

Characterizing age-dependency of cytokine levels in healthy children

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Objectives

- To characterize the age-dependency and normal ranges of eight cytokines in healthy children (IL-1ra, IL-2, IL-4, IL-6, IL-10, IP-10, IFN- γ and TNF- α), as well as the age-dependency of cytokine release following antigen stimulation *ex vivo*.

Methods

- Healthy children aged 0-12 years undergoing elective/diagnostic procedures were eligible for inclusion.
- Whole blood was stimulated *ex vivo* for 24 hours using Staphylococcus enterotoxin B (SEB), Phytohaem-agglutinin (PHA) or *Candida albicans*, or left unstimulated, and cytokine concentrations were measured using a Luminex bead-based multiplex assay (precision: 2-19%, lower limit of quantification LLOQ=3.2 pg/mL).
- Data was analyzed by censored mixed effect regression analysis in NONMEM version 7.3 to evaluate linear and non-linear relationships of cytokine levels with age.
- Simple log and box-cox transformations were compared for description of between-subject variability (BSV) using the likelihood ratio test, and evaluated using visual predictive check diagnostic.

Results

- A total of 271 children were included (median age 5.2 years, IQR 3.4-7.8; 74% male).

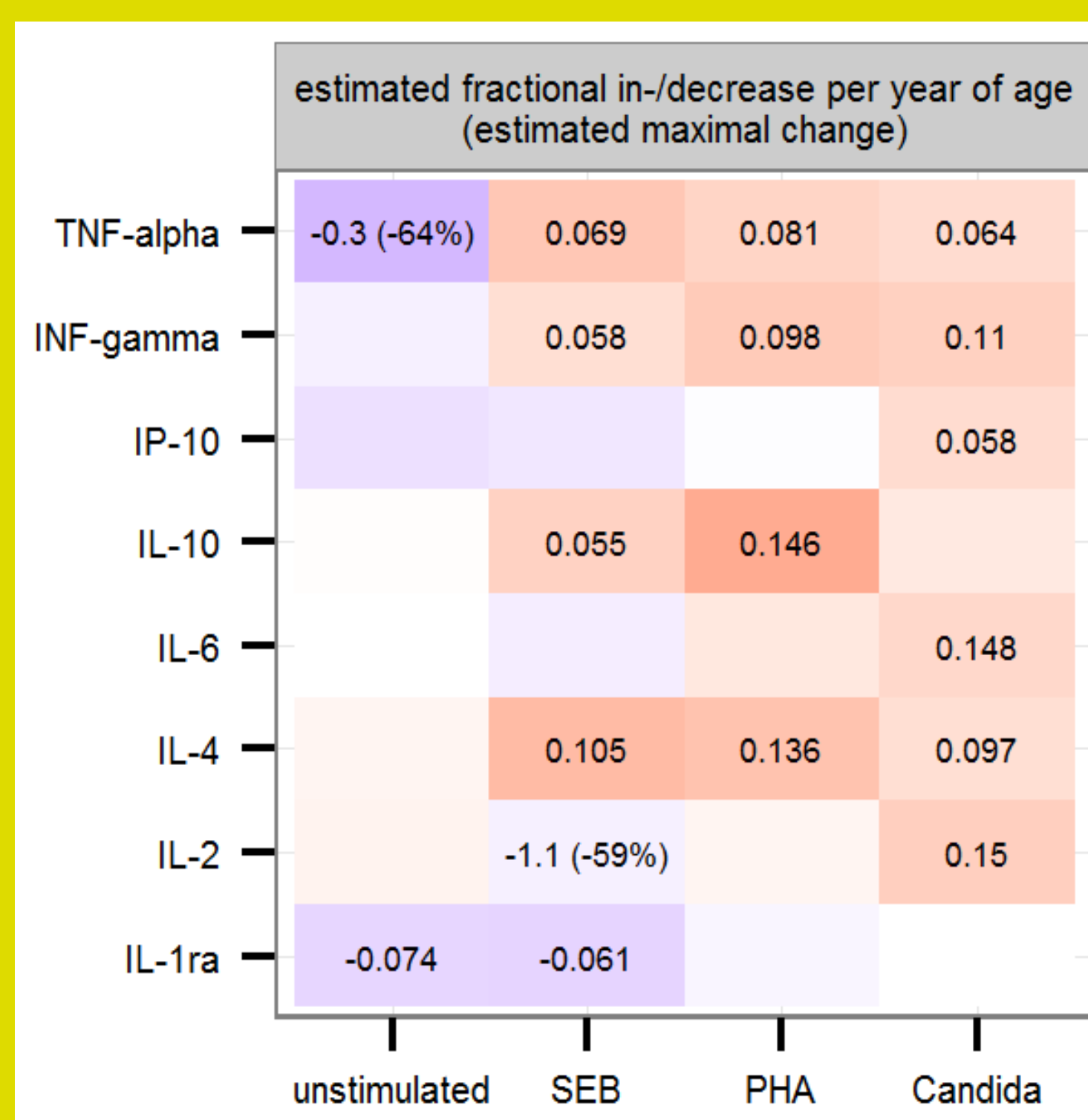


Fig. 1: Association of cytokine concentration with age under different conditions (i.e. unstimulated, or stimulated with the antigen SEB, PHA, or *Candida albicans*). **Blue:** decrease with age, **Red:** increase with age observed. **Numbers** indicate the estimated regression coefficient, which equals roughly the corresponding fractional in- or decrease with age (a maximal change could only be estimated for TNF- α in unstimulated samples and for IL-2 after SEB stimulation). **Empty fields** indicate that a significant change with age could not be estimated with good confidence ($p > 0.05$).

- Generally, **unstimulated** samples were **negatively correlated with age** (Fig. 1 & 2), while antigen-stimulated samples were mostly **positively correlated with age** (Fig. 1).
- An exponential (i.e. fractional) concentration change with age best described the observed age-dependency.
- BSV was large (74% to 240%), and was only to a small part (3-7%) explained by age. Statistical criteria suggested for some cytokines that a box-cox transformation could improve the description of BSV. A log-normal distribution appeared however sufficient in visual predictive check diagnosis (Fig. 2).
- Residual unexplained variability was 19%.

