ONLINE SUPPLEMENT

Ronald L. Simons et al. "Childhood Adversities Amplify the Association between Adult Stressors and Inflammation in a Domain Specific Manner: Nuancing the Early Life Sensitivity Model"

A. The Balance of Pro- to Anti-inflammatory Cytokines Predicts Illness

The ratio of pro- to anti-inflammatory cytokines has been linked to a variety of illnesses including cardiovascular disease (Anguera et al. 2002; Chen et al. 2010; Kelic et al. 2006; Libby et al., 2016;Taniguchi et al. 1999), cancer (Kumar et al. 2015; Lin and Lin, 2016), immunity (Dodoo et al. 2002; Sahiratmada et al. 2007), asthma (Gosset et al. 1999); substance abuse (Zago et al. 2016), and depression (Dhabhar et al. 2009; Taraz et al. 2012). Further, Calegari et al. (2018) recently reported that exercise training improves the IL-10/TNFα cytokine balance. All of this points to the importance of balance in the expression of pro- and anti-inflammatory cytokines. Based on this observation and as described in more detail in the measures section, the inflammatory index used in the present study involved summing across pro- and anti-inflammatory cytokines, respectively, and then calculating the ratio of these two summary scores.

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B. Psychometric Properties of the Cytokine Measure

Inflammation is a reflection of the activitation of the cytokine system which consists of a variety of pro- and anti-inflammatory cytokines. It is difficult to assess given its complex, multfaceted nature. Further, ELISA assays tend to suffer from limited reliability In the clinical setting, clinical centers routinely set their own diagnostic range for these assessments even when using the same diagnostic machinery as other centers. Indeed, when different batches of reagents are produced or choice of 510K approved platform is changed (e.g. from Bio-Rad to Roche) the normal range changes. In the research setting, the variability is probably even higher. For example, the results using a kit from one manufacturer to another manufacturer can vary dramatically. Finally, because of non-specific binding effects, the results obtained from multiplexing versus single plexing can differ. We assayed multiple cytokines in the current study in an attempt to minimize the variability of individual measurements on conclusions related to the relative inflammatory diathesis being assessed.

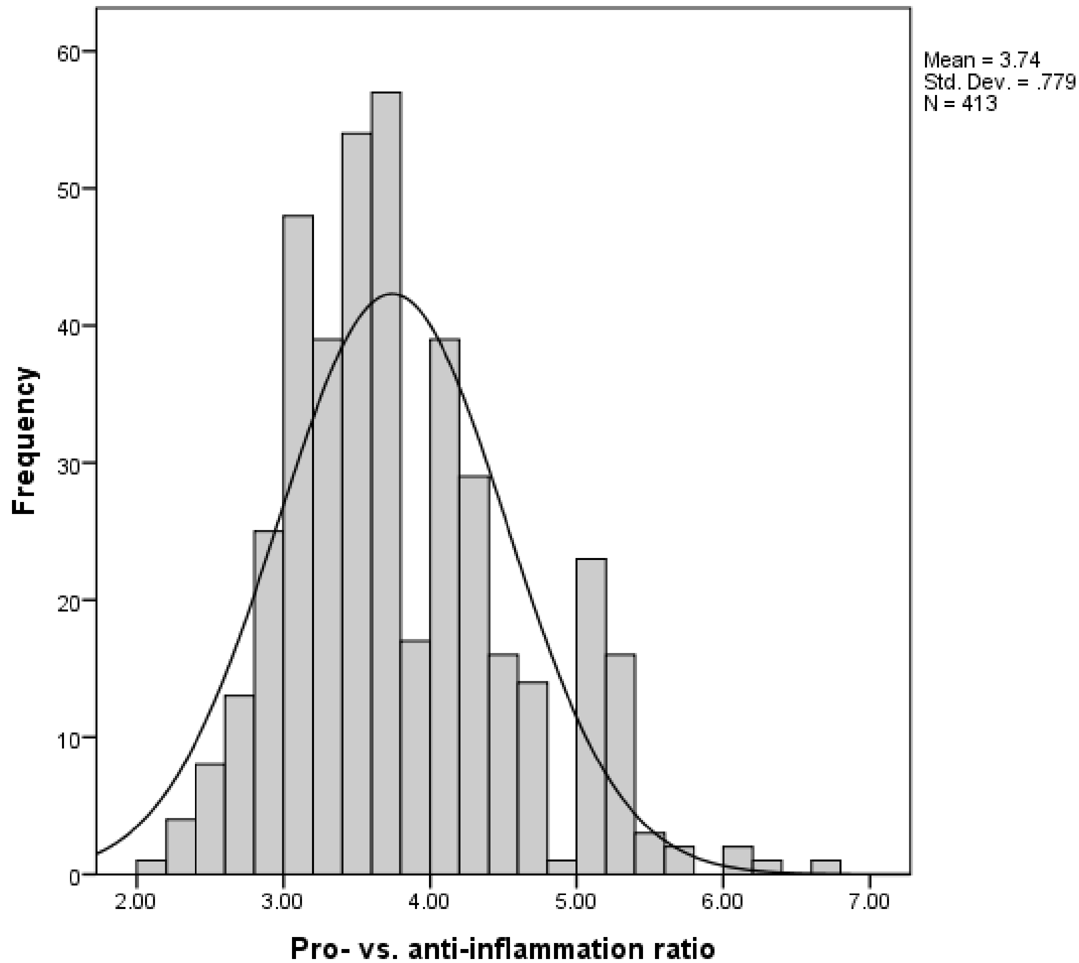
Cytokine measurement reproducibility was good (overall average intra-assay coefficients of variation for IL-1B, 3.2%; IL-4, 3.9%; IL-5, 4.4%; IL-6, 2.7% ; IL-7, 3.4%; IL-8, 6.2%; IL-10, 2.4%; IL-12, 3.2%; IL-13, 3%; IL-17, 3.5%; G-CSF, 4.4%; MIP-1β = 3.6%; TNF-α = 2.8%; IFN-γ, 3.6%). Raw scores for each cytokine are provided in Online Supplemental Table S1 which provides raw descriptive statistics for each inflammatory marker, including the range of values detected, the mean raw cytokine level and SD, and the coefficient of variability, and skewness. As expected, cytokine scores co-varied across all cytokines with an intra-class correlation of .740.

