



## Data Article

## Normative dataset for plasma cytokines in healthy human adults



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## ABSTRACT

We determined normative data for plasma cytokines established from a cohort of 126 carefully screened healthy adults aged 18 to 64 years. Participants were enrolled to ensure an even age and sex distribution and to include at least 30% non-Caucasians. Plasma cytokines for 18 analytes were tested by multiplex immunoassay. The data are presented by age cohort (18–29 years, 30–39, 40–49, and 50–66), as well as by sex and racial background. This dataset complements published normative ranges of cellular subsets generated by comprehensive polychromatic flow cytometry analysis of the healthy human immune system [1]. These data are available to researchers and have value as a reference range for research involving peripheral cytokines.

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## Specifications Table

Subject	Biology
Specific subject area	Human Immunology
Type of data	Table 1 Figures 3
How data were acquired	Plasma cytokines tested by multiplex immunoassay (MAGPIX, Luminex corporation, US)
Data format	Raw data and analyzed data. Normative ranges for plasma cytokines are presented in the format of Mean $\pm$ standard deviation (SD).
Parameters for data collection	Carefully screened 126 subjects through clinical and laboratory testing. Peripheral whole blood of subjects anticoagulated with ACD. This work was carried out in accordance with Declaration of Helsinki.
Description of data collection	Blood was centrifuged within 6 h after phlebotomy and aliquoted for long term storage at $-80^{\circ}\text{C}$ . The undiluted plasma was subsequently tested to measure the concentration of IL-2, IL-4, IL-5, IL-10, IL-6, IL-9, IL-12p70, TNF- $\alpha$ , GM-CSF, IL-1 $\beta$ , IFN- $\gamma$ , IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, and IL-27 by multiplex immunoassay.
Data source location	Institution: Duke University City/Town/Region: Durham Country: United States of America Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 36.0014 $^{\circ}$ N, 78.9382 $^{\circ}$ W
Data accessibility	The analyzed data are available in this article and the raw data set is in the repository [2]. Repository name: GitHub Data identification number: <a href="https://doi.org/10.5281/zenodo.4472598">https://doi.org/10.5281/zenodo.4472598</a> Direct URL to raw data: <a href="https://duke-hhis.github.io/reference-range/#/">https://duke-hhis.github.io/reference-range/#/</a>
Related research article	Yi JS, Rosa-Bray M, Staats J, Zakrotsky P, Chan C, Russo MA, et al. Establishment of normative ranges of the healthy human immune system with comprehensive polychromatic flow cytometry profiling. PLoS One. 2019;14(12):e0225512. <a href="https://doi.org/10.1371/journal.pone.0225512">https://doi.org/10.1371/journal.pone.0225512</a> . eCollection 2019.

## Value of the Data

- Normative ranges for plasma cytokines were established from a large population of rigorously screened healthy donors.
- Scientists performing cytokine research related to human immunology can benefit from the data provided.
- These normative ranges may be used as control data for experiments or for interpreting clinical laboratory results with varied age, race or sex human subjects.

## 1. Data Description

The dataset contains plasma cytokine concentrations from 126 healthy donors for IFN- $\gamma$ , TNF- $\alpha$ , IL-2, IL-5, IL-17, IL-18, IL-21, IL-22, GM-CSF, IL-1b, IL-4, IL-6, IL-9, IL-10, IL-12, IL-13, IL-23, IL-27. The raw dataset can be accessed on github, <https://duke-hhis.github.io/reference-range/#/>. Table 1 presents the normative value for these cytokines expressed as mean  $\pm$  standard deviation (SD). Reference values are provided by age groups (18–29, 30–39, 40–49, 50–66 years), sex, and race. GM-CSF, IL-1 $\beta$ , IL-4, IL-6, IL-9, IL-12, IL-13, IL-23, and IL-27 were not detectable in this population.

