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

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Voorwoord

In Nederland zijn naar schatting 25.000 tot 30.000 vrouwen met een siliconen-borstimplantaat. Wereldwijd hebben een tot twee miljoen vrouwen een borstimplantaat. Ongeveer tachtig procent van hen heeft een implantaat om de borst te vergroten, en ongeveer twintig procent om weggenomen borstweefsel als gevolg van borstkanker of een andere aandoening te compenseren.

Het is bekend dat er vrouwen zijn met siliconen-borstimplantaten die gezondheidsklachten ervaren. Zij hebben onder meer last van pijnlijke gewrichten en spieren, vermoeidheid, slaapstoornissen, en chronische hoofdpijn. Vaak zijn de klachten zoals die onder andere worden gemeld bij het Steunpunt voor Vrouwen met Siliconen-implantaten (SVS, Lelystad Haven, Nederland) zeer ernstig.

Wetenschappelijk onderzoek heeft echter geen relatie tussen siliconen-borstimplantaten en diverse aandoeningen kunnen aantonen. De Gezondheidsraad heeft in 1999 een rapport uitgebracht met daarin soortgelijke conclusies.

De commissie van de Gezondheidsraad benadrukt wel het belang van een goede voorlichting aan potentiële draagsters van siliconen-borstimplantaten over de mogelijke bijwerkingen en risico's. Ook moet er voorlichting gegeven worden over klachten die door sommige vrouwen ervaren worden waarvoor een relatie met siliconen-implantaten wetenschappelijk niet is aangetoond. Tevens wordt het opzetten van een landelijke registratie en nauwkeurige follow-up bepleit, zowel voor mogelijke algemene als lokale klachten/complicaties bij draagsters van een siliconen-borstimplantaat.

Het onderzoek dat beschreven is in dit rapport is geïnitieerd naar aanleiding van een publicatie in de wetenschappelijke literatuur dat de aanwezigheid van bepaalde antistoffen in het bloed gerelateerd zou zijn aan de ernst van de klachten/symptomen. Door het RIVM is onderzocht of dit ook in Nederland het geval is.

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Samenvatting

Het doel van het in dit rapport beschreven onderzoek is vast te stellen of er in Nederland een populatie vrouwen met een siliconen-borstprothese bestaat met ernstige ziekteverschijnselen, en een hoge prevalentie van antipolymeer antistoffen (APA). De aanwezigheid van deze antipolymeer antistoffen is in de literatuur beschreven bij vrouwen met ernstige ziekteverschijnselen en een siliconen-borstimplantaat (SBI), en bij ernstige fibromyalgie patiënten. Of deze antipolymeer antistoffen geïnduceerd zijn op basis van een immunologische antigeen specifieke reactie is niet bekend, dus de term polymeerbindende immunoglobulinen lijkt meer van toepassing.

Eerder werd reeds onderzoek verricht naar de prevalentie van APA bij vrouwen met een SBI en ernstige ziekteverschijnselen. Deelneemsters werden geselecteerd middels een vragenlijst op basis van zelfgerapporteerde klachten. De ernst van de ziekteverschijnselen werd hierna klinisch vastgesteld tijdens de studie. Toen werd bij slechts drie van de 42 onderzochte vrouwen met een SBI een positive waarde voor de APA test gevonden. Vrouwen met ernstige ziekteverschijnselen vormden echter slechts een klein deel van de onderzoeksgroep. Eerder gepubliceerde resultaten betreffende de aanwezigheid van polymeer bindende antistoffen bij vrouwen met een SBI en ernstige ziekteverschijnselen, konden derhalve noch bevestigd noch ontkend worden.

In het hier gepresenteerde onderzoek werd een meer uitvoerige vragenlijst gebruikt met vragen over spier- en gewrichtspijn, vermoeidheid, en vragen over het lichamelijke en psychosociale gevoel van welbehagen. Bovendien heeft de huisarts of de behandelend specialist een oordeel gegeven over de klinische symptomen van de SBI vrouwen gedurende de laatste twaalf maanden voor het onderzoek. De criteria voor opname in de studie waren gebaseerd op de bereidheid tot deelname van de vrouw, en de klinische evaluatie van de huisarts of behandelend specialist. Het doel was om minimaal 50 vrouwen met een SBI of blootstelling aan een SBI en ernstige ziekteverschijnselen, in de studie op te nemen. Omdat dit aantal niet bereikt werd met de vastgestelde selectiecriteria, werd een gedeelte van de vrouwen in de studie groep uitgenodigd op basis van de ernst van de klachten zoals door hen aangegeven op de vragenlijst.

In totaal namen 42 vrouwen deel aan de studie. De leeftijd varieerde van 31-73 jaar. Eenentwintig vrouwen werden in de studie opgenomen op basis van gegevens van de huisarts/specialist, en 21 vrouwen op basis van de vragenlijst. De gemiddelde blootstelling aan een SBI was 16 jaar. Bij de vrouwen werd klinisch onderzoek uitgevoerd en bloed afgenomen. Tevens werden bloedmonsters onderzocht van 80 vrouwen uit de algemene

bevolking in dezelfde leeftijdscategorie (studiegroep plus of min 5 jaar) met een leeftijd van 26-69 jaar.

De studiegroep werd ingedeeld in vier subgroepen met respectievelijk minimale, geringe, matige, en ernstige ziekteverschijnselen. De onderverdeling vond plaats op basis van enerzijds de afname van functionele capaciteit (zelfredzaamheid, wat kan men nog wel en niet meer doen in het dagelijkse leven), en anderszijds de algemene beoordeling door de studie-arts wat betreft de ziekte-activiteit en een algemene schatting van de pijn. De meeste vrouwen met SBI (31 van de 42) werden ingedeeld in de groep met minimale ziekteverschijnselen. Zes van de 42 vrouwen met SBI werden ingedeeld in de groep met geringe, twee in de groep met matige, en drie in de groep met ernstige ziekteverschijnselen.

