonshedders		IgA⁺ Shedders		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	p-value
EGF	289.1	220.1	184.6	183.8	589.8	380.5	**0.0042**
FGF	16.46	16.96	23.03	33.21	84.58	82.7	**<0.0001**
TGF-A	1.18	1.49	2.55	2.8	4.12	3.28	**<0.0001**
G-CSF	16.58	46.28	37.58	49.43	99.28	113.1	**<0.0001**
VEGF	50.36	50.83	58.46	71.48	144.7	157	**<0.0001**
GM-CSF	3.79	3.78	3.19	3.19	14.62	15.45	**<0.0001**
IL-3	0.05	0.04	0.03	0.07	0.17	0.175	**<0.0001**
IL-7	709.4	233.6	1.95	1.95	459.4	473.8	**<0.0001**

	Chemokines (Median, pg/mL)						
	IgA⁻ Shedders		IgA⁻ Nonshedders		IgA⁺ Shedders		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	p-value
Eotaxin	17.35	18.38	28.41	26.6	33.61	32.52	>0.9999
Fractalkine	144	141.8	163.9	144.9	340.3	355.4	**<0.0001**
CRO	9762	7274	5715	6253	12511	12046	**<0.0001**
MDC	1407	1334	541.9	578.4	2162	2044	**<0.0001**
MCP-1	262.4	269.2	332.4	295.2	454.4	483.9	**0.0785**
MCP-3	3.28	3.155	12.25	14.47	22.67	22.45	0.1312
MIP-1B	99.95	98.32	72.58	68.13	248.2	205.2	**<0.0001**
IP-10	1121	1428	890.9	1138	1730	2832	0.1676
IL-8	19.67	23.98	35.36	35.21	88.01	54.8	>0.9999

Interferon; **IL**; interleukin; **IP**: interferon-γ-inducible protein; **MIP**: macrophage inflammatory protein; **MCP**: monocyte chemoattractant protein; **GM-CSF**: granulocyte–macrophage colony-stimulating factor; **TGF**: transforming growth factors; **EGF**: epidermal growth factors; **FGF**: Fibroblast growth factors; **VEGF**: vascular endothelial growth factors; **G-CSF**: granulocyte-colony stimulating factor.

Vaccine responders had elevated **levels in some adaptive and pro-inflammatory but not anti-inflammatory cytokines**

