ORIGINAL RESEARCH

Long Term Assessment of Blood Indices and Immune Panel Profiling of Subjects Receiving Chiropractic Care: A Pilot Study

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ABSTRACT

Objective: A pilot study to evaluate the approach of monitoring immune status as a feasible means of assessing physiological health in longitudinal studies that seek to ascertain changes in patient health status under chiropractic care.

Methods: The study presents findings profiling blood indices and the immune status of 11 novice chiropractic subjects (7 males, 4 females) at baseline, with re-assessments at 3 months and 9 months duration. The New Zealand group was compared to several other non New Zealand healthy populations.

Results: Although significant changes occurred in blood indices and the immune profile in the present study group at 3 and 9 months re-assessments, the study values remained within the reference range for healthy adults. Significant positive correlations were seen for CD3% and CD8% at baseline and 3 months, suggesting a proportional increase or decrease over the range of values. It may be that the positive correlation is a reflection of the hosts' resistance to viral infection and destruction of virus infected cells. Negative correlations were observed for CD56% and CD20% and CD56% and CD3% at 3 months and 9 months. CD 56% was also negatively correlated with CD8% and CD4% at different sampling periods. The inverse relationship among these lymphocyte subpopulations may reflect a natural balancing or redistribution of the overall lymphocyte subpopulation as individual cell types respond to a variety of immune challenges. The subjects in this pilot study sustained physiological health from the standpoint of maintaining a panel of blood indices and lymphocyte markers within normal reference ranges throughout the 9 months period. Further, the subjects' blood indices and immune panels were comparable with population findings from other countries and ethnicities.

Conclusion: It is concluded that the approach of monitoring immune status is feasible as a means of assessing physiological health in longitudinal studies that seek to ascertain changes in patient health status.

Key Words: *Lymphocyte subpopulations, reference ranges, immune response, T lymphocytes, B lymphocytes, NK cells, CD4/CD8 ratio, chiropractic*

Introduction

This is the first of two papers investigating subjects, normal with respect to physiological and immunological indices, while receiving long term chiropractic care. This paper characterizes the immune panel of the 11 subjects completing the study, while the second paper reports on changes in the immune status in regard to the chiropractic care received.[1]

It is clear that a number of variables may affect immune status other than response to invading organisms. These variables are diverse and include such events as exercise, psychological stress, and several therapeutic modalities. As well, studies have shown that immune status is affected by age,[2,3] gender and race,[4,5] population,[6,7] mood,[8] smoking, quality of sample, and brand of monoclonal antibodies.[9] Others have found that age, ethnicity, smoking, and alcohol consumption[10] produce no statistically significant changes.

Murray et al.,[11] have shown a sharp rise in T suppressor/cytotoxic (CD8) and natural killer cells (CD56) as well as killer cell activity with moderate increases in T helper (CD4) and B cells (CD20) following strenuous exercise. These authors conclude that an increase in sympathetic activity might be responsible for the changes in immune cells as lymphoid tissue is richly

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innervated by sympathetic fibers. They state further, since T suppressor/cytotoxic cells have the greatest density of beta 2 adrenergic receptors, this could account for the greater rise in T suppressor/cytotoxic cells.

From the standpoint of sympathetic effects, a number of studies have looked at immune status in response to psychological stress. In that regard, while strenuous exercise enhances NK cell activity and elevates suppressor/cytotoxic cells, strenuous exercise under stress conditions, such as heat, lowers levels of NK killer cell activity.[12-14] As well, Manuck et al.,[15] showed a marked increase in CD8 lymphocytes in high reactors (measured though cardiovascular and catecholamine responses) to a modified Stroop color-word interference test and a mental arithmetic test compared to no change for low reactors. Both groups, however, had similar decrease in CD4 cells. Naliboff et al.,[16] using mental arithmetic as psychological stress with age as a factor divided females into a 21-41 and 65-85 age group. Younger subjects exhibited an increase in NK cell activity, numbers of circulating CD8 suppressor/cytotoxic T cells and NK lymphocytes, while older subjects showed no stress related NK activity. Older subjects, however, did experience increases in CD8 cells and NK lymphocytes, as did the younger subjects.

Brosschot et al.,[17] studied self-reported life stress to a brief mild interpersonal stressor (solve a 3-dimensional puzzle, not knowing it had no solution). Male teachers, 24-55 years old were assessed for CD8, CD4, NK, and B cells. Blood was drawn after a preliminary 30-minute rest session and 12 minutes after the test. The number of NK cells and CD8 cells appeared to increase, while the CD 4 level decreased.

Alternatively, Diego et al.,[18] showed an increase in NK cell number (CD56) and CD56+CD3- in HIV positive adolescents after receiving massage therapy twice weekly for 12 weeks. This group was matched with a similar group receiving relaxation therapy which showed no changes. The massage therapy group also showed an increased CD4/CD8 ratio and increase in CD4 cell number.

A similar study by Ironson et al.,[19] was conducted on 29 men, 20 HIV+ and 9 HIV-. A subset of 11 of the HIV+ men received a month of massage therapy and a month without therapy for comparison with the HIV- men who also received massage therapy for one month. Following the month of massage therapy, significant increases were observed in NK cell number, NK cell activity, soluble CD8, and CD8 cells. There were also significant decreases in cortisol and anxiety perception with an increase in relaxation, all of which were significantly correlated with increases in NK cell number.

Allen,[20] in a literature review, cited a number of earlier studies suggesting that the nervous system plays a role in the modulation of immune response. He concludes that there is a need for clinical trials to measure short and long terms effects of specific chiropractic care on immune status. He further concludes that these should include a broad range of parameters of immune competence accompanied by assessments of the clinical significance of the parameters.

Prior to consideration of clinical trials, the present pilot study seeks to assess the variability (range of normal) of the immune system while long-term care is being provided. This is viewed as important in light of the wide range of values for immune markers characteristically observed in healthy individuals. In agreement with Allen, this should be done to avoid possible error in assigning significance to immune responses that are actually within normal ranges.

