Serologic Evaluation of Women Exposed to Breast Implants

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ABSTRACT. ***Objective***. There continues to be uncertainty whether women with silicone breast implants experience activation of their immune system and show increased prevalence of serologic markers of connective tissue diseases. We conducted laboratory tests in a large number of women with and without breast implants, and in diabetic patients with presumed silicone exposure via insulin syringes.

Methods. Subjects were chosen from women enrolled in the run-in phase of the Women's Health Study (WHS, a randomized trial testing aspirin and vitamin E in preventing cardiovascular disease and cancer), and included 298 women without breast implants, 298 women with breast implants, and 52 diabetic patients diagnosed before age 30. Comparison groups were matched on age, race, date of blood provided to the WHS, and randomization status. We compared the proportion with abnormal results in 16 serologic tests among the 3 groups of women, stratifying by the matching factors. We also tested for monoclonal immunoglobulins by electrophoresis.

Results. For 14 of the 16 serologic tests, the proportions with abnormal results among the 3 groups of women were not significantly different. Of the remaining tests, C3 levels were decreased in 8 (2.7%) women without breast implants and 22 (7.4%) women with breast implants ($p = 0.003$). C4 levels were decreased in 31 (10.4%) women without breast implants and 48 (16.1%) women with breast implants ($p = 0.03$). Women without breast implants and diabetic patients did not differ significantly in the proportions having decreased C3 and C4 levels. Women with breast implants did not have higher frequency of monoclonal immunoglobulins detected by electrophoresis.

Conclusion. We found little evidence for activation of the immune system in women with breast implants. The clinical significance of isolated reductions in C3 and C4 levels, in the absence of other abnormalities such as elevated levels of antinuclear antibody, is unknown. (J Rheumatol 2001;28:1523–30)

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More than 1.5 million women in the United States received silicone breast implants for augmentation or reconstructive mammoplasty from 1962 until 1992, when the US Food and Drug Administration issued a moratorium on their use. Despite widespread concerns that implants may increase the risk of connective tissue diseases (CTD), possibly through activation of the immune system, large epidemiologic studies generally have failed to show higher rates of classic CTD among women with breast implants[1-16]. One study showed a modest increase in risk of self-reported CTD[17]. Another study of Medicare claims diagnoses revealed an association between breast implants and systemic lupus erythematosus[18]. A recent metaanalysis nevertheless concluded that the research to date provided no proof for an association between breast implants and CTD[19]. Attempts to find serologic evidence for activation of the immune system, or for markers of classic CTD, have produced conflicting results. Small studies of patients with breast implants who were referred for evaluation of rheumatic symptoms have reported increased frequencies of abnormal serologic markers, such as a positive test for antinuclear antibodies (ANA)[20-33]. Large studies, in which a comparison group was

included, have not confirmed the observations of the small studies[2,5,34]. The largest study to date noted no increased frequency of abnormal findings in 19 serologic tests conducted among women with silicone breast implants, except for a slightly increased frequency of antibodies to single stranded DNA[34]. A recent review by the Institute of Medicine concluded that based on existing studies, there is no evidence of an increased risk of CTD among women with breast implants, nor is there support for a role of silicone in activating the immune system[35].

To provide more information on immune activation and frequency of serologic markers of CTD among women with and without breast implants, we performed a series of 16 serologic tests and assessed presence of monoclonal immunoglobulins by electrophoresis among subjects selected from a large cohort of health professionals.

MATERIALS AND METHODS

Participants. The Women's Health Study (WHS) is a randomized, double blind, placebo controlled trial of low dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer among 39,876 female health professionals[36,37]. Between September 1992 and May 1995, letters of invitation to participate in the trial and questionnaires on sociodemographic characteristics, lifestyle habits, and medical history were sent to 1.76 million female health professionals in the United States and Puerto Rico. Women were asked to return the enrollment questionnaire, regardless of whether they were willing or eligible to participate in the trial. A total of 453,787 women returned the questionnaire. Of these, 65,169 were willing and eligible to be in the trial and were enrolled into the run-in phase. Women who reported cardiovascular disease and cancer other than nonmelanoma skin cancer were ineligible for the trial. In addition, women were excluded if they had serious illnesses that might affect their participation or compliance in the trial. After the run-in phase, 39,876 women who continued to be willing and eligible were selected for the actual trial.

We requested blood samples from the 65,169 women enrolled into the run-in phase of the WHS trial and obtained samples from 33,340 women (51%). Of these 33,340 women, 474 reported having had breast implants, but not silicone, paraffin, or collagen injections; additionally, they did not report a history of early onset (< 30 years) diabetes mellitus. We do not have information on whether the implants were silicone gel-filled or saline-filled, or about implant complications such as rupture. We randomly selected 300 women, who also provided blood specimens that arrived in adequate condition (see Blood collection and processing procedures, below), to be included in our present study, since *a priori* power calculations showed that we would have 80% power to detect a prevalence of antinuclear antibodies (ANA) of 30%, compared with 20% among an equal number of women without breast implants.

