Study to determine the presence of antipolymer antibodies in a group of Dutch women with a silicone breast implant

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Abstract

Objective

To determine whether there exists a population of Dutch women with a high prevalence of antipolymer antibodies (APA) and severe health complaints/symptoms, and exposure to a silicone breast implant (SBI). As the antigen-specific nature of the antipolymer antibody has not yet been established, we refer to the term polymer binding immunoglobulins.

Methods

The study population was selected from a voluntary registry of SBI recipients of a Dutch consumers organisation. The final selection was based on the severity of self-reported complaints in a questionnaire. A total of 42 SBI recipients were included in the study, clinically examined and blood samples were obtained.

Results

In 12 of 42 SBI recipients an increase in the level of polymer binding immunoglobulins was detected compared to a negative reference sample, 3 of these 12 showing a positive and 9 a weakly positive response. In 3 out of 12 non-SBI recipients, included for control on the performance of the APA assay, an increased level of polymer binding immunoglobulins was demonstrated, 2 of these 3 showing a positive and 1 a weakly positive response. The study population of SBI recipients was categorised in severity subgroups (limited, mild, moderate, advanced) based on the functional capacity and the physicians general assessment of pain and disease activity. Most (34 of 42) SBI recipients belonged to the limited severity subgroup.

Conclusion

Our methods failed to select a group of severely symptomatic Dutch SBI recipients reported to have a high prevalence of polymer binding antibodies. A discrepancy was present between the self reported severe complaints and the observed mild clinical symptoms. In the group of SBI recipients with self reported severe complaints recruited we did not find a high prevalence of polymer binding immunoglobulins. SBI exposure (mean 17 years) did not result in induction of polymer binding immunoglobulins in this minimal symptomatic study group.

Key words

Silicone breast implant, antipolymer antibodies (APA), polymer binding immunoglobulins.

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Introduction

Since the early nineties there has been serious concern over the health risks associated with silicone breast implants (SBI) both in the scientific literature and in the lay press. This has resulted in restriction of the use of silicone breast implants in the US in 1992, based on the lack of sufficient evidence for their safety (1). Their use is now restricted to clinical trials which include safety evaluation. The concern is especially focused on the potential interaction of silicones with the body, eventually leading to the expression of connective tissue diseases. In several case reports the occurrence of connective tissue diseases in SBI recipients was reported (reviewed in 2).

However, to date most large scale epidemiological studies (3-8) and systematic reviews of the scientific literature (9-11) have not provided evidence to support an association between SBI and connective tissue disease, although some studies could not rule out a small increased risk (12, 13). Furthermore, internationally several panels of experts set up to review the evidence relating to possible health risks associated with SBI, concluded that there is no scientific proof for a link between SBI and any connective tissue disease (14-17). The primary safety issue for silicone breast implants is considered to be the local reaction at the implant site. Therefore, there seems to be no major risk involved for systemic disease in the use of SBI.

It might be argued that the aforementioned study results do not rule out an association between connective tissue diseases and SBI. Rather than an established connective tissue disease, SBI may be associated with an atypical connective tissue disease (18). Terms used include, amongst others, 'undifferentiated connective tissue disease', 'human adjuvant disease', 'silicone poisoning', 'siliconosis', and '(silicone) associated connective tissue disease. Evidence for an association between SBI and such a syndrome is lacking, however (11, 18).

Another possible issue may be that rather than inducing disease silicones may act as a co-factor for the development of disease. If this is the case, silicones may then modify the expression or the severity of a disease in women who are already primed. Such activity may explain the co-incidence of disease and SBI. However, indications for such activity are also lacking (15).

Animal studies have revealed the potency of silicones to interact with the immune system, resulting in either enhancement or suppression of immune functions (19, 20). In a toxicological evaluation silicones were found to have only minimal general and immunotoxic effects, inducing minor natural killer (NK) cell suppression (19, 21, 22). Reduced NK cell activity was also reported in SBI recipients (23). Adjuvant activity (enhancement of immune responses) was observed in mice only when antigen (bovine serum albumin, BSA) was mixed with silicone gel (24-26), but not when mixed with silicone oil or elastomeric particles (25, 27). Silicone gel could replace complete Freund's adjuvant (CFA) to elicit autoantibodies to rat thyroglobulin in an animal model (24). However, antithyroglobulin antibody levels were lower compared to CFA. In addition, CFA induced thyroiditis whereas silicones did not. Similarly, CFA but not silicones can be used for the induction of adjuvant arthritis in an animal model (24). Thus, silicones have limited adjuvant activity that is seen only under specific experimental conditions.