Objective of the Present Study

This paper reports the physiological status (blood indices) and the immune profile of the subjects prior to and during a 9 months period of chiropractic care. A second objective of the present study was to draw initial comparisons of blood indices and standard lymphocyte markers with other non New Zealand populations.

Methods

Study Design and Measurements

The subjects were all receiving chiropractic care for the first time. Each patient was cared for according to standard clinic procedures at the New Zealand College of Chiropractic Health Center, in Auckland.[21] Human consent was obtained from each patient, and the study was reviewed by the Ethics Committee of the Auckland University of Technology. Student interns, supervised by duly registered professional chiropractic clinical staff members, provided chiropractic care.

Subjects were introduced to care through an initial chiropractic physical examination and placed on a plan of care unique to their initial chiropractic findings. Generally, throughout the study period, subjects visited the clinic 2-3 times per week during the first few weeks, increasing to monthly thereafter. During visits, patients were evaluated for the presence of vertebral subluxation [22] and received chiropractic adjustments when indicated. Subjects were formally reassessed at intervals of 3 and 9 months after the initial visit (baseline). At baseline, and at each formal reassessment, subjects were asked to complete a questionnaire designed to determine if they might be immune compromised.

Additionally, at baseline and at each formal reassessment, a qualified laboratory technician at Auckland Hospital drew peripheral blood samples. Laboratory tests included a complete blood count and immune panel of lymphocyte populations (lymphocyte markers) including: T cells (CD3, CD3%), T helper/inducer cells (CD4, CD4%), T suppressor/cytotoxic cells (CD8, CD8%), B cells (CD20, CD20%), NK cells (CD56,CD56%), and CD4/CD8 ratio. Complete blood count indices included hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), red cell count (RBC), red cell morphology, platelet count, white cell count (WBC), differential count for segmented neutrophils, basophils, eosinophils, monocytes, lymphocytes, and blood film for white blood cell morphology. Subjects' indices, including lymphocyte cell markers were determined by standard laboratory methods (flow cytometry for lymphocyte cell markers) and compared to established reference values used by Auckland Hospital.

Subjects

The pilot study, conducted between 4/November/99 and 13/December/00 (13 months), was initially comprised of 8 males and 8 females. Of the 16 subjects, data reported in this study is

Table 1. Present Study Demographic and Peripheral Blood Indices Compared to a Non-New Zealand Population

	Assessment Intervals				
	Baseline	**3 Months Duration**	**9 Months Duration**	**Reference Values**	**Comparison* Population**
Demographics					
Number of Subjects	(N=11)	(N=11)	(N=11)		(N=107)
Gender (M/F)	7/4				13/94
Age (years)**	22-52 (26)				15-75 (53)
Mean ± SD	29.5 ± 9.0				
Males (age)	22-52 (26)				
	29.6 ± 10.2				
Females (age)	22-36 (29.5)				
	29.3 ± 7.8				
Blood Index					
Hemoglobin	14.5 ± 13.1	14.4 ± 15.3	14.4 ± 14.1	11.5 - 16.5	13.0± 1.14
Platelet Count	270.0 ± 86.1	273.9 ± 64.1	273.3 ± 51.8	150 - 400	213.0 ± 48.0
WBC	6.6 ± 1.4	6.1 ± 1.0	6.2 ± 1.4	4.0 - 11.0	4.3 ± 7.0
Neutrophils	3.8 ± 1.1	3.2 ± 0.7	3.6 ± 1.0	2.0 - 7.5	2.1 ± 2.0
Total Lymphocytes	2.1 ± 0.6	2.0 ± 1.4	2.0 ± 0.7	1.5 - 4.0	1.8 ± 6.0

* Healthy adult population (Hove et al. Cytometry 1997 Jul 1; 28(3): 220-7). ** Age ranges are shown with the median in parentheses.

representative of 11 (7 males and 4 females) subjects that completed the duration of the study. The average age of the group was 29.5 ± 9.0 (range 22-52 yrs, median of 26). By gender, the age for males was 29.5 ± 10.2 (range 22-52 yrs, median 26 yrs) and 29.3 ± 7.8 (range 22-36, median 29.5 yrs) for the females. Not all subjects commenced the study at the same time, thus their respective baseline and reassessment values encompassed nine months each, but were staggered over the 13 months time frame.

Statistical Treatment of Data

Blood Indices and Lymphocyte Markers

Although the blood indices and immune lymphocyte markers were shown to be parametric, in addition to Pearson correlation coefficients and independent t-tests, the data for baseline and the two follow-up reassessments were compared by the Wilcoxon Signed Ranks test, which does not require an assumption of normal distribution, and tests for two-tailed significance among ranked pairs $P<0.05$.

To assess the clinical significance of changes in either the blood indices or lymphocyte markers mean values, the method of Cohen [23] was used to determine the effect size (mean 2 – mean 1/standard deviation of mean 1). By this method, 0.20 is considered a small clinical effect, 0.50 is considered moderate and 0.80 or greater is considered a large clinical effect.

Results

Population Demographics and Blood Indices

The eleven subjects (New Zealand) who were profiled according to gender, age and certain blood indices were compared to a healthy non-New Zealand population to ascertain the similarity of blood indices (Table 1). The comparison population

Table 2. Significant Differences in Blood Indices Across Time in Novice Chiropractic Patients

	Assessment Intervals			
	Baseline	**3 Months Duration**	**9 Months Duration**	**Reference Range**
Blood Index *				
Hemoglobin	14.8 ±1.41	14.3 ±1.56	14.4 ±1.41	11.5 -16.5
Baseline/3 Months		(p =0.00)**		
Baseline/9 Months			(p =0.00)	
Hematocrit	45.6 ±4.25	43.6 ±4.48	42.9 ±3.56	*36 - 47*
Baseline/9Months			(p =0.01)	
Mean Corpuscular Hemaglobin	29.6 ±1.40	29.1 ±1.44	29.7 ±1.15	*27 - 32*
3Months/9Months			(p =0.04)	
Red Cell Distribution Width	13.5 ±0.52	13.4 ±1.26	12.4 ±0.61	*11.5 -13.5*
Baseline/9Months			(p =0.00)	
3Months/9Months		(p =0.01)		
Basophils	10.0 ±3.30	8.0 ±4.03	6.0 ±1.43	*2 - 10*
Baseline/9Months			(p =0.01)	

* Hemoglobin (Hg) [g/100ml], Hematocrit (Hct) [cL/L], Mean Corpuscular Hemoglobin (Mcv) [Hg/RBC], Red Cell Distribution Width (RDW) [pulse/unit volume].

