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5747 '04 APR -9 10 10

April 8, 2004

Division of Dockets Management (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

Re: Docket # 2004D-0002 "New Draft Guidance Document for Breast Implants"

We would like to take this opportunity to commend the FDA for providing a comprehensive guidance document for approval of saline, silicone gel, and alternative breast implants and to provide suggested changes to this draft guidance document. We base our suggestions on over ten years of research concerning immunological aspects of silicone breast implants.

We would propose the following changes to Section 9.3:

rheumatic signs and symptoms

We would propose adding the measurement of average pain threshold of positive tender points as assessed by dolorimetry. Decreased pain threshold is a hallmark sign of fibromyalgia and adding this measure will provide increased sensitivity to identifying fibromyalgia in the study participants.

CTD evaluations

We would propose deleting "If indicated" for follow-up evaluations. The collection of data on serological information should be performed on all study participants. Collecting this data will provide increased sensitivity in identifying undiagnosed CTDs during the course of the study.

We would also propose that the analysis of anti-polymer antibodies (APA) be added to the serological evaluation of all study participants. In our research, we have found that APA are a silicone-associated immunological response and that the presence of these antibodies are clinically relevant in fibromyalgia. We have also found significant correlations between APA O.D. and scores for: stiffness; fatigue; limited activity; headache; anxiety and depression ( $p < 0.05$ ). We have provided a detailed description of our research concerning APA in the enclosed

2004D-0002

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information to support the proposed inclusion of the APA assay (aPAA) into the guidance document.

To correspond with our suggested changes to section 9.3, we would propose the following changes to Section 10.6:

The results of clinical evaluations including serological data should be presented. We propose that the data be reported for each cohort of patients as the mean of each test at each time point. In addition, the data should be presented for each cohort as the percent abnormal (out of normal range) at each time point.

The mean average pain threshold for each cohort for each time point should be presented.

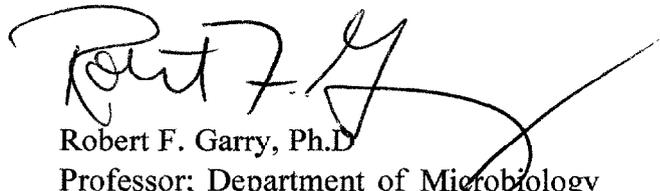
Analysis of the above data should include statistical analysis to determine if significant changes occur at each time point and if significant upwards or downwards trends occur over time.

Again, we would like to commend the FDA for the development of a comprehensive guidance document for breast implants. If you have any questions regarding our suggestions or the included information please contact us.

Sincerely,



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enclosures

**Summary Report  
and  
Supporting Documentation**

**Comments on: Docket # 2004D-0002  
“New Draft Guidance Document for Breast Implants”**

**Silicone, Fibromyalgia,  
and  
Anti-Polymer Antibodies**

**By**

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## Table of Contents

<b>1. Introduction .....</b>	<b>1</b>
<b>2. Background .....</b>	<b>1</b>
<b>2.1 Silicone .....</b>	<b>1</b>
<b>2.2 Fibromyalgia Syndrome .....</b>	<b>2</b>
<b>2.3 Silicone and FMS .....</b>	<b>4</b>
<b>3. The Anti-Polymer Antibody Assay .....</b>	<b>5</b>
<b>3.1 Initial Observations .....</b>	<b>5</b>
<b>3.2 Characterization of APA .....</b>	<b>8</b>
<b>4. Association of APA and Silicone .....</b>	<b>10</b>
<b>4.1 APA Seroreactivity in SBI Patients .....</b>	<b>10</b>
<b>4.2 APA Seroreactivity in Other Silicone Exposed Patients .....</b>	<b>12</b>
<b>5. Association of APA and FMS .....</b>	<b>14</b>
<b>5.1 Initial Study of APA seroreactivity in FMS, OA, and     Autoimmune Diseases .....</b>	<b>14</b>
<b>5.2 Association of APA Seroreactivity in FMS .....</b>	<b>15</b>
<b>5.3 APA Seroreactivity in FMS: Clinical Significance and     Identification of a Large Subgroup of APA Seropositive FMS     Patients .....</b>	<b>18</b>
<b>6. Discussion .....</b>	<b>29</b>
<b>7. Bibliography .....</b>	<b>31</b>

## APPENDICES

- Appendix 1:** Tenenbaum SA, Rice JC, Espinoza LR, Cuellar ML, Plymale DR, Sander DM, et al. Use of antipolymer antibody assay in recipients of silicone breast implants. *Lancet* 1997;349(9050):449-454.
- Appendix 2:** Wilson RB, Gluck OS, Tesser JR, Rice JC, Meyer A, Bridges AJ. Antipolymer antibody reactivity in a subset of patients with fibromyalgia correlates with severity. *J Rheumatol* 1999;26(2):402-7.
- Appendix 3:** VandeVord PJ, Gupta N, Wilson RB, Vinuya RZ, Schaefer CJ, Canady AI, et al. Immune reactions associated with silicone-based ventriculo-peritoneal shunt malfunctions in children. *Biomaterials* 2004;25(17):3853-3860.
- Appendix 4:** Praet SFE, van Blomberg M, Mulder JW, Geertzen R, Wilson R, Prins APA. Anti-polymeric antibodies, rheumatic complaints and silicone leakage of breast implants [abstract]. In: 14th European League Against Rheumatism Congress; 1999; Glasgow, Scotland; 1999.

## 1. Introduction

The anti-polymer antibody assay (aPAA) detects antibodies that bind to partially polymerized polyacrylamide in serum and other body fluids including cerebral spinal fluid. These antibodies were initially identified in silicone breast implant (SBI) patients and have been found to identify a group of SBI patients experiencing moderate to severe complications of a fibromyalgia-like illness (Appendix 1) (1). These antibodies have also been identified in a large group of fibromyalgia patients without implants and are associated with severity of illness in these patients as well (Appendix 2) (2). Further studies have revealed that these antibodies are also present in the majority of children with silicone-based ventriculo-peritoneal shunts who are experiencing sterile shunt malfunction (Appendix 3) (3). These patients experience many of the same local complications with their silicone-based tubing as those experienced by SBI patients. Taken together, these results indicate that these antibodies are associated with silicone exposure and fibromyalgia and suggest that a causal relationship exists between silicone exposure and fibromyalgia.

## 2. Background

### 2.1. Silicone

Over the last 40 years the use of silicones in the medical, pharmaceutical, cosmetic and food processing industries has become widespread. However, the safety of silicone remains an open question. The greatest controversy has surrounded the use and safety of silicones in breast augmentation. Breast augmentation using direct injection of paraffin, petroleum jelly and silicone gel into the breast began shortly after World War II in Japan and Korea. Many of the women undergoing these procedures developed local inflammatory complications and signs and symptoms suggestive of a systemic connective tissue disease. Physicians speculated that this syndrome developed as a consequence of activation of the immune system in response to the injected material and coined the term "human adjuvant disease"(4, 5).

In 1962, largely due to the presence of the local complications associated with the direct injection of silicone into the breast, the silicone gel implant was developed by enclosing the gel within a silicone elastomer shell(6). Again, anecdotal reports appeared in the literature suggesting that SBI patients were experiencing local complications similar to those found in patients receiving silicone injections, as well as signs and symptoms consistent with connective tissue diseases (CTDs)(7, 8). Recent epidemiological studies have confirmed that SBI patients experience severe local complications associated with their implants, but have failed to demonstrate a large association between SBI and well-defined CTDs(9-12). Unfortunately, these epidemiological studies have been limited in their ability to detect small increases in well-defined CTDs. In addition, they have not ruled out the possibility that symptomatic SBI patients have either a new CTD or an atypical CTD such as FMS(13).

The presence of local inflammatory reactions resulting from silicone injection or implantation suggests that there is an immunological response to silicone. However, the issue of whether silicone-implant exposure results in a specific antibody response has been very controversial, and there is conflicting evidence as to whether silicone itself is immunogenic or indirectly causes an immune response by acting as an adjuvant(14-23). To

determine if anti-silicone antibodies exist, several attempts have been made to develop specific assays that detect these antibodies, if they exist, in patients exposed to silicone implants(20, 24, 25). However, the assays that have been developed so far appear to be limited by the non-specific binding of proteins, including immunoglobulins, to silicones(26).

The presence of anti-silicone antibodies in silicone-exposed patients was first reported by Goldblum et al.(20). Using an ELISA-based assay to measure anti-silicone antibody binding to Silastic tubing, sera from two patients experiencing an inflammatory response associated with their silicone-based ventriculoperitoneal (VP) shunts were examined for the presence of anti-silicone antibodies(20). Both patients' sera were found to contain significantly higher amounts of IgG that bound to the tubing when compared to normal controls or to a group of shunt patients who were not experiencing inflammatory problems. The binding of IgG to the tubing could be reduced by pre-incubation of the sera with Silastic tubing, and Fab fragments prepared from one of the patient's purified IgG retained the ability to bind to the tubing. These results suggest that Goldblum et al.(20) had demonstrated specific binding of anti-silicone antibodies to Silastic tubing. However, a subsequent report by the authors(27), in abstract form only, suggested that the binding of IgG to the tubing was dependent on albumin concentration and as a result, was non-specific.

Wolf et al.(24) have reported the detection of anti-silicone antibodies using polydimethylsiloxane adsorbed to microtiter plates coated with bovine serum albumin. Using this assay, the investigators found that sera from silicone breast implant patients had higher levels of IgG binding than sera from control groups without breast implants. They also found that patients with clinically confirmed rupture or leakage of their implants demonstrated even higher levels of bound IgG compared to patients with no known rupture of their implants. Unfortunately, it was unclear if this assay was measuring specific binding since approximately half of the observed binding was non-specific(23).

## 2.2 Fibromyalgia Syndrome

Fibromyalgia Syndrome (FMS) is a common chronic disorder of widespread pain that afflicts millions of individuals(28). Associated signs and symptoms include tender points, fatigue, morning stiffness, sleep disorder, headache and cognitive problems(29). Not all of the signs and symptoms are present in every patient, and each individual patient may have different signs and symptoms at different times. The signs and symptoms are often debilitating, and they require major lifestyle changes in many patients.

Most patients report feeling some pain all the time, and many describe it as being "exhausting." The pain can vary, depending on the time of day, weather changes, physical activity, and the presence of stressful situations, and may be described as stiffness, burning, radiating, and aching. The pain is often more intense after disturbed sleep. The other major complaint is fatigue, which some patients report as being more debilitating than the pain. Fatigue and sleep disturbances appear to be almost universal in patients with FMS(28).

The American College of Rheumatology (ACR) first formally defined FMS in 1990(29). The ACR criteria for diagnosis requires that a patient manifest localized tenderness in at least 11 of 18 specific sites on the body (referred to as tender points) and a history of chronic wide-spread pain of greater than 3 months duration in order to receive a diagnosis of FMS. The disorder was at that time a source of controversy among physicians, and it remains so today. The ACR adopted its diagnostic criteria so that cohorts of FMS patients

could be identified for research purposes and that the disorder could be further characterized on a systematic basis. The fundamental issue upon which physicians continue to disagree is whether FMS is physically based or psychologically based. The absence to date of any laboratory evidence that FMS patients have a distinct physical disorder has done much to keep the controversy alive(30).

Using ACR criteria, estimates for the prevalence of FMS in the general population range from 2% to 10% with about a ten-fold higher incidence in women compared to men (31, 32). These estimates of the prevalence of FMS may be low since approximately 23% of the adult female population have 6 or more positive tender points(29) and between 15% and 20% of adult U.S. women experience chronic widespread pain for greater than 3 months duration(33). Patients with FMS account for 15-20% of the patients seen in rheumatology practices (34). In Canadian rheumatology practices FMS was found to be the second most common diagnosis, and the percentage of patients presenting with FMS was perceived by Canadian rheumatologists to be increasing (35).

