## t is illegal to post this copyrighted PDF on any website Disequilibrium of Cytokine Serum Levels in Levels in Provided 24.3% Hispanic, 44.3%

## Veterans With Chronic Schizophrenia Medicated With Antipsychotics: Association With Measures of Excitement and Hostility

To the Editor: In 2013, we published a study<sup>1</sup> of the levels of serum cytokines from 47 patients with schizophrenia and 20 healthy controls. We found significantly increased levels of GRO, MCP-1, MDC, and sCD40 L and significantly decreased levels of IFN-y, IL-2, IL-12p70, and IL-17 in schizophrenia patients compared to controls. We also identified a potential role for the IL-17 pathway in worsening of schizophrenia symptoms.<sup>1</sup> We now have expanded this study by enrolling an additional 24 patients. Further, we obtained sequential samples and psychometric measures from 35 patients at a second visit, and 18 patients at a third visit. We hypothesized that cytokines within the Th17 pathway and their ratios to each other would be linked to distinct symptoms of psychopathology as measured by Positive and Negative Syndrome Scale (PANSS)<sup>2</sup> scores. We utilized the total PANSS scores, general psychopathology scores, negative scale scores, and positive scale scores and the individual positive subscale symptoms: delusions, hallucinatory behavior, suspiciousness/persecution, conceptual disorganization, excitement, grandiosity, and hostility.

**Method.** In patients, symptom severity was evaluated using the PANSS. Cytokine/chemokine levels were tested with Millipore bead-based flow immunoassays in a Luminex 100 system (Luminex Corporation, Austin, Texas). The levels of 38 cytokines at each visit were correlated with PANSS scores. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc, Cary, North Carolina). A *P* value < .05 was considered to be statistically different for all comparisons.

white, 17.1% black, and 4.3% Asian. The mean±SD patient age was  $53.7\pm10.4$  years and control age was  $41\pm11.6$  years. Gender distribution was 84% male and 16% female. No statistically significant differences in gender, ethnicity, body mass index, or weight were found between groups. All patients had a longstanding diagnosis of chronic paranoid schizophrenia and were undergoing treatment with antipsychotic medications. Of the patients, 35 (49.29%) were also treated with antidepressants. The mean±SD PANSS scores in patients were 21.5+8.5 for positive symptoms,  $21.9\pm7$  for negative symptoms,  $40.8\pm11$  for general psychopathology symptoms, and  $83.7\pm24.2$  for total scores. Clinical factors of illness (currently hospitalized or not, in-patient or out-patient), treatment with antidepressants, history of posttraumatic stress disorder, or substance abuse did not associate with psychopathology.

By performing the Hochberg method for multiple testing, 4 molecules were found to be increased in patients across all 3 visits: GRO, MCP-1, MDC, and sCD40 L. Levels of IFN- $\gamma$  were decreased in patients compared to controls across all 3 time points. IL-17 levels were reduced only at visit 1, while levels of IL-4 were significantly decreased only at visit 3 (Table 1).

We established a significant correlation between IL-4 and hostility at visit 1 and visit 2 with adjusted *P* values of .0028 and .0152 using the Hochberg method. There was also a significant correlation between IL-1 $\beta$  and excitement at visit 1 with an adjusted *P* value of .0228 using the Hochberg method (Table 2).

The ratios of IL-2:IL-17, IL-4:IL-17, and IL-1 $\beta$ :IL-17 were associated with hostility in veterans with schizophrenia compared to control subjects. The ratio of IL-4:IL-17 was also associated with excitement. The adjusted *P* values of the above ratios using the Hochberg method were significant (Table 2).

Table 1. Cytokine Levels of Control Subjects and Veterans With Schizophrenia at Visits 1, 2, and 3 <sup>a,b</sup>										
					P Value (Hochberg) Control vs Schizophrenia					
		V	eterans With Schizophrer	nia						
Cytokine	Controls	Visit 1 (n = 70)	Visit 2 (n = 35)	Visit 3 (n = 18)	Visit 1	Visit 2	Visit 3			
Eotaxin	120±41.4	253±198	413±515	805±775	.0093*	.0034*	.0608			
	122 (101–139)	216 (119–320)	285 (152–484)	604 (96.0-1,137)						
GRO	78.9±42.3	$650 \pm 452$	1,033±891	783±826	.0033*	.0034*	.0035*			
	69.0 (56.6–112)	555(350-803)	857(405-1293)	548(418-795)						
IFN-γ	76.9±105.3	$14.0 \pm 25.1$	$23.5 \pm 49.7$	9.8±11.9	.0033*	.0448*	.0099*			
	38.4 (12.5-86.4)	6.2 (2.6-14.3)	6.8 (2.9-22.3)	5.4 (3.2–11.9)						
IL-1ra	$36.3 \pm 70.5$	36.3±59.7	$57.0 \pm 56.8$	92.6±112	.9236	.0297*	.1023			
	0.0 (0.0-55.2)	16.1 (0.0-40.6)	40.0 (18.0-102)	56.4 (7.3–144)						
IL-2	$5.5 \pm 10.2$	2.8±6.9	$6.5 \pm 15.3$	$3.1 \pm 5.6$	.0480*	.9806	.9820			
	3.2 (1.8-5.2)	0.0 (0.0-2.9)	0.0 (0.0-4.8)	0.0 (0.0-3.4)						
IL-4	6.8±9.4	6.7±17.7	5.6±17.7	$0.0 \pm 0.0$	.9236	.5740	.0068*			
	2.8 (0.0-12.2)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)						
IL-12p70	32.6±87.2	$5.1 \pm 13.8$	$5.5 \pm 7.6$	$5.4 \pm 7.8$	.0093*	.9308	.9820			
	5.5 (2.2–15.4)	0.0 (0.0-4.3)	2.0 (0.0-7.5)	2.2 (1.2-3.0)						
IL-17	$23.4 \pm 30.1$	7.8±17.2	15.2±31.6	12.3±29.0	.0033*	.9806	.6670			
	15.3 (4.4–23.6)	1.8 (0.0-6.8)	4.0 (1.3-13.5)	4.5 (0.0-9.7)						
MCP-1	287±117	548±271	781±392	$795 \pm 405$	.0033*	.0034*	.0035*			
	270 (212–337)	503 (369–658)	671 (473–1,112)	707 (481–1,240)						
MDC	650±283	1,268±379	$1,145 \pm 447$	1,211±346	.0033*	.0034*	.0035*			
	557 (434–741)	1,215 (1,020–1,510)	1,584 (1,031–1,817)	1,122 (901–1,404)						
sCD40 L	416±181	10,522±5,567	9,562±5,838	7,576±4,696	.0033*	.0034*	.0035*			
	361 (301–504)	14,202 (3,867–14,202)	10,610 (2,246–14,202)	9,092 (26,097-10,610)						