Also, there might be an immune response against the silicone material itself. The presence of anti-silicone antibodies in SBI recipients was reported by several authors (28-31). Part of these responses were found to be non-antigen (silicone) specific (32, 33). The binding of human and animal antibodies to silicone was demonstrated to be most probably dependent on the physical properties of serum proteins and consistent with the interaction of hydrophobic molecules (IgG) with hydrophobic surfaces (silicones) in an aqueous system (32). Controversial results were reported for cellular immune responses against silicones and/or derivatives and/or silicone associated antigens like silica (34-36).

Recently the presence of antipolymer antibodies (APA) was claimed in SBI

recipients (37) and fibromyalgia patients (38). There has been criticism regarding the methodology employed by Tenenbaum *et al*. (39-42). The chemical structure of the polymerised polyacrylamide used as antigen in the APA assay is unrelated to that of silicone. No evidence has been put forward for the antigen-specific nature of the immunoglobulin binding, and a possible cross reactivity between silicones and acrylamide was explained by structural similarity for the low molecular weight fractions (43). So the antigen specificity of the immunoglobulin binding in the APA assay in terms of 'antipolymer antibody' remains a question. Hence, we refer to "polymer binding immunoglobulins"(PBIs) to describe this activity in serum.

The prevalence of the PBIs was reported to be highest in SBI recipients and fibromyalgia patients with severe symptoms (37, 38). Although a diagnosis cannot be made based on the presence of these PBIs alone, the value of such an assay would be the objectivity of a laboratory test. The Independent Review Group (UK) stated that confirmation of the results in independent laboratories was needed before any conclusion could be drawn from the Tenenbaum study (14). Previously we demonstrated the reproducibility of the APA assay in our laboratory, and concluded that the assay could be used for the evaluation of the presence of polyacrylamide binding immunoglobulins in the serum of women with a silicone breast implant (44).

For an evaluation of the value of the APA assay a comparison is needed between symptomatic SBI recipients, asymptomatic SBI recipients, and symptomatic and asymptomatic non-SBI controls. However, starting such a large-scale epidemiological study is only warranted when the target population (severely symptomatic SBI recipients and APA positivity) can be recruited and identified. As a first step we tried in this study to identify whether there exists a group of Dutch women with a SBI and severe complaints and symptoms, and high values of polymer binding immunoglobulins.

Materials and methods

Study design

The study was conducted as a cross-sectional study in a population of women with SBI and complaints. Selection, blood sampling and laboratory assays were performed from May 1998 to November 1998. Participants were selected from a registry of SBI recipients (n = 3200) of one of the national consumer organisations (Consumentenbond, The Hague, The Netherlands). SBI recipients had registered because of the world-wide concern regarding the safety of SBI as extensively published in the international scientific and lay press. In this registry complaints were not systematically recorded. Approximately 600 SBI recipients registered complaints dealing with arthralgia and fibromyalgia, complaints with a high prevalence in the study of Tenenbaum (37). Travelling distance from the research centre was added to the selection criteria for the convenience of the participants.

The selected (n = 211) SBI recipients received a questionnaire by mail in order to record their complaints in more detail for further selection. The questionnaire included a set of questions on connective tissue related problems based on a screening questionnaire for detecting connective tissue diseases developed by Karlson *et al.* (45). The Shortened Fatigue Questionnaire (46), a validated questionnaire, was included to determine the intensity of the patient's fatigue. Further questions were SBI-specific, such as the date and reason for the implant, the date and reason in cases where the SBI was explanted, and specific SBI-related health problems. No information was obtained on the brand of the implant. One hundred and forty-three (68%) of the questionnaires were returned and 50 SBI recipients were selected for inclusion in the study. Based on the completeness of the answers in the questionnaire regarding arthralgia (score range 0-10) and fatigue items (score range 4-28), and the willingness and possibility of being present on the planned days for the physical examination, SBI recipients were selected to be participants in the study. Women who received a SBI after breast reconstruction because of breast cancer were not included.