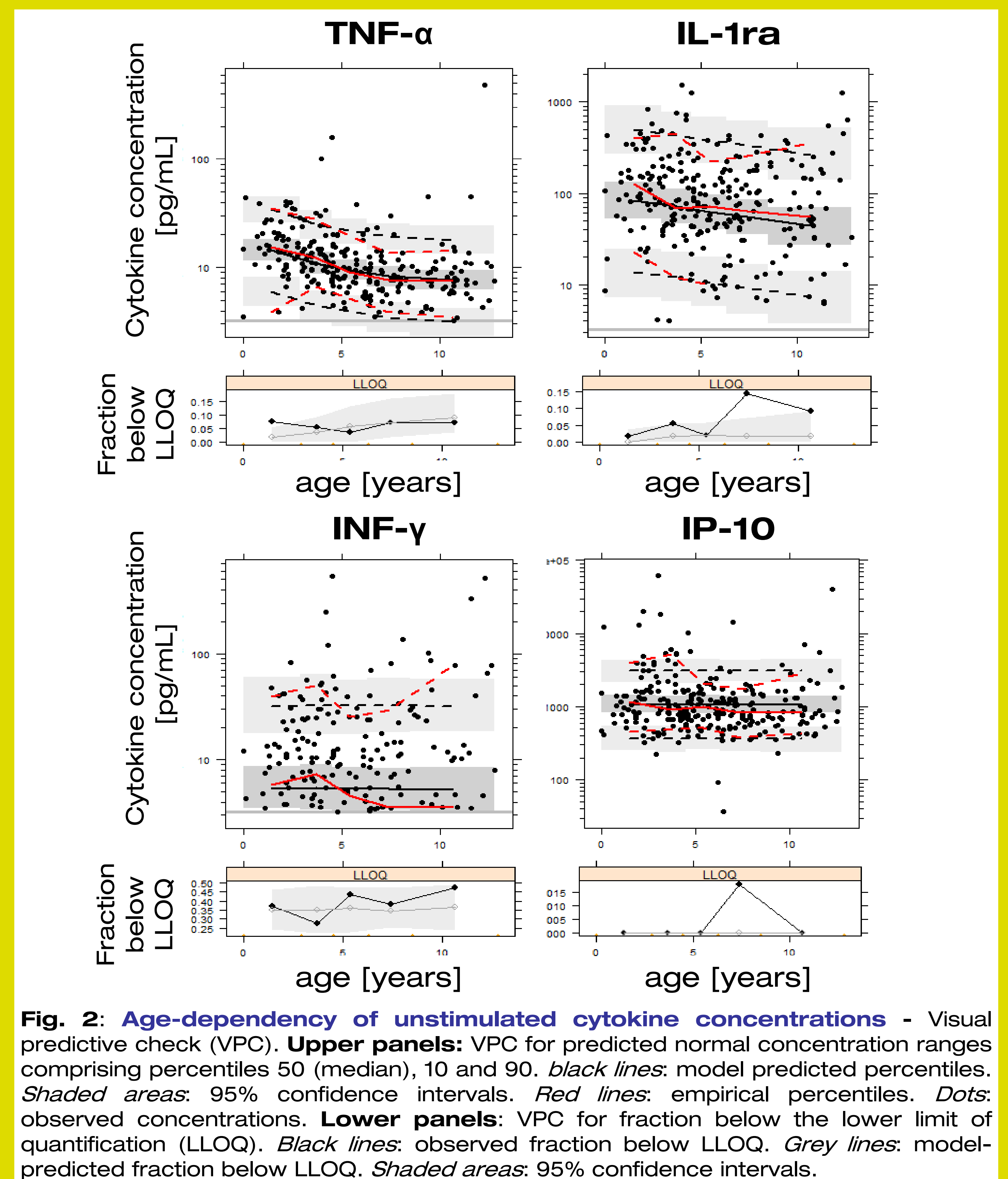


Fig. 2: Age-dependency of unstimulated cytokine concentrations - Visual predictive check (VPC). **Upper panels:** VPC for predicted normal concentration ranges comprising percentiles 50 (median), 10 and 90. **black lines:** model predicted percentiles. **Shaded areas:** 95% confidence intervals. **Red lines:** empirical percentiles. **Dots:** observed concentrations. **Lower panels:** VPC for fraction below the lower limit of quantification (LLOQ). **Black lines:** observed fraction below LLOQ. **Grey lines:** model-predicted fraction below LLOQ. **Shaded areas:** 95% confidence intervals.

- TNF- α** showed the strongest age-dependency, with a decline of **-0.3/year** (=26%/year, 'half-life' $t_{1/2}$ =2.3 years, baseline=19 pg/mL, lower asymptote=7 pg/mL, $p < 0.001$).
- IL-1ra** decreased by **-0.7/year** (=7%/year, $t_{1/2}$ =9 years, $p=0.008$), while a lower asymptote could not be quantified.
- INF- γ and IP-10:** An age-dependency could not be quantified for these cytokines, but was suggested for INF- γ by increasing proportions of data below the LLOQ in older age groups.
- IL-2, IL-4, IL-10, IL-6:** a high proportion ($\geq 70\%$) of samples was $< \text{LLOQ}$ and the age-dependency could therefore not be characterized.

Conclusions

- In healthy children unstimulated levels of TNF- α and IL-1ra decrease during the first 12 years of life.** The other cytokines did not show a clear association with age, but the detection of such an association may have been impeded by the large proportion of data below LLOQ.
- The importance of age-dependent levels of these two important pro-inflammatory and anti-inflammatory cytokines need to be further investigated.
- It may be relevant for choosing the optimal dose of immune-modulating drugs in pediatric patients.