** All p values represent significant difference from Baseline using a two tailed repeated measures Student's T- test and Wilcoxon Signed Ranks test.

was distributed as 13 males and 94 females with an older age range of 15-75 yrs and a median of 53 yrs.

The New Zealand population revealed higher values for Hb, platelet count, WBC, total lymphocytes and percentage of neutrophils compared to the non-New Zealand population. However, both populations fell within normal reference ranges.

Complete Blood Count

Red Blood Cells

Significant differences in certain blood indices for subjects in the present pilot study are shown in Table 2. Hemoglobin values were significantly decreased at both the 3 month (14.3 ± 1.56, p = 0.00) and 9 month (14.4 ± 1.41, p = 0.00) reassessment periods when compared with the baseline value (14.8 ± 1.41). Hematocrit values also decreased in the group from baseline (45.6 ± 0.04) to 3 months (43.6 ± 0.04), becoming significant by 9 months duration of care (42.0 ± 0.03, p = 0.01). As well, mean corpuscular volume decreased from baseline to 3 months (29.6 ± 1.40, 29.1 ± 1.44) but reversed by 9 months of care elevating significantly to 29.7 ± 1.15, p = 0.04. Red cell distribution width remained constant from baseline to 3 months duration (13.5 ± 0.52, 13.4 ± 1.26, respectively) but dropped significantly lower than baseline and the 3 months reassessment value to 12.4 ± 0.61 at 9 months duration of care (p= 0.00 and 0.01, repectively). Although significant changes occurred in the group, all values remained within the reference ranges.

Table 3. Range of Absolute Values* and Percentages for Certain Lymphocyte Cell Markers Contrasted to Reference Range Values**

	Sample Interval		
	Baseline	**3 Months Duration**	**9 Months Duration**
Cell Marker			
CD3	620-2528	809-3158	839-2203
Median	1490	1582	1498
CD3%	57-82	57-88	57-81
Median	72	75	74
Reference Range	780-2600		
CD4	441-1327	454-1993	503-1387
Median	887	936	938
CD4%	25-53	28-53	39-57
Median	47	45	47
Reference Range	500-1650		
CD8	168-1474	209-1193	250-882
Median	446	512	478
CD8%	16-42	15-38	16-35
Median	22	25	23
Reference Range	210-1200		
CD20	84-474	125-414	155-370
Median	247	246	271
CD20%	7-18	7-17	9-16
Median	12	12	13
Reference Range	80-600		
CD56	22-393	20-454	76-317
Median	215	177	181
CD56%	1-26	1-32	3-12
Median	12	10	8
Reference Range	40-500		
CD4:CD8 Ratio	0.6-3.1	0.7-3.3	1.4-2.9
Median	2.4	1.8	2.0
Reference Range	1.5-3.0		

**Absolute values for subjects and reference ranges (Auckland Hospital) are expressed as cells/microliter. Percentages are a ratio of the cell type to the total lymphocyte cell count.

Table 4. Absolute and Percentage Values* for Certain Lymphocyte Cell Markers in Novice Chiropractic Patients over Nine Months of Care

	Assessment Intervals		
	Baseline	**3 Months Duration**	**9 Months Duration**
Cell Marker			
CD3	1513 ± 595	1482 ± 647	1514 ± 444
Reference Range	780-2600		
CD3 %	73.3 ± 8.7	73.8 ± 7.2	73.8 ± 7.15
CD4	943 ± 299	940 ± 397	938 ± 256
Reference Range	500 -1650		
CD4 %	46.2 ± 6.1	46.9 ± 6.1	47.5 ± 5.7
CD8	509 ± 293	485 ± 260	478.4 ± 169.1
Reference Range	210-1200		
CD8%	23.4 ± 7.7	24.9 ± 6.7	23.2 ± 5.0
CD20	267 ± 126	248 ± 97	271 ± 56.2
Reference Range	80-600		
CD20 %	12.3 ± 3.1	12.3 ± 2.4	12.7 ± 1.9
CD56	178 ± 85	181 ± 96.4	181 ± 68.7
Reference Range	40-500		
CD56%	10.1 ± 7.3	10.6 ± 8.8	8.4 ± 2.6
CD4:CD8 Ratio	2.1 ± 0.7	2.1 ± 0.7	2.1 ± 0.4
Reference Range	1.5-3.0		

*Absolute values for subjects and reference ranges (Auckland Hospital) for healthy adults are expressed as cells/microliter. Percentages are a ratio of the cell type to the total lymphocyte cell count. Reference ranges for % values were not available for Auckland Hospital.

White blood cells

In regard to white blood cells, the number of basophils in the study group decreased from 10.0 ± 0.03 at baseline to a 3 months reassessment value of 8.0 ± 0.04. The 9 months basophils value for the study group (6.0 ± 0.01, p = 0.01) also decreased significantly from baseline. White blood cell indices reported as decreasing or increasing significantly nevertheless remained within the normal reference ranges.

Immune Status

Prior to subjects' peripheral blood draw, a questionnaire was administered to assist in determining if there were indications of immune compromise (Figure 1). There were no reports of any negative event or other factor(s) that would indicate a state of immune compromise.

Lymphocyte Markers

Table 3 shows the range of lymphocyte cell markers in the study population in relationship to the Auckland Hospital Clinical Laboratory reference values. Reference values were only provided for absolute cell marker counts, as the laboratory did not report reference values for percentage of total lymphocytes.

Cell Markers were generally within their respective reference range for absolute numbers. In regard to specific cell markers, values that were outside of the reference range reflected either a single subject or two (not always the same individual) within the group. That is, the CD3 reflected a value range of 620-2528 (reference range 780 – 2600). The value of 620 reflected a single subject as the remaining 10 subjects were within the reference range.

