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Autoimmune Technologies LLC
Applied Biomedical Science

SCIENCE SUMMARY

THE ANTI-POLYMER ANTIBODY ASSAY (APA ASSAY) and FIBROMYALGIA SYNDROME

The Anti-Polymer Antibody Assay, or APA Assay, detects an abnormal immune system response in most fibromyalgia patients. In one preclinical study, the titers of the antibodies detected correlated with nine separate clinical measures of fibromyalgia severity, including fatigue, stiffness, anxiety and depression. The APA Assay appears to be the first practical laboratory test for fibromyalgia, and the correlation between antibody titers and symptomatology appears to provide the first direct evidence that fibromyalgia syndrome is in fact a unique disease which is based on a physiological, and not a psychological, pathology.

INTRODUCTION

The Anti-Polymer Antibody Assay, or APA Assay, detects anti-polymer antibodies in the blood of most patients with fibromyalgia and fibromyalgia-like symptoms. Data presented in July 2004 at the International Myopain Society's Sixth World Congress on Myofascial Pain and Fibromyalgia described statistically significant relationships between the titers of these antibodies and nine different clinical measures of fibromyalgia severity. Previously published data strongly suggests that the presence of these antibodies distinguishes between patients with fibromyalgia and patients with connective tissue diseases such as systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, rheumatoid arthritis, and poly/dermatomyosis.

The Company continues to gather research data using the APA Assay and is preparing further publications. In addition, the Company is commencing FDA clinical trials of the APA Assay Test Kit for use as an aid in diagnosing fibromyalgia, in differentiating fibromyalgia from other disorders which have similar symptoms, and in identifying those fibromyalgia patients who are manifesting this unique, symptom-associated immune response.

The data presented in July 2004 was obtained from a study of well-characterized fibromyalgia patients which was conducted at the University of Texas Health Science Center in San Antonio (the "San Antonio study"). The San Antonio investigators were Yang-Ming Xiao, M.D., Ph.D., I. Jon Russell, M.D., Ph.D., and Joel E. Michalek, Ph.D. Dr. Russell is one of several world-recognized fibromyalgia experts, and Dr. Michalek is the principal investigator for the Air Force Ranch Hand Study, which is the largest and longest-running epidemiological study in the United States. Manuscripts describing the San Antonio study are now being readied for publication.

The APA Assay appears to be the first practical blood test for fibromyalgia. Until now, physicians have had only subjective measures available to assist them in identifying fibromyalgia patients. The Company believes the APA Assay to be a powerful tool which can be used by physicians to objectively identify fibromyalgia patients.

In part because of the historical absence of laboratory evidence to the contrary, many physicians currently feel that fibromyalgia is not a physically-based illness. These physicians instead believe that fibromyalgia is either a psychological disorder or that fibromyalgia symptoms are the product of some other disease which has not yet been correctly diagnosed. By directly associating immune response with symptomatology, the APA Assay demonstrates that fibromyalgia is in fact a unique physical disorder, or in lay terminology, "a real disease."

The data from the San Antonio study suggests that anti-polymer antibodies might play a direct role in the fibromyalgia disease process. Data from this study as well as from previously published studies indicate that approximately one-half to two-thirds of primary fibromyalgia patients test positive for anti-polymer antibodies. Primary fibromyalgia syndrome is believed by many researchers to have more than one cause, and it is entirely possible that fibromyalgia patients who test positive for anti-polymer antibodies represent a previously unrecognized majority group of fibromyalgia patients whose disease is the result of one particular cause.

Drugs which modulate a patient's immune response are frequently prescribed by physicians to help alleviate symptoms in autoimmune disease patients. The APA Assay shows that a symptom-associated abnormal immune response is present in APA-positive fibromyalgia patients, and physicians could choose to interpret this finding as an indication that such drugs might also be useful in treating these patients.

The titers of the antibodies detected by the APA Assay correlate with many measures of severity of symptoms of fibromyalgia. In contrast, other immunological test results rarely show any correlation at all between antibody titers and symptoms in any rheumatic disease. Because such correlations can in fact be explored by using the APA Assay, the test may also prove to be a valuable aid to physicians who wish to monitor symptom flare and other on-going conditions in their fibromyalgia patients.

