**Supplementary Table 1. Background characteristics at baseline, comparing children included and followed-up (analysed) with children included, but lost to follow-up**

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Analysed** | **Lost** |   |
| **Background parameter** | **Observations** | **p** |
| All, n | 146 | 112 |   |
| Male, % (n) | 45% (66) | 61% (68) | 0.01 |
| Age, days (10-90%-tile) | 291 (276-368) | 293 (275-420) | 0.57 |
| Dry season, % (n) | 49% (71) | 53% (59) | 0.52 |
| Age of mother, years (10-90%-tile) | 25 (19-36) | 25 (18-35) | 0.91 |
| Schooling of mother, years (10-90%-tile) | 6 (0-11) | 6 (0-11) | 0.92 |
| MUAC of mother, mm (sd) | 281 (38) | 280 (32) | 0.78 |
| Electricity in house, % (n) | 31% (44) | 30% (31) | 0.82 |
| Toilet inside, % (n) | 14% (20) | 12% (12) | 0.55 |
| Polio vaccine at birth, % (n) | 72% (105) | 82% (92) | 0.06 |
| Previous VAS, % (n) | 64% (94) | 55% (62) | 0.14 |
| MUAC, mm (sd) | 148 (11) | 157 (81) | 0.21 |
| Weight, kg (sd) | 8.7 (1.2) | 8.8 (1.3) | 0.56 |
| Height, cm (sd) | 72 (3) | 73 (3) | 0.15 |
| Axillary temperature >37°C, n (%) | 0% (0) | 1% (1) | 0.34 |
| Medicine use on day of enrolment, % (n) | 5% (7) | 5% (6) | 0.84 |
| Maternally reported symptoms |   |   |   |
| Cold (runny nose), % (n) | 31% (40) | 27% (27) | 0.52 |
| Coughing, % (n) | 38% (55) | 30% (34) | 0.22 |
| Diarrhoea, % (n) | 9% (13) | 16% (17) | 0.10 |
| Fever, % (n) | 8% (12) | 12% (13) | 0.36 |
| Vomiting, % (n) | 1% (2) | 2% (2) | 0.79 |
| Any of the symptoms above, % (n) | 50% (73) | 49% (55) | 0.89 |

The analysed group comprises infants with a valid measurement of plasma biomarker, *in vitro* cytokines and/or differential counts at baseline and follow-up. The lost group comprises infants from whom a blood sample was obtained at enrolment, but for various reasons, no valid blood sample was obtained and analysed at follow-up. The background information, statistical tests and presentation are similar to Table 1.

Statistical test using Chi2-test for categorical values; Kruskal-Wallis test for non-normally distributed numerical values; Student’s t-test for normally distributed numerical values.

n: number of individuals; sd: standard deviation; MUAC: mid-upper-arm-circumference; % is calculated in respect to the total number of individuals with the relevant information available.

**Supplementary Table 2: The effect of vitamin A supplementation on white blood cell counts, stratified by previous vitamin A supplementation and sex**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | No Previous VAS |   | Previous VAS |   |   |
|   | Male | Female |   | Male | Female |   |   |
|   | n=16 | n=11 |   | n=23 | n=23 |   |   |
|   | GMR (95% CI) | GMR (95% CI) | p1 | GMR (95% CI) | GMR (95% CI) | p1 | p2 |
| Total leukocytes | 0.79 (0.58-1.07) | **1.42 (1.08-1.87)** | **0.005** | 0.84 (0.72-0.99) | 1.04 (0.80-1.35) | 0.19 | 0.10 |
| Lymphocytes | 0.83 (0.66-1.05) | 1.36 (0.98-1.88) | 0.06 | 0.78 (0.63-0.98) | 0.94 (0.76-1.16) | 0.26 | 0.21 |
| Monocytes | 0.71 (0.49-1.01) | 1.40 (1.02-1.93) | **0.002** | 0.90 (0.72-1.12) | 1.18 (0.87-1.60) | 0.20 | 0.18 |
| Neutrophils | 0.63 (0.37-1.07) | 1.02 (0.60-1.74) | 0.27 | 1.09 (0.72-1.64) | 1.16 (0.73-1.84) | 0.81 | 0.39 |
| Eosinophils | 1.28 (0.73-2.26) | 1.01 (0.42-2.40) | 0.72 | 0.72 (0.46-1.12) | 0.94 (0.54-1.62) | 0.50 | 0.47 |
| Basophils | 0.73 (0.42-1.26) | **1.71 (1.21-2.41)** | 0.04 | 0.76 (0.46-1.27) | 1.27 (0.82-1.96) | 0.11 | 0.44 |