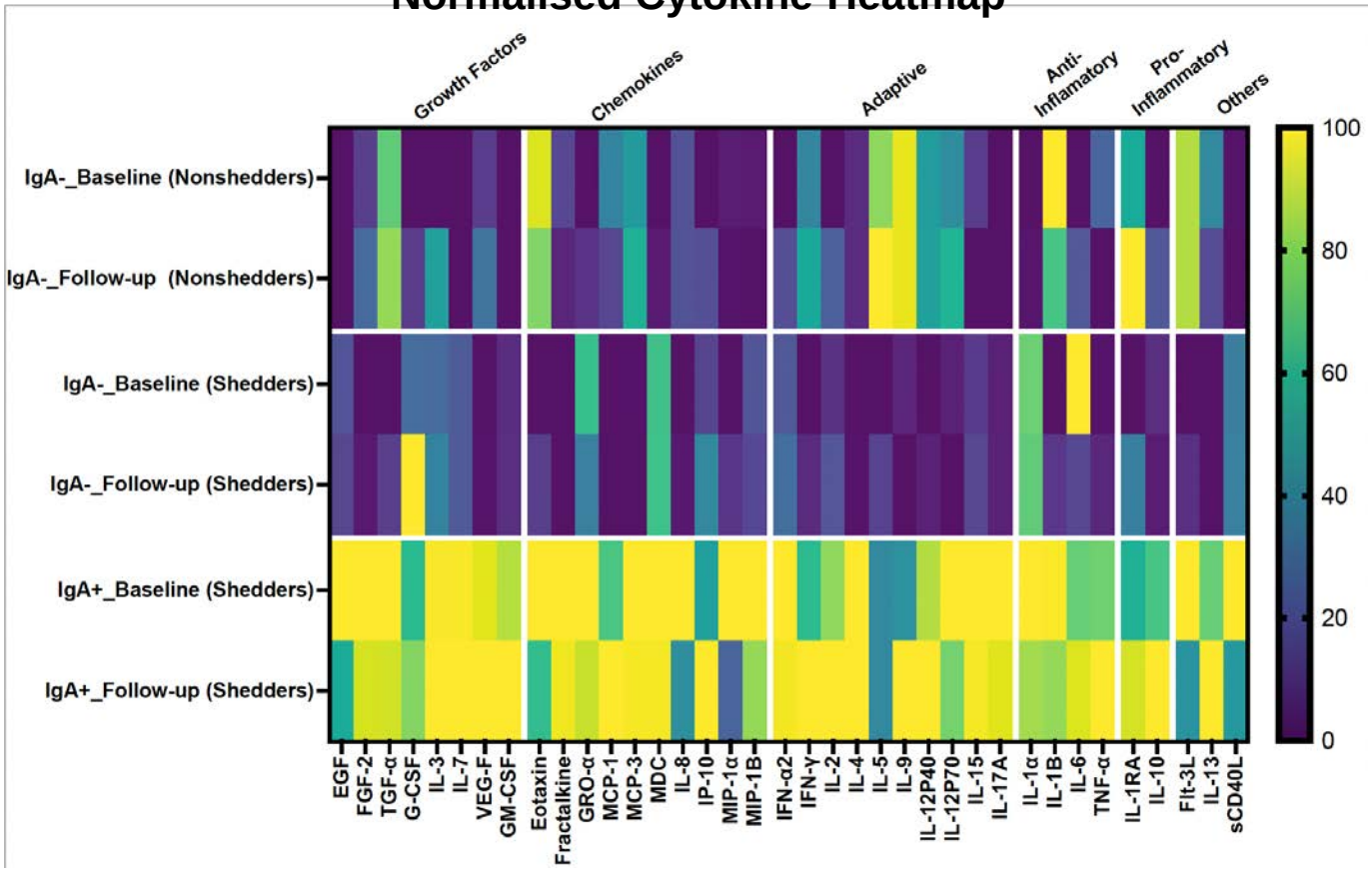


	Adaptive cytokines (Median, pg/mL)						
	IgA⁻ Shedders		IgA⁻ Nonshedders		IgA⁺ Shedders		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	p-value
IFN-A2	18.45	20.84	12.4	17.88	51.57	20.38	**<0.0001**
IFN-g	7.84	8.885	11.55	13.21	11.64	20.38	>0.9999
IL-2	0.49	0.53	0.39	0.57	1.3	1.25	**<0.0001**
IL-4	30.05	29.55	34.5	34.5	104.7	126.9	**<0.0001**
IL-5	0.46	0.56	1.18	1.35	0.96	1.04	>0.9999
IL-9	0.79	0.73	1.82	1.82	0.875	2.25	>0.9999
IL-15	1.05	1.15	1.37	1.04	4.25	4.305	**<0.0001**
IL-17A	1.3	1.35	1.21	1.21	5.41	5.41	<0.0001
IL-12P40	2.2	2.17	13.58	14.1	19.49	24.52	>0.9999
IL-12P70	1.15	0.88	2.22	2.8	4.4	4.01	**0.0021**