THE RESEARCH STUDIES

An article published in the Journal of Rheumatology[(1)] entitled "Anti-Polymer Antibody Reactivity in a Subset of Patients with Fibromyalgia Correlates with Severity" shows that 47% of patients with fibromyalgia and 61% of patients with severe symptoms of fibromyalgia were seroreactive on the APA Assay. The pain thresholds, as determined by dolorimeter scores, for seropositive patients in the study were significantly lower than those of seronegative patients, demonstrating that APA reactivity correlates with severity of symptoms. No other reported laboratory measure has been found to correlate with severity of symptoms in patients with fibromyalgia.

An earlier study of a different group of patients with fibromyalgia-like symptoms showed similar results. In an article published in The Lancet[(2)] entitled "Use of Anti-Polymer Antibody in Recipients of Silicone Breast Implants," 68% of patients with advanced fibromyalgia-like symptoms exhibited APA Assay seroreactivity. The results of these two studies suggest that the presence of anti-polymer antibodies may serve as a laboratory marker for an illness that is the same or similar in both groups of patients. The APA Assay was also shown to be a very specific test, with a level of APA reactivity among patients (such as lupus patients) known not to have any fibromyalgia symptoms of approximately 3%.

Both articles also present results showing that APA reactivity is markedly lower ($p<0.05$) in patients with diffuse connective tissue diseases than in patients with fibromyalgia or fibromyalgia-like symptoms alone. Such connective tissue diseases include rheumatoid arthritis, systemic lupus

erythematosus and systemic sclerosis/scleroderma, and it can be difficult, particularly in severe cases of fibromyalgia, to differentiate fibromyalgia from these diseases.

The San Antonio study data presented in July 2004 at the Sixth World Conference on Myofascial Pain and Fibromyalgia[3] is entitled "Anti-Polymer Antibodies Identify a Large Subgroup of Fibromyalgia Syndrome Patients." The conclusions recited in the abstract are as follows:

> *The presence and titer of APA correlate with clinical measures, suggesting that these antibodies identify a large, previously unrecognized subgroup of primary fibromyalgia syndrome (PFMS) patients and that APA may be important in the pathogenesis of that PFMS subgroup.*

In the San Antonio study, statistically significant correlations ($p \leq 0.05$) were found between the optical densities of the ELISA test results (*i.e.,* the antibody titers) and the following nine clinical measures of fibromyalgia severity:

Measure	Description	Scale
STIFF	Degree of stiffness severity (VAS*)	0 - 10
FELTINAM	Feeling from "good" to "bad" in the morning (VAS*)	0 - 10
HOWTIRED	Degree of fatigue severity (VAS*)	0 - 10
LIMITACT	Symptoms-limited-activity days experienced during the last week	0 - 7
HEADACHE	Days in which headache was experienced during the last week	0 - 7
ANXIOUS	Level of anxiety (VAS*)	0 - 10
DEPRESS	Level of depression severity (VAS*)	0 - 10
ZUNG	The Zung depression index	0 - 100
CESD	The Center for Epidemiological Studies depression index	0 - 60

* As marked by the patient on a visual analog scale

In addition, the correlation between antibody titers and a tenth clinical measure of fibromyalgia severity approached statistical significance at $p = 0.06$. This tenth measure was ABDMPAIN, which represents the number of days in which abdominal pain was experienced during the last week[3],[4].

Anti-polymer antibodies have also been shown to belong to the IgG2 subclass of human immunoglobulins, a subclass of IgG composed of antibodies that typically recognize non-peptide antigens[5].

In the San Antonio study, APA seroreactivity was found in approximately 26% of the population of relatively healthy normal controls. Subsequent analysis revealed that the APA-positive members of the control group exhibited a statistically-significant ($p \leq 0.05$) increase in pain sensitivity as measured by dolorimeter, which suggests that anti-polymer antibodies are present in individuals with mild manifestations of fibromyalgia. A high APA reactivity rate among individuals who consider themselves to be healthy corresponds with the high rate of subclinical fibromyalgia found in studies by Clauw[6] and by Wolfe *et al*[7], which approached 20% of the adult female population in the United States.

Anti-polymer antibodies were discovered at Tulane University Medical School, and the APA Assay and has been licensed to Autoimmune Technologies, LLC of New Orleans. The APA Assay is currently covered by U.S., European and Australian patents, and other patents are pending.

The APA Assay is now being manufactured in commercial ELISA kit form. Because the APA Test Kit is undergoing FDA clinical trials it cannot be distributed in the U.S., though it is available in other countries. Individual tests for anti-polymer antibodies can be conducted in nitrocellulose strip format by Autoimmune Technologies as a service to interested physicians and researchers for investigational use.

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