Diagnosing a patient complaining of signs and symptoms associated with a variety of rheumatic diseases and FMS is difficult and can be a long process. In fact, the average time between symptom onset and diagnosis is 5 years(36). Further complicating the diagnosis of FMS is the fact that many physicians do not adhere to the ACR diagnostic criteria. For example, the seventeenth (1999) edition of The Merck Manual of Diagnosis and Therapy includes no stipulation that any minimum number of tender points be evident for a patient to be given a diagnosis of fibromyalgia(37). The normal number of tender points is zero.

There are more than 100 different diagnoses of rheumatic diseases, and arthralgia is the most common manifestation, but muscles, skin, and blood vessels are often involved. Many of these conditions have overlapping constellations of symptoms, especially in the early stages of the disorder making a clear-cut diagnosis sometimes problematic. The classification criteria of arthritis and the connective tissue diseases of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis/dermato-myositis, vasculitis, Sjögren's syndrome, and other miscellaneous diseases are reviewed during patient work-up. Most diagnoses of rheumatic diseases require subjective and objective evidence of disease that is supported by laboratory or radiographic abnormalities. The basis for ACR classification criteria for rheumatic diseases includes symptoms (subjective complaints), signs of clinical disease, and laboratory testing.

Making a diagnosis of fibromyalgia is almost always an exclusionary process, because a physician makes the diagnosis only after ruling out all other disorders that are likely to be the cause of the patient's symptoms. It is especially important to rule out systemic lupus erythematosus, since this condition can be life-threatening(38). So far, no existing laboratory test has been useful in detecting any anomaly in fibromyalgia patients, and the absence of a meaningful laboratory test plays an important role in forcing exclusionary diagnoses. In addition, FMS is frequently misdiagnosed as polymyositis, rheumatoid arthritis, juvenile rheumatoid arthritis, or systemic lupus erythematosus due to the lack of objective markers resulting in inappropriate treatment and increased medical costs(39).

A recent study examining the costs of FMS found that overall, in the U.S., FMS accounts for approximately \$15 billion per year in direct medical costs alone. On an individual basis, the cost for FMS is more than \$2,200 per patient. The largest contribution to the direct costs is hospitalization and outpatient visits as FMS patients average 10

outpatient medical visits per year and 1 hospitalization every 3 years. The second largest contributor to medical costs associated with FMS is drug use(36).

Current FMS treatment modalities focus on managing the symptoms of the disorder. The physician, the patient, and sometimes a physical therapist may all play an active role in the management of FMS, and patients may benefit from a combination of exercise, medication, physical therapy, and relaxation. Studies have shown that aerobic exercise, such as swimming and walking, improves muscle fitness and reduces muscle pain and tenderness. Heat and massage may also give short-term relief. Anti-depressant medications may help elevate mood, improve quality of sleep, and relax muscles. Aspirin and other NSAIDs have not generally been shown to be effective in clinical trials but sometimes help individual patients(28, 40)

Current therapeutic regimens give no consideration to possible immune system involvement in FMS patients, and current laboratory tests provide no evidence of any such involvement. However, a recent study found that 5/10 FMS patients experienced significant improvement in signs and symptoms after a 16 week regimen of hydroxychloroquine, a drug that inhibits antigen presentation and is used to treat rheumatoid arthritis patients(41).

Failure to identify effective treatments for FMS patients is due, at least in part, to the fact that the etiology of this affliction, despite intense research, has not been elucidated, nor has the pathogenic mechanisms been determined(40). Recent studies have suggested that alterations in sleep (42), insulin-like growth factor I levels (43), tryptophan levels (44), and serotonin levels (45) are possible initiators of FM, but none of these alterations are found in all FMS patients. These results suggest that FMS may not be a distinct syndrome but may encompass several patient populations that have overlapping signs and symptoms. A recent study that 24% of FM patients report acute onset of disease following physical trauma and 14% report onset following emotional trauma furthers the concept that there are multiple subsets of FMS patients(46).

### 2.3. Silicone and FMS

Recent epidemiological studies have either failed to show significant increases in the incidence of classic autoimmune diseases in SBI-exposed individuals or have documented a small increased risk(9-12). However, several studies have suggested that SBI-recipients may experience a syndrome similar to those observed in patients with FMS (47-51). In addition, two studies have found that approximately 40% of SBI patients referred for evaluation met criteria for the diagnosis of FMS(52, 53). These studies suggest that there may be a casual association between SBI and FMS.

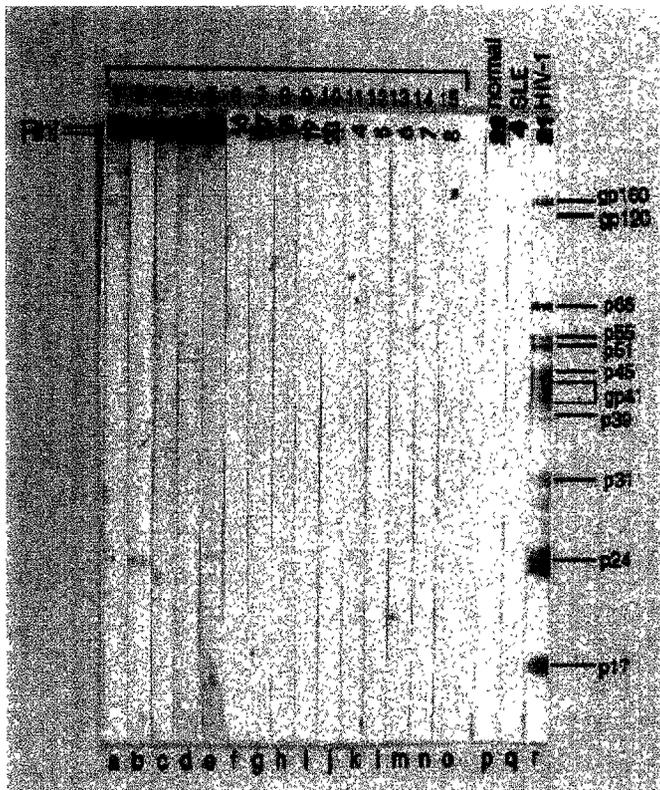
A causal association between silicone and FM is further suggested by a recent epidemiological study by Brown et al.(54). In their study, SBI patients with extracapsular leakage of silicone were found to be at a significantly higher risk of having physician-diagnosed FMS compared to SBI patients without leakage (24.7% vs. 10.7%;  $p < 0.004$ )(54). Though not compared in the study by Brown et al.(54), the rate of diagnosed FMS in SBI patients without leakage, 10.7%, is higher than the reported 3% prevalence of FMS in adult women in the general population(31). This suggests that the presence of silicone implants alone, without leakage, may also result in an increased risk of FMS.

### 3. The Anti-Polymer Antibody Assay

#### 3.1 Initial observations

To test whether patients experiencing silicone-implant complications produced specific antibodies to any of the common autoantigens recognized by patients with other autoimmune conditions (i.e. Sm, Scl-70, SS-A, etc.) or perhaps to retroviral antigens we used a HIV western blot to detect the presence of serum antibodies to proteins purified from human cells (Garry *et al.*, *Science* **250**, 1127-1129, 1990; reviewed in Garry *et al.*, *The Retroviridae*, vol. IV, pp. 491-603, 1995). We observed that recipients of silicone breast implants produced serum antibodies to certain autoantigens. The level of reactivity, particular to ribonuclear proteins, was higher than in the normal controls (55). However, there was no pattern diagnostic for other systemic autoimmune diseases, such as SLE.

In contrast to results with protein autoantigens, we found that patients experiencing complications following silicone-breast implantation produced serum antibodies that recognized a large molecular weight component (Rhl) present on commercially available immunoblot strips containing human cellular and HIV proteins (Figure 1).



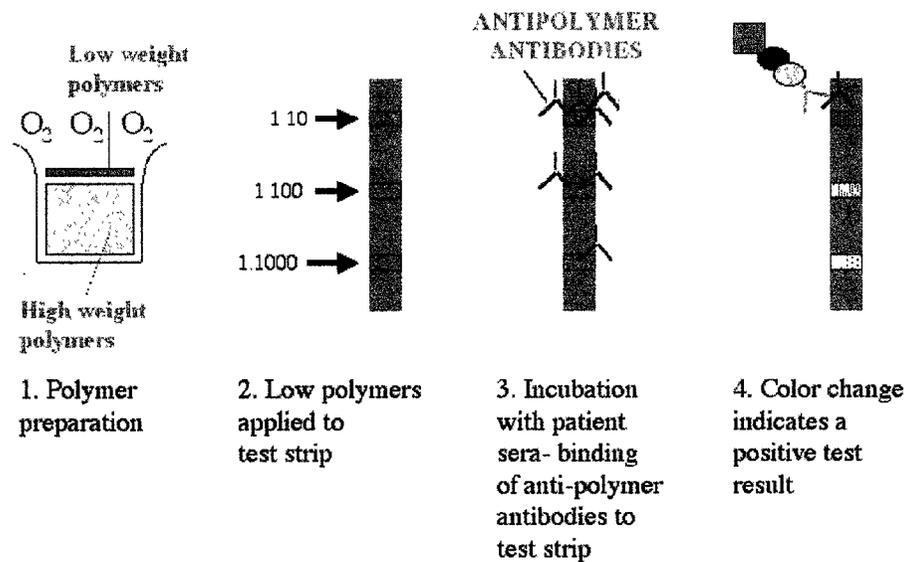
**Figure 1. HIV western blot analysis.** Sera from SBI patients (Lanes 1-15) were diluted 1:400 in blocking buffer and applied to commercially available HIV western blot strips. The strips were then processed as per the manufacturer's instructions. Controls included no sera (lane 16), sera from a normal control (Lane 17; Normal) and a patient with SLE (Lane 18, SLE) and HIV-1 positive control sera (Lane 19, HIV-1).

After further characterization, we have concluded that this antigen is not a protein, but a complex composed of partially polymerized acrylamide (PPA). Polyacrylamide gels

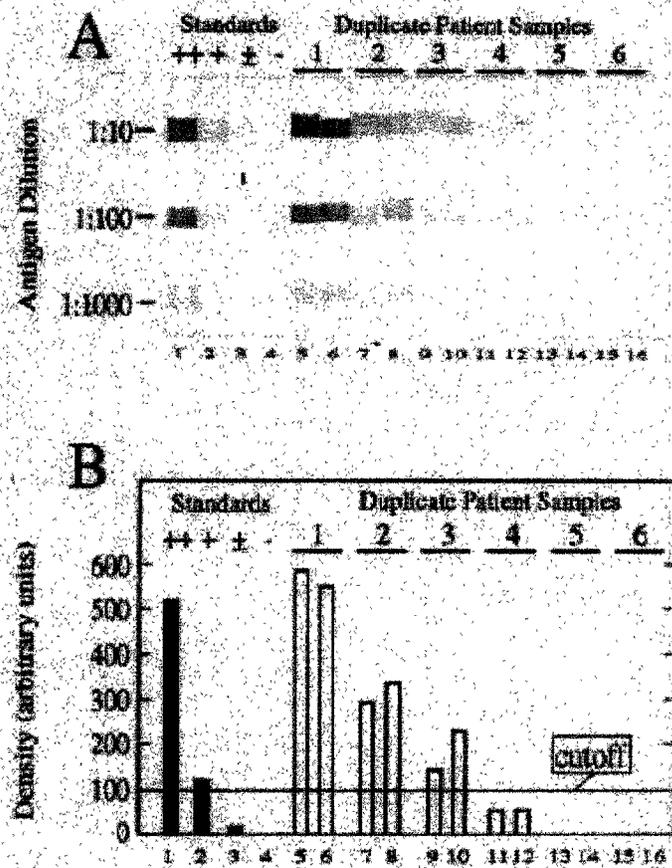
are formed as a result of free-radical polymerization. Compounds that can act as a free-radical “trap” inhibit the cross-linking procedure. Oxygen present in the air is such a compound and even after gel degassing, oxygen in the air can still inhibit complete polyacrylamide cross-linking at the gel-air interface. This results in a thin layer of PPA formed at the air-gel interface. Upon western blot transfer, the PPA is electrophoretically transferred to nitrocellulose, along with proteins in the gel.