The inclusion criteria for the current study were arthralgia in three or more joints with a severity score of ≥ 6 and fatigue with a score ≥ 23, as these complaints were reported to have a high prevalence in the study population of SBI recipients of Tenenbaum (37). Five women with complaints of pain in 1-2 joints were included to obtain a total of 50 participants. The medical history, physical examination and the presence of polymer binding immunoglobulins was examined in 42 SBI recipients, while 8 women could not participate due to reasons not related to the study (*e.g.* holidays, time and/or day proposed for physical examination not suitable). A full description of the study has been published as a report of the National Institute of Public Health and the Environment, Bilthoven, The Netherlands (47).

Clinical examination of participants

The medical history was taken and a complete physical examination was performed on the 42 SBI recipients by a registered rheumatologist (CAG). Information on prosthesis explant and local breast complications such as pain, capsular contraction, migration of the SBI, and so on were recorded. The systemic symptoms evaluated included fatigue, sleep disturbances, painful joints and muscles, muscle weakness, swollen joints, sicca symptoms (Schirmer test for tear production), morning stiffness, Raynaud's phenomenon, chronic headache and irritable bowel symptoms. Physical examination included a general examination with special focus on neurological signs and dermatological abnormalities (like rashes and scleroderma). The total tender joint count (53 joints), total swollen joint count (44 joints) (48) and tender points according to the American College of Rheumatology (ACR) criteria for fibromyalgia were included (49). A Schirmer test for the detection of Sjögrens syndrome was performed (50). The women were subgrouped according to the Steinbrocker classification for functional disability ranging from I (complete

functional capacity and ability to carry out all usual activities without handicap) to IV (largely or wholly incapacitated and able to carry on little or no self care) (51). General assessment of pain and disease activity of the SBI recipients was recorded on a visual analogue scale (VAS) of 100 mm, while the physician's general assessment of disease activity was recorded on a 5-point scale ranging from asymptomatic to very severe.

To determine severity subgroups similar to the Tenenbaum study (37), SBI recipients were further classified according to their disease activity and functional disability: limited (asymptomatic/mild disease activity and functional disability class I/II), mild (asymptomatic/mild disease activity and functional disability class III/IV), moderate (moderate/severe/very severe disease activity and functional disability class I/II) and advanced (moderate/severe/very severe disease activity and functional disability class III/IV). Autoimmune diseases were diagnosed according to the classification criteria for the specific diseases; criteria for systemic lupus erythematosis (SLE); rheumatoid arthritis (RA), scleroderma and fibromyalgia according to the ACR criteria (49, 52-54); and Sjögren's syndrome according to the criteria of Fox *et al*. (55).

Routine blood testing

Blood was obtained by routine procedures (vein puncture), serum was prepared by centrifugation, and peripheral blood lymphocytes (PBL) were harvested by centrifugal Ficoll separation. The white blood cell count (WBC), red blood cell count (RBC) and differentiation were performed in a Multispecies Haematology Analyser H1E (Bayer BV, Division Diagnostics, Mijdrecht, The Netherlands). Reference values for the upper and lower limits for women were established by the H1E users group in The Netherlands. Blood smears were prepared and routinely stained by May-Grünwald and Giemsa. Anti-nuclear antibody (ANA) assays were performed with a serum dilution of 1 in 40. Extractable nuclear antigens (ENA) according to the Ouchterloney-technique and Immunoblot according to the Western blot technique were performed following standard hospital procedures (University Medical Centre Utrecht, Utrecht, The Netherlands).

APA assay

Materials. Nitro-cellulose strips containing partially polymerised acrylamide (38), and reference serum samples were kindly provided by Dr. R.B. Wilson (Autoimmune Technologies, LLC, New Orleans, LA, USA). The reference serum samples comprised negative, weakly positive, positive and strongly positive serum samples. The strips were coated at three different sites with three dilutions of polyacrylamide in distilled water 1:1,000; 1:100; and 1:10 (37, 38).

