**Additional file**

**Table S1.** Sputum and serum mediators lower limit of detection (LLD) and lower limit of quantification (LLQ).

|  |  |  |
| --- | --- | --- |
|  | **Sputum**  | **Serum**  |
|  | LLQ | LLD | LLQ | LLD |
| **IL-1α** | 0.64 | 0.46 | 3.2 | 1.07 |
| **IL-1β** | 2.56 | 1.58 | 2.6 | 1.13 |
| **IL -2** | 0.64 | 0.453 | 3.2 | 2.8 |
| **IL-4** | 3.2 | 1.18 | 3.2 | 0.811 |
| **IL-5** | 0.64 | 0.476 | 3.2 | 0.677 |
| **IL-6** | 0.64 | 0.516 | 3.2 | 0.781 |
| **IL-6R** | 0.64 | 0.443 | 16 | 3.53 |
| **IL-8** | 2.56 | 0.617 | 2.6 | 0.818 |
| **IL-9** | 16 | 6.74 | 80 | 23 |
| **IL-10** | 3.2 | 2.17 | 3.2 | 1.07 |
| **IL-12p70** | 0.64 | 0.463 | 3.2 | 1.17 |
| **IL-13** | 16 | 6.75 | 80 | 33.5 |
| **IL-15** | 3.2 | 0.61 | 3.2 | 0.757 |
| **IL-17** | 16 | 4.25 | 16 | 8 |
| **IL-18** | 16 | 3.72 | 80 | 20.5 |
| **IL-23** | 400 | 180 | 2000 | 605 |
| **IL-33** | 10000 | 3265 | 10000 | 2000 |
| **CXCL9** | 0.64 | 0.247 | 3.2 | 0.783 |
| **CXCL10** | 12.8 | 3.2 | 12.8 | 6.92 |
| **CXCL11** | 3.2 | 1.24 | 16 | 3.26 |
| **CCL2** | 3.2 | 2.5 | 3.2 | 2.18 |
| **CCL3** | 16 | 13.2 | 80 | 25.5 |
| **CCL4** | 16 | 6.05 | 16 | 6.13 |
| **CCL5** | 3.2 | 1.21 | 16 | 4.56 |
| **CCL11** | 16 | 12.3 | 80 | 67.8 |
| **CCL13** | 16 | 13.4 | 16 | 6.05 |
| **CCL17** | 0.64 | 8.93 | 16 | 7.93 |
| **CCL26** | 3.2 | 0.932 | 3.2 | 1.28 |
| **TNF-α** | 0.64 | 0.31 | 3.2 | 0.953 |
| **TNF-R1** | 16 | 14.9 | 16 | 6.7 |
| **TNF-R2** | 0.64 | 0.524 | 80 | 0.519 |
| **VEGF** | 400 | 125 | 320 | 180 |
| **GMCSF** | 0.64 | 0.493 | 3.2 | 0.634 |
| **IFNγ** | 0.64 | 0.463 | 0.6 | 0.338 |
| **TSLP** | 80 | 2.37 | 400 | 295 |
| **EGF** | 6.4 | 2.36 | 6.4 | 1.18 |
| **NGF** | 6.4 | 2.35 | 6.4 | 1.35 |
| **SCF** | 6.4 | 3.22 | 6.4 | 3.26 |
| **ST2** | 32 | 25.6 | 160 | 40.7 |
| **CHTR** | 64 | 30.6 | 16000 | 3405 |
| **CEACAM-5** | Not done |  | 558 | 5 |
| **Periostin** | Not done |  | 4 | 2.46 |
| **DDP4** | Not done |  | 43.75 | 33.67 |

**Table S2.** Clinical characteristics at children assessed either at stable visits or following admission to hospital for an acute-severe exacerbation of asthma.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable**  | **Stable** **(n=17)** | **Acute-severe Exacerbation****(n=18)** | **P-value**¶ |
| **Male (n [%])** | 12 (71) | 13 (72) | 0.91 |
| **Age (year)** | 12 (0.7) | 9 (0.8) | 0.019 |
| **BMI (kg/m2)** | 21.4 (1.2) | 16.8 (0.7) | 0.003 |
| **GINA Treatment step (median [range])** | 4 (1 to 4) | 2 (1 to 4) | 0.001 |
| **Family history of asthma (n[%])** | 13 (100) | 7 (54) | 0.005 |
| **Hstory of eczema (n[%])** | 9 (56) | 9 (50) | 0.72 |

