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Irregularities in red cell distribution width and lymphocyte concentration in individuals with Chronic Fatigue Syndrome

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Abstract

Background: Chronic Fatigue Syndrome is a complex condition with no clear aetiology. There are inconsistent reports of abnormal red cell indices and variations in some immune cell subsets in CFS patients. **Aim:** Toexaminehaemato logical and immunological indices and the frequency of irregularities. **Methods:** Full blood counts and immune cell subsets of 24 Chronic Fatigue Syndrome individuals and 18 healthy, sedentary controls were analysed by cell sorter and flow cytometer. The frequencies of abnormal blood count and immunological indices, and health conditions in both groups were also recorded. **Results:** Total lymphocyte concentration was below-normal in 33.3% of CFS participants, with mean values significantly lower than the non-CFS group. CFS participants also had a lower $CD3^{+}CD4^{+}$ lymphocyte count ($p < 0.05$). Compared to non-CFS, the CFS group had significantly higher frequencies of above-normal red cell distribution width (RDW), above-normal erythrocyte mean cell volume (MCV), previous/current iron deficiency, cardiac symptoms, recurring sore throats and headaches, poor sleep, joint and back pain. **Conclusion:** Significantly higher frequencies of abnormal RDW, MCV and lymphocyte concentrations in CFS participants may be associated with iron deficiency and immune cell dysfunction.

Keywords:chronic fatigue; haematology; immune dysfunction; iron deficiency

1.0 Introduction

Chronic Fatigue Syndrome (CFS) or Myalgic Encephalomyelitis (ME), a complex disorder with no clear aetiology, affects the autonomic, immune, neuroendocrine and musculoskeletal systems(Loblay et al., 2002).Proposed mechanisms for CFS include: altered central nervous system function resulting from an abnormal immune reaction to an infectious agent; neuroendocrine disturbances and altered hypothalamic-pituitary axis function; and cognitive disturbances due to infection, severe stress or other stimuli (Lorusso et al., 2009).

There has been some evidence of abnormalred blood cell indices such as blood volume(Streeten and Bell, 1998), erythrocyte morphology(Mukherjee et al., 1987; Simpson 1997; Simpson and Herbison, 1997), and of normochromic normocytic anaemia (Winkler et al., 2004)in CFS patients but such findings are inconsistent across studies(Brenu et al., 2010). It has been suggested that abnormal erythrocyte shape and cell volume may be associated with altered micro-circulationand anaemia, and therefore implicated in the fatigue that CFS patients experience (Simpson and O'Neill, 2001). Altered erythrocyte deformability and rheology have implications for blood flow and volume, and oxygen, lactate concentration and potassium exchange (Mukherjee et al., 1987; Simpson and Herbison, 1997; Richards et al., 2007).Changes in erythrocyte shape, cell membrane and contents have also been identified in patients with multiple sclerosis and rheumatoid arthritis (Richards et al., 2007) but the extent of such findings in CFS is unclear.

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There is more consistent evidence of immune system dysfunction in CFS but the exact nature and degree of dysfunction remains ambiguous(Loblay et al, 2002; Bansal et al., 2011). It is uncertain whether immunological irregularities are a cause or product of CFS (Bansal et al., 2011). Significant differences in the concentrations of some leukocyte subsetsand in the activation of sub-populations oflymphocytes have been documented (Brenu et al., 2010; Peakman et al., 1997; Lorusso et al., 2009; Brenu et al., 2012) but other studies found no differencein lymphocyte sub-population counts between CFS and non-CFS individuals (Swannink et al., 1996; Robertson et al., 2005).Increased inflammatory markers and pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF) α have been reported in many studies, suggesting that low-grade systemic inflammation may be a characteristic of CFS (Lorusso et al., 2009).To investigate the extent of immunological and haematological irregularities in CFS, and the clinical significance thereof, we compared resting full blood counts and leukocyte sub-populations (absolute cell counts and percentages) inCFS and non-CFS individuals.

