***Supplementary Material***

**Men have higher cytokine production response upon stimulation with lipopolysaccharide than women: a pooled-analysis including 15 study populations.**

**Authors:** Karel G. M. Beenakker, Rudi G. J. Westendorp, Anton J. M. de Craen†, Sijia Chen, Yotam Raz, Bart E. P. B. Ballieux, Rob G. H. H. Nelissen, Alexander F. L. Later, Tom W. Huizinga, Pieternella E. Slagboom, Dorret I. Boomsma and Andrea B. Maier

|  |  |  |  |
| --- | --- | --- | --- |
| Name study population | Key ref. for methods |  | Cytokine production response |
|  | TNF-α, pg/ml |  | IL-6, pg/ml |  | IL-12, pg/ml |  | IL-1β, pg/ml |
|  | Women | Men |  | Women | Men |  | Women | Men |  | Women | Men |
| LPS, ng/ml | Median | Median |  | Median | Median |  | Median | Median |  | Median | Median |
| (IQR) | (IQR) |  | (IQR) | (IQR) |  | (IQR) | (IQR) |  | (IQR) | (IQR) |
| **General population** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Myoage young | n/a | 10 | 5.1 | 7.5 |  | 73.4 | 87.9 |  | 2.3 | 3.3 |  | 10.1 | 13.8 |
|  |  |  | (3.3-6.9) | (5.1-10.0) |  | (54.4-89.7) | (76.6-94.7) |  | (1.7-2.9) | (2.2-4.3) |  | (8.2-12.0) | (9.6-15.7) |
| YNTR | [[9](#_ENREF_8)] | 10000 | 7.0 (5.5-9.9) | 7.2 (6.0-10.4) |  | 64.4(53.0-90.5) | 72.2(61.0-95.8) |  |  |  |  | 5.8(4.0-7.5) | 6.6(4.6-8.9) |
| Meningitis relatives | [[10](#_ENREF_9)] | 10 | 4.4∞ (3.1-6.2) | 5.0∞(3.7-7.1) |  |  |  |  |  |  |  |  |  |
| MS-SLE relatives | [[1](#_ENREF_11)2] | 1000 | 7.6 (5.3-10.6) | 9.3(6.7-13.2) |  | 26.5(17.1-38.2) | 35.7(22.9-55.9) |  | 8.9(5.6-12.7) | 9.6(6.7-15.2) |  | 1.8(1.0-3.3) | 2.7 (1.6-5.1) |
| Ghana | [[4](#_ENREF_44)7] | 10 | 18.6(12.9-25.8) | 20.5(13.3-28.6) |  |  |  |  |  |  |  |  |  |
| ANTR | [[9](#_ENREF_8)] | 10000 | 5.9(4.8-7.9) | 7.3(5.4-10.3) |  | 63.8(52.3-80.1) | 71.4(57.8-105.7) |  |  |  |  | 4.8(3.5-6.8) | 6.2(4.5-8.5) |
| LLS | [[3](#_ENREF_31)3] | 10 | 8.1(5.7-11.0) | 9.1(6.4-13.7) |  | 82.8(67.2-103.4) | 94.1(78.5-120.1) |  | 4.6b(3.5-5.9) | 5.0b(3.9-6.7) |  | 10.1(7.4-13.1) | 12.1(9.4-16.0) |
| Myoage old | n/a | 10 | 7.0(5.1-10.5) | 7.6(5.9-9.5) |  | 72.1(54.8-92.6) | 75.7(60.0-93.1) |  | 2.3(1.8-3.5) | 3.0(1.9-4.0) |  | 9.7(7.1-12.1) | 11,5(9.9-14.7) |
| PROSPER | [[4](#_ENREF_45)8] | 10000 | 13.6(8.6-20.1) | 15.1(10.3-25.6) |  | 69.5(52.1-92.7) | 73.8(56.7-97.4) |  | 12.8(8.3-19.9) | 12.6(8.0-19.2) |  | 7.1(4.8-10.6) | 9.1(5.9-14.2) |
| Leiden 85-plus | [[4](#_ENREF_46)9] | 10 | 9.6(6.7-12.7) | 11.3(8.5-14.7) |  | 56.9(40.8-78.4) | 70.5(48.6-90.5) |  | 6.2(3.8-9.4) | 8.1(5.1-11.6) |  | 3.4(1.9-5.5) | 4.5(2.3-7.6) |
| **Specific diseases** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MS-SLE | [[1](#_ENREF_11)2] | 10 | 7.2(4.7-10.8) | 11.0(7.7-15.3) |  | 26.2(13.8-45.1) | 23.8(17.2-42.4) |  | 7.0ª(4.4-14.7) | 14.6ª(11.2-17.1) |  | 1.9(1.0-3.6) | 1.6(0.8-2.8) |
| BEST | [[5](#_ENREF_50)3] | 10 | 6.2 (3.9-9.5) | 9.06.4-10.8) |  | 108.3 | 102.5 |  |  |  |  | 1.4(0.6-2.9) | 1.6(0.8-4.2) |
| Cardiac surgery | n/a | 10 | 5.8(3.5-8.4) | 7.1(4.3-10.0) |  | 78.6(61.7-100.6) | 94.2(79.1-119.2) |  |  |  |  |  |  |
| GARP | [[3](#_ENREF_33)5] | 10 | 7.6(5.8-9.6) | 7.7(5.9-11.7) |  |  |  |  |  |  |  | 3.0(1.9-4.7) | 3.6(2.4-6.7) |
| PRALINE | n/a | 10 | 6.9(3.7-11.7) | 5.5(2.7-14.1) |  | 108.3(75.7-128.8) | 102.5(79.7-139.1) |  | 3.6(2.1-4.7) | 3.9(1.9-9.1) |  | 10.7(7.7-20.6) | 11.4(9.5-20.9) |
| Ref.: Reference, LPS concentration: whole blood samples were incubated for with lipopolysaccharide for 24 hours at 37°C. n/a: not available. \* LPS was incubated for 4 hours, ∞ LPS was incubated for 6 hours, ª 1000 ng/ml LPS was used, b 50000 ng/ml LPS was used. |