<sup>a</sup>Values are represented in pg/mL as mean  $\pm$  SD and median (interquartile range).

<sup>b</sup>Levels of cytokines were measured in plasma using Millipore bead arrays. The comparisons of cytokines between controls vs schizophrenia subjects at each visit were performed after logarithmic transformation. \* indicates that 11 of 38 cytokines became statistically significant after applying the Hochberg method (*P* values are corrected for multiple testing).

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Table 2. Correlation Between Cytokines and Cytokine Ratios and Symptoms of Psychopathology Established by the Positive Subscale PANSS Scores<sup>a</sup>

	Ln IL-2	Ln IL-4	Ln IL-6	Ln IL-1β	Ln IL-17
Visit 1 <sup>b</sup>					
Excitement	0.28000 .0189	0.29797 <b>.0122</b>	0.27120 <b>.0232</b>	0.39936 <b>.0006</b>	-0.01209 .9209
Hostility	0.21771 .0702	0.44011 <b>.0001<sup>d</sup></b>	0.10203 .4007	0.25255 .0349	-0.13880 .2518
Visit 2 <sup>b</sup>					
Hostility	0.22576 .1922	0.56555 <b>.0004</b> °	0.13259 .4477	0.37937 <b>.0246</b>	0.04244 .8087
	Ln IL-2:IL-17	Ln IL-4:IL-17	Ln IL-6:IL-17	Ln IL-1β:IL-17	
Visit 1 <sup>b</sup>					
Excitement	0.31617 <b>.0077</b>	0.27328 <b>.0221</b>	0.26692 .0255	0.37176 <b>.0015</b> <sup>f</sup>	
Hostility	0.37596 <b>.0013</b> 9	0.49189 <b>.0001</b> <sup>d</sup>	0.28851 <b>.0154</b>	0.41024 <b>.0004</b> e	
Visit 2 <sup>b</sup>					
Hostility	0.08957 .6089	0.39763 <b>.0180</b>	0.04764 .7858	0.08597 .6234	

<sup>a</sup>For each variable, the first row is the Pearson correlation coefficient and the second row is the P value for the test of the Pearson *r*. All *P* values were adjusted using the Hochberg method for multitesting of cytokines; bolding indicates statistical significance.

<sup>b</sup>Visit 1: n = 70; visit 2: n = 35.

 $^{c}P = .0228$ .  $^{d}P = .0038$ .  $^{e}P = .0152$ .  $^{f}P = .0464$ .  $^{g}P = .0432$ .

Abbreviation: PANSS = Positive and Negative Syndrome Scale.

Limitations of this study include the relatively small sample size. Also, we were unable to control for the effects of antipsychotic medications, which may modulate cytokine levels. Importantly, a higher proportion of Th17 cells and elevated plasma levels of IL-17 were described in drug-naive patients with a first episode of schizophrenia.<sup>3,4</sup> However, after 4 weeks of risperidone treatment, there was a trend toward decreased plasma levels of IL-17.<sup>3</sup>

Our findings of decreased levels of IL-4, IL-17, and IL12p70 are in agreement with previous studies.<sup>5,6</sup> When the levels of IFN- $\gamma$ and IL-2 are low, the production of IL-17 is suppressed.<sup>6</sup> Further, differentiation of naive T cells (To) to Th17 is mediated by IL-12 and IFN- $\gamma$ .<sup>6</sup> The low levels of IL-17 we observed could be influenced by the low levels of IL12p70, IL-2, or IFN- $\gamma$ .<sup>7</sup> Also, it is possible that the observed dysregulation of cytokines in veterans with chronic schizophrenia could be due to treatment with antipsychotics.

Importantly, levels of GRO, MCP-1, MDC, and sCD40 L were significantly elevated across all 3 visits. MCP-1 mediates the transendothelial migration of inflammatory cells across the blood-brain barrier and modulates local inflammatory response by inducing production of IL-4.<sup>8</sup> Increased levels of IL-4 may reduce the production of IL-17.<sup>7</sup>

In conclusion, our results indicate that there is a link between imbalance of cytokine levels and psychopathology in veterans with chronic schizophrenia treated with antipsychotics. When the psychopathology was analyzed by the separate items of the positive subscale of the PANSS, we found that serum levels of IL-4 may correlate with hostility and IL-1 $\beta$  may correlate with excitement. We suggest that such an approach should be explored further.

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