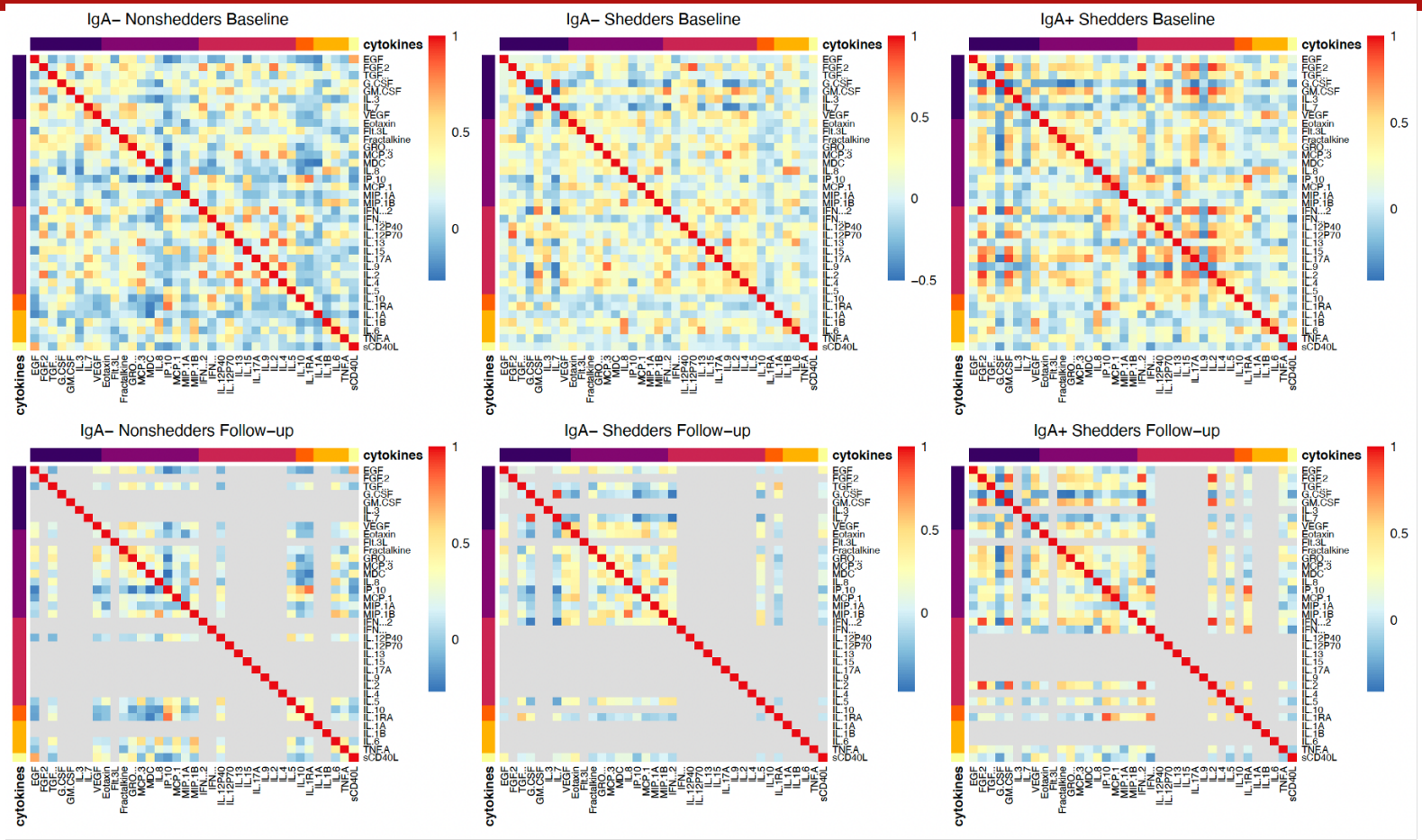
	Pro-inflammatory cytokines (Median, pg/mL)						
	IgA- Shedders		IgA- Nonshedders		IgA+ Shedders		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	p-value
IL-1A	54.59	55.06	3.24	4.33	103.5	63.05	**0.0017**
IL-1B	0.81	0.87	1.77	1.47	1.89	1.68	>0.9999
IL-6	8.19	3.19	1.81	3.23	8.07	8.54	**<0.0001**
TNF-A	18.18	18.04	19.7	18.2	23.53	28.1	>0.9999
	Anti-inflammatory cytokines (Median, pg/mL)						
IL-1RA	13.9	22.43	26.04	35.81	27.2	5.41	>0.9999
IL-10	8.285	8.08	6.66	8.215	12.1	15.08	0.1568
	Other cytokines (Median, pg/ul)						
sCD40-L	426	344.4	111.2	106.4	972.8	552.3	**0.0005**

