

# Activation of the coagulation system in Gulf War Illness: a potential pathophysiologic link with chronic fatigue syndrome A laboratory approach to diagnosis

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Most symptoms of Gulf War Illness (GWI) are similar to Chronic Fatigue Syndrome (CFS) and/or Fibromyalgia (FM). We investigated whether these symptoms are associated with an activated coagulation system as has been reported in some cases of CFS/FM. The coagulation assays include activation markers of the cascade, platelet activation and hereditary risk factors. Our findings show activation of the coagulation system in GWI. This evidence of a hypercoagulable state suggests that symptoms may be due to poor blood flow and, therefore, a basis for the potential utility of anticoagulant therapy. *Blood Coagul Fibrinolysis* 11:673- 678 (D 2000 Lippincott Williams & Wilkins.

**Keywords:** Gulf War Illness (Syndrome), Chronic Fatigue Syndrome, Fibromyalgia, soluble fibrin monomer, platelet activation, fibrin deposition, thrombophilia, hypofibrinolysis, endothelial cells

## Introduction

The existence of Gulf War Illness (GWI) has been controversial. It has been difficult to diagnose because no definite etiological agent has been isolated or a biochemical process defined [1]. GWI is responsible for physiological and mental stress symptoms that have required medical therapy. In many, it is a medical entity with neurological symptoms caused by sequential insults to the brain [2]. Because some of the symptoms and chronicity of GWI are similar to those of the Chronic Fatigue Syndrome/Fibromyalgia (CFS/FM) complex, and because immune system activation of coagulation has been observed in CFS/FM, we investigated coagulation and platelet activation in veterans with a diagnosis of Gulf War Illness. The sensitive new markers of coagulation activation assays included: fibrinogen, Prothrombin fragment 1 + 2, thrombin/ anti-thrombin complexes, soluble fibrin monomer and platelet activity index (PA) by flow cytometry using CD62P [with and without adenosine diphosphate (ADP) stimulation]. Predisposing hereditary factors include, in part, plasminogen activator inhibitor, factor 11 activity screening for the pro- thrombin gene mutation, AntiThrombin activity, protein C activity, protein S (PS) activity, APC resistance screening for factor V Leiden mutation, lipoprotein (a), Homocysteine and anti-B2GPI anti- bodies.

## Model

We propose that Gulf War Illness may be due, at least in part, to an intense immune response to viral, bacterial, chemical, and/or biological warfare antigens, following exposure to either intact pathogens or vaccines. This immune response may activate the coagulation system by the cross-reaction of anti- bodies against certain cell surface antithrombotic proteins. We hypothesize that the binding of anti- B2-GPI or anti-Annexin-V antibodies to their corresponding proteins on the endothelial cell (EC) surfaces exposes phosphatidylserine, a potent thrombogenic phospholipid, on the EC surfaces. Inflammatory responses, which include cytokine down regulation of the antithrombotic environment (Thrombomodulin and tPA) causes EC expression of prothrombotic tissue factor (TF) on the EC surface. TF and exposed PS allow binding of the coagulation tenase and prothrombinase complexes, resulting in thrombin generation. Fibrinogen is then cleaved by thrombin, forming soluble fibrin monomer (SFM). SFM forms dimer complexes, increasing blood viscosity, and is deposited on the affected EC surfaces. This 'fibrin deposition' could create local ischemia and pathology by blocking nutrient passage and oxygen delivery in the microcirculation. Cross-linked fibrin and full-scale clot formation do not occur in this model because the amount of thrombin formation is less than the amount needed for factor XIII activation [3]. This process may also decrease the local antithrombotic environment by inhibiting EC ability to express heparans, Thrombomodulin and Annexin 11 (inhibition of plasminogen activation). This process may be documented as below normal thrombin- AntiThrombin complex values in the circulating plasma. Using this model, the administration of anticoagulants (heparin or Coumadin) to GWI patients should decrease the coagulation activation, with potential relief of symptoms related to poor blood flow, fibrin deposition, and/or this activated coagulation or hypercoagulable state.