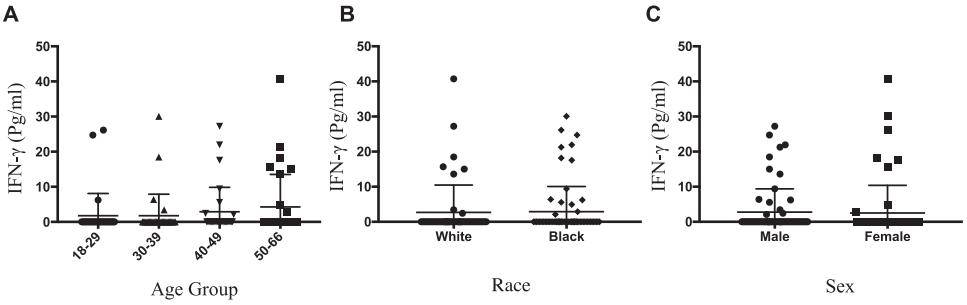
Fig. 1A shows the distribution of the cytokine IFN- $\gamma$  among 18–29, 30–39, 40–49, 50–66 age groups, and there were no significant differences among the age groups. Fig. 1B and 1C shows IFN- $\gamma$  concentrations presented by race, and sex, respectively. There were no statistically significant differences among the groups.

Fig. 2A shows distribution of cytokine TNF- $\alpha$  among 18–29, 30–39, 40–49, 50–66 age groups and there were no significant differences among them. Fig. 2B and 2C shows TNF- $\alpha$  concentrations presented by race, and sex, respectively. There were no statistically significant differences among the groups.

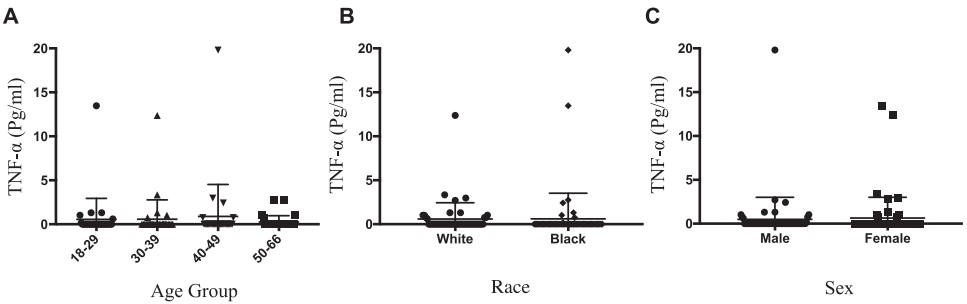
**Table 1**  
Dataset of plasma cytokines in healthy human adults.