Interferon; **IL**; interleukin; **IL1-RA**: IL-1 receptor antagonist; Tumor necrosis factor: **TNF;** Soluble CD40 ligand: **sCD40L**

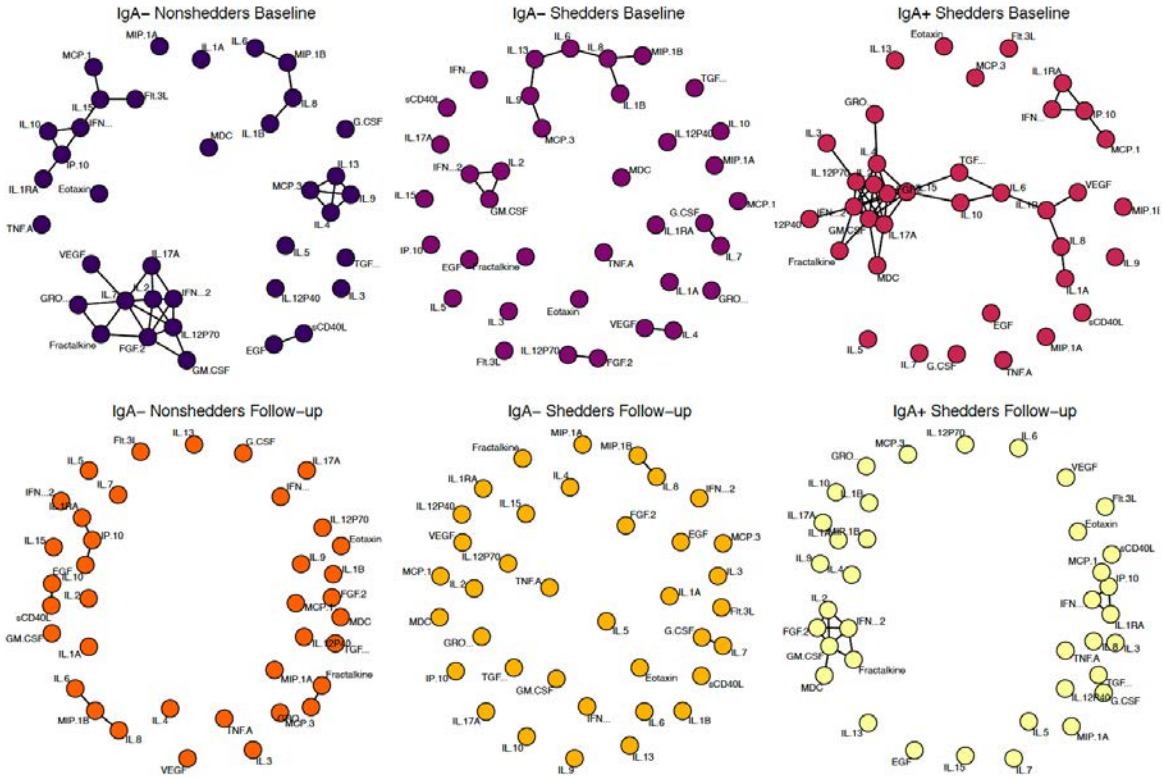
Normalised Cytokine Heatmap



Distinct cytokine profiles in children who seropositive and shed rotavirus (IgA^+ shedders) compared to IgA^- shedders and IgA^- nonshedders prior to vaccination