Geometric mean ratios (GMR) with 95% confidence intervals (CI) comparing infants receiving VAS with infants receiving placebo. A GMR > 1 can be interpreted as an increasing effect of VAS on the cell count.

Significant estimates after adjustment for multiple comparisons are highlighted in bold.

1 Analysis of interaction between VAS and sex.

2 Analysis of the three-way interaction between VAS, sex and previous VAS.

**Supplementary Table 3. The effect of vitamin A supplementation on *in vitro* cytokine responses**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   |   |   | **VAS** | **no VAS** | **VAS vs no VAS** |
|   |   |   | n=57 | n=63 | n=120 |
|   |   | Det | GM (95% CI)1 | GM (95% CI)1 | GMR (95% CI)2 |
| TNF-α | medium1\* | 33% | 35 (24-50) | 30 (20-44) | 1.23 (0.74-2.06) |
|   | LPS | 98% | 439 (311-618) | 614 (444-851) | 0.71 (0.44-1.14) |
|   | Pam | 90% | 146 (92-231) | 137 (88-214) | 1.12 (0.58-2.13) |
|   | medium3\* | 19% | 20 (12-33) | 20 (12-33) | 0.90 (0.44-1.85) |
|   | PHA | 69% | 20 (12-35) | 26 (16-44) | 0.75 (0.35-1.62) |
|   | Poly I:C\* | 23% | 21 (12-35) | 25 (16-39) | 0.81 (0.41-1.60) |
|   | BCG\* | 28% | 30 (20-45) | 28 (19-42) | 1.04 (0.59-1.86) |
|   | PPD | 63% | 20 (12-35) | 18 (11-31) | 1.04 (0.49-2.21) |
|   | OPV\* | 23% | 31 (21-47) | 16 (9-29) | 2.46 (1.24-4.89) |
|   | TT\* | 32% | 30 (20-45) | 34 (24-49) | 0.89 (0.53-1.51) |
|   | DT\* | 17% | 18 (10-34) | 19 (11-33) | 1.03 (0.43-2.43) |
| IL-10 | medium1 | 64% | 10 (7-16) | 6 (4-9) | 2.09 (1.19-3.67) |
|   | LPS | 100% | 228 (177-293) | 270 (212-343) | 0.89 (0.65-1.21) |
|   | Pam | 98% | 130 (96-178) | 148 (110-200) | 1.13 (0.81-1.59) |
|   | medium3\* | 41% | 41 (30-56) | 43 (32-58) | 0.97 (0.63-1.48) |
|   | PHA | 90% | 41 (29-58) | 50 (36-69) | 0.86 (0.52-1.43) |
|   | Poly I:C\* | 41% | 43 (32-59) | 41 (30-55) | 1.10 (0.72-1.68) |