We then randomly selected 300 matched comparison women (referred to as "women without breast implants") who did not report breast implants; silicone, paraffin, or collagen injections; or early onset (< 30 yrs) diabetes mellitus. These women were matched to women with breast implants on year of birth, race (white or other), date of return of blood sample to the WHS (within 12 mo), and randomization status (randomized into WHS or not).

We selected another comparison group, diabetic patients with age of onset < 30 years who did not report breast implants or silicone, paraffin, or collagen injections (referred to as "diabetics"). These women were presumed to be insulin dependent and exposed to low levels of silicone via insulin syringes[38,39]. Although the goal was to study 300 diabetics, only 56 women met these criteria. Of these, 53 satisfied the matching criteria listed above for women without breast implants and were included in this study.

One woman with breast implants, as well as her matched control without breast implants, did not provide sufficient blood for analyses. Due to an oversight, plasma from another woman with breast implants was not sent for analysis; thus, this subject and her matched control were excluded from the study. Since this matched control without breast implants also served as a matched control for one diabetic subject, this diabetic additionally was excluded. This left 298 women without breast implants, 298 women with implants, and 52 diabetics in our present study.

Blood collection and processing procedures. Women arranged to have their blood collected in sodium citrate and EDTA tubes, which were chilled and shipped by overnight carrier in cold packs to our laboratory. We recorded whether any samples arrived warm or were hemolyzed. Women whose blood samples arrived warm or hemolyzed were not eligible for study. On arrival, the samples were centrifuged and plasma was removed and stored in liquid nitrogen. Frozen samples selected for study were placed at –30°C overnight, then thawed at room temperature, and distributed into 1.2 ml tubes. These tubes were refrozen at –30°C and transported to the Clinical Immunology Laboratory at Massachusetts General Hospital for testing. Samples were identified by number only so that laboratory personnel were masked to the source of the samples.

Laboratory studies. All kit based assays were performed on plasma from the citrate anticoagulated blood. Assays were performed according to the directions of the manufacturer.

ANA were sought using HEp-2 cell substrate (HEp-2 cell cultures, IFA test system; Zeus Scientific Inc.). Samples yielding a positive ANA, defined as a titer of ≥ 1:40, then were assessed for antibodies to La, Sm, Sm-RNP, and Jo1 by ELISA (Varelisa, Pharmacia and Upjohn Diagnostics, Freiburg, Germany), and antibodies to double stranded DNA (dsDNA) by *Crithidia luciliae* assay (Quantafluor, Sanofi Diagnostics Pasteur Inc., Chaska, MN, USA).

All samples were assessed by ELISA for antibodies to Ro, single stranded DNA (ssDNA), and Scl-70 (Varelisa). We also tested all samples for rheumatoid factor (RF), complement components (C3, C4), and immunoglobulins (IgG, IgA, IgM) by nephelometry (Beckman Instruments, Fullerton, CA, USA). Abnormal levels for these tests were defined as > 30 units for RF, < 86 mg/dl for C3, < 20 mg/dl for C4, < 614 or > 1295 mg/dl for IgG, < 69 or > 309 mg/dl for IgA, and < 53 or > 334 mg/dl for IgM. Additionally, we determined the concentration of C-reactive protein (CRP) in all samples on the Behring BN II analyzer (Dade Behring, Wilmington, DE, USA); a concentration of > 0.8 mg/dl was considered abnormal.

Finally, we tested the samples for the presence of monoclonal immunoglobulins by electrophoresis. Fibrinogen was removed from the EDTA anticoagulated plasma by treatment with bovine thrombin (MDA Fibriquik, Organon Teknika Corp., Durham, NC, USA). In brief, 250 μl of plasma and 20 μl thrombin solution were mixed and held at 37° for 15 min. The precipitate was removed by centrifugation. The supernatant was treated with an additional 20 μl thrombin solution. After the second treatment, fibrinogen was no longer detectable by agarose gel electrophoresis and immunofixation using antifibrinogen antiserum (The Binding Site Inc., San Diego, CA, USA). Serum proteins were examined by agarose gel electrophoresis[40] and electrophoretically restricted bands in the gamma globulin zone were identified by immunofixation[40]. Two reviewers independently reviewed the gels, then compared results, and reached consensus regarding isotype and estimated concentration of monoclonal immunoglobulins. The designation "very low concentration monoclonal immunoglobulin" refers to monoclonal proteins in the range 12.5–25 mg/dl; "low concentration" 50 to several hundred mg/dl; "moderate concentration" 1000–2000 mg/dl; and "high concentration" 3000 mg/dl.