## Methods

The test group consisted of 33 veterans, comprising 27 military personnel activated and deployed during the Gulf War, three veterans activated but not deployed (AND), and three veterans activated and deployed to the Gulf after the war (ADA). This was compared with 33 age- and sex-matched healthy controls. The ill veterans participating in this study satisfied the diagnosis of GWI. Blood samples were drawn according to specific laboratory protocols and transported to HEMEX Laboratories (Phoenix, AZ, USA) for testing. Assays included: platelet activity index by flow cytometry using CD62P (Becton Dickinson, San Jose, California, USA) (with and without ADP stimulation); fibrinogen, Clauss method (reference range, 180- 310 mg/dl) (Dade Behring, Marburg, Germany); Prothrombin fragment I + 2 (F1 + 2), Enzygnost F1 + 2 (reference range, 0-1.1 nmol/i F1 + 2) (Dade Behring); thrombin-AntiThrombin complexes (TAT), Enzygnost TAT (1.0-4.1,ug/1) (Dade Behring); SFM, Berichrom FM (0-17 mg/1) (Dade Behring); plasminogen activator inhibitor (PAI-1), Chromolize PAII (0-15.5 U/ml) (Biopool Int., Ventura, California, USA); factor 11 activity, ThromboScreen III Deficient Plasma (60-120%) (Pacific Hemostasis, Middletown, Virginia, USA); AntiThrombin activity (AT), Berichrom AT III (75-125%) (Dade Behring); protein C activity (PC), Staclot PC (60-140%) (Diagnostica Stago, Asnieres, France); PS activity, Staclot PS (65-150%) (Diagnostica Stago); APC Resistance, CoaTest APC Resistance V (Chromogenix, Milano, Italy); Lipoprotein (a) (Lp(a)), TintElise Lp(a) (0-30 mg/dl) (Biopool Int.); Homocysteine, IMX System (0-13,umol/1) (Abbott Diagnostics, Deerfield, Illinois, USA); and anti B2GPI antibodies, Reaads B2GPI (0-20 G, A or M Units) (Corgenix Inc. Westminster, Colorado, USA). Student's t tests were calculated for test and control groups, and indicated different populations (based on one SD) due to some very high values beyond the reference ranges in the study group. Statistical P values were then determined using 2 X 2 chi-square analysis for each assay [4].

## Results

Results for the 33 veterans are reported as a qualitative score of coagulation activation tests in the immune System Activation of Coagulation (ISAC) panel as previously reported in this journal [5]. In addition, eight hereditary thrombosis risk factors were evaluated. An age-matched/sex-matched control group was tested for the same markers of coagulation activation. The average age of the patient group was 40 years versus 41 years in the control group. The ISAC Panel results are summarized in Tables 1-3. As previously reported by Berg *et al.* [51], the laboratory criterion for activation of coagulation in CFS/FM is two or more positive results on the ISAC panel. In this study, 22/33 or 67% of the GWI patient group scored positive in two out of five or more tests.

**Table 1.** Control versus patient group results (n) and significant difference (chi-square analysis) between groups

	Test							
	62P	+ADP	PA index	FIB	SFM	TAT	FI +2	
Reference range	0-27	40-80	Normal	180-310	0-17	1.0-4.1	0-1.1	
Control mean	18	53	0% positive	276	11	1.7	1.0	
Patient mean	34	38	52% positive	293	19	2.5	1.4	
	High	High	Positive	High	High	High	High	Two or more positive
Control	6/33	3/33	1/33	3/33	1/33	1/33	2/33	0/33
Patient	16/33	13/33	15/33	17/33	17/33	6/33	11/33	23/33
P value	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.10	< 0.005	< 0.001

ADP, Adenosine diphosphate; PA, platelet activity; FIB, fibrinogen; SFM, soluble fibrin monomer; TAT, thrombin -AntiThrombin complex; F1 + 2, fibrinogen 1 + 2.

**Table 2.** Coagulation activation compensation mechanisms associated with hereditary risk factors

	Number of ISAC tests positive			
	1/5	2/5	3/5	4/5
Increased PC, PS, and/or AT	6/10	5/10	6/11	0/1
Positive hereditary risk factors	8/10	5/10	5/11	1/1

PC, Protein C; PS, protein S, AT, AntiThrombin

**Table 3.** Risk factors identified with elevated protein C (PC), protein S (PS) and/or AntiThrombin (AT) levels

	No risk factors identified	Increased				
		Lp(a)	PAI-1	FII	Lp(a) and PAI-1	Lp(a) and FII
Increased PC, PS and/or AT	3/13	6/7	5/7	8/12	2/2	4/5

Lp(a), Lipoprotein (a); PAI-1, plasminogen activator inhibitor-1; FII, factor II.

