

Table 1

Referral Laboratories and Years when Acute Phase Proteins (APPs) and Cytokines were Assayed

Assays, Statistical Values, Mean Ages	Reported Assay Results in 1st and 2nd Female Sets				
	1st Set	2nd Set	All Females	All Males	Total Subjects
	NWU, 1992	BU, 1996		BU, 1996	
APPs:					
CRP (mg/L):					
Mean ± SE (n)	4.23 ± 1.91 (69)	3.15 ± 0.67 (71)	3.68 ± 1.00 (140)	3.50 ± 0.66 (85)	3.61 ± 0.67 (225)
Median; IQR	1.00; 1.00-2.00	1.10; 0.30-3.90	1.00; 1.00-2.00	1.90; 0.80-3.40	1.40; 0.90-2.70 [†]
CVs (%)	Not remitted	2.9		12.5	
ASAA (mg/L):	Not performed				
Mean ± SE (n)		17.2 ± 6.2 (73)	17.2 ± 6.2 (73)	4.0 ± 0.6 (85)	10.1 ± 2.9 (158)
Median; IQR		6.9; 3.7-11.6	6.9; 3.7-11.6	2.5; 1.6-3.9*	3.8; 2.1-7.2 [†]
CVs (%)		6.7		13.6	
Cytokines:	SLI, 1995	SLI, 1995	SLI, 1995	SLI, 1996	SLI
IL-6 (pg/ml):				Not performed	
Mean ± SE (n)	2.91 ± 0.60 (45)	2.71 ± 0.33 (62)	2.80 ± 0.31 (107)		2.80 ± 0.31 (107)
Median; IQR	2.10; 1.20-2.85	1.85; 1.30-3.45	1.90; 1.30-3.20		1.90; 1.30-3.20
CVs (%)	11.6	9.3	10.5		
IL-1β (pg/ml):					
Mean ± SE (n)	0.42 ± 0.01 (45)	0.89 ± 0.30 (62)	0.70 ± 0.18 (107)	0.73 ± 0.15 (80)	0.71 ± 0.12 (187)
Median; IQR	0.00; 0.00-0.70	0.40; 0.20-0.80	0.30; 0.01-0.70	0.00; 0.00-1.00*	0.30; 0.00-1.00 [†]
CVs (%)	30.0	14.2	22.1	24.8	23.3
IL-1ra (pg/ml):					
Mean ± SE (n)	558 ± 57 (43)	648 ± 55 (58)	610 ± 40 (101)	710 ± 90 (63)	649 ± 42 (164)
Median; IQR	447; 334-692	613; 351-784	503; 339-751	502; 258-902	502; 323-810
CVs (%)	15.6	8.1	11.9	20.5	15.2
TNF-α (pg/ml):					
Mean ± SE (n)	1.33 ± 0.14 (45)	3.01 ± 0.50 (62)	2.31 ± 0.31 (107)	2.15 ± 0.21 (75)	2.24 ± 0.20 (182)
Median; IQR	1.03; 0.62-1.80	1.57; 0.85-2.78	1.39; 0.76-2.27	1.90; 1.30-2.50*	1.60; 0.89-2.36 [†]
CVs (%)	17.9	18.1	18	18.70	18.3
sTNF RI (pg/ml):					
Mean ± SE (n)	792 ± 41 (42)	1193 ± 57 (62)	1031 ± 42 (104)	1713 ± 45 (75)	1317 ± 40 (179)
Median; IQR	753; 596-899	1141; 886-1353	921; 727-1236	1627; 1469-1905*	1306; 847-1642
CVs (%)	17.7	6.5	12.1	4.3	8.8
IL-2sRa (pg/ml):					
Mean ± SE (n)	939 ± 35 (41)	1176 ± 63 (62)	1081 ± 42 (103)	883 ± 54 (72)	1000 ± 34 (175)
Median; IQR	960; 720-1040	1091; 793-1392	1040; 779-1259	825; 601-993*	901; 720-1160
CVs (%)	5.6	11.9	8.8	8.4	8.6
Mean ages ± SEs [‡]	43.7 ± 1.2	44.0 ± 1.2	43.9 ± 0.9	41.8 ± 0.9	43.2 ± 0.7