A number of individuals had non-dectable levels for several of the cytokines (see Table S1). To an extent, this is to be expected given that the sample consists of young adults for whom low inflammation is a normal or baseline state. It is also the case, however, that multiplex platforms tend to have higher rates of none detection than the high sensitivity single-plex approach. Indeed most of the 14 cytokines included in our anallysis demonstrated a skewed distribution where many individuals had no detectable level, some had a dectable level, and a few had very high levels (see mean, SD, and skewness for each cytokine as reported in Supplemental Table 1 and distribution of scores in Figure 1).

Supplemental Table S1. Table of intra-assay coefficient of variation (CV%) at each standard, mean raw cytokine levels (and SD) for each cytokine, range of values detected, and skewness

Cytokine	S1	S2	S3	S4	S5	S6	S7	S8	Mean (SD) of CV	Assay working ranges, pg/m LLOQ		ULOQ	Mean (SD) of raw value pg/ml	% undetectable	Skewness
IL-1β	0.86	1.54	2.22	1.38	1.86	4.68	4.47	8.25	3.16 (2.50)	0.3	–	5,386	0.38 (3.15)	57.87	18.64
IL-4	1.32	1.17	2.79	2.11	4.49	4.22	6.01	8.88	3.88 (2.63)	0.2	–	3,484	0.13 (2.02)	92.74	20.07
IL-5	0.45	1.16	2.02	2.10	3.10	7.00	7.11	12.31	4.41 (4.06)	0.6	–	14,775	1.36 (7.55)	80.15	11.65
IL-6	0.84	1.31	1.27	1.81	2.63	3.89	4.90	4.72	2.67 (1.63)	1.3	–	23,266	13.18 (147.20)	82.81	17.68
IL-7	1.06	0.72	2.05	1.97	3.85	4.54	6.01	7.31	3.44 (2.39)	0.5	–	11,986	3.76 (14.64)	20.82	15.65
IL-8	4.43	3.60	5.14	2.33	5.81	6.93	9.48	11.96	6.21 (3.18)	1.3	–	25,143	14.83 (114.00)	9.20	17.02
L-10	1.88	1.16	1.10	2.35	1.84	2.92	3.66	4.05	2.37 (1.10)	3.4	–	55,895	36.70 (335.93)	18.89	14.25
IL-12	1.48	1.15	2.78	1.34	2.21	4.76	4.54	7.47	3.22 (2.21)	2.3	–	38,759	31.32 (424.74)	71.67	19.68
IL-13	0.46	1.30	0.94	0.74	2.54	4.93	5.80	7.51	3.03 (2.70)	0.3	–	5,925	2.38 (22.61)	47.70	17.67
IL-17	1.53	1.14	4.85	2.71	2.65	3.98	4.12	7.04	3.50 (1.92)	1.7	–	35,460	4.63 (18.49)	86.92	5.48
G-CSF	1.65	2.19	2.87	2.61	3.64	6.25	6.22	9.87	4.41 (2.81)	1.5	–	29,714	5.04 (26.91)	69.25	14.94
MIP-1β	0.98	1.20	2.78	1.47	3.55	3.47	5.24	9.94	3.58 (2.95)	0.2	–	4,584	82.49 (58.88)	1.21	3.17
TNF-α	1.29	1.05	1.37	2.20	2.74	3.47	3.83	6.76	2.84 (1.89)	3.0	–	52,606	12.57 (91.48)	33.90	16.24
IFN-γ	1.25	2.28	2.84	3.83	4.85	6.69	7.03	----	4.44 (2.20)	3.0	–	14,989	5.35 (58.34)	91.77	17.12

Supplemental Figure S1. Distribution of scores for the categorical ratio inflammatory index.



Supplemental Table S2. Correlation between Alternative measures of inflammation

	1	2	3
1. Categorical	——		
2. Ratio log transformed	.847**	——	
3. Difference log transformed	.915**	.933**	——
4. IL6+TNF/IL10	.670**	.553**	.668**

Note: N = 413; **$p \leq .01$; *$p \leq .05$ (two-tailed tests).