The amount and clarity of PPA present varied considerably on the commercially available immunoblot strips used in preliminary investigations. Therefore, we developed a PPA line-blotting procedure that is more consistent and sensitive in detecting antibodies that bind to PPA. Because of the polymer nature of the antigen, we have called the antibodies that recognize PPA anti-polymer antibodies (APA) and have called this test the Anti-Polymer Antibody assay (aPAA) (Figure 2 and 3).

## The anti-polymer antibody assay



**Figure 2. The Anti-polymer antibody assay.** Aliquots of PPA are sequentially diluted 10, 100, and 1000 fold and applied to nitrocellulose membranes and allowed to air dry. The nitrocellulose membranes are then cut into 4 mm wide strips and incubated with patient sera diluted in blocking buffer for 1 hr. Specifically bound immunoglobulins are then visualized by a series of reactions using biotinylated goat anti-human IgG, avidin-conjugated horseradish peroxidase, and the enzyme substrates hydrogen peroxide and 4-chloro-1-naphthol.



**Figure 3: aPAA Examples**

A: APA immunoblots produced with polymer diluted 1/10, 1/100, and 1/1000; serum from six SBI recipients (1-6) run in duplicate; ++ (strong positive), + (positive), ± (weakly reactive), and - (negative) sera run as assay controls; all sera diluted 1/400.

B: APA immunoblot Images analysed with NIH image 1.55; cut-off value defined by comparison with values obtained with sera from appropriate control populations. (from Tenenbaum *et al.*, *Lancet* 349, 449-454, 1997; see Appendix 1)

To define the specificity and sensitivity of the aPAA, an unblinded study was performed utilizing serum samples from a previously described cohort of SBI recipients experiencing local and systemic complications(53). The percentage of persons reporting complications following silicone breast implantation that were seroreactive on the aPAA was greater than 50% (Table 1). This was a significantly greater frequency of APA positivity than the prevalence of 9% in healthy blood donor sera obtained from the Tulane University Hospital blood bank (OR=17.0, 95% CI=7.55-46.7,  $p < 0.0005$ ). Sera from systemic lupus erythematosus (SLE) or rheumatoid arthritis patients (RA) demonstrated APA seroreactivity in less than 10% of the cases. Therefore, the presence of APA does not appear to be a general marker for rheumatic diseases(1).

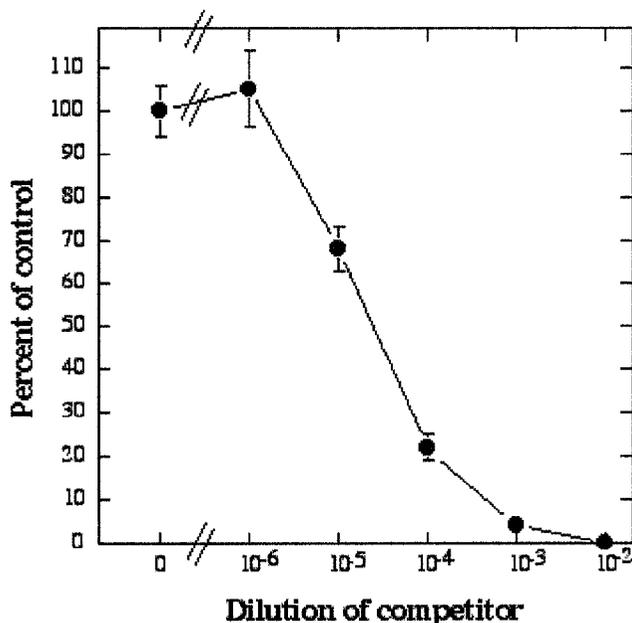
Table 1: Unblinded study of APA in SBI recipients, women with autoimmune disease, and healthy donors (from Tenenbaum *et al.*, *Lancet* 349, 449-454, 1997; see Appendix 1)

Group	Number positive for APA*
Healthy blood donors (n=100)	9 (9.0%)
SBI recipients (n=667)	363 (54.4%)
Systemic lupus erythematosus (n=205)	13 (6.3%)
Adult rheumatoid arthritis (n=92)	3 (3%)

\*Seroreactivity was defined by visual comparison to positive controls.

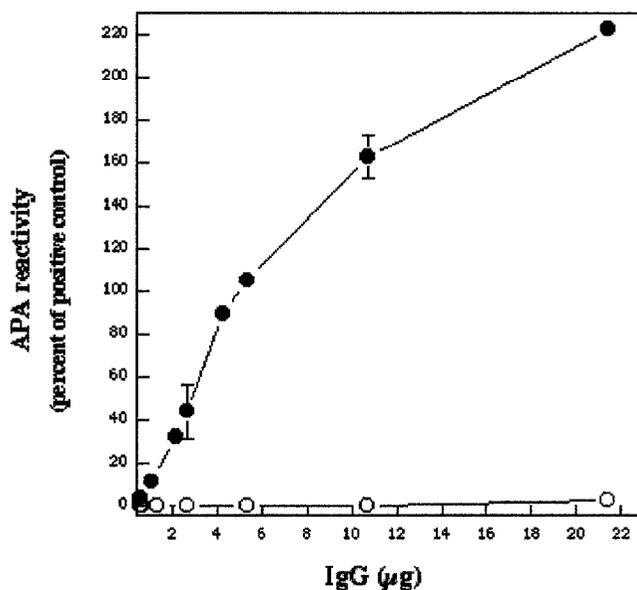
### 3.3. Characterization of APA

To demonstrate that the observed binding of antibody to partially polymerized acrylamide impregnated on nitrocellulose strips is specific, aPAAs were performed in which 10-fold serial dilutions of the antigen were included as a competitor during the incubation with control antisera. Each dilution was tested in triplicate, and the experiment was duplicated on a different day. The strips were processed and digitized, and the optical density for each band was determined. Data from the two experiments were combined (six data points for each dilution), and expressed as a percent of control without competitor. As shown in Figure 4, the data clearly show that excess partially polymerized acrylamide effectively competes for antibody binding to the assay strips and demonstrates that the aPAA is specific and reproducible.



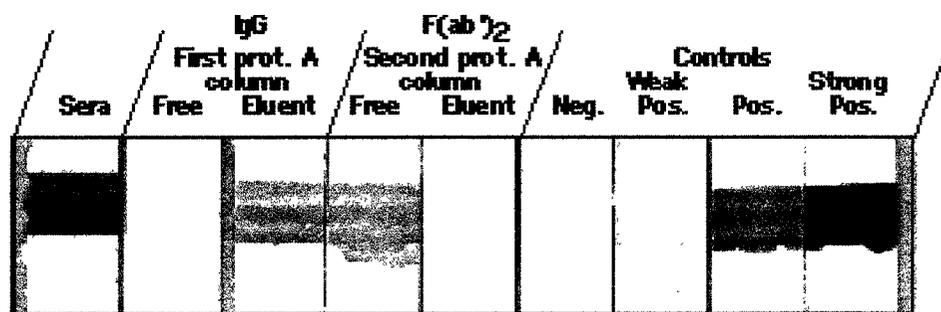
**Figure 4.** Partially polymerized acrylamide competition. Partially polymerized acrylamide stock solution was 10-fold serially diluted in blocking buffer. The dilution series was then applied to APA Assay strips in triplicate and positive control antisera was added to each strip (1:400 dilution). The strips were then processed for the APA assay. Data from two experiments were combined and expressed as a percent of controls that did not receive partially polymerized acrylamide. Error bars are SD/control x100. (from VandeVord et al. Biomaterials, in-press, 2004; Appendix 3)

To determine if the observed binding of APA to partially polymerized polyacrylamide was independent of total IgG concentration and the presence of non-IgG serum proteins or factors, total IgG was purified from both APA seropositive and seronegative SBI-patient sera samples using a protein A column. Aliquots containing up to 21.4  $\mu\text{g}$  of purified IgG from each of the samples were then analyzed on the APA assay. To exclude the possibility that any serum component or non-IgG-related protein was involved in binding of immunoglobulin to the strips, the blocking buffer used during this step in the APA assay was changed to a formulation that did not contain any serum or protein (PBS plus 0.1% Tween -20). (Goat serum is routinely used as a component of the blocking buffer in the APA assay.) As shown in Figure 5, purified IgG from a seropositive SBI patient retained the ability to bind to partially polymerized polyacrylamide, while purified IgG from an APA seronegative SBI patient failed to bind antigen at any concentration of IgG tested. These results demonstrate that the binding of APA-specific IgG to partially polymerized polyacrylamide was independent of total IgG concentration and independent of non-IgG related serum components, including albumin.



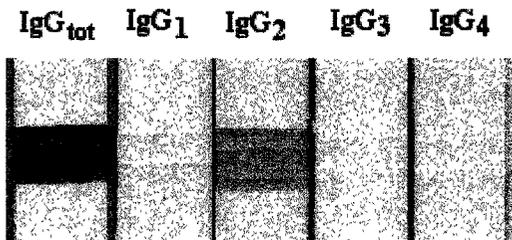
**Figure 5.** Purified IgG retains APA binding. Total IgG was purified from an APA positive and an APA negative patient sample using protein A columns. Aliquots of the purified IgG samples were diluted in PBS plus Tween-20 and applied in triplicate to APA immunoblot strips. Positive and negative controls were also included. Following the processing of the strips for the aPAA, the strips were digitized and the rO.D. determined. The data is expressed as a percentage of the mean positive control rO.D. Error bars are (SD/control) x100. (from VandeVord et al. Biomaterials, inpress, 2004; Appendix 3)

The specificity of APA binding to antigen was further characterized by testing the ability of purified (Fab')<sub>2</sub> fragments of total IgG from an APA seropositive serum sample to bind partially polymerized polyacrylamide. IgG was purified from a second seropositive sample using a protein A column and digested with pepsin to generate (Fab')<sub>2</sub>. Undigested IgG was removed using a second protein A column. Samples from the original sera, the flow-through from the first protein A column, the eluted IgG, the flow-through from the second protein A column containing the (Fab')<sub>2</sub>, and the undigested IgG were analyzed on the APA assay. As shown in Figure 6, both the purified IgG and the (Fab')<sub>2</sub> retained the ability to bind partially polymerized polyacrylamide demonstrating that the binding of APA to the antigen was not due to a non-specific interaction between the antigen and the Fc portion of IgG.



**Figure 6.** F(ab')<sub>2</sub> fragments retain APA binding. F(ab')<sub>2</sub> fragments were generated from a positive APA sera sample using a protein A column and immobilized pepsin. Aliquots of the starting sera, the first protein A column flow-through and eluted IgG fractions, and the second protein A column flow through (F(ab')<sub>2</sub> fragments) and eluted uncleaved IgG fractions were applied to APA immunoblot strips and processed for the aPAA. Strong positive, positive, weak positive, and negative controls were also assayed.