Quality control. Prior to this study, we carried out quality control testing. We selected 20 plasma samples from women enrolled in the run-in phase of the WHS, who did not report breast implants or silicone injections; further, they were selectively sampled because they reported rheumatoid arthritis with positive test for RF. (Samples used for quality control were not included in the actual study.) We split the 20 samples into 40 specimens

and tested for: ANA; anti-Ro, anti-ssDNA, and anti-Scl-70 antibodies; RF, C3, C4, IgG, IgA, IgM, and CRP; and monoclonal immunoglobulins by electrophoresis. Tests for anti-La, anti-Sm, anti-Sm/RNP, and anti-Jo1 then were performed on 5 samples (i.e., 10 test specimens) with a positive ANA. Laboratory personnel were unaware that the samples were split samples from 20 rather than 40 separate women.

There was complete agreement in the split samples on test results for ANA; anti-La, anti-Sm, anti-Sm-RNP, anti-Ro, and anti-Scl-70 antibodies (positive vs negative test); and C3, C4, IgM, and CRP (abnormal vs normal levels, as defined above). There was agreement for RF (positive vs negative test) in 18 of 20 split samples; 2 samples yielding discordant findings had results slightly above and slightly below the cutoff. For anti-ssDNA and anti-Jo1 antibodies (positive vs negative test), and IgG and IgA (abnormal vs normal levels), there was agreement in 80–95% of instances.

For monoclonal immunoglobulins ascertained by electrophoresis, 2 readers agreed on the results in 15 of 20 split samples (14 negative samples and 1 sample with a very low concentration immunoglobulin). For each of the remaining 5 samples, one reader interpreted the electrophoresis as showing a very low concentration band, the other reader as negative. In addition, 16 samples from patients at Brigham and Women's Hospital with known positive tests for RF were interspersed with samples from the actual study at random intervals. RF was correctly detected 100% of the time.

Statistical methods. We first examined several characteristics used for matching (age, race, randomization status) among the 3 groups of women to ensure that they were well matched. We also examined the proportions who were postmenopausal and who smoked, testing for differences using chi-squared tests.

We then compared the presence of ANA (detected on HEp-2 substrate), anti-Ro, anti-ssDNA, and anti-Scl-70 antibodies (detected by ELISA), and RF among women with and without breast implants, using a matched set analysis. We made similar comparisons between diabetics and women without breast implants. We compared ANA titer distributions between women with and without breast implants, and between diabetics and women without breast implants using stratified chi-squared tests for trend. Next, we examined the proportions of women in the 3 groups with abnormal levels of complement components, immunoglobulins, and CRP (defined above), again using matched set analyses. Because of significant differences between groups in the proportion with decreased levels of C3 and C4, further exploratory analyses were performed. In each of the 3 groups, we examined the number of women concordant and discordant for decreased levels of C3 and C4. We then restricted analyses to women with a positive ANA because patients with systemic lupus erythematosus (SLE) who have a positive ANA frequently undergo complement level testing to assess disease activity.

Finally, we compared the frequency, isotype, and approximate concentration of monoclonal immunoglobulins detected by electrophoresis among the 3 groups using matched set analyses.

RESULTS

The 3 groups of women were well matched with regard to age, race, and randomization status (Table 1). Among women with breast implants, the mean duration of implants was 14 (± 5.7) years. There were no significant differences among groups in the proportions of postmenopausal women and smokers.

We first examined the proportion of women in each group with a positive test for various autoantibodies (Table 2). Similar proportions of women in the 3 groups tested positively for ANA at various titers: 28.9% of women without breast implants, 28.9% of women with implants, and 26.7% of diabetics. There were no significant differences in the distribution of ANA titers among the 3 groups (Table 3). Among women who tested positively for ANA, we further tested for antibodies against La, Sm, Sm-RNP, and Jo1 (Table 2). Such antibodies were rarely found; thus, no statistical tests comparing groups were conducted.

We tested for antibodies to Ro, ssDNA, and Scl-70 among all women. These antibodies were found in all 3 groups with low frequency and there were no significant differences among groups. Antibodies to Ro were detected in 0–1.0% of women in the 3 groups (Table 2). Between 2.4 and 5.8% of women in the 3 groups had antibodies to ssDNA, while 0–1.0% had antibodies to Scl-70. Positive tests for RF were found in 3.2% of women without breast implants and 6.0% of women with implants. No diabetic tested positively for RF.

The proportions of women with decreased levels of C3 and C4, increased or decreased levels of IgG, IgA and IgM, and increased level of CRP are shown in Table 4. The proportions with abnormal levels, except for C3 and C4, did not differ among the 3 groups. C3 levels were decreased in 8 (2.7%) women without breast implants compared to 22 (7.4%) women with implants (p = 0.003). C4 levels were decreased in 31 (10.4%) women without breast implants compared to 48 (16.1%) women with implants (p = 0.03). Women without breast implants and diabetics did not differ significantly in the proportions having decreased C3 and C4 levels.