NWU = Northwestern University Immunology Research Laboratory, BU = Boston University Immunology Research Laboratory, SLI = Specialty Laboratories, Inc., Santa Monica, CA., SE = standard error of mean, IQR = interquartile range of median, CVs (%) = Intra-set percentile coefficients of variation of paired tests above the lowest reference calibration measure. * Spearman correlations between sexes, adjusted for age (p<0.050) and † Spearman correlations with age (p<0.050), see text for details. ‡ ANOVA of subgroup ages, p=0.078

Table 2

Acute Phase Protein and Cytokine Assay Results in Female, Male, and Combined Control vs pre-RA Subjects

Assays, Statistical Factors, and Ages	Normalized Assays in Females*		Reported Assays in Males		Control vs pre-RA Values	
	Control	pre-RA	Control	pre-RA	All Control	All pre-RA
CRP (mg/L):						
Mean ± SE (n)	2.78 ± 0.42 (111)	5.18 ± 1.92 (29)	2.56 ± 0.36 (68)	7.28 ± 2.87 (17)	2.70 ± 0.29 (179) [†]	5.95 ± 1.60 (46) [†]
Median; IQR	1.80; 1.10-2.70	1.70; 0.90-3.40	1.85; 0.80-2.90	2.60; 0.70-7.90	1.80; 0.90-2.80	1.80; 0.88-4.55
ASAA (mg/L):						
Mean ± SE (n)	19.90 ± 8.0 (56)	8.43 ± 1.46 (17)	3.91 ± 0.72 (68)	4.40 ± 1.29 (17)	11.13 ± 3.7 (124)	6.41 ± 1.02 (34)
Median; IQR	6.65; 4.03-12.23	7.50; 3.40-11.55	2.45; 1.60-3.78	3.50; 1.55-4.90	3.60; 2.03-7.05	4.00; 2.53-8.43
IL-6 (pg/ml):						
Mean ± SE (n)	2.58 ± 0.26 (82)	3.53 ± 1.07 (25)	Not Measured	Not Measured	2.58 ± 0.26 (82)	3.53 ± 1.07 (25)
Median; IQR	2.00; 1.30-3.05	1.60; 0.95-3.73			2.00; 1.30-3.05	1.60; 0.95-3.73
IL-1β (pg/ml):						
Mean ± SE (n)	0.90 ± 0.23 (82)	0.58 ± 0.11 (25)	0.70 ± 0.34 (64)	0.81 ± 0.50; (16)	0.82 ± 0.20 (146)	0.67 ± 0.16 (41)
Median; IQR	0.53; 0.10-0.97	0.50; 0.17-0.65	0.00; 0.00-1.00	0.50; 0.00-1.00	0.40; 0.0-1.00	0.50; 0.03-1.00
IL-1ra (pg/ml):						
Mean ± SE (n)	659 ± 50 (76)	553 ± 53 (25)	680 ± 102 (52)	853 ± 180 (11)	667 ± 51; (128)	645 ± 69 (36)
Median; IQR	516; 373-831	571; 331-742	427; 242-917	723; 441-828	504; 324-862	666; 377-790
TNF-α (pg/ml):						
Mean ± SE (n)	2.94 ± 0.32 (82)	3.85 ± 0.77 (25)	2.16 ± 0.26 (60)	2.11 ± 0.21 (15)	2.61 ± 0.22 (142)	3.19 ± 0.50 (40)
Median; IQR	2.24; 1.26-3.60	2.17; 1.44-4.86	1.90; 1.20-2.50	2.20; 1.40-2.90	2.00; 1.20-3.24	2.19; 1.43-3.54
sTNF RI (pg/ml):						
Mean ± SE (n)	1194 ± 46 (79)	1169 ± 61 (25)	1715 ± 51 (60)	1708 ± 102 (15)	1419 ± 41 (139)	1371 ± 68 (40)
Median; IQR	1109; 926-1310	1174; 1015-1322	1618; 1483-1883	1873; 1444-1988	1371; 1064-1656	1322; 1101-1762
IL-2sRα (pg/ml):						
Mean ± SE (n)	1150 ± 49 (79)	1222 ± 71 (24)	905 ± 64 (58)	793 ± 84 (14)	1046 ± 40 (137)	1064 ± 64 (38)
Median; IQR	1076; 853-1313	1213; 993-1445	825; 613-1022	844; 596-966	933; 756-1249	1081; 830-1328
Mean ages ± SEs	43.9 ± 1.0	43.8 ± 2.0	41.8 ± 1.1	41.6 ± 2.1	43.2 ± 0.8	43.1 ± 1.5

* 1st set values are normalized to 2nd set values within each test for later correlational analyses of the combined control and pre-RA female results