2.0 Methods

2.1 Participants

This current study presents the baseline data of a wider randomized controlled pilot trial where 24 CFS participants were recruited to investigate the effects of different exercise modalities on immune and haematological indices (Australia and New Zealand Clinical Trials Registry ACTRN12612001241820). The inclusion criteria for the CFS participants were a diagnosis of CFS from the participant's medical practitioner (according to the Centres for Disease Control criteria, Fukuda et al., 1994); an age between 16 to 80 years; having no medically-diagnosed cardiorespiratory, endocrine, metabolic condition or current musculoskeletal injury requiring the use of steroidal anti-inflammatory medications; the ability to communicate in English and to give informed consent. In addition, eighteen healthy, sedentary non-CFS participants were recruited as a control group for blood tests and immunological assays. All participants were recruited from the local community and university staff through advertisements at the Southern Cross University campus and Health Clinic, local medical clinics and hospitals, local newspapers, television and radio media. Southern Cross University Research Ethics Committee approved all study procedures (HREC ECN-13-066).

Allparticipants completed a full medical and health history questionnaire that included family history of chronic conditions, current medical conditions and symptoms, current medications and supplements, levels of physical activity and general health questions. The haematology and immunology outcome measures were respectively assessed with a full blood cell count and flow cytometric analysis of leukocyte subsets.

2.2 Full blood counts and flow cytometric analyses

All blood samples were coded at the collection site count (Northern Pathology, Lismore, Australia). Participants were advised not to exercise for 24 h prior and to avoid diurnal fluctuations, blood draws were completed between the hours of 08:00 and 10:00 am. Five mL of venous blood was drawn in an EDTA tube (BD Biosciences, Sydney, Australia) to provide samples for a full blood count(Northern Pathology, Lismore, Australia). A further five mL was collected in an EDTA tube (BD Biosciences, Sydney, Australia) for flow cytometric assays of $CD3^+CD4^+$ (T helper), $CD3^+CD8^+$ (cytotoxic effector) lymphocytes, $CD45^+$ (HLA marker), $CD3^-CD19^+$ (B cells) and $CD3^-CD56^+CD16^+$ (Natural Killer) cells. The absolute cell counts (per 10,000 events) and cell counts expressed as a percentage of total lymphocytes was measured by flow cytometer (FACSCantoII, BD Biosciences, Australia) using the BD Multitest 6-colour direct immune fluorescence TBNK reagent and BD Trucount tubes (BD Biosciences, Australia), and FACSCanto2 .1 software (BD Biosciences, Australia). Data was decoded and grouped for statistical analysis.

2.3 Statistical analyses

Data were analysed using IBM SPSS© Version 21 and are presented as the mean ± standard deviation. All data were examined for skewness and kurtosis to confirm normal distribution. Baseline means were compared using an Independent T-test. Percentages of the entire cohort (n=42), CFS (n=24) and non-CFS (n=18)groups were calculated accordingly. The frequencies of reported FBC values and medical conditions in both CFS and non-CFS were examined using Pearson's Chi-squared analysis to determine significant differences between groups. Significance was set at $p < 0.050$ for all statistical analyses.

3.0 Results

Data for both groups were normally distributed. There were no significant differences between groups with respect to age, gender, height and weight. The mean ages of the CFS and non-CFS groups were 50.9 ± 10 yr and 50.6± 10 yr respectively. The female-to-male ratios for CFS and non-CFS were17:7 and 13:5 respectively.The CFS group had a mean time since diagnosis of 2.9 ± 2.7 yr. The reported health conditions in both groups(Table 1) showed the CFS group had significantly greater frequencies of cardiac symptoms (e.g. heart murmur, palpitations, postural orthostatic tachycardia syndrome [POTS], arrhythmias) ($p = 0.004$); dizziness or fainting ($p = 0.011$); respiratory symptoms ($p = 0.031$); dyspnea, ($p = 0.026$); recurring sore throats ($p = 0.002$);recurring headaches ($p = 0.004$); poor sleep ($p = 0.016$); joint pain (e.g. inflamed joints, hip pain, knee pain) ($p < 0.001$); back pain (e.g. lumbar, thoracic, cervical) ($p = 0.001$).A previous or current medical diagnosis of iron deficiency/anaemia occurred in 45.8% of the CFS group, significantly greater ($p = 0.001$) than non-CFS.