|   | BCG | 62% | 8 (4-13) | 12 (7-20) | 0.62 (0.30-1.30) |
|   | PPD | 89% | 37 (26-54) | 38 (27-54) | 1.10 (0.64-1.88) |
|   | OPV | 54% | 7 (4-13) | 6 (3-10) | 1.75 (0.79-3.89) |
|   | TT | 63% | 8 (5-13) | 10 (6-15) | 0.98 (0.50-1.94) |
|   | DT\* | 39% | 41 (29-58) | 40 (28-55) | 1.08 (0.66-1.77) |
| IL-2 | medium3\* | 19% | 23 (14-37) | 17 (9-29) | 1.39 (0.66-2.92) |
|   | PHA | 73% | 17 (10-28) | 18 (11-28) | 1.01 (0.49-2.11) |
|   | Poly I:C\* | 16% | 23 (14-37) | 10 (5-22) | 2.02 (0.84-4.85) |
|   | BCG\* | 45% | 39 (28-54) | 52 (40-66) | 0.69 (0.46-1.03) |
|   | PPD | 59% | 11 (7-17) | 8 (5-12) | 1.28 (0.78-2.11) |
|   | OPV\* | 25% | 30 (20-45) | 21 (13-35) | 1.38 (0.72-2.62) |
|   | TT | 73% | 20 (12-31) | 25 (16-39) | 0.60 (0.34-1.07) |
|   | DT\* | 42% | 48 (35-65) | 40 (28-55) | 1.01 (0.67-1.53) |
| IL-5 | medium3\* | 27% | 25 (16-39) | 30 (20-44) | 0.82 (0.46-1.46) |
|   | PHA | 83% | 33 (20-57) | 31 (18-51) | 1.10 (0.51-2.36) |
|   | Poly I:C\* | 33% | 28 (18-44) | 39 (28-54) | 0.77 (0.46-1.30) |
|   | BCG\* | 38% | 35 (24-51) | 40 (29-55) | 0.88 (0.55-1.41) |
|   | PPD\* | 39% | 32 (22-47) | 48 (37-63) | 0.66 (0.42-1.04) |
|   | OPV\* | 33% | 33 (23-49) | 33 (23-48) | 0.98 (0.57-1.67) |
|   | TT | 59% | 6 (3-9) | 6 (4-10) | 0.79 (0.40-1.57) |
|   | DT\* | 50% | 55 (42-72) | 43 (32-59) | 1.22 (0.82-1.81) |
| IFN-γ | medium3\* | 24% | 20 (12-33) | 30 (20-44) | 0.67 (0.35-1.29) |
|   | PHA | 93% | 81 (51-131) | 70 (44-112) | 1.07 (0.54-2.13) |
|   | Poly I:C | 77% | 25 (14-46) | 29 (16-51) | 0.91 (0.39-2.08) |
|   | BCG\* | 49% | 48 (36-64) | 53 (42-68) | 0.89 (0.62-1.28) |
|   | PPD | 76% | 28 (17-45) | 22 (14-36) | 1.36 (0.72-2.57) |
|   | OPV\* | 29% | 30 (20-45) | 30 (20-44) | 1.02 (0.59-1.78) |
|   | TT | 59% | 9 (4-19) | 10 (5-21) | 1.10 (0.42-2.90) |
|   | DT\* | 29% | 27 (17-44) | 32 (22-48) | 0.78 (0.44-1.38) |