Although considerable variation occurred between assessment intervals, the study group exhibited mean cell marker values that all fell within the reference range for absolute values established by Auckland Hospital (Table 4).

A comparison of reference ranges for absolute cell markers values and percentage values from different populations is shown in Table 5. This information was provided in the absence of cell marker percentage reference values from Auckland Hospital. Populations as diverse as Italian, Kuwaiti, Caucasian, African-American, Hispanic Ethiopian, and Dutch are represented.

Mean cell marker percentages in the present study fell within or were close to the reference ranges for the wide diversity of populations of healthy adults described in Tables 5 and 6. The one or two individual absolute CD values in the present study (Table 3) that appeared either high or low for CD56 and CD3 were also out of reference range for the diversity of healthy adults populations shown in Table 5. The low absolute value for CD3 in the present study (Table 3, baseline), while out of range by Auckland Hospital reference values, would have been within normal limits for the Italian population represented in Table 5. As well, the low value for CD20 relative to the New Zealand range would have been in range for the Ethiopian and Dutch populations (Table 5). These authors were unable to find CD20% reference values in the literature for comparative purposes.

The 11 subjects in the present study, taken as a group, are compared to other populations in Table 6. Using available data for non New Zealand study populations, the New Zealand group was relatively consistent with regard to being higher, lower, or about equal in regard to the respective lymphocyte subpopulations. That is, the present study group was approximately the same with regard to CD3, CD3% (Italian and Saudi), and CD56% (Saudi) study populations. In regard to other populations, higher values for the present study group were seen for CD4 compared to Saudi and Ethiopian, CD4% compared to Saudi and Iranian, CD20 compared to Ethiopian and Greek, CD20% compared to Italian, CD4/CD8 ratio compared to Saudi and Ethiopian. Lower values for the present study group were seen for CD8 compared to Saudi, Ethiopian, CD8% compared

Table 5. Reference Ranges for Absolute and Percent Values* for Certain Lymphocyte Cell Markers in Healthy Adults from Different Populations

	Range[A] (Present Study)[1]	Other Population Ranges[B-D]		
Cell Marker	A	B	C	D
CD3	780 -2600	605 -2460[2]	830 -2710[3]	
CD3%	-	60 - 87[2]	64 - 85[3]	54 - 88[5]
				40 - 80[6]
CD4	500 -1650	493 -1666[2]	450 -1650[3]	522 -1594[4]
		259 -1919[5]	237 -1660[6]	
CD4%	-	32 - 61[2]	34 - 54[3]	32 - 59[4]
				33 - 60[5]
				28 - 55[6]
CD8	210 -1200	224 -1112[2]	290 -1170[3]	135 -1047[4]
CD8%	-	14 - 43[2]	20 - 42[3]	15 - 36[4]
				9 - 39[5]
				11 - 43[6]
CD20	80 - 600	51 -419[7]	110 -670[7]	
CD20%	-			
CD56	40 - 500		60 -580[3]	
CD56%	-		4 - 22[3]	
CD4:CD8 Ratio	1.5 - 3.0		0.8 -4.5[5]	0.7 -3.11[4]
			0.7 -3.20[6]	

* Values standardized to cells/microliter, % = ratio of cell type to total lymphocyte count.

1. Reference values from Auckland Hospital, Auckland, New Zealand
2. Santagostino, et.al., Haematologica (Italian population) 1999; 84:499-504
3. Kabba et al., Kuwait University, (Kuwait population) kaaba@hsc.kuniv.edu.kw
4. UTMB Lab Survival Guide.(mixture of races) utmb.edu/lsg/labsurvival guide/hem/cd4%20.html.
5. Howard et al. Clinical Cytometry; 1996 26:231-232 (Caucasian, Afro-American, Hispanic populations pooled).
6. Howard et al. Clinical Cytometry; 1996 26:231-232 (Asian population).
7. Tsegaye, et al., Ethiopian and Dutch populations. Clinical and Diagnostic Laboratory Immunology, May 1999; 6(3): 410-414.

to Italian and Iranian, CD56 compared to Saudi and Ethiopian, CD56% compared to an Iranian population.

In all instances, however, the lymphocyte marker percentages were in similar proportions. That is, in the group represented in the present study as well as the other populations represented in Table 6, lymphocyte subsets as a percent of total lymphocytes generally reflect similar proportions for CD3 (70.9% - 73.7%), CD4 (39.4% - 44.4%), CD8 (23.4% - 28.4%), with CD20 (8.5% - 12.4%), and CD56 (11.5% - 15.8%) being similarly matched. The ration for CD4/CD8 did vary from population to population ranging from the present study group of 2.1 to a low of 1.2 for the Ethiopian population.

Though the present pilot study was limited to 11 subjects, a gender comparison (Table 7) revealed that the 4 females had higher values than males over the three assessment intervals for CD3, CD3%, CD4, CD4%, CD8, and CD8%. Values for CD20, CD20%, and CD4/CD8 ratios were essentially the same for the 7 males and 4 females. Males exhibited higher values for CD56 absolute and CD56% at all 3 assessment periods. There were no significant differences, however, with the exception of an increase in the CD4% between baseline and 9 months assessment among the females (48.3 ± 3.6 to 50.3 ± 4.3, $P = 0.016$).

As well, when males and females in the present study group were compared to males and females in Iranian and Ethiopian populations (Table 7), present study males and females expressed higher CD4, CD20 than males and females in the Ethiopian population, but lower CD8 and CD56 levels. Compared to the Iranian study population genders, the present study group males and females expressed higher CD4% and CD4/CD8 ratios, but lower CD8% levels.