The following chemicals were used in the assay: NaCl (Sigma,Axel, The Netherlands), Tween® 20 (Merck-Schuchardt, Hohenbrunn, Germany), Tris buffer (Boehringer Mannheim,Almere, The Netherlands), goat serum (Biogenesis, Poole, UK), phosphate buffered saline (SVM, Bilthoven, The Netherlands), albumin, bovine fraction V (purity > 96%) (Sigma), methanol (Merck), 4-chloro-1-naphtol (purity > 97%) (Janssen Chimica, Beerse, Belgium), Perhydrol® 30% hydrogen peroxide (Merck), biotinylated goat-anti-human IgG (RPN 1186, Amersham Life Science, Little Chalfont, UK), avidin-horseradish peroxidase (P0347 DAKO, Glostrup, Denmark), distilled water (SVM),and milk powder (Campina Melkunie B.V., Eindhoven, The Netherlands).

Methods: All buffers used were prepared freshly and preservative was not added. The nitrocellulose strips were placed in a 20-well tray and washed in 2 ml of washing buffer (0.1 M NaCl, 0.3% Tween, pH 7.4) for 5 minutes at room temperature on a rocking platform.

After decanting the buffer, 2 ml of blocking buffer (80 mM NaCl, 16 mM Tris, 4% heat inactivated goat serum, 6% powdered milk) containing 5 l serum sample was added (1:400 dilution). Four reference samples - a strongly positive control, a positive control, a weakly positive control and a negative control - were included in each separate assay. Strips were incubated for 1.5 hours at room temperature on a rocking platform. After decanting the blocking buffer with serum, the strips were washed three times for 5 minutes with washing buffer at room temperature on a rocking platform.

After decanting the washing buffer, 2 ml blocking buffer with biotinylated anti-human-IgG (H+L,1:1,000 diluted) was added and the strips were incubated for 1 hour at room temperature on a rocking platform. The blocking buffer with antihuman IgG was decanted and the strips were washed with washing buffer as described above.

Avidine conjugated horseradish peroxidase (dilution 1:500) in 2 ml of BSA-blocking buffer (10 mM phosphate buffered saline, 1% BSA and 0.1% Tween, pH 7.4) was added and strips were incubated for 1 hour as described above.

After decanting the BSA blocking buffer with avidine conjugated horseradish peroxidase and washing the strips with washing buffer as described above, the strips were incubated for 15 minutes at room temperature on a rocking platform with detection buffer (30% methanol, 0.03% hydrogen peroxide, 3.4 mM 4- chloro-1-naphtol, 7 mM phosphate buffered saline, pH 7.4). The detection buffer was decanted and the strips were washed several times with distilled water. Strips were dried on filter paper and placed on a record sheet. The colour of the bands on the strips was quantified using a CCD camera and Molecular Analyst software (BioRad Laboratories, Hercules, CA, USA) and expressed as Optical Units (O.U.). The mean O.U. value for all three bands was determined for each serum sample. The extinction was compared to and corrected for the extinction of a blanco non-antigen coated site on the strip. The difference in extinction expressed in optical units between the antigen-coated site and the non-antigen-coated site is a measure for the immunoglobulins bound to the site. Each serum sample was investigated on three different days. The results in extinction of the test samples were expressed as percentage of the extinc-

tion of the positive reference serum sample.

The cut-off point for positivity was determined at 20% of the positive reference sample, 20 % being the mean plus three times the standard deviation of the negative reference serum (x ± SD, 6 ± 5) (Table V) when expressed as percentage of the positive reference serum. Values between 20% and 50% were designated weakly positive, and values above 50% positive. The upper value of the weakly positive response was determined at 50% of the positive reference being the mean plus three times the standard deviation of the weakly positive reference serum (mean ± SD, 18 ± 10, n = 6) (see Table V) when expressed as percentage of the positive reference serum. All serum samples were at least three times tested individually.

Results

Participant characteristics

The characteristics of the 42 SBI recipients are described in Table I. Most SBI recipients were older than 40. Most women (n = 37) had received their SBI before 1986, of which 19 before 1977. Cosmetic surgery was the reason for the SBI in the majority of the women (67%). Twenty (48%) of the SBI recipients had had their SBI explanted, in 8 of them the SBI was replaced, in most cases by a saline-filled breast implant with a silicone shell. Most explantations (n = 18) were performed after 1992, the year of the FDA ban on the use of SBI for cosmetic surgery (1). The mean duration of SBI exposure was 17.1 years for the total group when explantation was considered as the end of the exposure time, and 17.9 years when re-implants were included.