1 Geometric mean (GM, pg/mL) for vitamin A recipients or placebo recipients, respectively.

Det: Proportion (%) of measurements above lower limit of detection for the entire population at follow-up.

2 Geometric mean ratios (GMR) of cytokine responses to innate agonists and vaccine antigens comparing VAS with placebo. A GMR (or PR) > 1 can be interpreted as an increasing effect of VAS on the cytokine response.

\* Outcomes with ≥50% ND measurements were analysed with Poisson regression for the frequency of measurements above lower limit of detection, giving proportion ratios (PR). Significant estimates after adjustment for multiple comparisons are highlighted in bold.

**Supplementary Table 4. The effect of vitamin A supplementation on *in vitro* cytokine responses, stratified by previous vitamin A supplementation and sex**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   | **No Previous VAS** |   | **Previous VAS** |  |   |
|   |   | **Male** | **Female** |   | **Male** | **Female** |  |   |
|   |   | n=21 | n=21 |   | n=33 | n=45 |   |   |
|   |   | GMR (95% CI) | GMR (95% CI) | p1 | GMR (95% CI) | GMR (95% CI) | p1 | p2 |
| TNF-α | medium1\* | 0.48 (0.12-1.98) | 0.69 (0.28-1.68) | 0.66 | 1.53 (0.42-5.63) | 2.32 (1.00-5.41) | 0.63 | 0.97 |
|   | LPS | 0.52 (0.17-1.61) | 0.69 (0.22-2.19) | 0.65 | 0.97 (0.40-2.33) | 0.67 (0.31-1.43) | 0.50 | 0.52 |
|   | Pam | 0.24 (0.06-1.05) | 1.30 (0.25-6.64) | 0.18 | 1.50 (0.45-4.99) | 2.17 (0.80-5.90) | 0.62 | 0.33 |
|   | medium3\* | (inf-2.21) | 0.28 (0.03-2.30) |   | 0.86 (0.21-3.57) | 1.68 (0.58-4.84) | 0.46 |   |
|   | PHA | 0.54 (0.07-4.03) | 0.13 (0.02-0.82) | 0.38 | 0.80 (0.20-3.25) | 1.99 (0.64-6.19) | 0.28 | 0.16 |
|   | Poly I:C\* | 0.40 (0.05-2.98) | (inf-1.39) |   | 1.11 (0.26-4.74) | 1.21 (0.51-2.86) | 0.91 |   |
|   | BCG\* | 0.30 (0.04-2.31) | 0.37 (0.04-3.03) | 0.91 | 1.79 (0.51-6.28) | 1.41 (0.64-3.12) | 0.77 | 0.80 |
|   | PPD | 0.38 (0.06-2.34) | 0.36 (0.06-2.32) | 0.97 | 1.91 (0.44-8.36) | 1.57 (0.47-5.23) | 0.85 | 0.92 |
|   | OPV\* | (inf-5.82) | 2.02 (0.54-7.64) |   | 3.34 (0.89-12.48) | 3.60 (1.12-11.61) | 0.93 |   |
|   | TT\* | 0.68 (0.07-6.53) | 0.24 (0.03-1.78) | 0.48 | 1.45 (0.58-3.65) | 0.92 (0.44-1.89) | 0.44 | 0.72 |
|   | DT\* | (inf-9.71) | (inf-1.08) |   | 2.95 (0.33-26.66) | 1.50 (0.52-4.27) | 0.58 |   |
| IL-10 | medium1 | 0.65 (0.18-2.40) | 0.74 (0.22-2.55) | 0.84 | 3.60 (1.40-9.29) | 3.70 (1.38-9.91) | 0.97 | 0.92 |
|   | LPS | 1.36 (0.64-2.90) | 0.72 (0.34-1.54) | 0.32 | 0.98 (0.55-1.75) | 0.74 (0.44-1.22) | 0.46 | 0.61 |
|   | Pam | 0.73 (0.33-1.61) | 0.87 (0.37-2.07) | 0.90 | 1.63 (0.87-3.06) | 1.28 (0.75-2.19) | 0.54 | 0.57 |
|   | medium3\* | 0.47 (0.13-1.72) | 1.39 (0.44-4.40) | 0.31 | 1.02 (0.47-2.22) | 1.05 (0.52-2.11) | 1.00 | 0.31 |