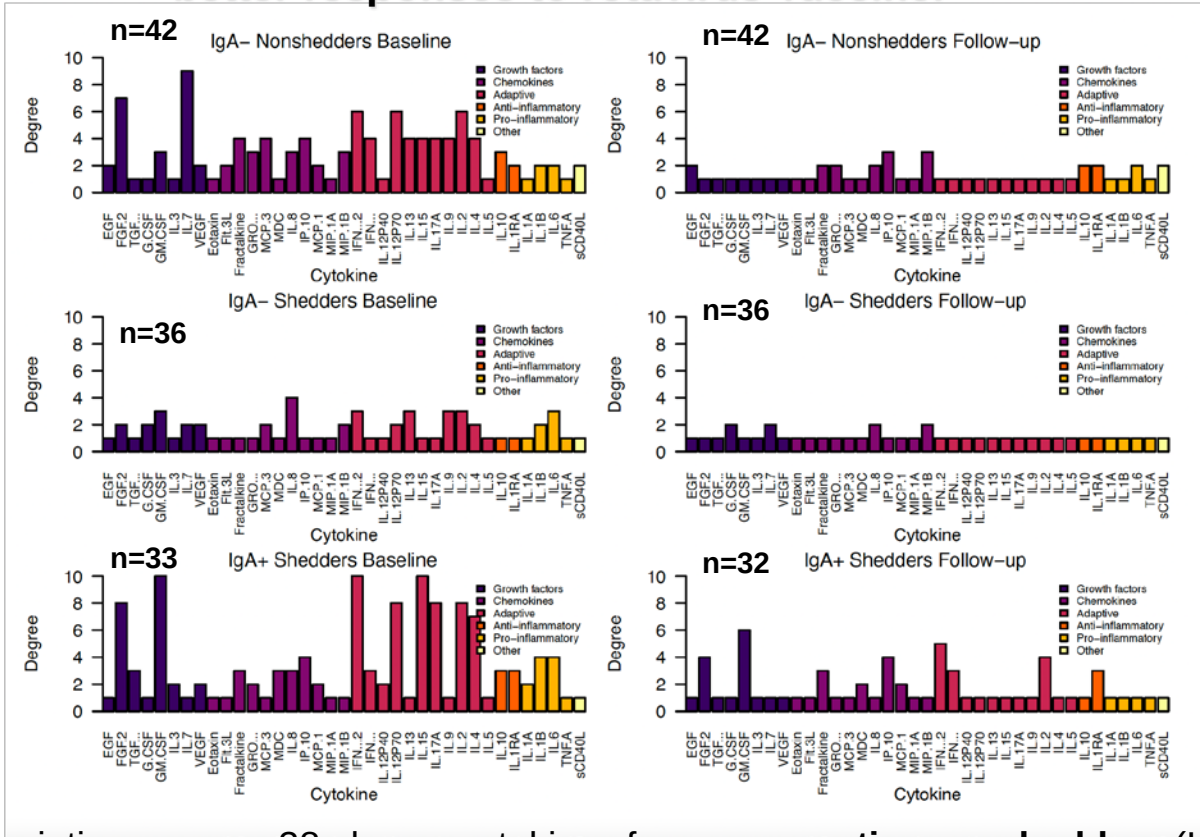


Correlogram: Cytokine interaction was positively correlated in rotavirus vaccine responders and mostly negatively correlated in poor vaccine responders.

