

Is chronic fatigue syndrome associated with platelet activation?

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Chronic fatigue syndrome (CFS) is a debilitating condition that has no known aetiology or pathophysiology. Recent investigations by other workers have suggested that individuals with CFS may have a hypercoagulable state. This study investigated various aspects of platelet activation and function in 17 patients with CFS and in 16 age-matched and sex-matched healthy controls. Platelet aggregation, platelet volume and coagulation tests were performed. Platelet aggregation was investigated by means of the photometric changes using citrated platelet-rich plasma, whole blood aggregation was calculated as the percentage fall in single platelet counts and the coagulation tests were performed on an automatic microcentrifugal analyser. A trend was observed for the patients to have lower aggregation results and a reduced mean platelet volume. However, this only reached statistical significance for one result; the rate of the aggregation slope by 1.0 $\mu\text{g/ml}$ collagen [CFS patients, 18 (9–28) versus controls, 32.5 (19–36); Mann–Whitney U test, $P = 0.029$]. No significant differences were found for any of the measurements of coagulation. These results are in contrast to previously reported findings. However, due to the heterogeneous nature of the disease, and the resulting lifestyles of the patients, caution should be taken when

comparing one group of patients with another. Nevertheless, we certainly found no evidence of increased platelet activation or of a hypercoagulable state in patients with CFS and, on the basis of these results, anti-platelet or anti-coagulant therapy is not warranted. *Blood Coagulation and Fibrinolysis* 17:89–92 © 2006 Lippincott Williams & Wilkins.

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Introduction

Chronic fatigue syndrome (CFS) is an illness of unknown aetiology associated with debilitating fatigue of at least 6 months duration and accompanied by a variety of symptoms suggestive of immune and neuroendocrine disturbances and abnormalities within the central nervous system. Although there are many reports pointing to chronic low-level immune activation within many CFS patients [1,2], no relationship has yet been established between such abnormalities and patients' symptoms. One group of researchers in particular [3], however, has hypothesized that CFS is a hypercoagulable state based on a model linking pathogen activated inflammation to cytokine modulation of the coagulation system [3,4]. Their model implicates immunological disturbances [2] and viral infections [5] are central to the hypercoagulable state, and they have also suggested that anticoagulant proteins such as protein C and protein S are associated with defects in fibrinolysis in this patient group [6]. They have further suggested that activation of coagulation may be a final common endpoint of a variety of CFS-like illnesses such as fibromyalgia and Gulf War Illness [7].

In support of this, our group has recently shown that patients with CFS had significantly elevated platelet poor plasma levels of transforming growth factor- β_1 and an increased expression of annexin V binding to neutrophils when compared with healthy, matched controls [8]. Transforming growth factor- β_1 is a multi-functional cytokine that is stored in the α granules of circulating platelets, is released during platelet activation and may be involved in platelet aggregation [9]. Annexin V is an anticoagulant protein that preferentially binds to negatively charged phospholipids such as phosphatidylserine, which is expressed on the outer leaflet of the cytoplasmic membrane during the apoptotic process. Phosphatidylserine can induce procoagulant responses [10] so an increased expression of annexin V could also reflect a procoagulant tendency. The observation of potential markers of platelet activation by us alongside the previous work by Berg and colleagues suggest that there may be altered platelet function in patients with CFS that requires further investigation.

In order to elucidate this further we set out to investigate platelet function in patients with a well-documented

Table 1 Demographics of subjects

	Patient group	Control group
<i>n</i>	17	16
Age [mean (range)] (years)	53 (26–69)	51 (26–66)
Sex (male : female)	8 : 9	8 : 8
Smoking (yes : no : ex)	1 : 14 : 2	1 : 13 : 2

history of CFS alongside a comparable group of healthy control subjects.

Methods

Nineteen patients who fulfilled the Centers for Disease Control 1994 criteria for CFS [11] and 16 age-matched and sex-matched healthy controls participated in this pilot study. Patients were enrolled from previously studied CFS patients held in a research database. Demographic details of subjects are presented in Table 1 and the duration of illness ranged from 3 to 22 years (mean, 12.5 years). Subjects who had received drugs known to have an effect on platelet function for up to 14 days prior to testing were excluded from the study, while all other medications were noted. None of the healthy control subjects were on any medication and all were matched for age, gender and smoking status. Two patients had the same age and gender, and were consequently matched with one similar control subject. All subjects gave written, informed consent and the local medical ethics committee approved the study.