|   | PHA | 0.81 (0.24-2.79) | 0.23 (0.07-0.77) | 0.22 | 0.85 (0.34-2.14) | 1.70 (0.77-3.76) | 0.23 | 0.07 |
|   | Poly I:C\* | 1.45 (0.46-4.60) | 0.65 (0.25-1.71) | 0.27 | 1.46 (0.59-3.61) | 1.11 (0.60-2.07) | 0.66 | 0.58 |
|   | BCG | 0.49 (0.08-3.22) | 0.25 (0.05-1.38) | 0.58 | 0.95 (0.27-3.40) | 1.06 (0.35-3.25) | 0.90 | 0.60 |
|   | PPD | 0.84 (0.24-3.02) | 1.13 (0.29-4.42) | 0.68 | 1.31 (0.47-3.64) | 1.11 (0.46-2.67) | 0.82 | 0.70 |
|   | OPV | 0.61 (0.11-3.41) | 1.57 (0.27-9.19) | 0.46 | 2.52 (0.67-9.42) | 2.77 (0.82-9.38) | 0.90 | 0.58 |
|   | TT | 0.24 (0.05-1.23) | 0.48 (0.11-2.16) | 0.59 | 1.26 (0.32-4.91) | 1.64 (0.57-4.77) | 0.74 | 0.76 |
|   | DT\* | 0.70 (0.17-2.93) | 0.71 (0.23-2.21) | 0.99 | 0.77 (0.27-2.24) | 1.86 (0.91-3.78) | 0.19 | 0.45 |
| IL-2 | medium3\* | 3.92 (0.48-31.66) | 1.37 (0.29-6.56) | 0.42 | 1.55 (0.31-7.90) | 0.87 (0.27-2.86) | 0.57 | 0.78 |
|   | PHA | 3.02 (0.55-16.61) | 0.37 (0.07-1.97) | 0.09 | 0.59 (0.17-2.12) | 1.29 (0.41-4.09) | 0.35 | 0.05 |
|   | Poly I:C\* | (0.20-inf) | (1.13-inf) |   | 0.78 (0.16-3.81) | 1.61 (0.48-5.46) | 0.52 |   |
|   | BCG\* | 0.62 (0.22-1.75) | 1.35 (0.51-3.58) | 0.27 | 0.54 (0.25-1.19) | 0.62 (0.32-1.19) | 0.78 | 0.47 |
|   | PPD | 1.70 (0.51-5.62) | 2.05 (0.62-6.76) | 0.84 | 0.80 (0.30-2.14) | 1.16 (0.50-2.66) | 0.56 | 0.86 |
|   | OPV\* | 0.88 (0.30-2.59) | 1.14 (0.22-5.78) | 0.75 | 0.64 (0.07-6.15) | 2.20 (0.78-6.24) | 0.42 | 0.54 |
|   | TT | 0.82 (0.19-3.50) | 1.41 (0.36-5.47) | 0.62 | 0.50 (0.17-1.49) | 0.40 (0.16-1.02) | 0.76 | 0.54 |
|   | DT\* | 1.78 (0.40-7.82) | 1.12 (0.47-2.68) | 0.59 | 0.51 (0.22-1.19) | 1.25 (0.68-2.31) | 0.10 | 0.18 |
| IL-5 | medium3\* | 0.38 (0.05-2.80) | 0.73 (0.22-2.39) | 0.57 | 0.50 (0.11-2.29) | 1.28 (0.57-2.87) | 0.29 | 0.84 |
|   | PHA | 3.50 (0.50-24.30) | 0.23 (0.03-1.50) | 0.07 | 0.83 (0.20-3.50) | 1.83 (0.52-6.41) | 0.41 | 0.04 |
|   | Poly I:C\* | (inf-1.16) | 1.52 (0.18-12.70) |   | 0.61 (0.27-1.36) | 1.04 (0.52-2.08) | 0.33 |   |
|   | BCG\* | 2.44 (0.57-10.37) | 1.11 (0.38-3.22) | 0.39 | 0.64 (0.18-2.23) | 0.68 (0.35-1.31) | 0.92 | 0.47 |
|   | PPD\* | 0.59 (0.14-2.48) | 0.42 (0.10-1.77) | 0.75 | 1.20 (0.51-2.86) | 0.53 (0.27-1.03) | 0.14 | 0.68 |
|   | OPV\* | 0.50 (0.05-4.58) | 1.81 (0.42-7.81) | 0.35 | 0.89 (0.39-2.02) | 0.95 (0.41-2.22) | 0.89 | 0.41 |
|   | TT | 0.81 (0.13-4.96) | 2.16 (0.42-11.08) | 0.48 | 0.58 (0.17-2.00) | 0.66 (0.22-2.02) | 0.86 | 0.55 |
|   | DT\* | 1.05 (0.33-3.33) | 1.21 (0.59-2.48) | 0.85 | 0.67 (0.26-1.71) | 1.79 (1.01-3.18) | 0.10 | 0.35 |
| IFN-γ | medium3\* | (inf-1.03) | (inf-0.59) |   | 1.02 (0.30-3.41) | 1.15 (0.47-2.79) | 0.90 |   |