Clinical features

Fatigue was reported by 95% of the women, myalgias by 74% and arthralgia by 100% (Table II). Pain of the shoulders was reported by 76% of the participants, whereas more than 60% reported pain of the neck, hand and knee joints. Approximately 50% reported morning stiffness and sleep disturbances and many women complained about rashes and dry eyes or mouth.

On physical examination (Table III) tender points were present in the majority of women (86%), while 20 (48%) of them had 11 or more tender points. The overall mean number of tender points of the study group was 8.7 ± 5.9 (n = 42). The Schirmer test identified 5/42 SBI recipients with tear production ≤ 5 mm in 5 minutes (Table III).

The distribution according to the Steinbrocker classification indicated that the majority of our study group belonged to a group with no or low disability, i.e. Steinbrocker score I and II. Only 6 SBI recipients had a Steinbrocker score of III, while none had a score of IV. The physician's general assessment of disease activity also showed that most SBI recipients belonged to a group with no or mild disease activity, n = 36 (85%) (Table III). There were no differences in age, year of SBI, duration of SBI exposure, or local complications (leakage and encapsulation) between the separate severity subgroups (data not shown).

Capsular contraction and prosthesis rupture or leakage, being local complications of SBI, were reported by 35 (83%) and 19 (45%) women, respectively (Table I). On physical examination capsular contraction was confirmed by the study physician in 18 women.

In 2 women (5%) rheumatoid arthritis was diagnosed; in one of them the rheumatoid arthritis pre-dated the SBI.

Table I. Characteristics of a group (N = 42) of SBI recipients with self-reported severe complaints: general characteristics.

Characteristics		Participants N (%)
Age	≤ 40	4 (10)
	41 - 50	19 (45)
	> 50	19 (45)
Year of first SBI	< 1977	19 (45)
	1977 - 1985	18 (43)
	> 1985	5 (12)
Reason for SBI	Cosmetic	28 (67)
	Fibrocystic	12 (29)
	Other/unknown	2 (5)
Explantation	Yes	20 (48) Replaced 8, not replaced 12
	No	22 (52)
Year of explantation (n=20)	< 1992	2 (10)
	≥ 1992	18 (90)
Capsular contraction	Reported by participants	35 (83)
	Diagnosed by study physician	18 (43)
Rupture/leakage	Reported by participants	19 (45)
Duration of SBI exposure		17.1 ± 6.5 17.9 ± 6.2 (including re-implant)

Table II. Characteristics of a group (N = 42) of SBI recipients with self reported severe complaints:inventory of complaints.

Complaints		Participants N (%)
Fatigue	Present	40 (95)
Arthralgia	None	0 (0)
	1 - 2 joints	5 (12)
	3 - 8 joints	34 (81)
	> 8 joints	3 (7)
Neck		26 (62)
Shoulder		32 (76)
Elbow		21 (50)
Wrist		16 (38)
Hand		28 (67)
Hip		19 (45)
Knee		27 (64)
Ankle		11 (26)
Foot		14 (33)
Spine		17 (41)
Morning stiffness > 1 hour		20 (48)
Myalgias		31 (74)
Sleep disturbances		22 (52)
Rashes		15 (36)
Dry eyes/mouth		15 (36)
Mouth ulcers		7 (17)
Muscle weakness		4 (10)
Fevers		2 (5)

Table III. Observations at physical examination of a group (N = 42) of SBI recipients with self reported severe complaints.Observations at physical examination.

Diagnosis	Classification	Incidence N	(%)
Tender points	None	6	(14)[a]
	1-6	11	(26)
	7-10	5	(12)
	11-18	20	(48)
Schirmer test	≤ 5 mm	5	
	> 5 mm	37	
Functional disability (Steinbrocker)	None/Limited (I)	15	(38)
	Mild (II)	21	(48)
	Moderate (III)	6	(14)
	Severe (IV)	-	
Physician's general assessment of disease activity	Asymptomatic	9	(21)
	Mild	27	(64)
	Moderate	4	(10)
	Severe	2	(5)
	Very severe	-	
Primary diagnosis	Rheumatoid arthritis	2	(5)
	Osteoarthritis	4	(10)
	Fibromyalgia	19	(45)
	None	17	(40)

Osteoarthritis was the primary diagnosis in 4 women (10%) and a diagnosis of fibromyalgia was made in 19 (45%) SBI recipients (Table III).