Table 6. Comparative Values* for Certain Lymphocyte Cell Markers in Adults in the Present Study and Other Healthy Populations

	Present Study [A]	Other Populations [B,C]	
Cell Marker	A	B	C
CD3	1539.1 ± 533.6	1532.9 ± 463.4[1]	1564 ± 485.0[2]
CD3 %	70.9 ± 8.0	73.7 ± 6.7[1]	72.9 ± 7.7[2]
CD4	937.3 ± 263.3	775.0 ± 225.0[3]	869.0 ± 310.0[2]
CD4 %	44.4 ± 7.5	42.1 ± 6.9[4]	39.4 ± 7.9[2]
CD8	539.4 ± 351.4	615.0 ± 278.0[1]	747.0 ± 333.0[3]
CD8%	23.4 ± 7.7	27.6 ± 7.5[1]	28.4 ± 8.5[4]
CD20	267.1 ± 118.5	191.0 ± 94.0.0[5]	230 ± 130.0[3]
CD20 %	12.4 ± 3.2	8.5 ± 3.0[6]	
CD56	214. 8 ± 101.2	262.0 ± 278.0[2]	250.0 ± 137.0[3]
CD56%	11.5 ± 6.7	11.7 ± 5.9[2]	15.8 ± 6.9[4]
CD4/CD8 Ratio	2.1 ± 0.7	1.6 ± 0.7[2]	1.2 ± 0.5[3]

*Values are expressed as cells/microliter. Percentages are a ratio of the cell type to the total lymphocyte count. Present Study (New Zealand population).

[1] Santagostino, et al., Italian population. Haematologica 1999; 84:499-504

[2] Abdulla, et al., Saudi population. Clinical and Diagnostic Laboratory Immunology, March 2002, 9 (2): 279-281.

[3] Tsegaye, et al., Ethiopian population. Clinical and Diagnostic Laboratory Immunology, May 1999; 6(3): 410-414.

[4] Shahghasempour et al., Iranian population. Pearl.sums.ac.ir/aim/0142.html

[5] Androtti, et al., Greek population. Haema 2003; 6(1): 54-60.

[6] Ferrari, et al., Italian population. Oncology Reports 2002; 9: 107-1113.

CD Marker Correlations

Table 8 shows Pearson correlation coefficients (r) that were significant at $p<0.05$ for lymphocyte markers as percents of the total lymphocyte count. As can be seen, positive and/or negative correlations were expressed between lymphocyte subpopulations both at the same and different assessment periods. That is, CD3% at baseline was positively correlated to CD8% at baseline ($r = 0.624$, $p = .040$) as well as both showing positive correlation at the 3 months assessment period ($r = 0.679$, $p = .022$). Moreover, CD3% at baseline was also positively correlated to CD8% at the 3months assessment period ($r = 0.630$, $p = .038$) and CD3% at the 3 months assessment period showed positive correlation to CD8% at the 9 months assessment period ($r = 0.654$, $p = .029$). In a complementary fashion, CD8% at baseline was positively correlated to CD3% at the 3 months assessment period ($r = 0.662$, $p = 0.027$) and CD8% at the 3 months assessment period showed a positive correlation with CD3% at the 9 months assessment period ($r = 0.614$, $p = 0.044$).

CD3% at baseline also showed a negative correlation to CD56% at the 3 months assessment period ($r = 0.864$, $p = .001$) while CD3% and CD56% were also negatively correlated at the 3 months assessment period ($r = -0.857$, $p = .001$) and the 9 months assessment period ($r = -0.737$, $p = .010$). CD 56% at baseline was also negatively correlated to CD8% at the 3 months assessment period($r = -0.647$, $p = .043$). CD4% at baseline showed a negative correlation to CD56% at the 3 months assessment period, while CD20 showed negative correlation with CD56% at baseline ($r = -0.672$, $p = .033$), 3 months assessment period ($r = -0.651$, $p = .030$), and at the 9 months assessment period ($r = -0.778$, $p = .039$).

Discussion

Blood Indices

Comparing blood indices, including lymphocyte subpopulations, over the course of the present study, served as one monitor of the physiological health of the eleven subjects. While the Auckland Hospital laboratory provided reference values for healthy adults, it is well known that values vary among populations for many indices. Thus, to characterize the study population globally, several comparisons with other populations were also made.

Relative to the complete blood count, the present population, when compared with another population of non-New Zealanders revealed higher values for hemoglobin, platelet count, white blood cell count, and total lymphocytes. These values were all within reference levels for healthy adults and do not have clinical significance. Rather, it is more likely that diet, and environmental factors may account for the geographical differences.

In regard to the indices of the complete blood count in the present study, the eleven subjects exhibited a significant decrease in hemoglobin between baseline, the 3 months and the 9 months re-assessment periods. Although the decrease was nu-

merically small, dropping by a mean percent of 3.3, it was consistent as the standard deviations were also quite small reflecting consistency among the subjects. However, the hemoglobin levels at baseline and 3 and 9 months were well within the reference range for healthy adults, and the clinical effects (effect size) were small (0.35, and 0.28).

As well, the hematocrit, mean corpuscular hemoglobin, red cell distribution width, and percentage of basophils also decreased at 3 months compared to baseline. None of these decreases were statistically significant and the clinical effect remained small with the exception of the decrease in basophils that expressed a small to moderate clinical effect, being 0.47, 0.35, 0.19 and 0.60 respectively. All values, however, remained within the reference values for healthy adults.

The Hematocrit, red cell distribution width, and number of basophils continued to decrease at the 9 months reassessment period being significantly lower than baseline values, but still within reference levels for healthy adults. The clinical effect, however, was large for red cell distribution width (2.11, where 0.80 is a large clinical effect), and decrease in basophils (1.21), and moderate for Hct (0.60). The 9 months level for mean corpuscular hemoglobin increased significantly from the 3 months level to slightly higher than the baseline value, expressing a small clinical effect (0.41) while red cell distribution width was significantly lower than the 3 month value resulting in a large clinical effect (0.80). The increase in mean corpuscular hemoglobin and decrease in red cell distribution width, however, were still within the reference values for healthy adults.

In evaluating the overall significance of these changes, clinical effect as well as statistical differences were considered. In that regard, the 3 month decrease in hemoglobin would not be considered clinically significant by virtue of the small clinical effect and the fact that it was still within normal limits, even though it was consistently found among the eleven subjects. The same would apply to the small decreases in hematocrit, red cell distribution width and number of basophils as these were not statistically significant and having only a small clinical effect. The moderate clinical effect associated with the decrease in basophils, compared to its borderline high count at baseline, suggests that the decrease might have been related to the season during which the values were obtained. The samples were collected in mid-November, which would be late spring/early summer in New Zealand. Since Basophils have been associated with allergic reactions [24] it may be that the population was experiencing normal seasonal adaptation to heavy pollen drifts. This is born out by the continued significant decrease at 9 months compared to the baseline value.