Table 1 Frequency of participant reported health conditions (current or previously diagnosed) and percentage of each group reporting the condition

Health and medical condition	CFS n=24 (% of group)	Non-CFS n=18 (% of group)
Smoker (previous or current)	11 (45.8)	3 (16.7)
Sleep disturbances or insomnia	10 (41.7)*	2 (11.1)
Iron deficiency or anaemia	11 (45.8)*	2 (11.1)
Cardiac symptoms	13 (54.2)*	2 (11.1)
Hypertension	2 (8.3)	1 (5.6)
Dizziness, fainting	10 (41.7)*	1 (5.6)
Fibromyalgia	3 (12.5)	0 (0)
Recurring sore throat	13 (54.2)*	0 (0)
Recurring headaches or migraine	10 (41.7)*	1 (5.6)
Type 1,type 2 or gestational diabetes	5 (20.8)	0 (0)
Asthma	7 (29.2)	2 (11.1)
Other pulmonary conditions (COPD, pneumonia)	8 (33.3)*	1 (5.6)
Dyspnea	9 (37.5)*	0 (0)
Thyroid disease	3 (12.5)	0 (0)
Osteoarthritis	7 (29.2)	2 (11.1)
Rheumatoid arthritis	3 (12.5)	0 (0)
Osteoporosis	1 (4.2)	0 (0)
Osteopenia	1 (4.2)	0 (0)
Leg cramps or pain	6 (25)	1 (5.6)
Inflamed/painful joints	17 (77.3)*	0 (0)
Back pain	15 (68.2)*	3 (16.7)[a]
Vestibular condition or vertigo	2 (8.3)	0 (0)
Gastrointestinal upsets	3 (12.5)	1 (5.6)
Previous cancer	1 (4.2)	1 (5.6)

* CFS group significantly different to non-CFS, $p < 0.050$; [a]Lower back pain only reported in non-CFS group.

Lymphocyte concentration was significantly lower in the CFS group compared to non-CFS but there were no other significant between-group differences with FBC indices(Table 2).The frequency of above-normal RDW (> 14.5 g/L) in the CFS group (33.3%, n = 8) was significantly higher than in the non-CFS group ($p = 0.030$). The frequency of above-normal MCV (>96 fL) was also significantly higher in the CFS group (25%, n = 6, $p = 0.022$). Below-normal leukocyte concentrations (< 4.0 x 10^9/L) were found in 8.3% (n = 2) of the CFS group and 5.3% of the non-CFS group. Thirty three percent (n = 8) of the CFS group had a below-normal total lymphocyte concentration (<1.5 x 10^9/L) compared to 10.5% of non-CFS ($p = 0.040$).

The CFS group $CD3^+CD4^+$ cell count (Table 3) was lower than the non-CFS ($p = 0.049$) but the concentrations of other lymphocyte sub-populations, absolute cell counts and percentages of positive cells, measured by TBNK flow cytometric analysis were not significantly different between groups.

Table 2 Full blood count indices in CFS and non-CFS groups.

Indice	Normal Reference Range Low - High	Non-CFS Mean ± SD (n=18)	CFS group Mean ± SD (n=24)
Hb (g/L)	120 – 150 (F) 130 – 170 (M)	135.6 ± 11.8	137.6 ± 12.2
Erythrocytes (10^{12}/L)	3.8 – 4.8 (F) 4.5 – 5.5 (M)	4.5 ± 0.4	4.5 ± 0.5
Haematocrit (%)	0.36 – 0.46 (F) 0.40 – 0.50 (M)	40.7 ± 4.0	41.2 ± 4.0
MCV (fL)	76 - 96	90.1 ± 4.1	91.2 ± 4.4
MCH (pg)	27 - 32	30.2 ± 1.3	30.6 ± 1.5
MCHC (g/L)	315 - 350	334.3 ± 8.1	335.0 ± 5.6
RDW (CV, %)	9.0 – 14.5	13.4 ± 0.7	14.0 ± 0.7
Leukocytes (10^9/L)	4.0 – 11.0	6.0 ± 1.6	6.3 ± 1.3
Neutrophils (10^9/L)	2.0 – 7.0	3.4 ± 1.0	3.6 ± 1.1
Lymphocytes (10^9/L)	1.5 – 4.0	2.8 ± 0.7	1.8 ± 0.5*
Monocytes (10^9/L)	0.2 – 1.0	0.5 ± 0.1	0.5 ± 0.1
Eosinophils (10^9/L)	0.0 – 0.5	0.2 ± 0.1	0.2 ± 0.1
Basophils (10^9/L)	0.0 – 0.1	0.02 ± 0.05	0.04 ± 0.05
Platelets (10^9/L)	150 – 400	248.8 ± 55.6	245.4 ± 72.9

* $p < 0.05$; (F) Female reference range; (M) Male reference range.