Clinical chemistry

No indications were found for general blood parameters, expressed as the mean of the study group, to be out of the normal range when compared to either a control group or reference values (data not shown). In some SBI recipients and controls, alterations outside the reference ranges were observed in white and/or red blood cell parameters. These alterations were generally only minimally outside the reference range.

Table IV shows the results of 3 separate tests for the detection of the polymer binding immunoglobulins level in the serum of the SBI recipients with a serum dilution of 1:400. Three samples (3/42, 7%) show a clear positive response and nine samples (9/42, 21%) a weakly positive response.

One of the women with a weakly positive response was the SBI recipient who was newly diagnosed with rheumatoid arthritis. In 4 women with a weakly positive response and one woman with a positive response no diagnosis was made.

In a group of 12 female laboratory workers whose serum were included as negative control samples for the APA assay, 2 (2/12, 17%) positive and 1 (1/12, 8%) weakly positive samples were detected (Table V). Although not physically examined, to our knowledge none of these laboratory workers were suffering from any connective tissue disease.

Laboratory tests and disease activity

Table VI shows the results of the APA, ANA and ENA assays in relation to the severity subgroups (disease activity and functional disability). The positive and negative sera of SBI recipients for the presence of polymer binding immunoglobulins were equally distributed over the separate severity subgroups. Similar results were obtained with regard to the presence of ANA. There was no difference in age, the reason for SBI, the number of reports of local complications (leakage and encapsulation), and the duration of SBI exposure between the positive and negative women (data not shown).

Table IV. Presence of polymer binding immunoglobulins in a group (N = 42) of SBI recipients with self reported severe complaints.

Sample no.	1 : 400 dilution	Primary diagnosis
R01	**28 ± 13**[a]	
R02	**67 ± 19**	Fibromyalgia
R03	16 ± 11	
R04	13 ± 12	Fibromyalgia
R05	7 ± 11	Osteoarthritis
R06	5 ± 5	Fibromyalgia
R07	6 ± 7	
R08	7 ± 7	Osteoarthritis
R09	14 ± 18	
R10	5 ± 4	Fibromyalgia
R11	5 ± 3	Fibromyalgia
R12	7 ± 3	Osteoarthritis
R13	9 ± 3	Osteoarthritis
R14	10 ± 6	Fibromyalgia
R15	17 ± 19	Fibromyalgia
R16	10 ± 9	
R17	**20 ± 10**	Fibromyalgia
R18	10 ± 12	
R19	9 ± 12	Fibromyalgia
R20	15 ± 14	Fibromyalgia
R21	**24 ± 8**	Rheumatoid arthritis
R22	8 ± 5	
R23	**22 ± 5**	Fibromyalgia
R24	13 ± 4	
R25	16 ± 5	Fibromyalgia
R26	**22 ± 10**	Fibromyalgia
R27	**24 ± 4**	
R28	**30 ± 2**	Fibromyalgia
R29	**25 ± 10**	
R30	**21 ± 4**	
R31	15 ± 3	
R32	15 ± 7	Fibromyalgia
R33	**263 ± 21**	Fibromyalgia
R34	**110 ± 5**	
R35	6 ± 8	
R36	7 ± 6	Fibromyalgia
R37	9 ± 7	
R38	12 ± 5	
R39	12 ± 9	Rheumatoid arthritis
R40	14 ± 13	Fibromyalgia
R41	17 ± 17	Fibromyalgia
R42	10 ± 10	

For legends see Table V.

Discussion

In our study we selected SBI recipients by the severity of self-reported complaints. However, our methods failed to recruit the study group of interest, namely SBI recipients who could be classified in the advanced severity subgroup. As a result we were not able to confirm the results of Tenenbaum *et al.* (37) regarding a high prevalence of polymer binding immunoglobulins in a population of SBI recipients with severe symptoms. This is most likely due to the different composition of our

Table V. Presence of polymer binding immunoglobulins in a group (N=12) serum of female laboratory workers.