The significant decreases in hematocrit and red cell distribution width at 9 months compared to baseline would likely be dismissed as being physiologically insignificant. The large clinical effects are likely due to the consistency of the population's decrease (small standard deviation). While a low hematocrit is associated with anemia, and red cell distribution width is low

Table 7. Gender Differences for Lymphocyte Cell Markers and Comparison to Available Cell Marker Data from other Populations

	Cell Markers					
	CD3	**CD3%**	**CD4**	**CD4%**	**CD8**	**CD8%**
Males						
Baseline	1398 ± 623	70.1 ± 9.2	878 ± 332	45.0 ± 7.2	459 ± 290	22.2 ± 6.2
3 months	1325 ± 397	70.6 ± 9.2	852 ± 228	45.7 ± 6.6	414 ± 182	22.0 ± 6.8
9 months	1459 ± 474	71.6 ± 7.9	885 ± 266	45.9 ± 6.1	452 ± 216	22.4 ± 6.5
Females						
Baseline	1713 ± 564	76.2 ± 5.3	1057 ± 225	48.3 ± 3.6*	596 ± 319	25.5 ± 7.3
3 months	1757 ± 962	78.3 ± 5.6	1096 ± 609	49.0 ± 5.2	609 ± 358	26.8 ± 7.6
9 months	1610 ± 434	77.8 ± 3.6	1031 ± 244	50.3 ± 4.3	523 ± 211	24.8 ± 5.9
Comparison Populations						
Males	1564 ± 485[2]	69.9 ± 6.3[1]	753 ± 227[2]	40.4 ± 6.9[1]	777 ± 362[2]	28.4 ± 8.3[1]
Females	1539 ± 423[2]	71.5 ± 6.3[1]	816 ± 218[2]	44.2 ± 6.4[1]	692 ± 269[2]	28.4 ± 8.9[1]
	CD20	**CD20%**	**CD56**	**CD56%**	**CD4/CD8 Ratio**	
Males						
Baseline	267 ± 145	12.5 ± 2.9	192 ± 85	11.5 ± 8.0	2.2 ± 0.7	
3 months	236 ± 83	12.5 ± 2.2	212 ± 127	12.9 ± 9.8	2.3 ± 0.7	
9 months	270 ± 70	12.8 ± 0.9	184 ± 77	8.8 ± 3.0	2.1 ± 0.3	
Females						
Baseline	266 ± 106	12.0 ± 4.0	154 ± 91	7.8 ± 6.2	2.0 ± 0.7	
3 months	269 ± 130	12.2 ± 3.2	128 ± 83	6.8 ± 5.9	2.0 ± 0.7	
9 months	272 ± 22	12.6 ± 2.9	175 ± 62	7.7 ± 1.8	2.1 ± 0.7	
Comparison Populations						
Males	186 ± 96[2]	9.0 ± 3.5[2]	277 ± 143[2]	11.7 ± 5.9[3]	1.6 ± 0.5[1]	
Females	203 ± 91[2]	12.8 ± 5.7[2]	258 ± 153[2]	14.7 ± 5.9[1]	1.7 ± 0.7[1]	

1. Shahghasempour et al., Iranian population. http://Pearl.sums.ac.ir/aim/0142/shahghasem0142.html.

2. Tsegaye et al., Ethiopian population. Clinical and Diagnositc Laboratory Immunology. May 1999; 6(3): 410-414.

3. Al Qouzi, et al., Saudi population. Clinical and Diagnostic Laboratory Immunology. March 2002; 9(2): 279-281.

* CD4% baseline interval vs 9 months, two tailed repeated sample (t-test), p = 0.016.

Table 8. Correlation Between CD Cell Markers over Three Assessment Periods in Novice Chiropractic Patients

	Assessment Interval			
	Baseline	3 Months Duration	9 Months Duration	Correlation Coefficient(r)
Positive Correlations				r *P**
1.	CD3% -CD8%			0.624(.040)
2.		CD3% - CD8%		0.679(.022)
3.	CD3%	CD8%		0.630(.038)
4.	CD8%	CD3%		0.662(.027)
5.		CD3%	CD8%	0.654(.029)
6.		CD8%	CD3%	0.614(.044)
Negative Correlations				
1.	CD3%	CD56%		-0.864(.001)
2.		CD3% -CD56%		-0.857(.001)
3.			CD3% - CD56%	-0.737(.010)
4.	CD56%	CD8%		-0.647(.043)
5.	CD4%	CD56%		-0.632(.037)
6.	CD20% - CD56%			-0.672(.033)
7.		CD20% -CD56%		-0.651(.030)
8.			CD20% -CD8%	-0.778(.039)

* Numbers in parentheses indicate p values for significant r coefficients.

in cases of macro or microcytic anemia, there were no other indications among the red blood cell indices that suggested anemia, nor were any abnormal morphology reports associated with the subjects. This is further supported by the fact that the hematocrit is normally three times the value of hemoglobin, which was the finding in this study. It is most likely, therefore, that the variations occurring were consistent within the population, thus rendering a large clinical effect, but not physiologically significant as borne out by the other blood indices.

Characterization of Immune Profile

In conditions that compromise the immune system it is clinically useful and important to assess patient progress or recovery. This is often done by monitoring changes in lymphocyte sub-populations such as T cells (CD3, CD3%), T helper/inducer cells (CD4, CD4%), T suppressor/cytotoxic cells (CD8, CD8%), B cells (CD20, CD20%), NK cells (CD56,CD56%), and the CD4/CD8 ratio.

That is, the establishment of reference values for healthy (non-diseased) adults is useful to signal potential problems with immune response if aberrant values appear. In the absence of aberrant values, attempts to describe or define a "healthy" immune system become more complex, as greater or lesser values do not necessarily reflect greater or lesser degrees of being "healthy." [25] This study points out the immense variation that can occur among circulating immune cells both within and between different adult, physiologically healthy populations.

