

Frequency of Deviant Immunological Test Values in Chronic Fatigue Syndrome Patients

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Of 11 immunological tests done on chronic fatigue syndrome patients and on fatigued controls, 3 tests (protein A binding, Raji cell, or C3 or C4 [deviant values in either complement component were counted as positive]) with deviant results discriminated best among the groups. Other tests, including immunoglobulin G subclasses, complement component CH50, interleukin-2, and anticardiolipin antibodies, did not discriminate well among the groups.

One hypothesis for the cause of chronic fatigue syndrome (CFS) is immunological dysfunction. Butressing this hypothesis are results of a host of papers reporting immunological abnormalities in CFS patients compared with either healthy controls or preexisting laboratory norms (1, 2, 4–6, 9, 13).

The purpose of this study was to compare clinically available immunological test results in CFS patients with those of a group of patients who were evaluated for fatigue but who did not fulfill Centers for Disease Control and Prevention (CDC) criteria for CFS. A third comparison group was made up of patients who fulfilled CDC criteria for CFS but who had a prior psychiatric history. The subjects were 119 patients referred to a tertiary university-based practice for evaluation of complaints of severe fatigue who either did or did not fulfill the formal case definition of CFS (8). CFS patients were stratified (i) by the absence or presence of a psychiatric history in the 5 years prior to onset of illness into CFS or CFS-psychiatric groups and (ii) by work status into working (even if only part-time) or disabled groups.

Sera were assayed by Roche Laboratories for circulating immune complexes (protein A binding [PAB], Raji cell, and C1q binding), for complement components (C3, C4, and CH50), for immunoglobulin G (IgG) subclasses (IgG1 and IgG3), and for interleukin-2. To evaluate the possibility that CFS was a variant of the antiphospholipid syndrome, sera were also assayed for anticardiolipin antibodies (IgM and IgG).

Our initial evaluation used data for all 11 tests to determine for each subject the percentage of tests that had deviant results. This was done by dichotomizing every test result as being within or outside the laboratory's published reference range for normals. Ranges were based on means \pm 2 standard deviations (SDs) for all tests except C3 (mean $-$ 1SD and $+$ 2SD) and C4 (mean $-$ 0.8SD and $+$ 1.5SD). Values outside the reference ranges were identified as "deviant." These were unidirectional for all variables except complement components.

One-tailed *t* tests were used to evaluate the groups for statistically significant differences in percentages of all 11 tests with deviant values. Comparisons of frequency data used the appropriate Fisher's exact or chi-square test. Finally, we used logistic regression (11) and recursive partitioning (3) to select a subset of tests that discriminated well among the groups.

Interleukin-2 data were not used in these modeling procedures because of missing values.

Sixty-four patients were classified as having CFS; 33% of this group was able to work at least part-time. Thirty-one patients were classified as being CFS-psychiatric; 13% of this group was able to work at least part-time. Significantly more CFS patients than CFS-psychiatric patients ($P = 0.025$) were able to remain working. Twenty-four patients were classified as "fatigued controls" because they did not fulfill the CFS case definition. Seven did not report a 6-month history of fatigue that reduced daily activity by more than 50%. The remaining 17 patients did have this major criterion for CFS (8) but endorsed few of the CDC minor-symptom criteria (range, 2 to 7; median = 4 symptoms). In addition, the examining physician's clinical impression was that each patient's symptom complex was consistent with some other diagnosis (i.e., depression in 11, anxiety in 2, hypochondriasis in 1, possible mild post-viral fatigue syndrome in 2, and post-polio syndrome/depression in 1).

The mean percentage of tests with deviant values in the CFS-working group ($18\% \pm 3\%$) was lower but not significantly different from that in either the CFS-disabled group ($23\% \pm 2\%$) or the controls ($16\% \pm 2\%$), while that of the CFS-disabled group was significantly different from that of the controls ($P = 0.017$). Table 1 shows the distribution of deviant values in the 11 tests following stratification for disability in the two patient groups. Because only four members of the CFS-psychiatric group were working, their data were not used further in our analyses. The CFS-psychiatric-disabled group had more deviant values ($22\% \pm 2\%$) than controls ($P = 0.043$). The two CFS-disabled patient groups did not differ from each other significantly. Fatigued controls tended to have the most deviant values in CH50 and in the IgG subclasses. Thus, these variables did not discriminate well among the groups.

Logistic regression and recursive partitioning indicated that PAB, Raji, C4, and anticardiolipin IgM antibodies were important variables for discriminating CFS-disabled patients from controls and PAB, Raji, and C3 were important for discriminating CFS-psychiatric-disabled patients from controls. Since our goal was to derive a set of tests that might identify all CFS patients, regardless of prior psychiatric history, we decided to drop the anticardiolipin IgM antibody data from further analysis because CFS-psychiatric-disabled patients had no abnormalities in this variable (Table 1).