Cytokines	Age (years)					Sex		Race	
	All (N = 126)	18–29 (N = 32)	30–39 (N = 33)	40–49 (N = 30)	50–66 (N = 31)	Male (N = 64)	Female (N = 62)	Caucasian (N = 51)	African-American (N = 68)
<b>IFN-<math>\gamma</math></b>	2.65 $\pm$ 7.23	1.79 $\pm$ 6.3	1.77 $\pm$ 6.11	2.88 $\pm$ 6.98	4.27 $\pm$ 9.25	2.78 $\pm$ 6.63	2.52 $\pm$ 7.86	2.68 $\pm$ 7.80	2.90 $\pm$ 7.16
<b>TNF-<math>\alpha</math></b>	0.57 $\pm$ 2.43	0.56 $\pm$ 2.39	0.57 $\pm$ 2.21	0.89 $\pm$ 3.64	0.27 $\pm$ 0.72	0.49 $\pm$ 2.51	0.64 $\pm$ 2.37	0.58 $\pm$ 1.85	0.61 $\pm$ 2.90
<b>IL-2</b>	0.35 $\pm$ 1.64	0.33 $\pm$ 1.89	0.15 $\pm$ 0.85	0.75 $\pm$ 2.28	0.21 $\pm$ 1.19	0.22 $\pm$ 1.27	0.48 $\pm$ 1.89	0.25 $\pm$ 1.31	0.47 $\pm$ 1.92
<b>IL-5</b>	0.34 $\pm$ 1.89	0.08 $\pm$ 0.45	0.12 $\pm$ 0.71	0.52 $\pm$ 1.99	0.67 $\pm$ 3.16	0.24 $\pm$ 1.38	0.44 $\pm$ 2.31	0.08 $\pm$ 0.57	0.57 $\pm$ 2.51
<b>IL-10</b>	0.16 $\pm$ 0.99	0.07 $\pm$ 0.36	0.01 $\pm$ 0.04	0.01 $\pm$ 0.08	0.54 $\pm$ 1.93	0.01 $\pm$ 0.05	0.31 $\pm$ 1.39	0.21 $\pm$ 1.23	0.13 $\pm$ 0.83
<b>IL-17</b>	1.23 $\pm$ 7.90	0.50 $\pm$ 2.85	0.50 $\pm$ 2.88	1.24 $\pm$ 6.81	2.73 $\pm$ 13.95	0.58 $\pm$ 4.67	1.89 $\pm$ 10.23	1.85 $\pm$ 11.07	0.89 $\pm$ 4.96
<b>IL-18</b>	3.22 $\pm$ 7.19	2.07 $\pm$ 5.56	2.88 $\pm$ 5.99	3.54 $\pm$ 7.70	4.48 $\pm$ 9.19	4.05 $\pm$ 7.72	2.37 $\pm$ 6.54	3.54 $\pm$ 8.00	3.11 $\pm$ 6.86
<b>IL-21</b>	10.27 $\pm$ 115.03	0.06 $\pm$ 0.32	0.00 $\pm$ 0.00	10.04 $\pm$ 0.24	41.65 $\pm$ 231.91	0.00 $\pm$ 0.00	20.88 $\pm$ 163.97	25.35 $\pm$ 180.80	0.02 $\pm$ 0.16
<b>IL-22</b>	3.97 $\pm$ 29.81	1.09 $\pm$ 6.17	0.75 $\pm$ 4.31	2.98 $\pm$ 15.52	11.35 $\pm$ 57.73	0.07 $\pm$ 0.53	8.01 $\pm$ 42.29	6.78 $\pm$ 45.01	2.28 $\pm$ 11.63
<b>GM-CSF</b>	-	-	-	-	-	-	-	-	-
<b>IL-1<math>\beta</math></b>	-	-	-	-	-	-	-	-	-
<b>IL-4</b>	-	-	-	-	-	-	-	-	-
<b>IL-6</b>	-	-	-	-	-	-	-	-	-
<b>IL-9</b>	-	-	-	-	-	-	-	-	-
<b>IL-12</b>	-	-	-	-	-	-	-	-	-
<b>IL-13</b>	-	-	-	-	-	-	-	-	-
<b>IL-23</b>	-	-	-	-	-	-	-	-	-
<b>IL-27</b>	-	-	-	-	-	-	-	-	-

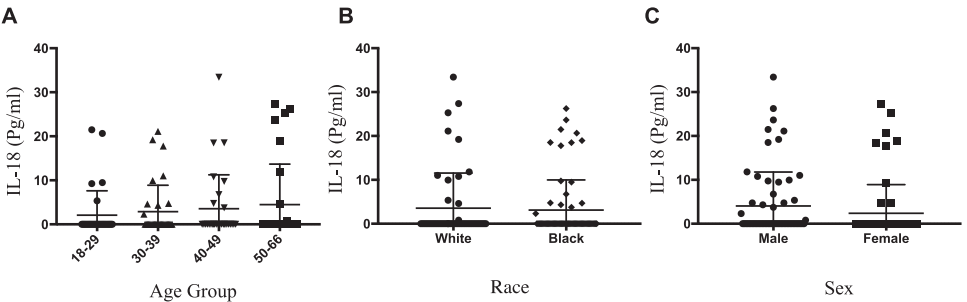
“-” represents cytokines that were not detected on the assays, cytokines unit – pg/mL.



**Fig. 1.** IFN- $\gamma$  concentrations among age, race and sex groups. A, IFN- $\gamma$  concentrations among age groups, including 18–29 years, 30–39 years, 40–49 years and 50–66 years. B, IFN- $\gamma$  concentrations by race groups, black represents participants with African ancestry. C, IFN- $\gamma$  concentrations between sex groups.



**Fig. 2.** TNF- $\alpha$  concentrations among age, race and sex groups. A, TNF- $\alpha$  concentrations among age groups, including 18–29 years, 30–39 years, 40–49 years and 50–66 years. B, TNF- $\alpha$  concentrations by race groups, black represents participants with African ancestry. C, TNF- $\alpha$  concentrations between sex groups.



**Fig. 3.** IL-18 concentrations among age, race and sex groups. A, IL-18 concentrations among age groups, including 18–29 year, 30–39 year, 40–49 year and 50–66 year. B, IL-18 concentrations by racial groups, black represents participants with African ancestry. C, IL-18 concentrations between sex groups.