This is in part explained by considering the body's natural and normal immune response to a number of variables such as exercise, psychological stress, allergens, seasonal shifts in viral populations (i.e., "flu") and other common infections. Since people encounter any number of these "stressors," the immune system responds accordingly. Changes occurring in the cells described above can vary greatly in a healthy human. Thus, on any given sampling, based on the "stressors" that have been encountered, a person could express a wide range for any of cell type involved in the normal immune response. For example, CD4 cells lead the attack on invading organisms and hence might be high at a time of initial infection, but drop off when CD8 suppressor cells have ended the immune response, consequently rising in number. Thus, in the absence of sure knowledge regarding a person's status relative to everyday stressors, presuming that a higher or lower number of these cells indicates a healthy or unhealthy immune system could likely be incorrect since these cells could readily fluctuate during the course of natural adaptation to "stressors." Published reference values provided by Auckland Hospital in New Zealand indicate that within normal limits CD3 and CD4 cells fluctuate by as much as 2.4 fold (244%), CD8 by 4.7 fold (471%), CD20 by 6.5 fold (650%), and CD56 by as much as 11.5 fold (1150%). Moreover, within that spectrum of fluctuation one individual may express a healthy immune system with lymphocyte marker levels far lower or higher than another individual with an equally healthy immune system. These authors consider it to be important that these concepts be considered when interpreting changes in immune profiles in healthy individuals.

This study has provided evidence that the wide range of lymphocyte markers found in the eleven subjects is typical of a population of healthy subjects. This is substantiated by a number of observations and comparisons. Assessments of the population in this study (for baseline, 3 and 9 months reassessment periods) fall within the reference range for physiologically healthy (non-diseased) adults.

In the present study, gender differences were apparent. This is also seen in other non-New Zealand study populations. Comparisons with these populations substantiate variations among humans. There are, however, some commonalities. For example, females and males in the New Zealand study group reported higher absolute CD4 and CD20 compared to a study population of Ethiopians. Moreover, in both populations, females had higher levels than males. As well, the proportion of CD markers is the same in all of the populations compared to the New

Zealand study group (Italian, pooled Caucasian, African-American, Hispanic, Kuwaiti, Iranian, Italian, Asian, Ethiopian and Dutch). That is, proportions descended from CD3 (highest), to CD4, CD8, with CD20 and CD56 being in the lowest proportion.

Thus, while considerable variations occur within and between populations, the relative proportions between the CD markers seem common. As well, gender differences may or may not be consistent from race to race or population to population within the same race. The range of "healthy" appears broadly described indicating that the consistency of reference values for non-diseased adults within any given population is the most definitive index of the health of that population.

Immune Response

It is clear that different health care approaches and modalities such as exercise, and "relaxation" approaches such as massage therapy appear to elicit an immune response. Studies described in the Introduction point out that CD4 and CD8 cells, and in some instances CD56 and CD20 cells increase in different proportions based on the study reported, while essentially the reverse is reported under psychological stress.

The majority of the studies mentioned above were conducted on adults presumed to be healthy (disease free), usually investigating an immediate or short-term immune response following a particular event. This investigation was consistent with that concept, but varied in regard to intent. The present study was designed to provide characterization of the immune status within a group, not as an immediate or short-term response to any particular event, but rather as a longitudinal profile over a period of time during which chiropractic care was a component of the group's lifestyle. In that regard, it has been shown that within the group followed over 9 months, the immune profile remained essentially constant and well within reference levels for healthy adults in New Zealand and to a large extent in comparison with a diversity of other non-New Zealand populations.

Inter-relationships between Lymphocyte subpopulations

Significant Positive Correlations

This study has also provided information regarding the interrelationship of immune cell levels that could be useful in future studies that attempt to understand the interactions of the lymphocyte markers in health and disease. In this regard, significant positive and negative correlations were shown for several of the lymphocyte sub-populations over the 9 months duration. All correlations were determined for absolute values expressed as a percent of the total lymphocyte count, as that expression is more stable or less noisy due to the wide ranges incurred with absolute values.

The positive correlation pattern between CD3% and CD8% suggests that these two cell types are strongly linked. There appears to be no change in the relationship between baseline and 3 months, as 3 months levels are also significantly correlated with their respective counterparts at baseline. However, the two are not directly correlated at 9 months, but find significant correlation with their respective counterparts at the 3 months reassessment period. The lack of significant correlation at the 9 month interval may be explained by the fact that among the 11 subjects, 7 did not exhibit changes of the same magnitude at 9 months compared to the levels at 3 months for CD3% whereas the CD8% values remained essentially the same. Thus, an overall shift downward in the group relative to CD3% occurred, though not to the point of statistical significance from the 3 months level, but sufficient to lose its significant correlation with CD8%.

Within this subject group, since the levels of each cell marker remained within reference limits, it is not likely that the changes have clinical significance, but do suggest a direct link between the levels of production between the two cell markers. CD3 and CD8 cells are both important in host resistance to viral infection and killing of cells infected with virus. It may be that the two cell types act synergistically, increasing or decreasing proportionally, even if they are distinct quantitatively.

Significant Negative Correlations

Significant negative correlations seen for CD20% and CD56%, CD3% and CD 20% were observed. While this pilot study cannot provide any specific substantiation, it may be that the inverse relationships between these cell markers reflect a natural balancing or redistribution of the overall lymphocyte subpopulation as individual cell types respond to a variety of immune challenges.

Although absolute values for any given subject varied considerably within the group, percent changes were generally fairly close (0% - 25%). Two subjects, however, expressed unusual spikes in activity at the 3 months sampling period compared to baseline. One subject exhibited spikes of 80% (CD4) returning to 25%, 79% (CD8) returning to 25%, 83% (CD20) returning to 8 % and 77% (CD3) returning to 23% at the 9 months sampling period. In this particular case, the blood draw was taken shortly before the subject commented on taking two weeks leave for not "feeling, quite right," even though there had been no report of any negative events on the subject's screening questionnaire. A second subject experienced a dramatic 800% increase in CD56 cell count. Interestingly, this subject had a low normal of 50 cells/microliter CD56 count at baseline where 40-500 represented the reference range. The blood draw for the 3 months sample followed shortly after this subject received an adjustment. Because of earlier reports of lymphocyte responses following an adjustment, care was generally taken to adjust the patient after their blood draw to avoid short-term effects. In this instant the results may be indicative of the individual's response to the adjustment, as has been previously reported.[26] The subject had returned to a cell count of 116/microliter at the 9 months sampling period. All other cell markers were stable.