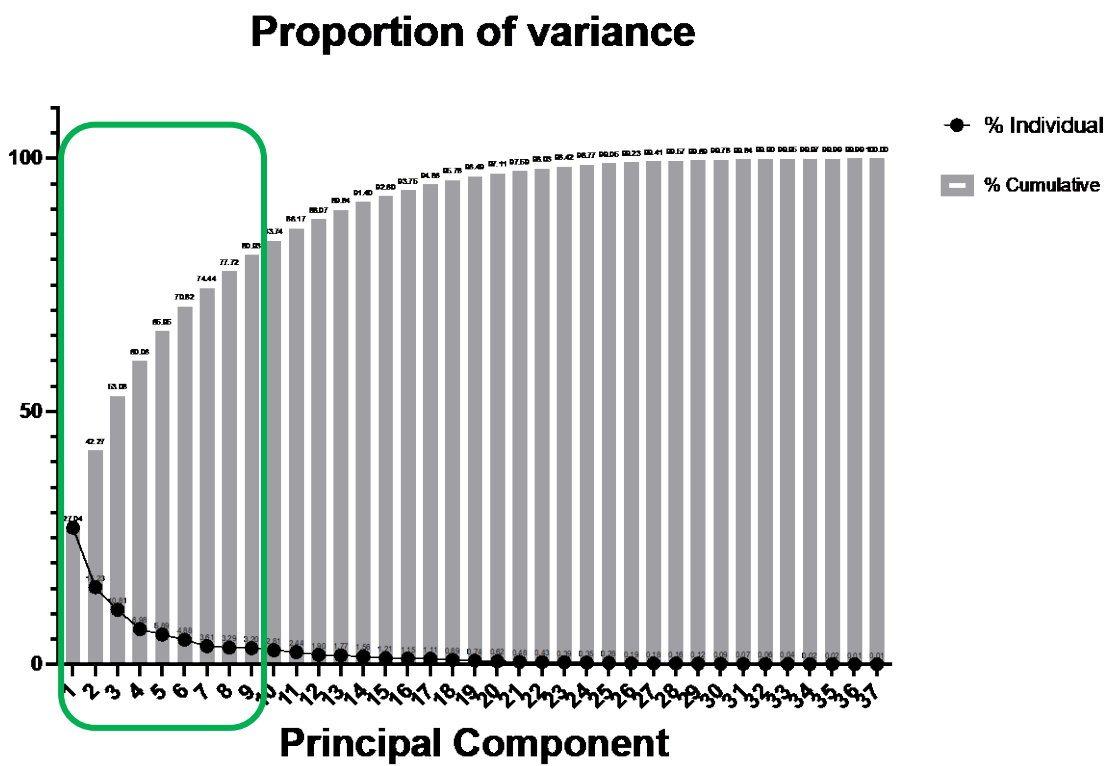


Network graphs associations among 38 plasma cytokines for **seronegative nonshedders** (IgA$^-$/RV1-), **seronegative shedders** (IgA$^-$/RV1+) and **seropositive shedders** (IgA$^+$/RV1+). The shorter the connecting line between the circles, the greater the magnitude of the correlation