Ten of the military subjects were positive in only one out of five tests, and one was negative for all five. In the veteran group that scored one out of five test positivity, eight out of 10 (80%) had positive hereditary thrombosis risk factors and six out of the 10 (60%) had compensatory anticoagulation mechanisms manifested as either elevated AntiThrombin, protein C or protein S levels. In contrast, the control group had no samples that were positive for two or more out of five tests, and only 10 of the 33 had one positive test. The rest of the controls were negative in all tests. The veteran that scored negative had taken numerous antibiotics for several years.

The hereditary risk factors of hypercoagulability can be divided into two groups based on the model of Glueck and Triplett and coworkers [6]; thrombophilia and hypofibrinolysis. Thrombophilia patients respond more favorably to anticoagulation than hypofibrinolysis patients. These categories may be useful in predicting treatment responses. Out of the 33 veterans, 20 (61%) had positive hereditary defects. Eight out of 33 (24%) were positive for thrombophilia risk factors and seven of 33 (21%) of the patients were positive for hypofibrinolysis. Five out of 33 patients (15%) had a risk factor in each group. This last combination was either increased Lp(a) and/or PAI-I with increased factor 11 levels. One patient had a Homocysteine level of 20.9 (reference range, 0-13). Sixteen out of 33 (48%) had evidence of activation of anticoagulation pathways as demonstrated by elevated protein C, protein S and/or AntiThrombin activity (see Table 3). This is probably a compensatory response that attempts to down regulate the hypercoagulable state that results from significantly increased fibrinolysis inhibitors or thrombophilia factors. This has also been observed in CSF/FM patients [5].

Thirteen of the veterans (27%) had normal protein levels in the hereditary risk factors screened. Nevertheless, 11 of these 13 patients (85%) had two or more activated coagulation markers (positive ISAC panel results). Three out of this group of 11 had increased protein C or protein S levels to compensate for the activated coagulation system. Two patients (6%) that had no detectable protein abnormalities had platelet activation. It is possible that these patients may have had other protein abnormalities, such as heparin cofactor 11, C4b binding protein, plasminogen, histadine-rich glycoprotein, factor XII, soluble Thrombomodulin, dysfibrinogen, and/or tissue factor.

## Discussion

### *Control group*

Civilian controls were selected instead of military controls for several reasons related to vaccination policy and other military environment exposures. A soldier who received the recent anthrax vaccination and developed Fibromyalgia tested 1/5 positive in this study. We also tested a civilian who received only a squalene injection. This person tested 2/5 positive in a preliminary study. Squalene antibodies were present in the Gulf War veterans [7] and there is speculation that squalene is the adjuvant in the recent anthrax vaccinations. The series of anthrax is mandatory for all soldiers. Therefore, the appropriate controls for the anticoagulation study are civilians until these issues are resolved.

### *Patient information*

All the veterans diagnosed with GWI in this study satisfied the diagnosis of Gulf War Syndrome by the Merck manual according to their physicians' or medical records elsewhere [8]. An in-depth survey of all medications, prescriptive or over-the-counter, was not attempted because some of the patients had participated in experimental Gulf War protocols that involved placebos. The number of positive tests in some patients who actively sought treatment was less than the untreated patients, implying beneficial responses from prescription medicines that could ameliorate the coagulation activation.