The findings of this pilot study must be cautiously interpreted as the size of the subject population lacked statistical power, increasing the probability of a type I or type II error. As well, there was no complementary control group, although each subject could be viewed as their own control in this study design. In consideration of these factors, it is suggested that there is, nevertheless, substantial evidence that the population remained physiologically healthy over the 9 months duration of the study.

Summary and Conclusions

A pilot study was completed at the New Zealand College of Chiropractic in Auckland, New Zealand in 2000. The study followed 11 novice chiropractic subjects (7 males, 4 females) as

outpatients over the course of 9 months. A summary of the objectives of the study and conclusions are as follows:

To monitor physiological status through blood indices and the immune competence of subjects prior to and during a 9 months period while receiving chiropractic care.

To draw initial comparisons of blood indices and standard lymphocyte markers with other non-New Zealand populations.

Blood Indices Summary: The initial physiological status of the population was assessed through a complete blood count including Hb, Hct, MCH, MCV, RDW, RBC count, Platelet count, WBC count, differential count for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and blood smear for morphology. Values for all of these parameters were within reference range for healthy adults at baseline and the two subsequent reassessment periods. Though likely to be physiologically and clinically insignificant (normal reference ranges and small effect sizes), a statistically significant decrease compared to baseline was seen for Hb at the 3 and 9 months reassessments, MCV at the 9 months reassessment, and number of basophils at the 9 months reassessment. RDW was also statistically decreased from baseline as well as the 3 months reassessment period, while Hct was increased at 9 months compared to the 3 months reassessment period. The decrease in basophils is attributed to possible decrease in allergic response to seasonal pollen counts as the baseline sample was collected in mid to late Spring, the 3 months value in Summer and the 9 months value in late Fall.

The present population, when compared with another population of non-New Zealanders revealed higher values for hemoglobin, platelet count, white blood cell count, and total lymphocytes. The values were all within reference levels and do not likely have any physiological significance. Rather, it is more likely that diet, and environmental factors account for the geographic differences as will be pointed out in the following section.

Conclusion(s): It was concluded by history and baseline blood indices that the subjects represented a healthy population exhibiting no clinically significant physiological changes detectable through the complete blood count.

Immune Status Summary: Specific immune cell markers (well established through clinical studies as representative indicators of immune competence) were monitored. These included T cells (CD3, CD3%), T helper/inducer cells (CD4, CD4%), T suppressor/cytotoxic cells (CD8, CD8%), B cells (CD20, CD20%), NK cells (CD56,CD56%), and CD4/CD8 ratio. Values for these parameters were monitored at baseline, 3 months and 9months after commencing chiropractic care. Findings indicated that:

Although fluctuations occurred, the study group of 11 subjects all remained within the reference range established by Auckland Hospital in New Zealand for each of the 3 sampling periods for all immune cell markers studied. Comparisons were made with other population studies from Ethiopia, Kuwait, India, Iran, Italy, Asia, Caucasian-American, Afro-American, Hispanic American pooled, Saudi, and Greece. While ranges and population means varied from population to population, the present study subjects were within reference ranges for all populations. Gender trends did vary by population with some common elements shared by all, but no uniform pattern was discerned. In the New Zealand study group females had higher CD3, CD3%, CD4, CD4%, CD8 and CD8% levels. Males and females reflected similar levels for CD20, CD20%, but were higher for CD56 and CD56% among the males. CD4/CD8 ratios were the same for females and males in the present study. It was further observed that in all populations studied the proportions of lymphocyte subsets as a percent of total lymphocytes were distributed as CD3 (70.9% - 73.7%), CD4 (39.4% - 44.4%), CD8 (23.4% - 28.4%), with CD20 (8.5% - 12.4%), and CD56 (11.5% - 15.8%) showing a similar range of percentages. CD4/CD8 ratios, however, did vary from population to population ranging from the present study group of 2.1 to a low of 1.2 for the Ethiopian population.

Significant positive and negative correlations between lymphocyte cell types revealed that CD3% and CD8% were positively correlated at baseline and 3months, suggesting a direct proportional production over the range of values found within the study group. These markers were also correlated indirectly from one sample period to the next, suggesting loss of level primarily with regard to CD3% at baseline. However, at all sampling periods the same positive correlation trend was observed. It may be that the positive correlation is tied to their immune functions of providing host resistance to viral infection and destruction of virus infected cells.

Negative correlations were found for CD56% and CD20% across all sample periods. As well, CD56% was negatively correlated with CD3% at 3 months and 9 months, but reciprocally correlated at baseline and 3 months. This appeared to be due to a reduction of CD3% at baseline and a lowering of CD56% at 3 months. CD56% was also negatively correlated to CD8% and CD4% at different sampling periods. It may be that the inverse relationships between CD56%, CD3%, CD4%, CD8% and CD20% reflect a natural balancing or redistribution of the overall lymphocyte subpopulation as individual cell types respond to a variety of immune challenges.

Conclusion(s): It is concluded that long term monitoring of blood indices and immune status is feasible, and suggested, for any study considering health care as an intervention or variable. Further, it is concluded that the present study group maintained a healthy immune profile throughout the duration of the study. This is based on no variations outside reference values for healthy New Zealand adults, an the consistency of the immune profile across time within the study group. Significant positive and negative correlations between cell markers signify normal interactions, likely associated with balancing the extent of response among cell types to a variety of immune challenges.

This pilot study has also provided some preliminary information regarding blood indices and the characterization of an immune profile that may be useful for comparisons to other non-New Zealand population studies regarding immune status. Limited numbers of subjects, however, preclude definitive conclusions. Larger studies that investigate the parameters presented herein will be necessary to verify the conclusions presented in this study.

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