Table 1. Characteristics of subjects.

	Women without Implants, N = 298	Women with Implants, N = 298	Diabetics, N = 52
Mean age (yrs)	50.8	50.8	49.9
Caucasian, %	96.6	96.6	98.1
Randomized into Women's Health Study trial, %	84.9	84.9	86.5
Postmenopausal, %	41.6	36.6	44.2
Cigarette smokers, %	11.4	15.8	15.4

No significant differences comparing women with vs women without breast implants, and diabetics vs women without breast implants.

Table 2. Frequency (%) of positive tests to various autoantibodies in women without breast implants, women with breast implants, and diabetics.

	Women without Implants, N = 298	Women with Implants, N = 298	p†	OR (95% CI)*	Diabetics, N = 52	p††	OR (95% CI)
Autoantibody							
ANA	86 (28.9)	86 (28.9)	1.00	1.00 (0.80–1.25)	14 (26.9)	0.85	1.08 (0.51–2.29)
Anti-La*	1 (1.2)	0	—	—	0	—	—
Anti-Sm*	0	0	—	—	0	—	—
Anti-Sm-RNP*	0	0	—	—	0	—	—
Anti-ds-DNA*	0	0	—	—	0	—	—
Anti-Jo1	1 (1.2)	1 (1.2)	—	—	0	—	—
Anti-Ro	2 (0.7)	3 (1.0)	0.66	1.50 (0.25–8.87)	0	0.32	—
Anti-ssDNA	7 (2.4)	13 (4.4)	0.16	1.86 (0.79–4.38)	3 (5.8)	0.08	3.00 (0.70–12.93)
Anti-Scl-70	1 (0.3)	3 (1.0)	0.32	3.00 (0.35–25.84)	0	—	—
RF	9 (3.2)	17 (6.0)	0.30	1.56 (0.68–3.57)	0	0.16	—

OR: odds ratio; 95% CI: 95% confidence interval.
*Conducted only among women with positive ANA.
†Women with breast implants vs women without implants, adjusted for age, race, date of blood sample return, and randomization status.
†† Diabetics vs women without breast implants, adjusted for age, race, date of blood sample return, and randomization status.

Table 3. Frequency (%) of positive tests for antinuclear antibody (ANA) at various dilutions of serum from women without breast implants, women with breast implants, and diabetics.

	Women without Implants, N = 298	Women with Implants*, N = 298	Diabetics†, N = 52
ANA titer			
Any positive ANA	86 (28.9)	86 (28.9)	14 (26.9)
1:40	9 (3.0)	7 (2.4)	0 (0)
1:80	21 (7.1)	26 (8.7)	2 (3.9)
1:160	26 (8.7)	25 (8.4)	6 (11.5)
1:320	14 (4.7)	16 (5.4)	4 (7.7)
1:640	8 (2.7)	7 (2.4)	0 (0)
1:1280	5 (1.7)	2 (0.7)	0 (0)
1:2560	1 (0.3)	0 (0)	1 (1.9)
> 1:2560	2 (0.7)	3 (1.0)	1 (1.9)

*Chi-squared test for trend, adjusted for age, race, date of blood sample return, and randomization status, p = 0.93, compared to women without breast implants.
†Chi-squared test for trend, adjusted for age, race, date of blood sample return, and randomization status, p = 0.81, compared to women without breast implants.

On further inspection, among women without breast implants, 6 had decreased levels of both C3 and C4, 25 had normal C3 but decreased C4 levels, while 2 had decreased C3 but normal C4 levels (kappa coefficient = 0.28). Among women with breast implants and diabetics, the corresponding distributions were 16, 32, and 6 (kappa coefficient = 0.40) and 6, 6, and 1, respectively (kappa coefficient = 0.56). Among women with a positive ANA, the proportions of women with decreased C3 or C4 levels did not differ significantly among the 3 groups, using Fisher's exact test (data not shown).

Women in the 3 groups did not differ significantly in the proportions with decreased or increased IgG, IgA, and IgM levels (Table 4). Women without implants were somewhat more likely to have increased CRP levels than women with breast implants (13.5% vs 8.8%; p = 0.06); there was no significant difference between women without implants and diabetics.

Monoclonal immunoglobulins also were sought by high resolution agarose gel electrophoresis. A somewhat higher proportion of women without breast implants were found to have monoclonal immunoglobulins, compared to women with implants (15.8% vs 10.4%; p = 0.06) (Table 5). There was no difference between women without breast implants and diabetics. Women with breast implants were less likely than women without implants to have monoclonal IgG lambda proteins (1.7% vs 5.0%; p = 0.03); they also were less likely to have very low or low concentration of monoclonal immunoglobulins (6.4% vs 11.1%; p = 0.04). Finally, there were no significant differences in the occurrence of multiple monoclonal proteins among the 3 groups (Table 6).