Fig. 3A shows distribution of cytokine IL-18 among 18–29, 30–39, 40–49, 50–66 age groups and there were no significant differences among them. Fig. 3B and Fig. 3C shows IL-18 concentrations presented by race, and sex, respectively. There were no statistically significant differences among the groups.

## 2. Experimental Design, Materials and Methods

### 2.1. Enrollment and subject eligibility

Healthy volunteers, who were new donors or had not donated blood or plasma in 6 months or longer, and meeting specific eligibility criteria were recruited at Biomat USA (Grifols) plasma donor centers in North Carolina between February 2015 to October 2016. There was a standardized screening process to determine donor eligibility for plasma donation as described previously [1]. Clinical data was entered into a REDCap database. This study was approved by the Duke local and Copernicus central IRBs and informed consent was obtained from all participants.

### 2.2. Blood collection and plasma processing

Prior to the plasma donation, approximately 50 mL of blood was collected from each donor in Acid Citrate Dextrose tubes (BD Vacutainer, Franklin Lake, NJ) and transported at room temperature to the Duke Immune Profiling Core (DIPC). Within 6 h, blood was centrifuged at 20 °C, 800xg for 20 min. 5 ml of plasma was recovered and aliquoted in 1 mL increments, and subsequently stored long term at –80 °C.

### 2.3. Multiplex immunoassay

Donors' plasma samples were used undiluted to measure the concentrations of IL-2, IL-4, IL-5, IL-10, IL-6, IL-9, IL-12p70, TNF- $\alpha$ , GM-CSF, IL-1 $\beta$ , IFN- $\gamma$ , IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, and IL-27. Multiplex immunoassay was performed according to the manufacturer's procedures (ThermoFisher EPX180–12,165–901). Magnetic beads were diluted and added to a 96-well flat bottom plate. Plasma or serially diluted standards were added to all wells in duplicate. The plate was placed on a shaker for 30 min at room temperature and 500 rpm, then moved to 4 °C overnight. The next day, detection antibodies were added and the plate was placed back on the shaker at room temperature for 30 min. Streptavidin-PE was then added to each well and shaken following above procedures. Prior to cytokine measurement, Magpix drive fluid was added to the samples (Luminex).

### 2.4. Statistical analysis

Multiplex raw data was transformed to numeric data according to the standard agent fluorescent values. Student *t*-tests or ANOVA tests were employed to compare two groups or three or more groups, respectively, and an alpha level of 0.05 was defined for statistical significance. Data are presented as means and standard deviations. All the graphs were produced in Prism7 (GraphPad Software, La Jolla, CA, USA).

## CRedit Author Statement

**Yingkai Li:** Conceptualization, Formal analysis, Visualization, Writing- Original draft preparation; **John S. Yi:** Conceptualization, Methodology, Formal analysis, Resources, data collection; Writing- critical review and editing; **Melissa A. Russo:** Data collection, Investigation, Data curation; Writing- critical review and editing; **Marilyn Rosa-Bray:** Supervision; Methodology, Investigation, Writing- critical review and editing; **Kent J. Weinhold:** Conceptualization, Resources, Writing- critical review and editing; **Jeffrey T. Guptill:** Conceptualization, Methodology, Resources data collection; Writing- critical review and editing; Supervision; Project administration, Funding acquisition. All authors reviewed and accepted the manuscript.

## Declaration of Competing Interest

JTG has received personal compensation from Grifols, Inc for consulting activities unrelated to this project. The authors declare that they have no other known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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- [1] J.S. Yi, M. Rosa-Bray, J. Staats, P. Zakrotsky, C. Chan, M.A. Russo, et al., Establishment of normative ranges of the healthy human immune system with comprehensive polychromatic flow cytometry profiling, PLoS ONE 14 (12) (2019) e0225512. eCollection 2019, doi:[10.1371/journal.pone.0225512](https://doi.org/10.1371/journal.pone.0225512).
- [2] Repository of normative range of normative dataset for plasma cytokines in healthy human adults. <https://duke-hhis.github.io/reference-range/#/>, doi:[10.5281/zenodo.4472598](https://doi.org/10.5281/zenodo.4472598).