Mean (SEM) unless otherwise stated

**Table S3.** Geometric mean (95% CI) sputum mediator concentrations (pg/ml) for all first stable and first exacerbation visits.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **All first stable (n=89)** | **First stable for subjects assessed at exacerbation (n=31)** | **Exacerbation (n=31)** | **P-value\*** | **P-value\*\*** |
| **IL-1α** | 51 (41 to 64) | 43 (30 to 68) | 63 (34 to 118) | 0.44 | 0.16 |
| **IL-1β** | 65 (49 to 87) | 70 (45 to 108) | 155 (68 to 353) | **0.011↑** | **0.026↑** |
| **IL-2** | 0.8 (0.6 to 1.0) | 0.8 (0.5 to 1.3) | 1.3 (0.6 to 2.5) | 0.11 | 0.26 |
| **IL-5** | 2.7 (1.9 to 3.9) | 4.4 (2.4 to 7.8) | 2.6 (1.4 to 5.1) | 0.94 | 0.19 |
| **IL-6** | 42 (30 to 59) | 50 (29 to 89) | 103 (55 to 192) | **0.01↑** | **0.042↑** |
| **IL-6R** | 207 (168 to 256) | 270 (183 to 397) | 436 (288 to 659) | **0.001↑** | **0.012↑** |
| **IL-8** | 3002 (2240 to 4024) | 3562 (2179 to 5822) | 5144 (3212 to 8237) | 0.061 | 0.09 |
| **IL-15** | 1.4 (1.1 to 1.9) | 1.8 (1.2 to 2.7) | 2.4 (1.5 to 3.9) | **0.041↑** | 0.34 |
| **IL-18** | 25.0 (19.8 to 31.0) | 28 (19 to 42) | 56 (32 to 100) | **0.001↑** | **0.004↑** |
| **CXCL9** | 567 (393 to 818) | 714 (340 to 1503) | 1806 (922 to 3538) | **0.002↑** | **0.014↑** |
| **CXCL10** | 707 (515 to 970) | 882 (491 to 1586) | 1362 (719 to 2583) | **0.047↑** | 0.28 |
| **CXCL11** | 53 (37 to 77) | 66 (34 to 129) | 67 (24 to 187) | 0.6 | 0.99 |
| **CCL2** | 274 (219 to 344) | 345 (212 to 562) | 445 (278 to 716) | **0.042↑** | 0.4 |
| **CCL3** | 312 (24 to 41) | 39 (23 to 64) | 37 (20 to 67) | 0.62 | 0.89 |
| **CCL4** | 364 (259 to 511) | 410 (227 to 741) | 688 (366 to 1292) | 0.066 | 0.12 |
| **CCL5** | 8.2 (6.4 to 11.0) | 9.6 (6.0 to 15.0) | 20 (11 to 36) | **0.001↑** | **0.014↑** |
| **CCL11** | 55 (43 to 69) | 60 (40 to 92) | 47 (30 to 73) | 0.5 | 0.33 |
| **CCL13** | 21 (17 to 26) | 26 (18 to 36) | 14.9 (11.0 to 20.0) | 0.1 | **0.009↓** |
| **CCL17** | 26 (20 to 34) | 30.2 (18.0 to 51.0) | 20.8 (13.0 to 34.0) | 0.37 | 0.22 |
| **CCL26** | 10.0 (7.4 to 14.0) | 11.0 (6.8 to 19.0) | 8.7 (4.7 to 16.0) | 0.63 | 0.42 |
| **TNFα** | 2.9 (2.0 to 4.0) | 3.7 (2.1 to 6.6) | 14.0 (5.5 to 36.0) | **<0.0001↑** | **0.004↑** |
| **TNF-R1** | 474 (375 to 600) | 606 (390 to 942) | 953 (564 to 1605) | **0.006↑** | **0.032↑** |
| **TNF-R2** | 220 (164 to 296) | 307 (180 to 524) | 673 (376 to 1203) | **<0.0001↑** | **0.005↑** |
| **VEGF** | 1373 (1163 to 1622) | 1514 (1123 to 2040) | 1441 (1092 to 1902) | 0.77 | 0.74 |
| **EGF** | 370 (298 to 458) | 362 (198 to 663) | 460 (344 to 616) | 0.22 | 0.69 |
| **CHTR** | 90798 (62916 to 131036) | 135247 (69719 to 262365) | 213581 (100828 to 452422) | **0.028↑** | 0.14 |