DISCUSSION

We found limited evidence for activation of the immune system among women with breast implants. There were no significant differences between women with and without breast implants in the proportions with abnormal results for 14 of 16 serologic tests (ANA; antibodies to La, Sm, Sm-RNP, Jo-1, dsDNA, Ro, ssDNA, Scl-70; RF; IgG, IgA, IgM; CRP). Further, women with breast implants did not have higher frequency of monoclonal immunoglobulins detected by electrophoresis than women without implants. There were no significant differences between diabetics and women without breast implants for any of the above tests.
We did find, however, that the proportions of women with

Table 4. Frequency (%) of abnormal complement levels, immunoglobulin levels, and C-reactive protein in women without breast implants, women with breast implants, and diabetics.

	Women without Implants, N = 298	Women with Implants, N = 298	p*	OR (95% CI)*	Diabetics, N = 52	p†	OR (95% CI)†
Test							
Decreased C3	8 (2.7)	22 (7.4)	0.003	2.75 (1.42–5.34)	7 (13.5)	0.10	3.50 (0.80–15.27)
Decreased C4	31 (10.4)	48 (16.1)	0.03	1.55 (1.04–2.31)	12 (23.1)	0.07	2.40 (0.93–6.20)
Decreased IgG	6 (2.0)	11 (3.7)	0.20	1.83 (0.73–4.60)	3 (5.8)	0.56	1.50 (0.38–5.94)
Decreased IgA	8 (2.7)	5 (1.7)	0.41	0.63 (0.21–1.89)	0	0.32	—
Decreased IgM	7 (2.4)	5 (1.7)	0.56	0.71 (0.23–2.24)	0	0.08	—
Increased IgG	35 (11.7)	36 (12.1)	0.90	1.03 (0.66–1.61)	8 (15.4)	0.59	1.33 (0.46–3.83)
Increased IgA	49 (16.4)	39 (13.1)	0.25	080 (0.54–1.18)	11 (21.2)	0.13	2.20 (0.79–6.16)
Increased IgM	11 (3.7)	15 (5.0)	0.41	1.36 (0.65–2.87)	0	—	—
Increased CRP	40 (13.5)	26 (8.8)	0.06	0.65 (0.42–1.01)	11 (21.2)	0.41	1.38 (0.65–2.91)

OR: odds ratio; 95% CI: 95% confidence interval.
*Women with breast implants vs women without implants, adjusted for age, race, date of blood sample return, and randomization status.
†Diabetics vs women without breast implants, adjusted for age, race, date of blood sample return, and randomization status.

Table 5. Frequency (%) of women without breast implants, women with breast implants, and diabetics who have monoclonal immunoglobulins detected by electrophoresis.

	Women without Implants, N = 298	Women with Implants, N = 298	Diabetics, N = 52
Test			
Any monoclonal immunoglobulin present	47 (15.8)	31 (10.4)	7 (13.5)
Isotypes present			
IgG kappa	21 (7.1)	14 (4.7)	4 (7.7)
IgG lambda	15 (5.0)*	5 (1.7)	2 (3.9)
IgM lambda†	1 (0.3)	2 (0.7)	0
Concentration of monoclonal immunoglobulin			
Very low or low	33 (11.1)*	19 (16.4)	6 (11.5)
Moderate or high	3 (1.0)	0	0

*Women with breast implants vs women without implants, adjusted for age, race, date of blood sample return, and randomization status, $p < 0.05$.
†No IgM kappa monoclonal proteins detected.

Table 6. Frequency (%) of presence of one or more low or very low concentration monoclonal immunoglobulins detected by electrophoresis in women without breast implants, women with breast implants, and diabetics.

	Women without Implants, N = 298	Women with Implants, N = 298	Diabetics, N = 52
Number of immunoglobulin bands			
3	0	2 (0.7)	0
2	15 (5.0)	5 (1.7)	1 (1.9)
1	18 (6.0)	12 (4.0)	5 (9.6)

No significant differences comparing women with vs women without breast implants, and diabetics vs women without breast implants, adjusted for age, race, date of blood sample return, and randomization status.

reduced levels of complement components, C3 and C4, were higher in the breast implant group compared to women without implants. The majority had isolated reduced levels of C3 or C4, rather than reduced levels of both. The most common finding was a decreased level of C4. Women without breast implants and diabetics did not differ significantly in the proportions having decreased C3 or C4 levels.

The reason(s) for the reduced levels of C3 and/or C4 in women with breast implants is (are) uncertain. Because multiple tests were carried out, significant differences could have occurred by chance. Complement components, such as C3 and C4, may be consumed during periods of active inflammation in diseases such as SLE, post-streptococcal glomerulonephritis, serum sickness, and chronic obstructive pulmonary disease[41], resulting in reduced C3 and C4 levels.