### *Coagulation and cellular anatomy*

Exposure to viral, bacterial, chemical, biological warfare pathogens and/or vaccine adjuvants induces an immune response. Normal immunoglobulin (Ig)M and IgG antibody formation leads to protection against such pathogens. IgA antibodies are formed from interaction of lymphoid elements in the mucosal membranes with the pathogens. We postulate that this is the case with exposures of the veterans in this study. Of the veterans who were tested for anti-B2-GPI antibodies (n = 5), those who had positive platelet activation had positive anti-B2-GPI IgA antibodies (n = 3) while those who had normal platelet activation had negative IgA antibodies (n = 3). In a previous study of Antiphospholipid antibodies in Gulf War Veterans, there was no significant positivity for either lupus anticoagulant or

anti-phosphatidylserine antibodies [9]. Anti-B2-GPI antibodies were tested, but only for the IgG type. Further studies of IgA positivity may yield better data about the concept of an air-borne pathogen or mucosal membrane exposure in these veterans. The EC is a connecting point between pathogen-activated inflammation and the coagulation system, and is part of the defensive host response. During inflammation, cytokines modulate the coagulation system by down regulating the expression of Thrombomodulin on EC surfaces and eliminating the anticoagulant environment by blocking the activation of protein C [10]. At the same time, these cytokines induce expression of TF on the EC surfaces, which promotes a procoagulant environment. Thus, both TF and PS promote the binding of the tenase and prothrombinase complexes for the local generation of thrombin. TF expression can also decrease local fibrinolysis by inducing EC to produce PAI-I instead of tPA. This leads to fibrin build-up instead of fibrin removal. Another result of fibrin deposition is the effect of diminishing capillary size, which may compromise erythrocyte integrity or impair the rate of delivery of oxygen and nutrients to the surrounding tissues.

### *GWI, CFS and FM*

There appears to be significant overlap, in the symptoms of Gulf War Illness, CFS and Fibromyalgia [11]. Perhaps FM is the peripheral manifestation of the clotting cascade activation with SFM generation and fibrin deposition blocking oxygen and nutrient passage to the muscles, nerves and tendons. The CFS may be the central nervous system manifestation of the clotting activation creating lack of blood flow to the brain due to hyperviscous blood, and, therefore, disturbance of the chemicals and hormones within the brain [12]. Here the causes could be viral, bacterial, chemical, toxin-related or lymphocytic transference. It is possible that the lymphocytes or antibodies found in bodily fluids could influence another person's immune system by transference [13]. Live infectious vaccines have been developed that can transfer immunity from one individual to another [14]. An injected live vaccine, such as smallpox or polio, could possibly be activated if the patient was immunocompromised [15]. The injection of an experimental adjuvant can immunocompromise or immunostimulate, depending on factors such as the dosage [16]. The toxins or contaminants within the anthrax or other vaccines could possibly be an infectious etiology [17].

### *GWI and genetics*

Urnovitz *et al* have found that Chromosome 22q11 genes rearrange themselves when exposed to toxins and infection, and that this rearrangement may be the cause for many of the neurological problems in GWI [18]. The gene for the coagulation protein heparin cofactor 11 (HCII) is also located on chromosome 22q11 [19]. This same gene rearrangement on C22 has been recently reported to cause low levels of HCII in the plasma. HCII is a protein that can inactivate fibrin-bound thrombin, whereas AT is ineffective against fibrin-bound thrombin. Thrombin may be bound to endothelial cells where there is 'fibrin deposition' on capillary walls. This potential HCII defect may well explain the hypercoagulable state in patients where no protein defects have been found to date.

## **Conclusion**

The symptoms of CFS/FM and GWI are very similar. The positive ISAC testing of the GWI patients parallels the results seen in CFS/FM patients. The hereditary risk profile is also positive in both. GWI may be a unique subset of CFS/FM. The recent study by Zhang *et al.* [20] indicates the immune function tests of lymphocyte subpopulations as well as cytokines of the ill veterans differ from the immune panel of CFS/FM patients. The pathophysiology remains constant; cytokines activating antibodies that bind to the EC and activate platelets and the coagulation cascade. Vaccines may be the cause of the hypercoagulable state in the AND and ADA groups of veterans. There is most likely a genetic predisposition to developing adverse reactions towards vaccinations since over 60% of the ill Gulf War veterans in our study have positive hereditary risk factors. There may also be other contributing factors to the Gulf War Illness that may have caused the illness or worsened the pre-existing disease. Activation of coagulation may be a final common endpoint for differing etiologies of GWI or CFS.

Coagulation activation is the central focus of the GWI, in diagnosis and probably the target for treatment. Further research into the cause of platelet activation is needed to determine if there is an infectious etiology. An in-depth study of the anti-B2GPI-IgA antibody in the GW veterans and their families needs to be addressed.

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