Platelet function assessments were carried out on platelets obtained from venous blood sampled from the ante cubital fossa using a 19-gauge butterfly, and anti-coagulated with 3.2% (0.109 mol/l) tri-sodium citrate 1 : 9 (v:v), with the first 5 ml being discarded. All blood samples were taken at the same time of day. Light tourniquet pressure was applied if required to assist venepuncture. The pressure was released for at least 10 s prior to the blood being drawn.

Five millilitres were used for whole blood aggregation. It has been reported that patients with CFS have non-discoidal and less deformable red blood cells [12,13] and that the red blood cell is a known to release ADP, which induces activation and aggregation of platelets. In order to investigate the influence of the red and white blood cells on platelet aggregation, whole blood aggregation was measured using 0.5 µmol/l ADP and 0.6 and 1.0 µg/ml collagen as agonists. Spontaneous aggregation was also measured using a previously described method [14].

Platelet-rich plasma (PRP) aggregation was measured from 10 ml citrated blood using the method as described by Born [15] and is a direct assessment of platelet function. PRP platelet counts were standardized to between $300 \pm 50 \times 10^9/l$, and if necessary the PRP

was diluted with the subject's platelet-poor plasma. The platelets were stimulated with 2, 1 and 0.5 µmol/l ADP (Trinity Biotech, Bray, Ireland) and 2, 1 and 0.5 µg/ml collagen (Technoclone, Dorking, UK). Five minutes after stimulation, final aggregation (percentage) and the slope of aggregation (rate of aggregation) were obtained for each concentration.

The mean platelet volume was measured as described by Bancroft *et al.* [16] from a small sample of the PRP. The mean platelet volume is a marker and possibly a determinant of platelet function, in that large platelets are more active than normal-sized platelets, and it is also a measure of platelet size reflecting changes in either the level of platelet stimulation or the rate of platelet production [16].

The final 5 ml blood was used to investigate coagulation pathways. The prothrombin time and activated partial thromboplastin time, measures of extrinsic and intrinsic coagulation pathways, respectively, as well as the International Normalized Ratio and fibrinogen were measured on an Instrumentation Laboratory Automated Coagulation Laboratory using reagents from Instrumentation Laboratory (Birchwood, Warrington, UK).

Results

Two patients were withdrawn from the study, one because of problems associated with taking blood and one because it was discovered after the study that the patient had measles at the time of the study visit.

Two CFS patients were on a low dose (10 mg) of amitriptyline taken at night for sleeping problems, two patients were on thyroxine, one patient was on diuretic medication, two further patients were on paracetamol-based drugs for pain relief and two patients were on hormone replacement therapy. Two patients were on more than one medication.

Due to the relatively small sample size and large spread of results, Mann–Whitney U non-parametric tests (using SPSS for Windows version 10) were applied as statistical tests to compare the results between the CFS patients and controls (Table 2).

No statistically significant differences were observed between the groups for whole blood aggregation, platelet volume or coagulation. Only one significant difference (out of a possible eight) was found for PRP aggregation, with patients having a significantly lower rate of aggregation (slope) [median (inter-quartile range) for CFS patients, 18 (9–28) versus 32.5 (19–36) for the control group, $P = 0.029$]. In all but one result (PRP aggregation with 0.5 µmol/l ADP) the patients had a lower median value than the control group, but these results failed to reach statistical significance.

Table 2 Platelet activation and coagulation results for chronic fatigue syndrome (CFS) patients and controls