Sample number	1 : 400 dilution
C01	11 ± 12[a]
C02	7 ± 8
C03	6 ± 10
C04	3 ± 3
C05	6 ± 3
C06	7 ± 7
C07	9 ± 10
C08	**76 ± 19**
C09	**40 ± 22**
C10	11 ± 11
C11	**51 ± 15**
C12	3 ± 3
Reference samples	
Strongly positive	173 ± 16[b]
Positive	100 ± 0
Weakly positive	18 ± 10
Negative	6 ± 5
Blank serum control	8 ± 11

a) Mean ± s.d.,expressed as percentage of positive (reference) sample, n = 3; Two series of assays were performed each on 3 separate days, series 1 sample numbers R1-R34, and series 2 sample numbers R35-R42 and C1-C12. In bold face weakly positive and positive samples value ≥ 20% of positive reference.
b) Mean ± s.d. n = 6, determined on 6 separate days in 6 separate assays. For blank serum control values with n=5 the mean± sd is 3 ± 3, when one of the results of blank serum control (30%) is considered outlier.

study population, lacking SBI recipients with severe symptoms. The results of our selection procedure, however, might indicate that such a population represents a small part of the total population of SBI recipients with complaints. Similar to the results of Tenenbaum (37), we observed a low prevalence of polymer binding immunoglobulins in our limited and mild severity subgroups, and in the non-SBI laboratory workers.

Some of our methods need to be discussed. A cross-sectional study design was used with a study group which did not comprise a representative sample of all SBI recipients with rheumatic complaints, but rather a highly selected subgroup with complaints. The selection was based on their own reporting to a Dutch consumer organisation (Consumentenbond) of having complaints. This database was established by the Consumentenbond in order to represent Dutch women in the ongoing law suits in the United States. Therefore it is likely that in this database there is an over-representation of SBI recipients with complaints. This register however gave us the opportunity to select in a short time period a sufficient number of women to perform a pilot study.

A first general selection for mailing of the questionnaire was done on the basis of the (not systematically) registered complaints dealing with arthralgia and fibromyalgia, as these complaints had a high prevalence in the study of Tenenbaum (37). For pragmatic and convenience reasons the questionnaire was sent out to women who were living a reasonable distance from the study centre. We had a response rate of 68% to our questionnaire. In this way a study population was selected with a high score of self-reported arthralgia and fatigue complaints. Since the purpose of this study was to confirm that the APA assay was able to give high values among women with an SBI and severe complaints/symptoms, there was no need to have a random sample but only to have a group of SBI recipients with severe complaints and symptoms. We were not able to confirm the results of Tenenbaum *et al.* (37) in a group of SBI recipients with severe symptoms. Was this a true finding or is there an alternative explanation? We created severity subgroups using the physician's assessment of disease activity and functional disability parameters. A similar approach was used by Tenenbaum *et al.* (37).

Participants in our study were selected on the basis of self-reported complaints of arthralgia and fatigue. Only SBI recipients reporting severe complaints were selected. It seemed likely that severe complaints would lead to a high score for functional disability and disease activity. In such a population the prevalence of polymer binding immunoglobulins was found to be highest

Table VI. Presence of polymer binding immunoglobulins in a group (N = 42) of SBI recipients with self reported severe complaints in relation to severity of symptomatic disease.

Severity classification	Assay polymer binding immunoglobulins[a]			Antinuclear antibodies (ANA)[b]			Extractable nuclear antigens (ENA)
	N	< 20[c]	20 - 50	> 50	Negative	Possible	Positive	Positive
	42	30 (71)[d]	9 (21)	3 (7)	24 (57)	8 (19)	10 (24)	11 (26)
Severity subgroups (functional disability and disease activity)[e]								
Limited	34	24 (70)	8 (24)	2 (6)	18 (53)	8 (24)	8 (24)	9
Mild	2	1 (50)	-	1 (50)	2 (100)	-	-	-
Moderate	2	2 (100)	-	-	1 (50)	-	1 (50)	1
Advanced	4	3 (75)	1 (25)	-	3 (75)	-	1 (25)	1

a) Polymer binding immunoglobulins determined in 1:400 serum dilution;
b) ANA positive, positive reaction in 1:40 serum dilution, ANA possible, no clear distinctive positive or negative reaction;
c) Value expressed as percentage of positive control reference (=100%);
d) Within parentheses percentage (%).
e) Severity subgroups: Limited: Steinbrocker I/II and asymptomatic/mild disease activity.
Mild = Steinbrocker III/IV and asymptomatic/mild disease activity; Moderate = Steinbrocker I/II and moderate/severe/very severe disease activity;
Advanced = Steinbrocker III/IV and moderate/severe/very severe disease activity.