**Supplementary table 1A:** Crude cytokine production response values in men and women in the included study populations.

|  |  |  |  |
| --- | --- | --- | --- |
| Name study population | Key ref. for methods |  | Cytokine production response |
|  | IL-1RA, pg/ml |  | IL-10, pg/ml |  | IFN-γ, pg/ml |  | GM-CSF, pg/ml |
|  | Women | Men |  | Women | Men |  | Women | Men |  | Women | Men |
| LPS, ng/ml | Median | Median |  | Median | Median |  | Median | Median |  | Median | Median |
| (IQR) | (IQR) |  | (IQR) | (IQR) |  | (IQR) | (IQR) |  | (IQR) | (IQR) |
| **General population** |  |  |  |  |  |  |  |  |  |  |  |  |
| Myoage young | n/a | 10 | 26.6(21.8-30.9) | 34.3(29.5-45.5) |  | 5.2(4.8-6.6) | 6.7(5.2-8.6) |  | 2.4(1.4-8.8) | 3.3 (2.4-4.8) |  | 0.13(0.09-0.22) | 0.16(0.09-0.28) |
| YNTR | [[9](#_ENREF_8)] | 10000 | 31.4(24.9-38.9) | 32.9(26.0-39.9) |  | 2.3(1.9-3.0) | 2.2(1.8-2.7) |  |  |  |  |  |  |
| Meningitis relatives | [[10](#_ENREF_9)] | 10 |  |  |  | 2.2(1.6-3.1) | 2.8(1.6-3.9) |  |  |  |  |  |  |
| MS-SLE relatives | [[1](#_ENREF_11)2] | 1000 | 17.0(11.8-27.5) | 19.6(12.9-29.1) |  | 1.1(0.7-1.5) | 1.2(0.8-1.6) |  |  |  |  |  |  |
| Ghana | [[4](#_ENREF_44)7] | 10 |  |  |  | 3.6(2.2-4.8) | 4.1(2.4-6.1) |  | 2.7(1.7-3.7) | 2.6(1.8-3.7) |  |  |  |
| ANTR | [[9](#_ENREF_8)] | 10000 | 35.4(27.8-41.8) | 33.6(29.0-39.3) |  | 2.1(1.7-2.5) | 1.9(1.5-2.6) |  |  |  |  |  |  |
| LLS | [[3](#_ENREF_31)3] | 10 | 60.4(49.4-77.2) | 68.9(52.5-88.8) |  | 4.3(3.2-5.4) | 4.3(3.2-5.7) |  | 1.7(0.8-4.1) | 1.8(0.6-4.7) |  | 0.15b(0.09-0.29) | 0.11b(0.07-0.20) |
| Myoage old | n/a | 10 | 25.4(20.4-31.3) | 37.0(25.7-43.8) |  | 5.0(3.6-7.6) | 5.3(4.0-7.2) |  | 1.6(0.7-2.6) | 1.4(1.0-2.7) |  | 0.09(0.06-0.14) | 0.07(0.04-0.09) |
| PROSPER | [[4](#_ENREF_45)8] | 10000 | 32.9(26.4-47.0) | 37.5(29.7-53.8) |  | 1.5(1.0-2.2) | 1.6(1.2-2.3) |  |  |  |  |  |  |
| Leiden 85-plus | [[4](#_ENREF_46)9] | 10 | 35.8(26.7-44.7) | 38.9(31.3-49.4) |  | 0.7 (0.5-1.0) | 0.8(0.5-1.2) |  | 0.13(0.04-0.40) | 0.14(0.05-0.51) |  |  |  |