The subclass of APA IgG was determined using biotinylated mouse monoclonal antibodies specific for each of the four human IgG subclasses. Sera from ten APA seropositive SBI samples were pooled to provide a representative sample and applied to APA test strips. The strips were then processed as usual except that an anti-human IgG subclass-specific monoclonal antibody was substituted, at a 1:100 dilution, for the secondary goat-anti-human total IgG antibody. As shown in Figure 7, the IgG that bound to partially polymerized polyacrylamide was predominately of the IgG2 subclass. A positive control consisting of nitrocellulose strips impregnated with human total IgG was used to assess the ability of the monoclonal antibodies to bind to IgG and be detected in the assay. On the control strips, all monoclonal antibodies except the monoclonal specific for IgG4 produced intense bands (data not shown). The reaction product produced by the IgG4 monoclonal antibody was weaker, but was clearly detectable. The difference in intensity may be related to the lower concentration of IgG4 normally present in human sera (56).



**Figure 7.** Determination of IgG subclass of APA. APA strips were incubated with serum from an APA positive patient as described in the methods. To determine the subclass of APA IgG, the strips were subsequently incubated with biotinylated mouse monoclonal antibodies specific for each human subclass of IgG. As a control total human IgG ( $IgG_{tot}$ ) was detected as described in the methods using goat anti-human IgG.

The results from the analysis of APA presented here demonstrate that the binding of APA to partially polymerized polyacrylamide is specific, independent of immunoglobulin levels and other serum factors, including albumin, and limited to the  $(Fab')_2$  fragment of IgG. Furthermore, the determination that the IgG subclass of bound APA IgG is predominately IgG2 further indicates that the binding of APA is specific.

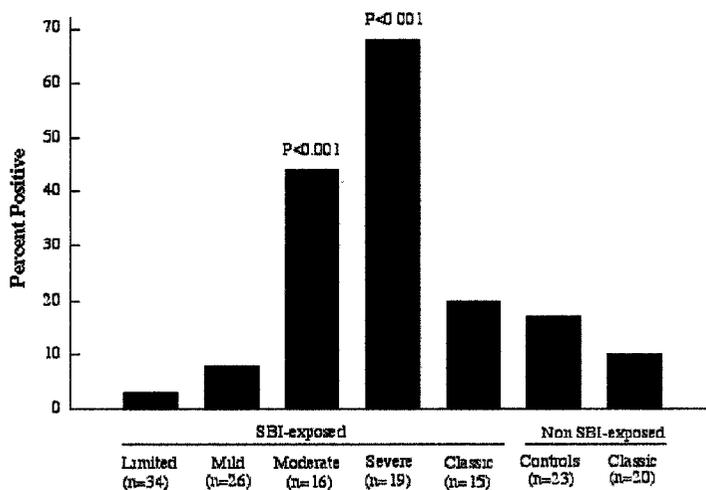
## 4. Association of APA and Silicone

### 4.1 APA seroreactivity in SBI patients

Based on the preliminary finding of the presence of APA in SBI patients, a blinded, single-center study was conducted to further characterize the APA assay and begin to examine the populations that are positive for the presence of APA. SBI recipients manifesting a range of signs and symptoms were recruited to determine whether or not the presence of APA correlates with severity of clinical complications. SBI exposed and non-exposed patients with specific autoimmune diseases, including SLE and Sjögren's syndrome, and healthy control subjects were also recruited. A history and physical was completed on all study participants and used to group the SBI recipients who did not meet criteria for specific autoimmune diseases as to the severity of their signs and symptoms and functional capacity (please see Tenenbaum *et al.*, *Lancet* **349**, 449-454, 1997 for a further discussion of the demographics of our study population; Appendix 1).

The serum samples obtained for this study were tested using the aPAA run with various dilutions of antigen and serum. At optimum serum (1:400) and antigen dilutions (1:10), objectively quantitated APA reactivity increased with severity of symptoms (limited = 1/34, 3.0%; mild = 2/26, 8%; moderate = 7/16, 44%; advanced = 13/19, 68%), a trend that was statistically significant ( $p < 0.001$ ) (Figure 8). When compared with the incidence of seroreactivity in SBI exposed women in the limited group, the incidence of APA reactivity was significantly higher in SBI-exposed individuals with moderate ( $p < 0.01$ , OR=25.7 95% CI=2.73-241) or advanced ( $p < 0.001$ , OR=71.5 95% CI=7.68-665) musculoskeletal and other signs and symptoms.

Neither SBI-exposed nor non-SBI exposed individuals with classic autoimmune diseases or SBI-exposed individuals with limited/mild signs and symptoms had significantly elevated APA seroreactivity relative to controls.



**Figure 8. Anti-polymer antibodies in SBI-recipients: results of a blinded trial.** aPAA were performed on SBI recipients with limited to advanced complications determined by Physician's Global Assessment and functional disability rating. SBI exposed and non-exposed patients with classic autoimmune diseases and non SBI-exposed controls were also tested. The p values refer to the comparisons with SBI recipients in the limited group (from Tenenbaum *et al.*, *Lancet* 349, 449-454, 1997; see Appendix 1).

These results were the first evidence from a blinded study for the existence of a laboratory marker that correlates with the severity of musculoskeletal and other signs and symptoms in SBI recipients. This study indicates that the aPAA, which measures antibody reactivity to PPA, a complex synthetic polymer, may objectively contribute to distinguishing between SBI recipients with limited to mild signs and symptoms from SBI recipients with moderate to advanced signs and symptoms. The correlation between the results of the APA assay with severity indicates that APA seropositivity is not merely a marker for exposure to silicone from SBI.

A second preliminary study of the association of APA and SBI was undertaken to evaluate the relation between APA, rheumatic complaints and extra-capsular leakage of silicone from SBI (Appendix 4)(57). In this study, it was found that APA levels increased after surgery, and that the presence of APA was associated with histologically proven extra-capsular leakage of silicone from the implant.

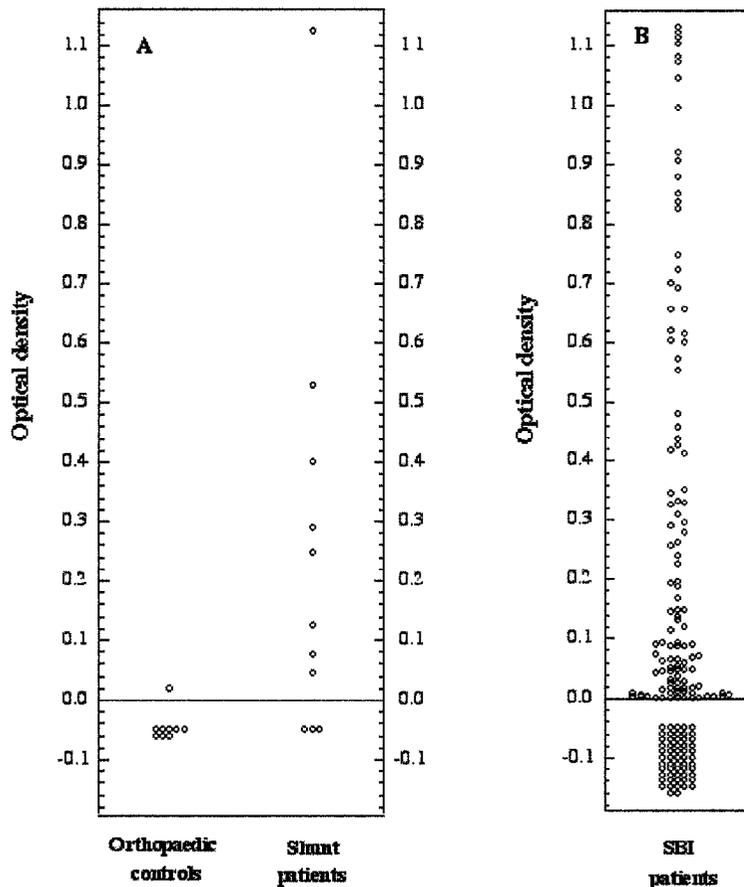
#### 4.2 APA seroreactivity in other silicone implant exposed patients

As discussed, Tenenbaum et al (1) demonstrated that APA are present in the majority of symptomatic SBI patients, however, it was unknown if the presence of APA was limited to SBI patients or if APA are present in other silicone-implant-exposed patient populations. To examine this question, we have analyzed sera from patients with silicone-based ventriculoperitoneal (VP) shunts. Similar to the situation with SBI, many VP shunt patients experience problems attributed to their implant including sterile malfunction, which has been proposed by Grower et al (58) to result from a delayed hypersensitivity to silicone, and undergo repeated replacement of their silicone implant. Several case reports of patients requiring multiple shunt revisions have demonstrated that shunt-related problems including skin erosion over the sight of the tubing, extrusion of the shunt, and shunt failure, resolve following replacement of the silicone shunt with a polyurethane-based device (59, 60).

Serum samples were obtained from VP shunt patients (n=11) undergoing revision of their shunts due to infection (n=2), elective lengthening (n=2), or sterile shunt malfunction (n=7). Control sera samples (n= 9) were obtained from orthopedic surgery patients. All samples were coded and blinded prior to shipment for APA analysis. In addition to the sera from VP shunt patients, banked sera from 168 SBI patients were also analyzed for APA seroreactivity. These sera were from a collection of sera from >1000 physician and attorney referred SBI patients evaluated at several rheumatology clinics in the northeastern US. These patients had one or more symptoms that were thought by either the patient or physician to be related to their implants. The 168 samples were picked without regard to any criteria except that a single rheumatologist had seen them all.

Following analysis, the prevalence of seroreactivity was found to be significantly higher in the shunt patients overall (8/11; 73%) as compared to the controls (1/9; 11%;  $P<0.01$ ) and similar to the prevalence observed in the SBI patients (111/168; 66%) (Figure 9). Five out of 7 of the patients with sterile malfunction and 2 out of 2 of the patients with infection were positive. One of the 2 patients undergoing elective lengthening was weakly positive.

Clinical data on the patients was mostly unremarkable except for the presence of increased eosinophils in one patient with a sterile malfunction of their VP shunt, who also was the highest responder on the aPAA. The presence of increased eosinophils has been observed in shunt (60) and SBI patients (61) reported to have "silicone allergy". No correlation of APA results with immunoglobulin profiles or levels was observed. Time since last revision ranged from less than 1 month for the 2 patients with infection, between 1 month and 39 months for the sterile malfunction patients (mean of 11.3 months) and greater than 48 months for the 2 patients with revision for elective lengthening was observed. Two of the patients experiencing sterile shunt malfunction were remarkable in that one has had 97 revisions since 1992 and another, a 4-year-old patient with congenital hydrocephalus, has had 35 revisions.



**Figure 9. rO.D. Scatter plots.** Sera from VP shunt patients and controls (A) and SBI patients (B) were diluted 1:200 and applied to APA immunoblot strips. The strips were incubated for 90 min with gentle rocking. Specifically bound immunoglobulins were then visualized by a series of reactions using biotinylated goat anti-human I g G , avidin-conjugated horseradish peroxidase, and the enzyme substrates hydrogen peroxide and 4-chloro-1-naphthol. The strips were then scanned and digitized. Mean pixel data was converted to rO.D., and the data was plotted versus category.

In a second in-press study of APA in silicone-based VP shunt patients (see VandeVord et al. *Biomaterials*, in-press, 2004; Appendix 3)(3), sera was obtained from 39 children (female n=18, male n=21) requiring surgical shunt revision. Patients ranged from 2 to 20 years of age, with the average age as 10.5 years. Three groups were identified based on the diagnosis for surgical revision: sterile malfunctions (n=24), malfunctions due to infectious causes (n=8), and elective shunt lengthening (n=7). All VP shunting systems were silicone based and from the same manufacturer. In this study an ELISA-based aPAA was used to detect APA.