†

IQR= interquartile range of median. p=0.051 (t-test of log-transformed normalized variables, with unequal variances assumed)

Table 3**Immune Network Correlations of Female Pre-Rheumatoid Arthritis (Top) and Control (Bottom) Cohort Subjects using Spearman Age-Adjusted Values**

<u>Immunomarkers</u>		<u>CRP</u>	<u>ASAA</u>	<u>IL-1β</u>	<u>IL-6</u>	<u>IL-1ra</u>	<u>TNF-α</u>	<u>sTNF RI</u>	<u>IL-2sRa</u>
CRP:	r		0.649	0.213	0.492	0.360	0.203	0.406	0.165
	p		0.012	0.329	0.017	0.092	0.353	0.054	0.463
	n		15	24	24	24	24	24	23
ASAA:	r	0.446		-0.444	-0.139	0.122	0.118	-0.046	-0.109
	p	0.001		0.129	0.650	0.692	0.701	0.88	0.722
	n	55		14	14	14	14	14	14
IL-1β:	r	0.089	0.097		0.252	0.257	0.242	0.323	0.114
	p	0.439	0.531		0.235	0.225	0.255	0.124	0.606
	n	79	45		25	25	25	25	24
IL-6:	r	0.263	0.179	0.348		0.447	0.136	0.749	-0.246
	p	0.020	0.246	0.001		0.028	0.525	<0.001	0.258
	n	79	45	82		25	25	25	24
IL-1ra:	r	0.300	0.225	0.434	0.546		0.482	0.356	-0.103
	p	0.010	0.164	<0.001	<0.001		0.017	0.088	0.639
	n	73	41	76	76		25	25	24
TNF-α:	r	0.299	0.119	0.226	0.304	0.400		0.180	0.049
	p	0.008	0.442	0.043	0.006	<0.001		0.400	0.832
	n	79	45	82	82	76		25	24
sTNF RI:	r	0.265	0.341	0.157	0.481	0.471	0.216		0.177
	p	0.022	0.024	0.170	<0.001	<0.001	0.058		0.419
	n	76	45	79	79	75	79		24
IL-2sRa	r	0.079	0.192	0.023	0.172	-0.021	0.035	0.451	
	p	0.052	0.212	0.841	0.131	0.862	0.763	<0.001	
	n	76	45	79	79	75	79	79	

Table 4

Immune Network Correlations of Male Pre-Rheumatoid Arthritis (Top) and Control (Bottom) Cohort Subjects using Spearman Age-Adjusted Values

<u>Immunomarkers</u>		<u>CRP</u>	<u>ASAA</u>	<u>IL-1β</u>	<u>IL-6$^{\phi}$</u>	<u>IL-1ra</u>	<u>TNF-α</u>	<u>sTNF RI</u>	<u>IL-2sRα</u>
CRP:	r		0.647	-0.232		0.432	0.246	0.342	-0.153
	p		0.007	0.405		0.212	0.397	0.232	0.619
	n		17	16		11	15	15	14
ASAA:	r	0.408		-0.432		0.639	-0.167	0.336	0.036
	p	0.001		0.108		0.047	0.569	0.240	0.906
	n	68		16		11	15	15	14
IL-1β:	r	0.212	0.121			-0.637 *	0.400	-0.263	0.458
	p	0.095	0.347			0.048	0.157	0.364	0.116
	n	64	64			11	15	15	14
IL-6$^{\phi}$:	r								
	p								
	n								
IL-1ra:	r	0.005	0.276	0.059			-0.482	0.676	0.086
	p	0.975	0.050	0.681			0.158	0.032	0.813
	n	52	52	52			11	11	11
TNF-α:	r	0.335	0.001	0.000		-0.012		0.391	0.480
	p	0.009	0.997	0.998		0.935		0.167	0.097
	n	60	60	60		52		15	14
sTNF RI:	r	0.313	0.229	0.171		0.118	0.244		0.479
	p	0.016	0.081	0.195		0.411	0.063		0.098
	n	60	60	60		52	60		14
IL-2sRα:	r	0.275	0.282	0.252		0.052	0.195	0.433	
	p	0.039	0.034	0.089		0.718	0.145	0.001	
	n	58	58	58		52	58	58	

* Negative (p<0.050) correlation observed in pre-RA, not found in control subjects

$^{\phi}$ Assayed only in females

Table 5

**Immune Network Correlations of Total Pre-Rheumatoid Arthritis (Top)
and Control (Bottom) Cohort Subjects using Spearman Age- and Sex-Adjusted Values**