| **Specific diseases** |  |  |  |  |  |  |  |  |  |  |  |  |
| MS-SLE | [[1](#_ENREF_11)2] | 10 | 19.7(12.0-34.4) | 20.7(13.9-30.9) |  | 1.0(0.5-1.4) | 1.0(0.7-1.5) |  |  |  |  |  |  |
| BEST | [[5](#_ENREF_50)3] | 10 | 25.9(18.7-31.0) | 37.1(28.5-50.3) |  | 0.5(0.3-0.9) | 0.8(0.4-1.1) |  |  |  |  |  |  |
| Cardiac surgery | n/a | 10 |  |  |  | 4.2(3.3-5.6) | 4.6(3.6-6.3) |  |  |  |  |  |  |
| GARP | [[3](#_ENREF_33)5] | 10 | 23.0(18.1-28.4) | 24.8 (19.6-30.4) |  | 0.7(0.5-1.0) | 0.8(0.6-1.1) |  |  |  |  |  |  |
| PRALINE | n/a | 10 | 50.8(35.5-61.0) | 45.0(31.6-61.6) |  | 4.6(2.8-6.1) | 4.5(2.4-5.2) |  | 0.6(0.2-1.6) | 1.0(0.6-2.6) |  | 0.05(0.03-0.09) | 0.05(0.03-0.14) |
| Ref.: Reference, LPS concentration: whole blood samples were incubated for with lipopolysaccharide for 24 hours at 37°C. n/a: not available. \* LPS was incubated for 4 hours, ∞ LPS was incubated for 6 hours, ª 1000 ng/ml LPS was used, b 50000 ng/ml LPS was used. |

**Supplementary table 1B**: Crude cytokine production response values in men and women in the included study populations.

**Supplementary table 2**. Pooled leukocyte concentration and differentiation in men and women of three independent study populations (Myoage young, Myoage old and LLS).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Men mean (95% CI) |  | Womenmean (95% CI) |  | p-value |
| Leukocytes \*109/L | 6.08 (5.34 to 6.82) |  | 5.87 (4.89 to 6.85) |  | 0.29 |
|  Neutrophils \*109/L | 3.58 (3.08 to 4.08) |  | 3.25 (2.56 to 3.94) |  | 0.008 |
|  Lymphocytes \*109/L | 1.79 (1.58 to 2.00) |  | 1.99 (1.73 to 2.25) |  | <0.001 |
|  Monocytes \*109/L | 0.53 (0.49 to 0.57) |  | 0.46 (0.41 to 0.50) |  | <0.001 |
|  Eosinophils \*109/L | 0.15 (0.15 to 0.17) |  | 0.15 (0.13 to 0.17) |  | 0.12 |
|  Basophils \*109/L | 0.03 (0.02 to 0.03) |  | 0.03 (0.02 to 0.04) |  | 0.25 |
| Estimates were calculated using a meta-analysis model with random effects and corrected for age and family relationships between subjects, CI: confidence interval. |