In 30 of the 39 patients (77%), antibodies to partially polymerized acrylamide were detected in the sera. There were differences amongst the three groups, with the average OD reading being highest in the infection group ( $2.47 \pm 0.55$ ), followed by the malfunction group ( $1.25 \pm 0.32$ ) and the elective lengthening group ( $0.74 \pm 0.24$ ). Statistical significance occurred between the infection and malfunction groups ( $p < 0.041$ ) and between the infection and the elective lengthening groups ( $p < 0.018$ ).

Implantation time and number of revision surgeries were statistically significant when evaluating between groups. As expected, the implantation time was significantly less for the infection and malfunction groups as compared to the elective lengthening group ( $p < 0.001$ ). The number of revision surgeries per each patient was also less for the elective lengthening group (4.71) as compared to the malfunction group (16.62) and the infection group (34.71).

The finding of APA in patients who have silicone-based VP shunts demonstrates that anti-polymer antibodies are not just associated with silicone breast implant exposure, and

supports a hypothesis that exposure to silicone of various types (breast implants, gel, tubing, etc.) can induce a unique immunological response: production of APA. In addition, similar to the findings of Tenenbaum et al (1), the majority of symptomatic SBI patients were seroreactive on the assay further demonstrating the reproducibility of the APA assay and supporting Tenenbaum et al's findings.

Several possibilities exist which may explain the presence of antibodies to partially polymerized polyacrylamide in silicone-implant-exposed patients. First, the generation of APA may result from molecular mimicry (62) and represent an immune response directed against silicone, byproducts of silicone catabolism, or another component of silicone implants that cross-reacts with partially polymerized polyacrylamide. If this is the case, then this antigen should compete with partially polymerized polyacrylamide for binding of antibody. Experiments are underway to determine if common silicone compounds can compete in the aPAA, however, due to the nonspecific binding of proteins to silicones (26), direct competition may be difficult to determine. Second, silicone may function as an adjuvant and/or physically interact with cellular components present in the surrounding tissue (63-65). This interaction may result in the structural alteration of the silicone or the cellular components such that they resemble partially polymerized polyacrylamide to the immune system. Third, because silicone and partially polymerized polyacrylamide are both cross-linked polymers, it is possible that any antigenic relatedness between these substances results from the type and degree of cross-linking, and not from the chemical composition of the polymer. Fourth, it is also possible that through epitope spreading (66) an initial antibody response to silicone may be expanded to other epitopes, some of which are shared by partially polymerized polyacrylamide. Despite our lack of understanding of how silicone exposure results in the development of antibodies that recognize PPA, the results presented here clearly show that, in addition to SBI patients, APA are found in another silicone-implanted patient population strongly suggesting that the production of APA is a silicone-associated response.

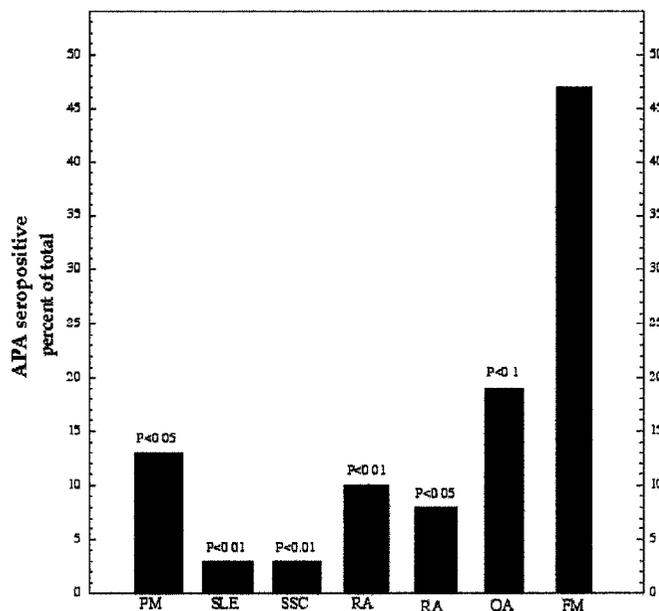
## **5. Association of APA and FMS**

### **5.1 Initial study of APA seroreactivity in FMS, OA, and Autoimmune Diseases**

As reported by Tenenbaum et al (1), the majority of symptomatic SBI patients experiencing symptoms of a non-classical and atypical connective tissue disease were positive on the APA assay, while few of the SBI patients with limited to no symptoms and those patients who met criteria for classical autoimmune diseases were positive on the APA assay. Because many of the symptoms reported by SBI patients appear to be similar if not identical to those observed in FMS patients (52, 53, 67), the prevalence APA seroreactivity in FM patients and autoimmune disease control groups was determined (2)(see Wilson et al. *J. Rheum.* **26**, 402-407, 1999; Appendix 2).

Sera from FMS patients (n=47), osteoarthritis patients (OA, n=16), and rheumatoid arthritis patients (RA, n=13) were analyzed for the presence of APA. Patients with implants of any kind and patients with concurrent autoimmune conditions were excluded. Banked sera from autoimmune disease controls including poly/dermatomyositis (PM, n=15), RA (n=30), systemic lupus erythmatosus (SLE, n=30), and systemic sclerosis (SSC, n=30) were also analyzed.

As shown in Figure 10, the prevalence of APA seroreactivity in FMS patients (47 %) was found to be significantly higher compared to RA patients (8 %,  $p > 0.05$ ). Also, the prevalence of seroreactivity in the FM group was higher than the OA group (19 %) with the difference approaching statistical significance ( $p < 0.1$ ). Upon examination of the rO.D. values two of the three APA positive OA samples had low levels of seroreactivity (rO.D. of  $< 0.016$ ). In the case of the banked autoimmune disease sera, the prevalence of APA reactivity in the RA (10%), SLE (3%), SSC (3%) and PM (13%) groups was less than 15%. Overall, the prevalence of APA seroreactivity in FM patients was 4-5 fold higher compared to the autoimmune disease groups examined ( $p < 0.05$ , all comparisons).



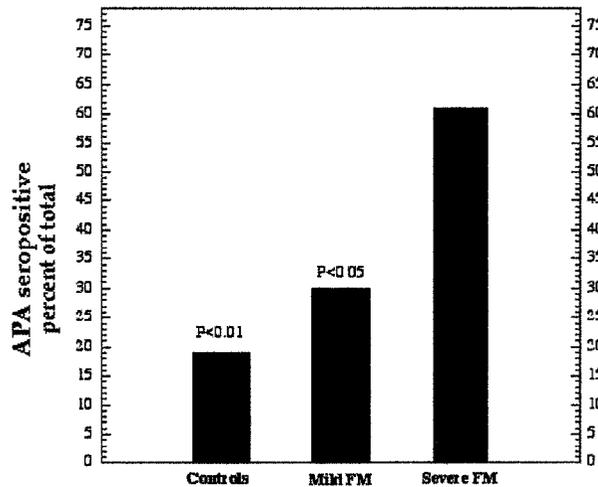
**Figure 10. APA seroreactivity is associated with FM.** Banked sera from autoimmune disease control samples from Cooperative Systemic Studies of Rheumatic Disease unit at the University of Utah (PM, SLE, SSC, and RA) and samples from the Arizona Rheumatology Center (RA, OA, and FM) were analyzed for the presence of APA. Samples with an rO.D.  $> 0.000$  were considered positive. Results are presented as the percent of seroreactive samples in each category. The strength of binary relations was tested by one-way ANOVA. (from Wilson et al. *J. Rheum.* 26, 402-407, 1999; Appendix 2)

## 5.2 Association of APA seroreactivity and severity in FMS

The results of Tenenbaum et al. (1) demonstrated that the prevalence of APA correlated with the severity of symptoms in SBI patients. To determine if the prevalence of APA seroreactivity was higher in FMS patients with more severe symptoms, banked sera samples from patients with mild symptoms and from patients judged to have more severe manifestations of FMS were obtained and analyzed.

Sera samples from FMS patients ( $n=28$ ) assessed by a rheumatologist as severe based on high scores on analog pain scales and a maximum number of tender points despite treatment with analgesics, anti-depressants, and physical therapy were analyzed for APA seroreactivity. The prevalence of APA seroreactivity in this group, as shown in Figure 11, was 61% (17/28). Sera samples ( $n=37$ ) from FMS patients assessed as mild based on moderate dolorimeter scores as a group and because they were enrolled in a previous study as a drug-free control group, were analyzed for APA seroreactivity. Sera samples from normal controls ( $n=21$ ) were also obtained and blinded with the FMS samples. The prevalence of APA seroreactivity in the mild FMS patients was 30% (11/37) and 19% (4/21) in the controls (Figure 11). Thus, the prevalence of APA seroreactivity observed in the severe FM patients

was significantly higher than that found in the mild FM patients ( $p < 0.05$ ) and controls ( $p > 0.01$ ).



**Figure 11. APA seroreactivity in normal controls and mild and severe FM patients.** Banked sera from FM patients assessed as severe and mild and from controls were analyzed for the presence of APA. Samples with an rO.D. > 0.000 were considered positive. The data are presented as the percent of seroreactive samples in each category. The strength of binary relations was tested by one-way ANOVA. (from Wilson et al. *J. Rheum.* 26, 402-407, 1999; Appendix 2)

To further determine if APA seroreactivity is correlated with FM severity, mean threshold and tolerance dolorimeter scores for both control and tender point sites of mild patients were obtained by chart review for the majority of the mild FMS patients (32/37) and normal controls (13/21). Scores were obtained by dolorimeter evaluation at 18 tender points and 4 control points using a Chatillon dolorimeter (68) and determining the pressure (range 0-3.8 kg/cm<sup>2</sup>/sec) when the patient began to experience pain (threshold) and when the pain became intolerable (tolerance). The mean dolorimeter scores for the FMS patients were compared vs. APA O.D. to determine if there was a difference in score for APA positive FMS patients and APA negative FMS patients. APA seroreactive patients tended to have a lower threshold and tolerance dolorimeter score at both tender points and control points. To determine if this decrease in dolorimeter scores in the APA positive FMS patients was significant, the means for each of the dolorimeter scores of the two populations were compared. Both mean threshold and mean tolerance dolorimetry scores were significantly lower ( $p < 0.05$ ) in the seropositive mild FMS patients compared to the seronegative patients (Table 2). Thus, the mean tender point threshold score for the APA seroreactive patients was 27% less than the score for the APA negative patients, and tolerance scores for the APA seroreactive patients were 23% less than the mean score of the APA negative patients. Threshold and tolerance scores were also lower for the control points in the APA seroreactive patients, but the difference was not statistically significant ( $p > .05$ ).

The data shows that the prevalence of APA seroreactivity was higher in the patients with more severe manifestations of FM, and indicates that the aPAA is identifying a subgroup of FM patients, who as a group, tend to have a lower threshold and tolerance to pain. This is the first report of an objective laboratory measure found to correlate with severity in FM patients.

Table 2. Mean dolorimetry score for the mild FM group (see Wilson et al. J. Rheum. 26, 402-407, 1999; Appendix 2)

Sample	n	Mean Dolorimeter Score (kg/cm <sup>2</sup> /sec ± SE)			
		T-thresh	T-toler	C-thresh	C-toler
APA neg.	22	1.83 ± .08 <sup>1</sup>	2.53 ± .11 <sup>2</sup>	2.45 ± .17 <sup>4</sup>	3.25 ± .20 <sup>4</sup>
FM APA pos.	11	1.33 ± .21 <sup>1</sup>	1.95 ± .25 <sup>2</sup>	1.96 ± .29 <sup>4</sup>	2.50 ± .32 <sup>4</sup>
Total	33	1.66 ± .10 <sup>3</sup>	2.33 ± .12 <sup>3</sup>	2.28 ± .15 <sup>5</sup>	2.88 ± .15 <sup>5</sup>
Controls Total	13	2.94 ± .15 <sup>3</sup>	3.41 ± .11 <sup>3</sup>	3.45 ± .13 <sup>5</sup>	3.72 ± .07 <sup>5</sup>

<sup>1</sup> p=0.02 for comparison between APA neg. and APA pos.