**\***p-value for unpaired comparison between all first stable and first exacerbation visits; **\*\***p-value for paired comparison between first stable and first exacerbation visits; ↑ increase in mediator concentration; ↓ decrease in mediator concentration.

**Table S4.** Geometric mean (95% CI) serum mediator concentrations (pg/ml) for all first stable and first exacerbation visits.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **All first stable (n=101)** | **First stable for subjects assessed at exacerbation (n=37)** | **Exacerbation (n=37)** | **P-value\*** | **P-value\*\*** |
| **IL-1β** | 5.9 (4.8 to 7.1) | 6 (4.4 to 8.3) | 3.2 (2.3 to 4.6) | **0.002↓** | **0.001↓** |
| **IL-5** | 5.6 (4.4 to 7.1) | 6.1 (4.1 to 9.2) | 4.0 (2.8 to 5.5) | 0.092 | 0.07 |
| **IL-8** | 14 (12 to 15) | 14 (12 to 16) | 12.0 (9.5 to 15.0) | 0.31 | 0.32 |
| **IL-18** | 346 (313 to 382) | 406 (343 to 479) | 391 (342 to 447) | 0.16 | 0.62 |
| **CXCL9** | 63 (54 to 74) | 73 (58 to 91) | 78 (61 to 100) | 0.14 | 0.88 |
| **CXCL10** | 124 (111 to 138) | 137 (114 to 164) | 168 (132 to 214) | **0.01↑** | 0.11 |
| **CXCL11** | 129 (111 to 150) | 150 (115 to 196) | 217 (162 to 290) | **0.001↑** | 0.15 |
| **CCL2** | 660 (601 to 725) | 664 (588 to 749) | 567 (494 to 651) | 0.082 | **0.003↓** |
| **CCL4** | 197 (174 to 223) | 202 (172 to 238) | 181 (147 to 223) | 0.49 | 0.21 |
| **CCL11** | 910 (807 to 1026) | 987 (815 to 1195) | 831.9 (684.0 to 1011.0) | 0.43 | 0.082 |
| **CCL17** | 790 (636 to 982) | 949 (634 to 1418) | 644 (474 to 874) | 0.31 | **0.027↓** |
| **CCL26** | 19 (15 to 25) | 22 (15 to 33) | 18 (11 to 31) | 0.87 | 0.4 |
| **TNFα** | 6.7 (5.1 to 8.8) | 7.1 (4.8 to 10.3) | 5.7 (4.0 to 8.2) | 0.51 | 0.3 |
| **TNF-R1** | 4071 (3805 to 4357) | 4249 (3891 to 4640) | 3932 (3532 to 4378) | 0.59 | 0.052 |
| **TNF-R2** | 5509 (5138 to 5907) | 5737 (5123 to 6425) | 5233 (4544 to 6027) | 0.47 | 0.069 |
| **VEGF** | 926 (798 to 1076) | 880 (672 to 1154) | 767 (574 to 1024) | 0.21 | 0.36 |
| **EGF** | 600 (488 to 737) | 578 (382 to 875) | 412 (271 to 626) | 0.071 | 0.27 |
| **SCF** | 90 (79 to 102) | 92 (72 to 117) | 90 (75 to 107) | 0.99 | 0.81 |
| **ST2** | 88 (73 to 105) | 76 (57 to 102) | 117 (84 to 162) | 0.098 | **0.033↑** |
| **CHTR** | 128633 (103578 to 159749) | 130046 (82144 to 205880) | 127970 (90452 to 181050) | 0.98 | 0.77 |
| **Periostin** | 5.7 (5.0 to 6.5) | 6.1 (5.0 to 7.3) | 4.6 (3.7 to 5.8) | 0.12 | **0.004↓** |
| **DDP4** | 446 (421 to 472) | 433 (393 to 477) | 440 (401 to 484) | 0.82 | 0.68 |