<u>Immunomarkers</u>		<u>CRP</u>	<u>ASAA</u>	<u>IL-1β</u>	<u>IL-6$^{\Phi}$</u>	<u>IL-1ra</u>	<u>TNF-α</u>	<u>sTNF RI</u>	<u>IL-2sRα</u>
CRP:	r		0.499	-0.120	0.470	0.405	0.214	0.338	0.153
	p		0.005	0.473	0.024	0.019	0.203	0.041	0.380
	n		32	40	24	35	39	39	37
ASAA:	r	0.358		-0.353	-0.113	0.396	-0.036	0.217	-0.160
	p	<0.001		0.066	0.713	0.061	0.860	0.277	0.435
	n	123		30	14	25	29	29	28
IL-1β:	r	0.173	0.093*		0.182	-0.203	0.159	0.001	0.243
	p	0.040	0.339		0.396	0.250	0.340	0.996	0.153
	n	143	109		25	36	40	40	38
IL-6$^{\Phi}$:	r	0.261	0.181	0.313		0.443	0.128	0.749	-0.248
	p	0.021	0.239	0.004		0.030	0.552	<0.001	0.254
	n	79	45	82		25	25	25	24
IL-1ra:	r	0.164	0.266	0.210*	0.538		0.342	0.363	0.053
	p	0.071	0.011	0.019	<0.001		0.048	0.035	0.771
	n	125	93	128	76		36	36	35
TNF-α:	r	0.310	-0.120	0.100	0.304	0.207		0.257	0.105
	p	<0.001	0.227	0.237	0.006	0.020		0.119	0.543
	n	139	105	142	82	128		40	38
sTNF RI:	r	0.285	0.296	0.146	0.481	0.319	0.180		0.181
	p	0.001	0.002	0.088	<0.001	<0.001	0.036		0.212
	n	136	105	139	79	127	139		38
IL-2sRα	r	0.230	0.284*	0.127	0.164	0.007	0.097	0.423	
	p	0.008	0.004	0.142	0.151	0.937	0.264	<0.001	
	n	134	103	137	79	127	137	137	

* Positive correlations in control, but negative in pre-RA subjects, p<0.050

Φ Assayed only in females

Table 6

**Immune Network Correlations of Total Female (Top) and Male (Bottom)
Cohort Subjects using Spearman Age-Adjusted Values**

<u>Immunomarkers</u>	<u>CRP</u>	<u>ASAA</u>	<u>IL-1β</u>	<u>IL-6</u>	<u>IL-1ra</u>	<u>TNF-α</u>	<u>sTNF RI</u>	<u>IL-2sRα</u>
CRP:	r	0.471	0.064	0.322	0.323	0.266	0.273	0.096
	p	<0.001	0.526	0.001	0.101	0.007	0.006	0.345
	n	70	103	103	97	103	100	99
ASAA:	r	0.482	-0.051	0.111	0.232	0.111	0.268	0.114
	p	<0.001	0.704	0.406	0.091	0.406	0.042	0.394
	n	85	59	59	55	59	59	59
IL-1β:	r	0.107	0.001	0.341	0.367*	0.232	0.183	0.047
	p	0.346	0.993	<0.001	<0.001	0.017	0.064	0.640
	n	80	80	107	101	107	104	103
IL-6$^{\Phi}$:	r				0.509	0.284	0.525	0.065
	p				<0.001	0.003	<0.001	0.519
	n				101	107	104	103
IL-1ra:	r	0.125	0.332	0.001		0.427 [†]	0.428	-0.040
	p	0.335	0.008	0.991		<0.001	<0.001	0.697
	n	63	63	63		101	100	99
TNF-α:	r	0.303	-0.020	0.060	-0.017		0.219	0.037
	p	0.009	0.865	0.611	0.897		0.026	0.710
	n	75	75	75	63		104	103
sTNF RI:	r	0.303	0.267	0.051	0.176	0.302		0.409
	p	0.009	0.021	0.666	0.171	0.009		<0.001
	n	75	75	75	63	75		103
IL-2sRα	r	0.186	0.237	0.242	0.046	0.254	0.509	
	p	0.121	0.047	0.042	0.723	0.033	<0.001	
	n	72	72	72	63	72	72	

Positive and stronger correlations in total female than male subjects, * p<0.019, † p<0.004

$^{\Phi}$ Assayed only in females