<sup>2</sup> p=0.03 for comparison between APA neg. and APA pos.

<sup>3</sup> p<0.001 for comparison between FM patients and controls

<sup>4,5</sup> Not statistically significant

<sup>x</sup> Only 2 of the 14 patients were APA pos.

### 5.3 APA seroreactivity in FMS: Clinical significance and identification of a large subgroup of APA seropositive FMS patients

Our previous work, both published and unpublished, discussed here and published work by others(69) suggests that FMS does not consist of one population of patients but encompasses several groups of patients with an overlapping constellation of signs and symptoms. To further investigate the possibility that the presence of APA identifies a subset of FMS patients, we have established a collaboration with I. Jon Russell, MD PhD. Dr. Russell is an established FMS researcher and clinician and co-author of the ACR criteria for FMS(29). During his studies directed at understanding the role of spinal fluid neurochemicals on the pathogenesis of fibromyalgia, Dr. Russell has established an extensive frozen sample base from well characterized FMS patients and controls(70). In addition to determining if APA identifies a subset of FMS patients, this study was undertaken to determine the clinical relevance of APA in FMS patients and to obtain performance characteristics of an APA ELISA kit.

#### *Study design*

Serum samples were provided from Dr. Russell's extensive frozen sample bank. The serum samples tested in this study were originally collected in the course of a series of ongoing studies approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Clinical information was collected systematically by questionnaire and physical examination. In addition, samples of cerebral spinal fluid (CSF), serum, and 24-hour urine were obtained and stored frozen at  $-70^{\circ}\text{C}$  and not thawed until needed for laboratory evaluation. Serum samples used in this study included those from 136 FMS patients and 62 HNC. Patients diagnosed with FMS and lacking other painful or inflammatory (rheumatic) conditions comprised the FMS group. Patients in the FMS group met the classification criteria for FMS established by the ACR(29). Healthy individuals who did not have symptomatic musculoskeletal pain and who did not meet ACR criteria for fibromyalgia syndrome were assigned to the healthy normal control (HNC) group.

A series of validated outcome measures derived from self-report questionnaires and examination measures of pain threshold were used to assess the clinical status of the patients or normal controls with regard to symptoms characteristic of FMS at the time of the phlebotomy (see Table 3). Some of the clinical measures may appear redundant because, at the time this study was started, it was not clear which self-assessment instruments were best for the assessment of FMS patients. Healthy subjects were instructed to respond to the same questions as those completed by the FMS patients to avoid missing values and to document their own current health status.

A tender point evaluation by palpation and dolorimetry allowed comprehensive assessment of the examination component of the ACR diagnostic criteria and documentation of the average pain threshold (APT). The total number of tender points (of 18 ACR Tender Points) painful to four kilograms of digital pressure (TTPPAL) were recorded. Also by the palpation examination, the severity of the tenderness was recorded using a three-point scale (0=no pain experienced; 1=pain experienced but no physical response was apparent; 2=pain experienced and a physical response such as a wince or withdrawal was observed; 3=pain experienced and a dramatic response was observed.) to quantify the patients verbal and physical response to four kg of palpation pressure at each of the 18 ACR tender points (TP)(29). The severity scores at each of these sites were summed to determine the tender

point index (TPI), ranging from 0 to 54(71). Finally, a Fischer dolorimeter(72) was used to measure the amount of pressure the subject would accept at each of the FMS tender point before the pressure sensation changed to pain. This value, ranging from 0 to 13 at 18 tender points, was averaged to provide the value referred to as the average pain threshold (APT)(45).

**Table 3.** Primary and secondary outcomes with best to worst ranges

Name	Description	Scale	Range
<i>Primary outcome measure</i>			
OD	Optical Density	B-W	-0.02 to 4.1
<i>Secondary Outcome measures</i>			
PAIN	VAS for pain severity	B-W	0 to 10
HOWBAD	VAS for pain severity	B-W	0 to 10
STIFF	VAS for stiffness severity	B-W	0 to 10
DURSTIFF	Duration of morning stiffness (hours)	B-W	0 to 8
FELTINAM	VAS for feeling good in the morning	B-W	0 to 10
FEELGOOD	Felt good during the last week (days)	W-B	0 to 7
HOWTIRED	VAS for fatigue severity	B-W	0 to 10
LIMITACT	Symptoms limited activity during the last week (days)	B-W	0 to 7
HAQ	Health Assessment Questionnaire score	B-W	0 to 3
HEADACHE	Headache during the last week (days)	B-W	0 to 7
ABDMPAIN	Abdominal pain during the last week (days experienced)	B-W	0 to 7
ANXIOUS	VAS for anxiety	B-W	0 to 10
HASSLE	Hassle questionnaire score	B-W	0 to 250
STATE	Limited Spielberger current anxiety status	B-W	21 to 84
TRAIT	Limited Spielberger usual anxiety status	B-W	9 to 36
DEPRESS	VAS for depression severity	B-W	0 to 10
ZUNG	Zung depression index	B-W	0 to 100
CESD	Center for Epidemiological Studies Depression index	B-W	0 to 60
SEFUNCAV	Self efficacy for control of reduced function	B-W	0 to 100
SEOTHRAV	Self efficacy for control of other problems	B-W	0 to 100
SEPAINAV	Self efficacy for control of pain	B-W	0 to 100
TTPPAL	Total number of tender points by palpation	B-W	0 to 48
TPI	Tender point index by palpation examination	B-W	0 to 54
APT	Average pain threshold by dolorimeter (kg)	W-B	0 to 13
SP	Substance P in CSF (fmol/ml)	B-W	0 to 83
NGF	Nerve growth factor in CSF (pg/ml)	B-W	0 to 250

Laboratory evaluations for this study were limited to measurements of APA, substance P, and nerve growth factor. Cerebral spinal fluid (CSF) substance P (SP) levels were measured in nearly all of the subjects and reported in fmol/ml. The results clearly showed significantly higher levels of SP in the primary FMS patients than in the healthy normal controls. The purpose for including that measure in the present study is merely to allow correlation analysis with the levels of APA titers. Similarly, nerve growth factor (NGF) was measured in the CSF of a subset of the patients for another study protocol and reported as pg/ml. This measure was found to be significantly elevated in the CSF of primary FMS patients and is included in the present report to allow correlational analysis with APA titers.

***Reliability of the OD measurement:***

To assess the reliability of the APA ELISA Assay kit, forty-six samples in this study received paired OD measurements; the first measurement was made in San Antonio, and the second in New Orleans. The results of these paired measurements are summarized in Table 4. The reliability of the OD measurement was assessed with the coefficient of reliability (also known as the intraclass correlation coefficient). A graphical summary is provided with an errors-in-both-variables regression line(73) overlaid on a plot of paired OD measurements (Figure 12).

**Table 4.** Paired optical density measurement summary

Statistic	First OD Measurement	Second OD Measurement
n	46	46
Mean	0.541	0.486
Standard deviation	0.743	0.704
Minimum	-0.020	0.000
First quartile	0.020	0.050
Median	0.170	0.155
Third quartile	0.890	0.620
Maximum	2.730	2.800

The coefficient of reliability for these paired data is 0.97 (95% CI 0.95 to 0.98). The errors-in-both-variables regression line, intercept =  $-0.025$  (95% CI  $-0.083$  to  $0.033$ ), slope= $0.945$  (95% CI  $0.881$  to  $1.01$ ), was not significantly different from the ideal line (intercept=0 and slope=1). These results demonstrate that the APA ELISA kit was highly reliable.

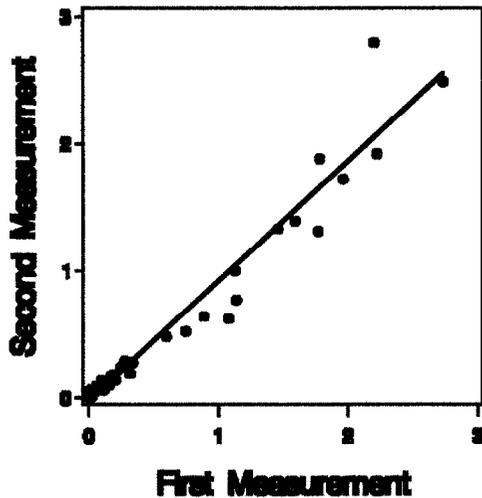


Figure 12: Paired OD measurements with the overlaid errors-in-both-variables line.

**Primary Outcome Measure (OD)**

The distributions of OD are summarized in Table 5 by group. No patients were missing an OD measurement. Both distributions were right-tail skewed, indicated by the mean exceeding the median in each group. The percentage of FMS patients with OD exceeding the HNC median was 92.7%. Repeated contrasts with HNC found a significant increase in FMS ( $p < 0.001$ ) with regard to the percentage of patients with OD exceeding the HNC median. The percentage of patients exceeding the HNC third quartile was 43.4% in the FMS group. Repeated contrasts with HNC found a significant increase in FMS ( $p = 0.01$ ) with regard to the percentage of patients OD exceeding the HNC third quartile.

Table 5. OD distribution summaries by group\*

Statistic	HNC	FMS
n	62	136
Mean	0.178	0.239
Standard deviation	0.512	0.622
Minimum	-0.02	-0.006
First quartile	0.001	0.021
Median	0.009	0.042
Third Quartile	0.05	0.117
Maximum	2.418	4.085

\*HNC=healthy normal controls, FMS=fibromyalgia syndrome

**Correlation and Slopes:**

Pearson’s correlation was used to assess the relation between OD and each of the 26 secondary outcome measures. Because OD distributions were right-tail skewed, OD distribution was log-transformed to approximate normality prior to computing the

correlation. The Pearson correlation between OD, and each of the 26 secondary outcome variables is shown in Table 6.

**Table 6.** Pearson correlation between optical density and secondary outcomes

Outcome	n	HNC		n	FMS	
		Corr	p		Corr	p
PAIN	62	-0.147	0.25	136	0.065	0.45
HOWBAD	62	-0.143	0.27	136	0.117	0.18
STIFF	62	-0.059	0.65	135	<b>0.227</b>	<b>0.008</b>
DURSTIFF	62	<b>0.346</b>	<b>0.006</b>	136	-0.04	0.65
FELTINAM	62	0.07	0.59	136	<b>0.226</b>	<b>0.008</b>
FEELGOOD	62	0.07	0.59	135	0.018	0.84
HOWTIRED	62	0.107	0.41	136	<b>0.187</b>	<b>0.03</b>
LIMITACT	62	-0.049	0.71	135	<b>0.194</b>	<b>0.02</b>
HAQ	62	-0.154	0.23	136	0.037	0.66
HEADACHE	62	0.018	0.89	136	<b>0.213</b>	<b>0.01</b>
ABDMPAIN#	62	-0.035	0.79	136	0.164	0.06
ANXIOUS	62	0.032	0.81	136	<b>0.214</b>	<b>0.01</b>
HASSLE	62	0.073	0.57	135	0.03	0.73
STATE	62	-0.144	0.27	136	-0.032	0.71
TRAIT	62	0.057	0.66	136	0.085	0.32
DEPRESS	62	-0.002	0.99	136	<b>0.193</b>	<b>0.02</b>
ZUNG	62	-0.065	0.62	135	<b>0.256</b>	<b>0.003</b>
CESD	62	-0.049	0.7	136	<b>0.186</b>	<b>0.03</b>
SEFUNCAV	62	-0.091	0.48	134	0.074	0.4
SEOTHRAV	62	0.075	0.56	135	-0.006	0.95
SEPAINAV	62	0.044	0.74	135	-0.059	0.49
TTPPAL	62	-0.009	0.95	136	0.108	0.21
TPI	62	-0.068	0.6	136	0.031	0.72
APT	62	<b>-0.264</b>	<b>0.04</b>	136	0.016	0.85
SP	60	0.094	0.48	134	-0.004	0.96
NGF	34	0.109	0.54	27	-0.138	0.49

\*Corr=Pearson correlation with OD, HNC=healthy normal controls, FMS= fibromyalgia syndrome. OD and NGF were log transformed prior to computing correlation. Bold font indicates a correlation significantly different from zero. # Approached significance.