In this study, positive tests for ANA were found in similar frequencies in all 3 groups. Among those with a positive ANA, there was no difference in the frequency of decreased C3 and/or C4 levels; nor did we detect anti-dsDNA antibodies in any subject. Therefore, it is unlikely that there was a disproportionate number of cases of undiagnosed SLE among the women with breast implants compared to the controls. It is also unlikely that more women in the breast implant group had one of the diseases listed above, because only apparently healthy women (women with SLE were specifically excluded) were eligible to be included in the Women's Health Study.

C4 exists as 2 isotypes: C4A and C4B, encoded at 2 loci on chromosome 6[42]. There is a high frequency of null alleles at both loci in the population; these alleles are responsible for a variable reduction in serum C4 levels[43]. Whether women with C4 deficiency elect to have augmentation mammoplasty more often than women with 4 normally functioning alleles is not known. Finally, even under carefully controlled storage conditions, the third component of complement undergoes degradation that is enhanced by storage duration. Breakdown products of C3 may not "register" in the nephelometric assay for native C3. We do not know the *in vitro* factors that might account for enhanced degradation of C3 in blood from women with implants compared to controls. Enhanced degradation might have occurred *in vivo* as a result of enzymatic action on native C3 mediated by cells coating the silicone capsule.

The findings in our study agree, for the most part, with those of the Nurses' Health Study, the largest study to have examined the serologic profile of women with breast implants[34]. Of 19 serologic tests conducted among 200 women with breast implants and 500 women without implants in that study, only antibodies to ssDNA were found in a higher proportion of women with breast implants[34]. In our study, we did not observe a significantly higher frequency of antibodies to ssDNA among women with breast implants. In contrast to our study, the Nurses' Health Study found similar proportions of women with and without breast implants to have reduced levels of C3 or C4. No other prospective cohort study of women with breast implants has assayed complement components.

In both the present study and the Nurses' Health Study, similar proportions of women with and without breast implants had a positive test for ANA (Table 3). This observation is in contrast to the higher proportion of positive tests for ANA seen in small groups of patients with breast implants who were referred for evaluation of rheumatic symptoms[20-33]. This discrepancy is likely to reflect the selected patient population in the small studies, rather than an unusually low proportion of women testing positive for ANA in our study. The proportion of women testing positive for ANA is similar to reported rates in control groups from other breast implant studies [28], as well as to the rates reported from 15 laboratories that studied the range of ANA in "healthy" individuals[44].

We were interested in the presence of monoclonal immunoglobulins among women with breast implants because of a hypothesis that silicone gel implants may increase the risk of multiple myeloma[45]. In genetically susceptible strains of mice, the injection of silicone gel into the peritoneal cavity induces the development of plasmacytomas[46]. Additionally, there have been case reports of multiple myeloma developing at an unusually early age in women with breast implants[25,47,48]. In our study, using a high resolution electrophoretic procedure for the detection and identification of monoclonal immunoglobulins, we were unable to find such proteins more frequently in women with breast implants. Moderate or high concentrations of monoclonal immunoglobulins were found in none of the women with, and 3 women without, breast implants. Based on the information we had, we could not determine whether these individuals also had a detectable lymphoproliferative process. Low or very low concentration monoclonal immunoglobulins were present in a lower proportion of women with breast implants than women without implants; the clinical significance of low and very low concentration monoclonal immunoglobulins is uncertain.

One limitation of our study is that we did not ascertain whether the breast implants that women received were silicone gel filled or saline filled. However, all breast implants, regardless of filling, are encased in a silicone envelope, so all women with breast implants would have been exposed to silicone. Further, we assumed rather than directly ascertained that women with diabetes diagnosed prior to age 30 would be insulin resistant and require insulin injections, with concomitant silicone exposure via syringes. We did not test for anticollagen antibodies reported in other studies[20,32] because the assay was not available in our laboratory. We specifically excluded women with self-reported SLE from the Women's Health Study, which limited our ability to study SLE in all 3 of our groups. It should be noted, however, that we were unable to detect an increased risk of SLE in women with breast implants in a previous study[17]. In addition, we ascertained only the diagnosis of CTD, not specific symptoms. We asked participants about the diagnosis of rheumatoid arthritis, SLE, polymyositis, dermatomyositis, Sjogren's syndrome, scleroderma, or other CTD, including mixed CTD. We did not ask about clinical symptoms of CTD or about the diagnosis of fibromyalgia. Finally, only 51% of women enrolled into the run-in phase of the WHS provided a blood sample. However, this low proportion is unlikely to have biased our findings, since similar proportions of women with and without breast implants (51.1% and 51.3%, respectively) provided blood samples.