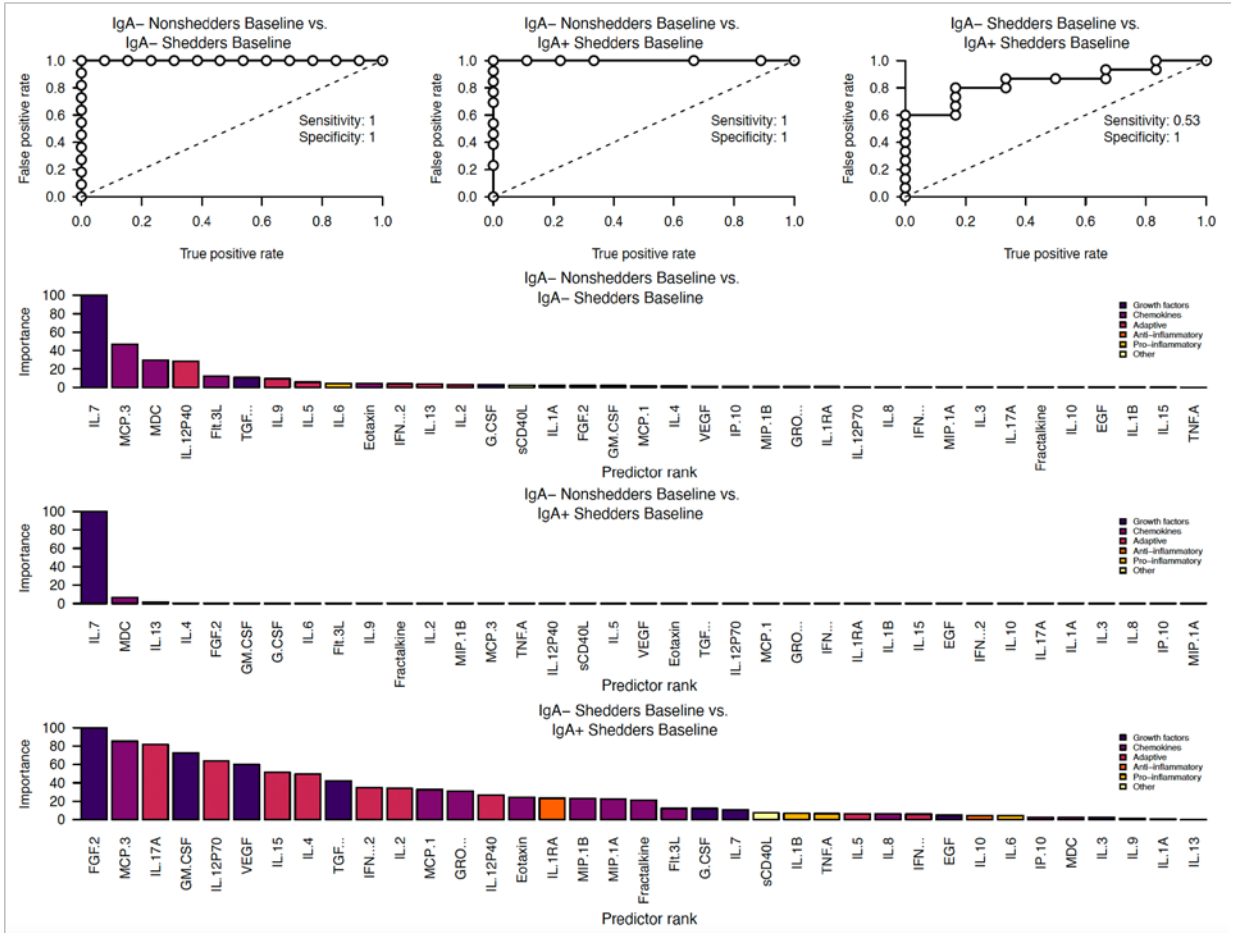
Cytokine networks driven by GM-CSF, INF-gamma and IL-15 were correlated with better responses to rotavirus vaccine.



Degrees of associations among 38 plasma cytokines for **seronegative nonshedders** (IgA$^-$/RV1-), **seronegative shedders** (IgA$^-$/RV1+) and **seropositive shedders** (IgA$^+$/RV1+) children.



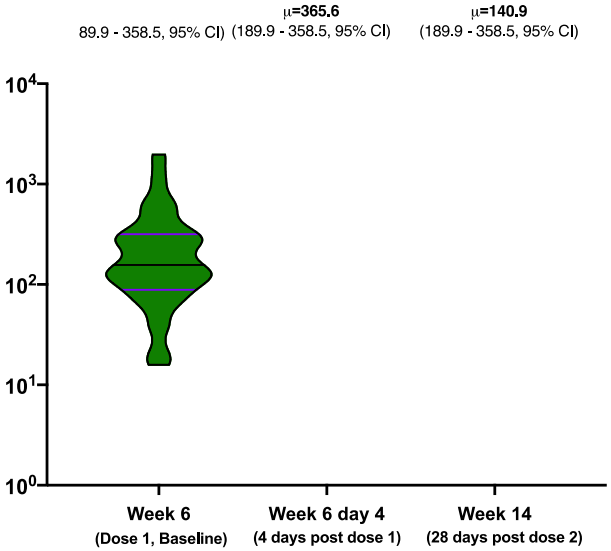
Scree plot demonstrating that 9 cytokines explains almost 80% of the variations observed between the cytokine profiles of IgA^{+} shedders, IgA^{-} shedders and IgA^{-} nonshedders