In the FMS group, STIFF, FELTINAM, HOWTIRED, LIMITACT, HEADACHE, ANXIOUS, DEPRESS, ZUNG, and CESD, were significantly and positively correlated with OD. Correlation of OD with APT, as observed in our earlier work (2) was not expected in this study, since the FMS patient population used here was highly selected based on TPI and APT to ensure a uniform population for Dr. Russell's studies. The means and SEM for TPI and APT in the FMS group were  $32.46 \pm 0.69$  and  $2.35 \pm 0.14$ , respectively. The correlation between APA OD and 9 clinical measures demonstrates that the presence of APA is clinically relevant to FMS and is not an epiphenomenon. This is the first instance of the titer (OD) of an antibody correlating with clinical parameters in FMS.

In HNC, DURSTIFF was significantly and positively correlated with OD and APT was significantly and negatively correlated with OD. Subsequent analysis revealed that the significant correlation observed for DURSTIFF in the HNC groups was an outlier-induced effect. The correlation between APT (average pain threshold) indicates that there may be a subgroup of the HNC population that is different from the rest of the HNC population.

Unadjusted analyses of covariance models were applied to estimate the slope relating OD (in log units) and each of the 26 secondary outcome variables and to compare the slope the FMS group with the slope in the HNC group. The slopes relating STIFF and OD in the FMS (0.41) and in the HNC groups (-0.07) were borderline significantly different ( $p=0.06$ ). The slopes relating LIMITACT and OD in the FMS (0.40) and in the HNC groups (-0.06) were borderline significantly different ( $p=0.08$ ). The slopes relating ZUNG and OD in the FMS (2.45) and in the HNC groups (-0.48) were significantly different ( $p=0.02$ ). The slopes relating CESD and OD in the PFMS (2.20) and in the HNC groups (-0.26) were borderline significantly different ( $p=0.08$ ). The slopes relating APT and OD in the FMS (0.01) and in the HNC groups (-0.49) were significantly different ( $p=0.01$ ).

#### ***Sensitivity and Specificity.***

The sensitivity and specificity of OD were computed under a range of definitions corresponding to a range of cut points (C). The percentage of patients in each of the four groups with  $OD > C$  are given in Table 7, for C ranging from 0 to 0.20 in increments of 0.01. The sensitivity of the OD measurement, relative to a cut point C, was defined as the percentage of FMS patients with  $OD > C$ . The specificity of the OD measurement was defined as the percentage HNC with  $OD \leq C$ . Sensitivity and specificity were computed for C ranging between 0 and 0.20 in increments of 0.01. Based on the sensitivities and specificities in Table 7 and the representation in Figure 13, OD was dichotomized to positive ( $OD > 0.03$ ) and negative ( $OD \leq 0.03$ ). With this definition, 58.1% of PFMS patients and 25.8% of HNC were APA positive; APA and group (HNC, PFMS) were significantly associated ( $p < 0.001$ ).

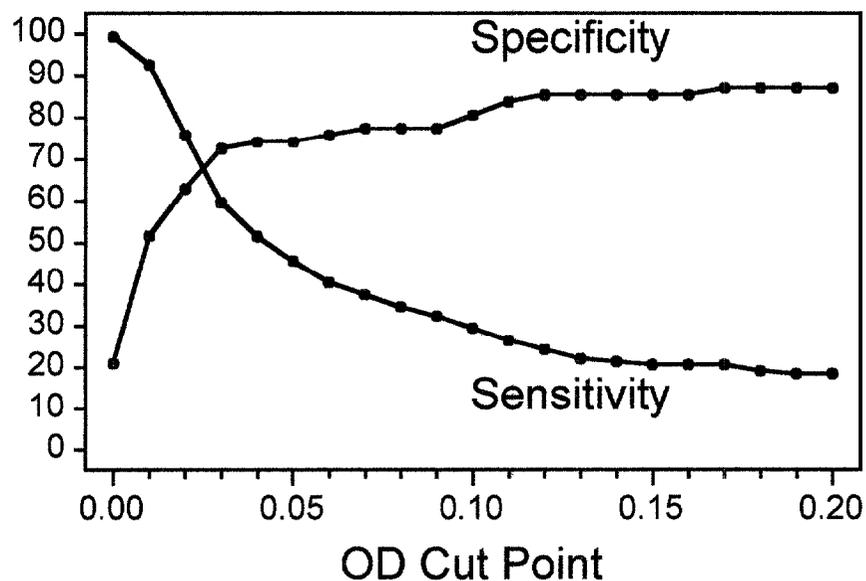
**Table 7.** The percentage of patients with OD greater than a cut point C by patient group and sensitivity and specificity at each cut point.

OD Cut Point (C)	HNC	FMS	Sensitivity*	Specificity†
0.00	75.8	98.5	98.5	24.2
0.01	46.8	91.2	91.2	53.2
0.02	33.9	75.0	75.0	66.1
0.03	25.8	58.1	58.1	74.2
0.04	25.8	51.5	51.5	74.2
0.05	24.2	43.4	43.4	75.8
0.06	24.2	40.4	40.4	75.8
0.07	22.6	37.5	37.5	77.4
0.08	22.6	33.8	33.8	77.4
0.09	21.0	32.4	32.4	79.0
0.10	19.4	28.7	28.7	80.6
0.11	16.1	26.5	26.5	83.9
0.12	14.5	24.3	24.3	85.5
0.13	14.5	22.1	22.1	85.5
0.14	14.5	21.3	21.3	85.5
0.15	14.5	20.6	20.6	85.5
0.16	14.5	20.6	20.6	85.5
0.17	12.9	20.6	20.6	87.1
0.18	12.9	19.1	19.1	87.1
0.19	12.9	18.4	18.4	87.1
0.20	12.9	18.4	18.4	87.1

\*Sensitivity is the percentage of PFMS patients with OD>C.

†Specificity is the percentage of HNC with OD≤C.

**Figure 13.** OD sensitivity and specificity for FMS.



***Secondary measures in APA positive ( $OD > 0.03$ ) and negative ( $OD \leq 0.03$ ) populations***

HNC and FMS populations were dichotomized based on APA OD. Patients and controls with an  $OD > 0.03$  were considered “APA positive” while those with an  $OD \leq 0.03$  were considered “APA negative”. Contrasts with each of the 26 outcome measures were performed for the FMS and HNC groups. In the case of FMS, STIFF ( $p=0.03$ ), ANXIOUS ( $p=0.02$ ), and DEPRESS ( $p=0.04$ ) were significantly increased in APA positive FMS patients versus APA negative patients. In the HNC group, APT ( $p=0.002$ ), as measured by dolorimeter, was significantly decreased in APA positive controls compared to APA negative controls. FELTINAM ( $p=0.07$ ), a measure of fatigue after sleep, was increased in APA positive controls, and the increase approached significance. These results confirm that APA identifies a subgroup of FMS that accounts for almost 60% of the FMS patients in this study population. The results in the HNC group demonstrates that APA identifies a subgroup of individuals that are different than the other HNC individuals and indicates that the “false” positive rate observed (25.8%) in this study is not an accurate measure of the true false positive rate of the APA ELISA kit.

Further analysis was performed to demonstrate the association of APA OD with subgroups of HNC and FMS groups. Plotting secondary outcomes against each other revealed patterns of APA positive and APA negative controls and patients. Specifically, FELTINAM and HOWTIRED, both measures of types of fatigue, demonstrated a difference between APA positive and APA negative controls. To take advantage of these patterns in HNC, abnormality was defined as being  $FELTINAM > 4$  and  $HOWTIRED > 4$  and the relation between APA and abnormality was assessed with Fisher’s exact test. The results are summarized in Table 8. Four of 16 APA positive HNC (25%) and 1 of 46 APA negative HNC patients (2.2%) were abnormal, a significant difference ( $p=0.01$ ).

**Table 8.** Contrast of HNC APA positive and HNC APA negative on the proportion abnormal with abnormal defined by  $FELTINAM > 4$  and  $HOWTIRED > 4$ .

APA	N	Abnormal	Percent	p
Negative	46	1	2.2	
Positive	16	4	25.0	0.01

In FMS a difference was found between FELTINAM, HOWTIRED, and STIFFNESS. In this case, abnormality was defined as being  $FELTINAM > 8$ ,  $HOWTIRED > 8$  and  $STIFFNESS > 8$  and the relation between APA and abnormality was again assessed with Fisher’s exact test. The results are summarized in Table 9. Thirty-two of 79 APA positive FMS (40.5%) and 9 of 57 APA negative FMS patients (15.8%) were abnormal, a significant difference ( $p=0.002$ ).

**Table 9.** Contrast of FMS APA positive and FMS APA negative on the proportion abnormal with abnormal defined by  $FELTINAM > 8$  and  $HOWTIRED > 8$  and  $STIFFNESS > 8$ .

APA	N	Abnormal	Percent	p
Negative	57	9	15.8	
Positive	79	32	40.5	0.002

A pattern of increased HOWTIRED, FELTINAM, and STIFFNESS was found in APA positive PFMS. The proportion of FMS patients with values greater than 8 in all three of these variables was significantly increased among those positive for APA. A similar pattern was found in HNC, with a significantly greater proportion of APA positive patients exhibiting both HOWTIRED and FELTINAM greater than 4. These results further demonstrate that the presence of APA identifies a subgroup of members in the HNC and FMS groups.

***Discriminate Analysis within FMS to Predict OD>0.03***

Logistic regression models were fit within FMS to determine how well the independent variables predict APA positive, where APA positive was defined as OD>0.03. Within the FMS group the number of subjects was large enough to fit full models including all main effects and all interactions with age. Models were fit in four steps: (1) A full main effects model including all secondary outcomes without reduction, (2) A full interaction model including all secondary outcomes and the interaction of each secondary outcome with age without reduction, (3) A reduction of the full main effects model using backward elimination, (4) A reduction of the interaction model using backward elimination. The SAS program defaults were used in the backward selection process; only those outcomes contributing significantly to the model ( $p<0.05$ ) were retained. If an interaction term was retained, then each corresponding main effect was retained, regardless of the significance of the main effect. The four logistic models are summarized in Tables 10, 11, 12 and 13. The sensitivity, specificity, and false positive and negative rates of each model are presented in Table 14.