both in SBI recipients and fibromyalgia patients (37, 38). However, on physical examination few women could be classified as being functionally disabled and/or expressing moderate or severe disease activity. Therefore, we were not able to assess the prevalence of polymer binding antibodies in the subgroup of SBI recipients we intended to study. Although our selection procedure was highly biased towards obtaining SBI women with severe complaints and symptoms, we were unable to do so. We must conclude that the inclusion criteria we used were not appropriate for our intended selection. This might be an explanation why we were not able to reproduce the results of Tenenbaum (37). Another reason might be that the subset of SBI recipients in the advanced severity subgroup represents a very small subset of all SBI recipients and that the Dutch cohort is just too small to identify this group. Also in the Tenebaum study the advanced severity subgroup comprised only 20% of the SBI population investigated (37).

Approximately 50% of the women had their SBI explanted, of whom 40% (8 of 20) received a second implant. As severity of the complaints was the primary reason for selection, this explains why women in whom the SBI was explanted were included in the study. Next, our exclusion criteria also did not include pre-existent rheumatic diseases. As a result we failed to exclude a woman whose rheumatoid arthritis predated her SBI. We realise that the data on this SBI recipient are not informative, but believe that it has no impact on our results.

Finally, one might criticise the serum dilution and cut-off point used for evaluation of the data. For determining positivity cut-off points the values (mean plus 3 times the standard deviation) of the negative and weakly positive reference serum samples were used. The borders of the categories (negative < 20%, weakly positive 20% - 50%, positive > 50%) were established with n = 6 at a serum dilution of 1:400. Tenenbaum based the cut-off value on the optical density that captured the largest number of affected patients but the smallest number of control samples (37). As we had reference samples for negative, weak, and positive responses available, we used the results of these reference samples (e.g., mean ± 3 x SD) to define our cut-off level at 50% of the positive reference. Besides the positive responses in SBI recipients (3/42, 7%) and non-SBI laboratory workers (2/12, 17%), also weak positive responses could be established, in both SBI recipients (9/42, 21%) and non-SBI laboratory workers (1/12, 8%).

The number of fibromyalgia diagnoses was rather high in our selected study population, which is not surprising as our population was primarily selected on the basis of arthralgia and myalgia. Nineteen of the 42 women in the study group were diagnosed as fibromyalgia patients. Six of these 19 (32%) fibromyalgia diagnosed women had polymer binding immunoglobulins in their serum (positive n = 2 and weakly positive n = 4), which is in the same range as that reported by Wilson *et al.* (38) for women with mild fibromyalgia. Many of the women in the study population showed myalgia in the thoracic and shoulder areas. These complaints are possibly more closely related to the surgery performed on the breast than to the presence of a silicone breast implant (6).

Whether an immune reaction against silicone in terms of an antigen specific response can occur is not clear. Both for humoral and cellular responses controversial results have been reported (28-36). Our entire study population had a mean exposure time to the silicone breast implant of 17 years. Therefore, it is not likely that exposure to silicones as such is relevant for the induction/presence of polymer binding immunoglobulins, as we then should have found a higher number of positive responses in our study population. However, in this regard a difference between symptomatic and asymptomatic women cannot be excluded. It might be that as yet unknown individual parameters are regulating the induction and binding of the polymer binding immunoglobulins.

In conclusion, we were unable to confirm a high prevalence of polymer biding immunoglobulins in SBI recipients with severe symptoms, due to the lack of a large proportion of symptomatic SBI recipients in our study population. Although selected based on self-reported severe health complaints, only a few of the women in our study were diagnosed by the study physician to be functionally disabled and/or to have high disease activity. Further studies are needed to assess whether the presence of polymer binding immunoglobulins shows high(er) levels and prevalence among women with severe functional disability and/or high disease activity. Only when such a (sub)group of SBI recipients can be identified, will a study aimed at answering the question: "Can the presence of polymer binding immunoglobulins discriminate between women with SBI and severe symptoms as compared to women with SBI and no symptoms, women with other rheumatic diseases without SBI and healthy controls without SBI and without symptoms?" be warranted.

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