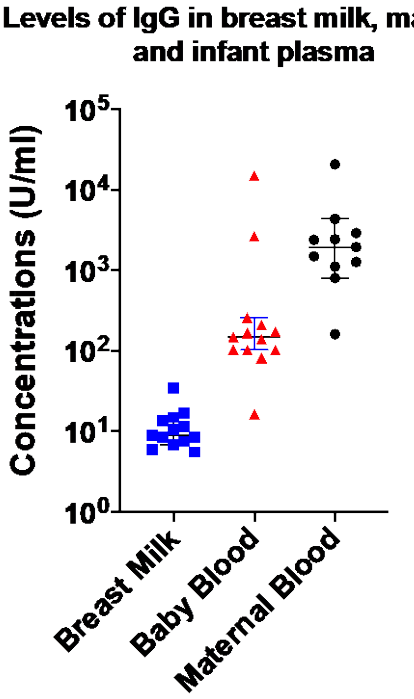


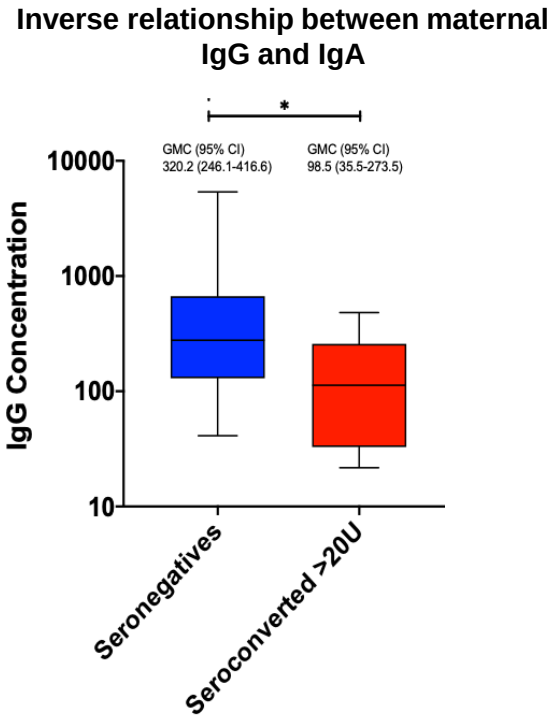
- Pre-existing **IL-7** before receipt of rotavirus vaccine is associated with poor vaccine response.
- High levels of **FGF, MCP-3, IL-17A** and **GM-CSF** are associated with better vaccine response.

Random forests analysis to predict cytokines associated with seropositivity and rotavirus vaccine shedding

Study confounders:
High levels of rotavirus specific maternal antibodies







Conclusions

- Children who respond to rotavirus vaccine have distinctive cytokine profiles.
- IL-7, FGF, MCP-3, IL-17A and GM-CSF are important cytokines that could be used to predict rotavirus vaccine response in children prior to vaccination.
- The identified growth factors and chemokines could be used as adjuvants in the formulation of next generation rotavirus vaccines as in other pathogens.

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Data analysis

- xPONET and Millipore Belysa curve fitting software was used to normalise Luminex data (Merck Life Sciences, UK).
- GraphPad Prism was used to analyse cytokine variations between vaccine responders and non-vaccine responders (ANOVA) on normally distributed data and non-parametric test on data that was not normally distributed. Significance differences were calculated using Tukey and Dunn’s test.
- R used to generate cytokine networks, heatmaps, random forest analysis