**Table 10.** Main effects logistic model to predict OD>0.03 in FMS

Source	Coefficient	Std Error	Chi-square	p-value
Intercept	-0.671	3.967	0.03	0.87
AGE	-0.0399	0.0251	2.52	0.11
PAIN	-0.2969	0.1518	3.82	0.05
HOWBAD	0.184	0.1772	1.08	0.3
STIFFNESS	0.3659	0.1598	5.24	0.02
DURSTIFF	0.0667	0.1387	0.23	0.63
FELTINAM	-0.1037	0.1847	0.32	0.57
FEELGOOD	0.1214	0.1402	0.75	0.39
HOWTIRED	-0.1071	0.1653	0.42	0.52
LIMITACT	0.1068	0.1189	0.81	0.37
HAQ	-0.707	0.4607	2.35	0.12
HEADACHE	0.0888	0.1036	0.73	0.39
ABDMPAIN	-0.0751	0.1099	0.47	0.49
ANXIOUS	0.1434	0.1342	1.14	0.29
HASSLE	-0.0093	0.0047	3.86	0.05
STAIT	-0.0068	0.0437	0.02	0.88
TRAIT	0.0397	0.0777	0.26	0.61

**Table 10.** continued

Source	Coefficient	Std error	Chi-square	p-value
DEPRESED	0.0467	0.1249	0.14	0.71
ZUNG	0.0274	0.0343	0.64	0.42
CESD	-0.0234	0.0324	0.52	0.47
SEFUNCAV	-0.0087	0.0134	0.42	0.52
SEOTHRAV	0.0039	0.0164	0.06	0.81
SEPAINAV	-0.0139	0.0161	0.74	0.39
TTPPAL	0.0007	0.1554	0.00	1.00
TPI	0.0344	0.0318	1.17	0.28
TPA	0.1877	0.256	0.54	0.46

**Table 11.** Interaction logistic model to predict OD>0.03 in FMS

Source	Coefficient	Std error	Chi-square	p-value
Intercept	-28.2168	71.7308	0.15	0.69
AGE	0.6598	1.5011	0.19	0.66
PAIN	-5.4314	2.3258	5.45	0.02
HOWBAD	-2.275	2.5045	0.83	0.36
STIFFNESS	-0.2019	2.1208	0.01	0.92
DURSTIFF	4.7378	2.6444	3.21	0.07
FELTINAM	-1.1304	2.6621	0.18	0.67
FEELGOOD	5.3342	3.1191	2.92	0.09
HOWTIRED	1.937	2.2309	0.75	0.39
LIMITACT	-4.645	2.1591	4.63	0.03
HAQ	25.6928	10.4646	6.03	0.01
HEADACHE	-7.6618	2.2164	11.95	<0.001
ABDMPAIN	2.8448	1.6348	3.03	0.08
ANXIOUS	3.6933	2.0643	3.2	0.07
HASSLE	-0.3286	0.1107	8.81	0.003
STAIT	-0.6755	0.6735	1.01	0.32
TRAIT	-0.7593	1.5286	0.25	0.62
DEPRESED	-0.2575	1.7227	0.02	0.88
ZUNG	-0.6355	0.5903	1.16	0.28
CESD	1.8595	0.7796	5.69	0.02
SEFUNCAV	0.144	0.1865	0.6	0.44
SEOTHRAV	-0.4794	0.2236	4.6	0.03
SEPAINAV	0.206	0.2093	0.97	0.32
TTPPAL	6.1176	3.009	4.13	0.04
TPI	-0.0523	0.4764	0.01	0.91
TPA	7.4617	4.2596	3.07	0.08
AGE*PAIN	0.0786	0.0438	3.22	0.07
AGE*HOWBAD	0.0765	0.0534	2.06	0.15
AGE*STIFFNESS	0.0247	0.0455	0.3	0.59
AGE*DURSTIFF	-0.097	0.0534	3.3	0.07
AGE*FELTINAM	0.0163	0.0545	0.09	0.76

**Table 11.** continued

Source	Coefficient	Std error	Chi-square	p-value
AGE*FEELGOOD	-0.1009	0.063	2.57	0.11
AGE*HOWTIRED	-0.0433	0.0459	0.89	0.35
AGE*LIMITACT	0.1078	0.0452	5.68	0.02
AGE*HAQ	-0.5835	0.2201	7.03	0.008
AGE*HEADACHE	0.1616	0.0452	12.77	<0.001
AGE*ABDMPAIN	-0.0591	0.0327	3.27	0.07
AGE*ANXIOUS	-0.071	0.0437	2.64	0.1
AGE*HASSLE	0.0064	0.0022	8.29	0.004
AGE*STAIT	0.0157	0.014	1.26	0.26
AGE*TRAIT	0.0168	0.0295	0.32	0.57
AGE*DEPRESED	0.014	0.0352	0.16	0.69
AGE*ZUNG	0.0138	0.0116	1.43	0.23
AGE*CESD	-0.0417	0.0163	6.5	0.01
AGE*SEFUNCAV	-0.0047	0.004	1.43	0.23
AGE*SEOTHRV	0.0103	0.0046	4.93	0.03
AGE*SEPAINAV	-0.0052	0.0045	1.32	0.25
AGE*TPPAL	-0.1396	0.0651	4.59	0.03
AGE*TPI	0.0044	0.0101	0.19	0.66
AGE*TPA	-0.1537	0.0885	3.02	0.08

**Table 12.** Reduced main effects model to predict OD>0.03 in FMS

Source	Coefficient	Std error	Chi-square	p-value
Intercept	-1.0992	0.7805	1.98	0.16
PAIN	-0.2264	0.1042	4.72	0.03
STIFFNES	0.3078	0.1263	5.94	0.01
ANXIOUS	0.2007	0.0878	5.23	0.02
HASSLE	-0.0084	0.0035	5.87	0.02

**Table 13.** Reduced interaction model to predict OD>0.03 in FMS

Source	Coefficient	Std error	Chi-square	p-value
Intercept	-2.4032	3.2063	0.56	0.45
AGE	0.0159	0.0619	0.07	0.8
PAIN	-0.2205	0.1098	4.03	0.04
STIFFNESS	0.3865	0.1361	8.06	0.005
HEADACHE	-1.2623	0.5226	5.83	0.02
ANXIOUS	1.4684	0.4963	8.75	0.003
HASSLE	-0.0107	0.0039	7.46	0.006
AGE*HEADACHE	0.0265	0.0104	6.45	0.01
AGE*ANXIOUS	-0.0264	0.0097	7.32	0.007

**Table 14.** Summary of four logistic regression models to predict OD>0.03 in FMS

## a) Sensitivity and specificity

Model	APA Positive Predicted/Observed	Sensitivity	APA Negative Predicted/Observed	Specificity
Full main effects	45/79	57.0%	22/57	38.6
Full interaction	44/79	55.7%	33/57	57.9%
Reduced main effects	56/79	70.9%	26/57	45.6%
Reduced interaction	59/79	74.7%	34/57	59.7%

## b) False positive and false negative rates

Model	Predicted positive/ Observed negative	False positive rate	Predicted negative/ Observed positive	False negative rate
Full main effects	35/57	61.4%	34/79	43.0%
Full interaction	24/57	42.1%	35/79	44.3%
Reduced main effects	31/57	54.4%	23/79	29.1%
Reduced interaction	23/57	40.4%	20/79	25.3%

To summarize, in PFMS patients, significant correlations were found between APA O.D. and scores for: stiffness; fatigue; limited activity; headache; anxiety and depression ( $p<0.05$ ). In NC, a significant correlation was found between APA O.D. and lower average pain threshold ( $p=0.04$ ). At a cut point of O.D.>0.03, the sensitivity of the aPAA for FMS was 58.1% and the specificity was 74.2% ( $p<0.001$ ). APA-positive PFMS patients had significantly higher mean scores for stiffness severity, anxiety, and depression compared to APA-negative PFMS patients ( $p<0.05$ ). A logistic regression model was found to predict APA-positive PFMS patients with a sensitivity of 74.7% and a specificity of 59.7% ( $p<0.05$ ). Thus, the presence and titer of APA correlate with clinical measures suggesting that these antibodies identify a large, previously unrecognized subgroup of PFMS patients. In addition, these results suggest that APA may be important in the pathogenesis of that PFMS subgroup

## 6. Discussion

Our results demonstrating an association between silicone-implant exposure and APA raises the question of how APA develop in a non-implant-exposed population of APA-positive FMS patients(2). The question of exposure may be addressed by the ubiquitous distribution of silicones in our environment. Silicones are found in cosmetics, foodstuffs,

lubricants, and pharmaceuticals, in addition to medical implants. It is possible that exposure to silicones from these sources provides the stimulus for the production of APA. If this is the case, production of APA is likely limited to a genetically susceptible population since APA are only found in a subset of FMS, SBI, and VP shunt patients despite widespread exposure to silicones. The possibility that responses to silicones may be genetically restricted is also supported by the observation of Young et al.(74) that symptomatic women with SBI are more likely to possess HLA DR53 and DR 7 than healthy women with implants. Interestingly, Young et al.(74) also found a significantly increased prevalence of DR53 and DR 7 in women with FMS but no implants.

If, as proposed, APA is a silicone-associated response, then the presence of APA in symptomatic SBI patients with signs and symptoms similar to FMS(1) and in FMS patients without silicone implants(2) strongly suggests that low level exposure to silicone in our environment is involved in the development of FMS. It is unlikely that the observed association of APA with silicone and FMS is an epiphenomenon, since we have found, as discussed, that the titer of APA correlates with nine clinical measures in FMS patients including measures of fatigue, stiffness, disability, and several measures of depression. A causal association between silicone and FM is also supported by a recent epidemiological study by Brown et al.(54). In their study, SBI patients with extracapsular leakage of silicone were found to be at a significantly higher risk of having physician-diagnosed FMS compared to SBI patients without leakage (24.7% vs. 10.7%;  $p < 0.004$ )(54). Interestingly, in a preliminary study we have also found an increased prevalence of APA in SBI patients with extracapsular leakage of silicone(57) (see Appendix 4). Though not compared in the study by Brown et al.(54), the rate of diagnosed FMS in SBI patients without leakage, 10.7%, is higher than the reported 3% prevalence of FMS in adult women in the general population(31). This suggests that the presence of silicone implants alone, without leakage, may also result in an increased risk of FMS.

Previous studies examining the association between connective tissue disease and silicone breast implants have not found an association between FMS and SBI(9-11); however, these studies, by design, excluded a diagnosis FMS or other atypical connective tissue diseases(11). FMS was excluded because of the lack of objective criteria to diagnose FMS. As established by the American College of Rheumatology, a diagnosis of FMS is based upon the presence of widespread pain for at least 3 months and the presence of tenderness at 11 out of 18 defined tender points(29). Due to the lack of objective laboratory markers, the existence of FMS as a distinct diagnostic entity has been questioned and some physicians regard FMS as a psychosomatic illness(75, 76). Our finding of APA in FMS patients (2) and the determination that APA titer correlates with clinical measures in FMS patients, as discussed, indicates that FMS is not psychosomatic and is a physiologically based illness.

The determination that APA are predominately of the IgG2 subclass may provide additional avenues of investigation and insight into the pathophysiology of silicone-associated FMS. For instance, in humans, the antibody response to non-peptide antigens is predominately an IgG2 response(77), and very little is known about how an immune response to these antigens is elicited(78). What is known is that antigen presentation of non-peptide antigens occurs through a non-MHC mediated pathway involving the CD1 family of glycoproteins(78) and that CD1 positive natural T cells play a key role in the cytokine-dependent differentiation of Th1 and Th2 effector cells(79). Since alterations in Th1/Th2 ratios and cytokine profiles have been implicated as stressors of the hypothalamic-pituitary-adrenal axis which has been proposed to play a role in the development of FMS(80), it is

possible that chronic stimulation of CD1 cells by the antigen that elicits the APA response leads to the development of FMS.

In conclusion, we have shown that the binding of APA is specific and reproducible, and that the majority of patients with VP shunts requiring revision of their devices develop APA as do the majority of symptomatic women with silicone breast implants. We have also shown that APA are present in a large percentage of FMS patients, identify a large subgroup of patients within the FMS population, and correlate with a variety of clinical measures. These results support the hypothesis that in a subset of the population silicone exposure induces a unique immunological response, production of APA, and that both the exposure and the immunological response to that exposure are linked to the development of FMS

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