Table 3 Absolute counts and percentages of leukocytes and lymphocyte sub-populations in CFS and non-CFS groups.

Variable	Non-CFS group Mean ± SD (n=18)	CFS group Mean ± SD (n=24)
$CD3^+$ count	1621.3 ± 561	1469.1 ± 441
$CD3^+$ %	74.0 ± 8	72.0 ± 7
$CD3^+CD8^+$ count	476.3 ± 166	495.1 ± 144
$CD3^+CD8^+$ %	21.6 ± 7	24.9 ± 9
$CD3^+CD4^+$ count	1124.4 ± 199	924.4 ± 179*
$CD3^+CD4^+$ %	48.5 ± 9	46.4 ± 6
$CD3^+CD4^+CD8^+$ count	19.6 ± 11	19.1 ± 14
$CD3^+CD4^+CD8^+$ %	1.0 ± 0.5	1.0 ± 0.8
$CD16^+CD56^+$ count	348.4 ± 167	286.0 ± 160
$CD16^+CD56^+$ %	15.7 ± 7	14.6 ± 7
$CD19^+$ count	240.4 ± 126	202.3 ± 121
$CD19^+$ %	10.7 ± 3	10.1 ± 4
$CD45^+$ count	2229.1 ± 687	1983.7 ± 544
CD4/CD8 ratio	2.4 ± 1	2.3 ± 1

Sub-population % for lymphocytes refers to the % of 10,000 total lymphocyte events.$CD3^+CD4^+$ count, * $p < 0.05$

4.0 Discussion

This study investigated the frequencies of irregularities in haematological and immunological indices in individuals with CFS compared to non-CFS. A secondary aim was to compare the frequencies of health conditions and symptoms between the two groups. It was clear that the characteristic symptoms of CFS (Loblay et al., 2002; Faulkner and Smith, 2008) occurred in large percentages of the diagnosed participants, irrespective of their age and time since diagnosis. However, given that the CFS group displayed haemoglobin and erythrocyte concentrations within the respective normal ranges, it was unexpected to find a high percentage of the CFS group with either a previous or current diagnosis of iron deficiency/anaemia, and with elevated RDW and MCV. Iron deficiency is not included in the CDC criteria for CFS but some previous studies have found abnormal erythrocyte morphology, RDW and MCV in CFS patients (Simpson and Herbison, 1997; Roberts et al., 1998; Simpson and O'Neill, 2001; Richards et al., 2007) , although the relationship between these findings and CFS path physiology and symptoms remains unclear.

More than 30% of the CFS group had above-normal RDW and 25% had above-normal MCV. The higher RDW in CFS patients is consistent with a study by Roberts et al. (1998)but contradicts other findings(Richards et al., 2007; Niblett et al., 2007). RDW is an indicator of greater variation in erythrocyte size (anisocytosis) and is increased in iron or folate deficiency(Dabbah et al., 2010). Compared to other haematological parameters, RDW becomes abnormal earlier in nutritional deficiency anaemias(Turgeon 2012). In CFS patients, the high RDW may indicate anisocytosis(Simpson and O'Neill, 2001),and furtherclinical investigations would be recommended given the high percentage of our CFS participants with a history of, or presenting, anaemia/iron deficiency(Sarma 1990; Winkler et al., 2004; Roberts et al., 1998).The significantly greater frequency of high MCV(average volume of red blood cells) in the CFS group may also be associated with underlying iron deficiency because MCV isalso considered a means of identifying anaemias, folate/vitamin B12 deficiencies and other erythrocyte disorders (Perkins 2009).

Abnormal erythrocyte morphology has been associated with oxidative damage (Richards et al., 2007; Kennedy et al., 2005), decreased deformability (Richards et al., 2007) and increased numbers of stomatocytes(Simpson and Herbison, 1997; Simpson and O'Neill 2001; Roberts et al., 1998), which may be linked to poor microcirculation, impaired oxygen and metabolic waste exchange and muscular fatigue in CFS patients (Mukherjee et al., 1987; Simpson and Herbison, 1997; Richards et al., 2007).Interestingly, elevated RDW is a strong predictor of mortality in patients with coronary disease and heart failure (Pascual-Figal et al., 2009; Dabbah et al., 2010), pulmonary hypertension and renal disease (Hampole et al., 2009)and in the elderly, irrespective of whether the patients have anaemia or not (Lam et al., 2013). The strong association between high RDW and poor clinical outcomes for chronic conditions is not well understood but reasons may include a RDW response to blood loss, inflammatory cytokine effects on iron metabolism and bone marrow function, and adrenergic activation effects on bone marrow responses (Dabbah et al., 2010). Although we excluded CFS patients with serious diagnosed cardiopulmonary conditionsfrom the study, CFS is a complex condition in which there isconsiderable evidence of cardiovascular deconditioning (Hurwitz et al., 2010), irregularities of the cardiovascular, autonomic and immune systems(Winkler et al., 2004; Hurwitz et al., 2010), and of chronic, low-grade inflammation(Maes et al., 2012).It may be beneficial to further investigate abnormal red cell indices in CFS patients (Jason et al., 2001).