|   | PHA | 4.74 (0.95-23.65) | 0.09 (0.02-0.43) | 0.003 | 0.44 (0.14-1.42) | 3.97 (1.44-10.90) | 0.005 | **0.00002** |
|   | Poly I:C | 6.44 (0.96-43.41) | 0.22 (0.03-1.45) | 0.008 | 0.48 (0.11-2.16) | 1.27 (0.34-4.78) | 0.34 | 0.01 |
|   | BCG\* | 0.87 (0.43-1.74) | 0.16 (0.02-1.05) | 0.10 | 1.17 (0.61-2.22) | 1.19 (0.64-2.22) | 0.98 | 0.13 |
|   | PPD | 1.85 (0.40-8.57) | 0.43 (0.09-2.16) | 0.11 | 0.95 (0.26-3.43) | 2.72 (0.94-7.86) | 0.23 | 0.07 |
|   | OPV\* | 1.00 (0.19-5.18) | 0.41 (0.11-1.52) | 0.37 | 0.79 (0.21-2.91) | 1.62 (0.75-3.50) | 0.37 | 0.22 |
|   | TT | 0.43 (0.05-3.81) | 0.24 (0.02-2.49) | 0.73 | 1.90 (0.35-10.43) | 2.47 (0.57-10.60) | 0.81 | 0.66 |
|   | DT\* | 0.71 (0.08-6.52) | 0.30 (0.05-1.97) | 0.57 | 0.77 (0.29-2.02) | 1.06 (0.46-2.42) | 0.62 | 0.48 |
| TNF-α:IL-103 | LPS | 0.48 (0.15-1.58) | 0.95 (0.28-3.20) | 0.40 | 1.03 (0.41-2.57) | 0.87 (0.39-1.94) | 0.79 | 0.43 |
|   | PAM | 0.45 (0.13-1.49) | 1.43 (0.37-5.54) | 0.25 | 0.86 (0.34-2.20) | 2.00 (0.92-4.34) | 0.17 | 0.77 |
|   | PHA | 0.71 (0.12-4.13) | 0.20 (0.04-1.02) | 0.42 | 0.96 (0.24-3.78) | 1.41 (0.52-3.78) | 0.66 | 0.25 |
|   | PPD | 0.38 (0.09-1.53) | 0.23 (0.05-1.16) | 0.60 | 1.95 (0.63-6.03) | 2.13 (0.76-5.97) | 0.91 | 0.65 |
|   | group | 0.51 (0.27-0.98) | 0.58 (0.29-1.14) | 0.61 | 1.11 (0.67-1.85) | 1.47 (0.97-2.23) | 0.48 | 0.78 |
| IFN-γ:IL-53 | PHA | 0.94 (0.13-6.79) | 0.51 (0.08-3.45) | 0.36 | 0.60 (0.14-2.53) | 2.21 (0.65-7.53) | 0.18 | 0.26 |
|   | TT | 0.37 (0.03-4.78) | 0.03 (0.00-0.85) | 0.23 | 5.48 (0.79-38.22) | 4.15 (0.84-20.48) | 0.82 | 0.37 |
|   | group | 0.74 (0.19-2.93) | **0.21 (0.06-0.76)** | 0.11 | 1.07 (0.38-2.97) | 2.49 (1.03-6.00) | 0.23 | 0.08 |

Geometric mean ratios (GMR) of cytokine responses to innate agonists and vaccine antigens comparing VAS with placebo, stratified by previous VAS and sex. A GMR (or PR) > 1 can be interpreted as an increasing effect of VAS on the cytokine response. Significant estimates after adjustment for multiple comparisons are highlighted in bold.

For some outcomes among infants not having received previous VAS, there were n=0 detectable measurements in either the VAS or the placebo group, and the effect of VAS could therefore not be analysed with Poisson regression; instead confidence intervals were obtained using log-likelihood estimation. Inf: infinitesimal.

1 Analysis of interaction between VAS and sex.

2 Analysis of the three-way interaction between VAS, sex and previous VAS.

3 Geometric mean ratio-ratios analysed for distributions with <50% ND measurements. ‘Group’ designates ratios of cytokine responses analysed collectively for all stimulations with <50% ND observations.

**\*** Outcomes with >50% ND measurements were analysed with Poisson regression