In conclusion, based on 16 tests for serologic markers of CTD, complement components, immunoglobulin levels,

and CRP, we found little evidence of activation of the immune system in women with breast implants. Further, women with breast implants did not have a higher frequency of monoclonal immunoglobulins detected by electrophoresis.

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REFERENCES

1. Burns CJ, Laing TJ, Gillespie BW, et al. The epidemiology of scleroderma among women: assessment of risk from exposure to silicone and silica. J Rheumatol 1996;23:1904-11.
2. Edworthy SM, Martin L, Barr SG, Birdsell DC, Brant RF, Fritzler MJ. A clinical study of the relationship between silicone breast implants and connective tissue disease. J Rheumatol 1998;25:254-60.
3. Englert H, Morris D, March L. Scleroderma and silicone gel breast prostheses — the Sydney study revisited. J Rheumatol 1998;25:254-260.
4. Friis S, Mellemkjaer L, McLaughlin JK, et al. Connective tissue disease and other rheumatic conditions following breast implants in Denmark. Ann Plast Surg 1997;39:1-8.
5. Gabriel SE, O'Fallon WM, Kurland LT, Beard CM, Woods JE, Melton LJ. Risk of connective-tissue diseases and other disorders after breast implantation. N Engl J Med 1994;330:1697-702.
6. Giltay EJ, Bernelot Moens HJ, Riley AH, Tan RG. Silicone breast prostheses and rheumatic symptoms: a retrospective follow up study. Ann Rheum Dis 1994;53:194-6.
7. Goldman JA, Greenblatt J, Joines R, White L, Aylward B, Lamm SH. Breast implants, rheumatoid arthritis, and connective tissue diseases in a clinical practice. J Clin Epidemiol 1995;48:571-82.
8. Hochberg MC, Perlmutter DL, Medsger TA Jr, et al. Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). Arthritis Rheum 1996;39:1125-31.
9. Nyren O, Yin L, Josefsson S, et al. Risk of connective tissue disease and related disorders among women with breast implants: a nation-wide retrospective cohort study in Sweden. BMJ 1998;316:417-22.
10. Park AJ, Black RJ, Sarhadi NS, Chetty U, Watson AC. Silicone gel-filled breast implants and connective tissue diseases. Plast Reconstr Surg 1998;101:261-8.
11. Sanchez-Guerrero J, Colditz GA, Karlson EW, Hunter DJ, Speizer FE, Liang MH. Silicone breast implants and the risk of connective-tissue diseases and symptoms. N Engl J Med 1995;332:1666-70.
12. Schusterman MA, Kroll SS, Reece GP, et al. Incidence of autoimmune disease in patients after breast reconstruction with silicone gel implants versus autogenous tissue: a preliminary report. Ann Plast Surg 1993;31:1-6.
13. Strom BL, Reidenberg MM, Freundlich B, Schinnar R. Breast silicone implants and risk of systemic lupus erythematosus. J Clin Epidemiol 1994;47:1211-4.
14. Weisman MH, Vecchione TR, Albert D, Moore LT, Mueller MR. Connective-tissue disease following breast augmentation: a preliminary test of the human adjuvant disease hypothesis. Plast Reconstr Surg 1988;82:626-30.
15. Wells KE, Cruse CW, Baker JL Jr, et al. The health status of women following cosmetic surgery. Plast Reconstr Surg 1994;93:907-12.
16. Williams HJ, Weisman MH, Berry CC. Breast implants in patients with differentiated and undifferentiated connective tissue disease. Arthritis Rheum 1997;40:437-40.
17. Hennekens CH, Lee IM, Cook NR, et al. Self-reported breast implants and connective-tissue diseases in female health professionals. A retrospective cohort study. JAMA 1996;275:616-21.
18. Greenlander S, Finkle WD. A retrospective cohort study of implanted medical devices and selected chronic diseases in Medicare claims data. Ann Epidemiol 2000;10:205-13.
19. Janowsky,EC, Kupper LL, Hulka BS. Meta-analyses of the relation between silicone breast implants and the risk of connective-tissue diseases. N Engl J Med 2000;342:781-90.
20. Bar-Meir E, Teuber SS, Lin HC, et al. Multiple autoantibodies in patients with silicone breast implants. J Autoimmun 1995;8:267-77.
21. Bridges AJ, Conley C, Wang G, Burns DE, Vasey FB. A clinical and immunologic evaluation of women with silicone breast implants and symptoms of rheumatic disease. Ann Intern Med 1993;118:929-36.
22. Brunner CA, Feller AM, Groner R, Dees E, Biefel K, Biemer E. Increase of immunologically relevant parameters in correlation with Baker classification in breast implant recipients. Ann Plast Surg 1996;36:512-8.
23. Claman HN, Robertson AD. Antinuclear antibodies and breast implants. West J Med 1994;160:225-28.
24. Cuellar ML, Scopelitis E, Tenenbaum SA, et al. Serum antinuclear antibodies in women with silicone breast implants. J Rheumatol 1995;22:236-40.
25. Garland LL, Ballester OF, Vasey FB, et al. Multiple myeloma in women with silicone breast implants. Serum immunoglobulin and interleukin-6 studies in women at risk. Curr Top Microbiol Immunol 1996;210:361-6.
26. Peters W, Keystone E, Snow K, Rubin L, Smith D. Is there a relationship between autoantibodies and silicone-gel implants? Ann Plast Surg 1994;32:1-5.
27. Press RI, Peebles CL, Kumagai Y, Ochs RL, Tan EM. Antinuclear autoantibodies in women with silicone breast implants. Lancet 1992;340:1304-7.
28. Sanchez-Guerrero J. Autoantibody testing in patients with silicone implants. N Am Clin Lab Med 1997;17:341-53.
29. Silverman BG, Brown SL, Bright RA, Kaczmarek RG, Arrowsmith-Lowe JB, Kessler DA. Reported complications of silicone gel breast implants: an epidemiologic review. Ann Intern Med 1996;124:744-56.
30. Smalley DL, Levine JJ, Shanklin DR, Hall MF, Stevens MV. Lymphocyte response to silica among offspring of silicone breast implant recipients. Immunobiology 1996;196:567-74.
31. Teuber SS, Rowley MJ, Yoshida SH, Ansari AA, Gershwin ME. Anti-collagen autoantibodies are found in women with silicone breast implants. J Autoimmun 1993;6:367-77.
32. Zandman-Goddard G, Blank M, Ehrenfeld M, Gilburd B, Peter J, Shoenfeld Y. A comparison of autoantibody production in asymptomatic and symptomatic women with silicone breast implants. J Rheumatol 1999;26:73-7.
33. Zazgornik J, Piza H, Kaiser W, et al. Autoimmune reactions in patients with silicone breast implants. Wien Klin Wochenschr 1996;108:781-7.
34. Karlson EW, Hankinson SE, Liang MH, et al. Association of silicone breast implants with immunologic abnormalities: A prospective study. Am J Med 1999;106:11-9.
35. Institute of Medicine Committee on the Safety of Silicone Breast Implants. Bondurant S, Ernster V, Herdman R, editors. Safety of silicone breast implants. Washington, DC: National Academy Press; 1999.