We foundthat a high percentage of the CFS group showed below-normal total lymphocyte concentrations and lower $CD3^+CD4^+$ lymphocyte counts compared to non-CFS. The CFS group had a significantly lowertotal lymphocyte concentration and a lower $CD4^+$ lymphocyte count, which may have clinical significance in terms of the ability of CFS patients to respond to infections (Lorusso et al., 2009).A high percentage of our CFS group reported recurring sore throats, indicative of upper respiratory tract infection (URTI). Recurring sore throats and inflamed lymph nodes are common symptoms of both CFS and URTI, and there is considerable evidence that CFS patients suffer from significantly more recurring episodes of URTI and other infectious illnesses compared to healthy controls (Loblay et al., 2002; Faulkner and Smith 2008). We reason that a lower $CD4^+$ cell count might indicate an impaired T-helper lymphocyte response, as these cells produce interleukin-2 when activated and are integral to lymphocyte sub-set activation and proliferation (e.g. T lymphocytes and NK cells)(Robertson et al., 2005). Our results do not agree with other studiesthatfound no differences between thedistributions of lymphocyte sub-populations in CFS patients and controls (Brenu et al., 2010; Swanink et al., 1996; Robertson et al., 2005; Hassan et al., 1998).

Significant differences in the concentrations of some leukocyte subsets (e.g. neutrophils) and in the activation of NK, T-helper ($CD3^+CD4^+$) and cytotoxic ($CD3^+CD8^+$) lymphocytes have been reported in individuals with CFS (Brenu et al., 2010; Peakman et al., 1997; Lorusso et al., 2009; Brenu et al., 2012) but the link between these findings and clinical status or outcomes for CFS patients is still unclear. A possible reason for immune suppression and increased susceptibility to infections in CFS individuals is psychological stress, causing alterations in the HPA-axis and autonomic nervous system (Faulkner and Smith, 2008). Alterations in leukocyte intracellular signalling may also affect immune cell function (Bowlus et al., 2003; Aluwhalia et al., 2009).

There is an established link between iron deficiencyand impaired cell-mediated and innate immunity (Aluwhalia et al., 2009). Both T- and B- lymphocyte activation and proliferation are compromised in iron-deficient individuals (Klecha et al., 2005), with sedentary, older individuals more at risk (Aluwhalia et al., 2009). Iron is a vital component of the enzyme ribonucleotide reductase, involved in DNA production and cell division; iron is also necessary for the activation of protein kinase C as part of the tyrosine kinase-inositol triphosphate intracellular signalling pathway in immune cells (Bowlus et al., 2003; Klecha et al., 2005; Aluwhalia et al., 2009). Some studies have shown no difference in the concentrations of lymphocyte subsets in iron-deficient individuals compared to iron-sufficient although there was reduced cell proliferative capacity (Aluwhalia et al., 2009) but others have reported decreased total lymphocyte and subset concentrations, and impaired granulocyte respiratory burst capacity (Santos and Falcao, 1990). It is possible that iron deficiency is linked to lower $CD4^+$ lymphocyte concentrations and counts in CFS individuals.

The inconsistencies in findings between some CFS studies may be due to the relatively small sample sizes,the heterogeneity of CFS cohorts, andto methodological differences between studies. The inconsistenciesdo highlight the need for the larger studies of immune parameters and clinical outcomes in CFS patients.

Conclusions

This study confirms irregularities in some haematological and immune indices in CFS patients although the association with CFS symptoms remains unclear. Abnormal RDW and MCV should be further investigated to rule out iron deficiencies.

Conflict of interest statement

The authors declare no competing financial interests.

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