Because the only remaining difference in the choice of tests between the CFS and CFS-psychiatric groups was in the type of complement component associated with deviant values, our

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TABLE 1. Distribution of test results for CFS and control groups

Test ^a	Distribution of results											
	Above normal				Normal				Below normal			
	CSF-working	CSF-disabled	CSF-psychiatric-disabled	Fatigued controls	CSF-working	CSF-disabled	CSF-psychiatric-disabled	Fatigued controls	CSF-working	CSF-disabled	CSF-psychiatric-disabled	Fatigued controls
aCL IgG Abs	0	3	2	2	21	40	24	20	NA ^b	NA	NA	NA
aCL IgM Abs	1	5	0	0	20	38	26	22	NA	NA	NA	NA
C3	0	2	3	0	20	35	20	23	1	6	4	1
C4	0	2	2	0	19	38	25	24	2	3	0	0
CH50	4	4	3	2	11	26	18	16	6	13	6	5
IL-2	3	5	1	1	14	32	21	16	NA	NA	NA	NA
IgG1	NA	NA	NA	NA	14	35	23	17	6	8	4	7
IgG3	NA	NA	NA	NA	14	25	17	12	6	18	10	12
PAB	5	18	15	2	16	24	12	19	NA	NA	NA	NA
Raji	5	13	8	4	16	29	19	18	NA	NA	NA	NA
C1q	1	6	4	2	20	37	23	20	NA	NA	NA	NA

^a aCL IgG Abs, anticardiolipin IgG antibodies; aCL IgM Abs, anticardiolipin IgM antibodies; C3, C3 complement component; C4, C4 complement component; CH50, CH50 complement component; IL-2, interleukin-2; PAB, circulating immune complexes by protein A binding assay; Raji, circulating immune complexes by Raji cell replacement method; C1q, circulating immune complexes by C1q enzyme assay.
^b NA, not applicable.

next step was to explore the frequency of deviant tests for just PAB, Raji, and either C3 or C4. To increase sensitivity, we assessed the frequency of finding deviant results in all possible combinations of these three tests for the different patient groups (Table 2). Importantly, no fatigued control patient ever had deviant results on two tests and only four of the CFS-working patients had at least one pair of tests with deviant results in both tests. In contrast, 12 and 8 of the CFS-disabled and CFS-psychiatric-disabled patients, respectively, had pairs of tests with deviant results; this distribution of deviant results was statistically significant ($P = 0.044$).

The goal of this study was to arrive at a small group of tests that could differentiate the case-defined CFS patient from one not fulfilling the case definition. Our statistical techniques for exploring our data suggested a panel of three tests: the PAB or Raji cell assays of circulating immune complexes and complement component C3 or C4.

Because fatigued controls did have abnormalities in some of these tests (Table 1), we elected to see whether combinations of deviant test results served to discriminate the groups better.

TABLE 2. Number of patients having deviant results for combinations of three immunologic tests

Patient group	No. of patients with deviant results for:			
	PAB and Raji ^a	PAB and C3 or C4 ^b	Raji and C3 or C4 ^c	All three tests ^d
CFS-disabled ($n = 42$)	2	7	3	0
CFS-working ($n = 21$)	1	1	2	0
CFS-psychiatric-disabled ($n = 27$)	2	4	2	0
Fatigued controls ($n = 21$)	0	0	0	0

^a Number of patients in each group having deviant values in both of these tests for immune complexes.

^b Number of patients in each group having deviant values in immune complexes by the PAB method and in either C3 or C4 complement components.

^c Number of patients in each group having deviant values in immune complexes by the Raji cell displacement method and in either C3 or C4 complement components.

^d Number of patients having deviant values in both the PAB and Raji cell methods for quantifying immune complexes and in either C3 or C4 complement components.

This was the case: 29% of CFS-disabled and 30% of CFS-psychiatric-disabled patients were identified by these tests in comparison to none of the fatigued control group (Table 2). We must emphasize that this analysis was retrospective and only identified less than one-third of the most disabled CFS patients. An important next step will be to see whether the same results occur prospectively.

One surprise was the relatively high frequency of deviant values in the fatigued controls. The probable explanation for this is that these patients were not, in fact, healthy controls but rather had a mild form of CFS. One important conclusion from this study is that one can differentiate such patients from those with classical CFS. The recent move to relax the criteria for diagnosing CFS occurred because there were problems in differentiating between these groups (7).

Concerning CFS patients without prior psychiatric histories, the reduced set of three laboratory tests also allowed us to differentiate disabled patients from those who could work at least part-time. The CFS patient that could work resembled the fatigued control rather than the patient in the CFS-disabled group. These data suggest that the three laboratory tests used here may be nonspecific measures of illness severity rather than markers of any specific immunological dysfunction. This impression is reinforced by the fact that the assay method for quantifying circulating immune complexes that was most often positive, the PAB, is less specific than the other tests (10). Future research is needed to determine whether our findings are specific to CFS or are seen in other severely disabling diseases in which fatigue is prominent.

Another important finding is that these tests also differentiated CFS patients with psychiatric problems occurring prior to onset of their illness from fatigued controls. The existence of prior psychiatric problems is an exclusion criterion in the research case definition of CFS (12). However, there are data indicating that patients with psychiatric diagnoses have an increased risk of concurrent, serious medical conditions (14). Our data lead us to endorse stratifying CFS patients by prior psychiatric status rather than excluding them in subsequent studies to determine the cause of this illness.

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