	CFS patients	Controls	P value
Whole blood aggregation (%fall)			
ADP 0.5 $\mu\text{mol/l}$	53 (44–60)	60.5 (31–73)	0.533
Collagen 1.0 $\mu\text{g/ml}$	56 (35–70)	75.5 (66–79)	0.081
Collagen 0.6 $\mu\text{g/ml}$	29 (15–57)	36 (29–56)	0.331
Spontaneous	43 (26–58)	39 (29–55)	0.970
Platelet-rich plasma aggregation			
% aggregated collagen 2.0 $\mu\text{g/ml}$	72 (69–76)	76 (72–80)	0.110
% aggregated collagen 1.0 $\mu\text{g/ml}$	43 (16–65)	66 (38–72)	0.184
% aggregated collagen 0.5 $\mu\text{g/ml}$	19.5 (12–63)	58 (24–67)	0.138
Slope collagen 2.0 $\mu\text{g/ml}$	40 (32–45)	42.5 (38–44)	0.444
Slope collagen 1.0 $\mu\text{g/ml}$	18 (9–28)	32.5 (19–36)	0.029
Slope collagen 0.5 $\mu\text{g/ml}$	15 (5–29)	20.5 (12–28)	0.361
% aggregated ADP 2.0 $\mu\text{mol/l}$	69 (50–74)	70 (51–75)	0.958
% aggregated ADP 1.0 $\mu\text{mol/l}$	20 (11–63)	59 (15–68)	0.363
% aggregated ADP 0.5 $\mu\text{mol/l}$	8 (6–13)	5.5 (3–15)	0.345
Slope ADP 2.0 $\mu\text{mol/l}$	35 (33–42)	38 (32–44)	0.817
Slope ADP 1.0 $\mu\text{mol/l}$	24 (21–31)	27.5 (22–36)	0.423
Slope ADP 0.5 $\mu\text{mol/l}$	10 (9–14)	18 (10–21)	0.053
Platelet volume			
Mean platelet volume	6.9 (6.5–7.5)	7.5 (6.9–7.8)	0.191
Skew	1.1 (0.9–1.3)	1.0 (0.9–1.2)	0.326
Coagulation			
Prothrombin time (s)	13.9 (13.1–14.4)	13.7 (13.2–14.3)	0.780
International Normalized Ratio	0.92 (0.86–0.96)	0.9 (0.9–1.0)	0.696
Activated partial thromboplastin time (s)	31.9 (30.8–32.7)	30.6 (28.2–32.1)	0.119
Fibrinogen (g/l)	2.80 (2.62–3.34)	2.89 (2.7–3.3)	0.985

Data presented as median (inter-quartile range) using the non-parametric Mann–Whitney U-test.

Discussion

The CFS patients in this study showed no evidence of increased platelet activation or hypercoagulability when compared with healthy matched control subjects. These results contrast with previously reported findings in CFS patients and bring into question the conclusion by Berg and colleagues [3,4] that CFS is an immune system activation of coagulation.

It is worth examining factors that might have contributed to these contrasting results; that is, the results reported here showing no differences in coagulation pathways between CFS patients and matched controls and those by Berg's group showing hypercoagulation in CFS subjects. In the first place CFS is a heterogeneous condition, and it is possible that we are looking at an entirely unrelated cohort of CFS patients with a different aetiology and symptom expression to that of Berg *et al.* Our CFS patient population has been strictly selected to fulfil the specific criteria of the Centers for Disease Control 1994 definition [11] and are representative of the large cohort of patients in our research database. In the paper by Berg *et al.* [3], however, they conducted "a blinded prospective study of 54 CFS and/or fibromyalgia patients and 23 controls", and this is clearly a broader case-definition than that used by us. Indeed, we have recently reported that fibromyalgia patients are biologically different to CFS patients in a number of key ways [17]. In a further paper, Brewer and Berg [18] investigated 30 patients with CFS who have at least had one positive blood culture for active human herpesvirus-6, and they report that samples were taken in the morning and that

none of the patients were on anticoagulant therapy. The results in this second study were reported as being "positive" from a panel of tests, with no results or equivalent control data provided.

Of further concern is the fact that there is no information about age, gender, smoking status, current medications or blood sampling methods in the Berg *et al.* paper, and all of these are known to affect coagulation pathways. It should also be stressed that there are gender differences in platelet aggregation, with higher levels being reported in women [19]. Although CFS female patients outnumber males by approximately 3:1 [20], we had equal number of both sexes within our patient and control populations. Similar information on gender ratios is not available in the research reported by Berg *et al.*

Exercise also influences platelet activity, especially in those who lead a sedentary lifestyle when compared with those who are physically trained [21], and an increase in platelet–platelet aggregates occurs after exercise [22]. This is of particular significance in a patient population who, by definition, have "a substantial reduction in previous levels of occupational, educational, social or personal activities" [11]. CFS is also characterized by post-exertional malaise with patients having less intense and shorter activity peaks followed by longer rest periods [23], often for as long as 24 h after the activity. Such an activity cycle might actually be expected to reduce platelet aggregation given the finding that 45 min of supine rest in a calm environment led to a highly significant decrease in platelet activation [24].

In general, although there were no significant differences between platelet aggregation in our CFS population when compared with matched control subjects, the trend was for lower activity and caution is required when interpreting data from this patient group given the gender bias and the fact that many patients have a sedentary lifestyle.

This study was planned to further investigate the work by Berg and colleagues and the tenuous observations made by us from early work. On the whole no significant differences in platelet function were found between the patients with CFS and control subjects. CFS is, however, a heterogeneous illness and patients have a variety of adapted lifestyles, so caution is needed when comparing one group of patients with another.

In this study there is certainly no evidence of increased platelet activation or that a hypercoagulable state exists in patients with CFS, and, on the basis of these results, a study of anti-platelet therapy in CFS patients is not warranted.

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