36. Buring JE, Hennekens CH, for the Women's Health Study Research Group. The Women's Health Study: Summary of the study design. J Myocardial Ischemia 1992;4:27-9.
37. Buring JE, Hennekens CH, for the Women's Health Study Research Group. The Women's Health Study: Rationale and background. J Myocardial Ischemia 1992;4:30-40.
38. Chantelau EA, Berger M. Pollution of insulin with silicone oil, a hazard of disposable plastic syringes. Lancet 1985;1:1459.
39. Collier FC, Dawson AD. Insulin syringes and silicone oil. Lancet 1985;2:611.
40. Keren DF. High resolution electrophoresis and immunofixation. Techniques and interpretation. In: Anonymous. Boston: Butterworth Heinemann; 1994.
41. Miller RD, Kueppers F, Offord KP. Serum concentrations of C3 and C4 of the complement system in patients with chronic obstructive pulmonary disease. J Lab Clin Med 1980;95:266-71.
42. Moulds JM, Warner NB, Arnett FC. Complement component C4A and C4B levels in systemic lupus erythematosus: Quantitation in relation to C4 null status and disease activity. J Rheumatol 1993;20:443-7.
43. Hauptmann G. Inherited deficiency of the fourth component of human complement. Immunodef Rev 1988;1:3-22.
44. Tan EM, Feltkamp TEW, Smolen JS, et al. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 1997;40:1601-11.
45. Salmon SE, Kyle RA. Silicone gels, induction of plasma cell tumors, and genetic susceptibility in mice: a call for epidemiologic investigation of women with silicone breast implants. J Natl Cancer Inst 1994;86:1040-1.
46. Potter M, Morrison S, Weiner F, Zhang XK, Miller FW. Induction of plasmacytomas with silicone gel in genetically susceptible strains of mice. J Natl Cancer Inst 1994;86:1058-65.
47. Tricot GJ, Naucke S, Vaught L, Vesole D, Jagannath S, Barlogie B. Is the risk of multiple myeloma increased in patients with silicone implants? Curr Top Microbiol Immunol 1996;210:357-9.
48. Silverman S, Vescio R, Silver D, Renner S, Weiner S, Berenson J. Silicone gel implants and monoclonal gammopathies: three cases of multiple myeloma and the prevalence of multiple myeloma and monoclonal gammopathy of undetermined significance. Curr Top Microbiol Immunol 1996;210:367-74.