**\***p-value for unpaired comparison between all first stable and first exacerbation visits; **\*\***p-value for paired comparison between first stable and first exacerbation visits;↑ increase in mediator concentration; ↓ decrease in mediator concentration.

**Table S5.** ROC area under the curve (AUC) (95% CI) for sputum and serum mediators between first stable and first exacerbation visits.

|  |  |  |
| --- | --- | --- |
|  | **Sputum** | **Serum** |
|  | **(Stable=89; Exacerbation=31)** | **(Stable=101; Exacerbation=37)** |
| **IL-1α** | 0.53 (0.39 to 0.67) | BLQ |
| **IL-1β** | 0.60 (0.47 to 0.74) | 0.33 (0.22 to 0.44) |
| **IL-2** | 0.57 (0.45 to 0.7) | BLQ |
| **IL-5** | 0.50 (0.37 to 0.62) | 0.37 (0.26 to 0.48) |
| **IL-6** | 0.64 (0.53 to 0.76) | BLQ |
| **IL-6R** | 0.69 (0.57 to 0.81) | ALQ |
| **IL-8** | 0.61 (0.49 to 0.73) | 0.47 (0.35 to 0.58) |
| **IL-15** | 0.62 (0.49 to 0.75) | BLQ |
| **IL-18** | 0.65 (0.53 to 0.78) | 0.59 (0.48 to 0.70) |
| **CXCL9** | 0.67 (0.56 to 0.78) | 0.64 (0.52 to 0.76) |
| **CXCL10** | 0.61 (0.48 to 0.73) | 0.63 (0.51 to 0.74) |
| **CXCL11** | 0.51 (0.37 to 0.64) | 0.71 (0.59 to 0.83) |
| **CCL2** | 0.60 (0.47 to 0.72) | 0.42 (0.31 to 0.53) |
| **CCL3** | 0.52 (0.39 to 0.66) | BLQ |
| **CCL4** | 0.61 (0.48 to 0.73) | 0.47 (0.36 to 0.59) |
| **CCL5** | 0.67 (0.55 to 0.80) | BLQ |
| **CCL11** | 0.46 (0.33 to 0.58) | 0.46 (0.35 to 0.57) |
| **CCL13** | 0.40 (0.30 to 0.51) | ALQ |
| **CCL17** | 0.43 (0.31 to 0.55) | 0.44 (0.34 to 0.55) |
| **CCL26** | 0.46 (0.34 to 0.58) | 0.49 (0.37 to 0.61) |
| **TNFα** | 0.68 (0.56 to 0.80) | 0.48 (0.38 to 0.59) |
| **TNF-R1** | 0.63 (0.51 to 0.76) | 0.45 (0.34 to 0.56) |
| **TNF-R2** | 0.70 (0.58 to 0.82) | 0.45 (0.33 to 0.58)  |
| **VEGF** | 0.51 (0.39 to 0.64) | 0.44 (0.33 to 0.55) |
| **EGF** | 0.56 (0.43 to 0.69) | 0.37 (0.26 to 0.49) |
| **SCF** | BLQ | 0.53 (0.42 to 0.63) |
| **ST2** | BLQ | 0.58 (0.46 to 0.71) |
| **CHTR** | 0.61 (0.48 to 0.73) | 0.50 (0.37 to 0.62) |
| **Periostin** | Not done | 0.43 (0.30 to 0.54) |
| **DDP4** | Not done | 0.47 (0.36 to 0.58) |

BLQ = Below limit of quantification; ALQ = Above limit of quantification.