Vijf van de 42 SBI draagsters (11.9%, 95% betrouwbaarheidsinterval 4.0%-25.6%) hadden een positieve waarde voor de aanwezigheid van polymeerbindende immunoglobulinen (APA) in het serum. Alle APA positieve reacties werden waargenomen bij vrouwen in de groep met minimale ziekteverschijnselen. Bij vier SBI draagsters werd een zwak positieve waarde gevonden. Een van de drie vrouwen ingedeeld in de groep met ernstige ziekteverschijnselen had een zwak positieve APA waarde.
In de controle groep werd bij vijf van de 80 vrouwen (6.3%, 95% betrouwbaarheidsinterval 2.1%-14.0%) een positieve APA waarde waargenomen, en bij één een zwak positieve waarde. Het geringe verschil tussen de SBI draagsters en de controle groep is statistisch niet significant.

Dit onderzoek kan de resultaten van Tenenbaum over de aanwezigheid van “antipolymeer antistoffen” in het bloed bij vrouwen met een SBI en ernstige symptomen noch bevestigen noch ontkennen. Het feit dat het niet mogelijk bleek in twee studies voldoende vrouwen met een SBI en ziekteverschijnselen op te nemen, geeft aan dat het aantal vrouwen met een SBI en ernstige symptomen in Nederland gering is. Ook bij een normale populatie Nederlandse vrouwen wordt er een behoorlijk aantal vrouwen met een positieve waarde voor antipolymeer antistoffen in het bloed gevonden. Dientengevolge kan het gebruik van de ‘antipolymeer antistof’ test niet aanbevolen worden als hulpmiddel bij de klinische evaluatie van vrouwen met een SBI en ernstige klachten/symptomen.

Summary

The presence of antipolymer antibodies (APA) was reported in women with severe symptoms and a silicone breast implant and in severe fibromyalgia patients. Whether these antipolymer antibodies are induced as an immunological antigen specific reaction is not known, so the term polymer binding immunoglobulins seems to be more appropriate. The focus of this report was to determine whether there exists a population of symptomatic silicone breast implant (SBI) recipients with a high prevalence of polymer binding immunoglobulins in the Netherlands.

Previously a study was conducted to establish the prevalence of APA in a group of severely symptomatic SBI recipients. Study participants were selected on basis of self reported complaints in a questionnaire. The severity of disease was clinically determined during the study. Only three of 42 SBI recipients were positive for the APA assay. However, we were unable to include a large proportion of severely symptomatic SBI recipients in that study population. So, the results regarding the presence of polymer binding immunoglobulins in symptomatic SBI recipients could not be confirmed nor excluded.

For the present study an extended questionnaire was used which included a screening questionnaire for connective tissue diseases, the Shortened Fatigue Questionnaire, and the RAND 36 item Health Survey. In addition, the general practitioner (or consulted specialist) was asked to fill out a questionnaire in order to form an opinion about the disease status of the women during the last twelve months. The inclusion criteria for the present study were based on willingness of the women to participate, and on the clinical evaluation of the general practitioner/specialist. The aim of the study was to recruit 50 women with a SBI or exposure to a SBI, and severely symptomatic disease. As this number of participants was not reached using the predetermined selection criteria, part of the women in the study group were selected on basis of self reported complaints recorded in the questionnaire.

A total of 42 SBI recipients were included in the study age ranging from 31 – 73 years. Twenty-one participants could be recruited via the physicians information, while 21 participants were included based on the questionnaire. The mean SBI exposure was 16 years. The participants were clinically examined and serum samples were obtained. Serum samples of eighty women age 26 – 69 years (study group plus or minus five years) were evaluated as controls for the presence of polymer binding immunoglobulins as well.

The study population of SBI recipients was categorized in severity subgroups based on the functional capacity, and the study physicians general assessment of pain and disease activity.

Most (31 of 42) SBI recipients belonged to the limited severity subgroup on an increasing scale of limited, mild, moderate and advanced. Six were categorized in the mild, two in the moderate, and three in the advanced severity subgroup.

Five of the 42 SBI recipients (11.9%, 95% confidence interval 4.0%-25.6%) showed a positive response for polymer binding immunoglobulins (APA) in their serum. All APA positive participants were classified in the limited severity subgroup. Four SBI recipients showed a weakly positive response. One of the three participants in the advanced severity subgroup had a weakly positive APA value.
In the control group five of the 80 women (6.3%, 95% confidence interval 2.1%-14.0%) showed a positive reaction for polymer binding immunoglobulins (APA), while one control sample was weakly positive.
The small difference between the SBI recipients and the control group is not statistically significant.

In conclusion, although we cannot confirm nor negate the results of Tenenbaum on the presence of antipolymer antibodies in symptomatic SBI recipients, our failure in two studies to recruit symptomatic SBI recipients suggests that the population of severely symptomatic SBI recipients in the Netherlands is rather small. In addition, also in the normal population a substantial number of positive reacting women were observed. Hence, we cannot recommend the use of the APA assay for diagnostic purposes in the clinical evaluation of SBI recipients with severe complaints/symptoms.

1. Introduction

Since the early nineties there has been serious concern on the health risks associated with silicone breast implants (SBI) both in the scientific literature and in the lay press. This has resulted in a restriction on the use of silicone breast implants in the US in 1992, due to the lack of sufficient evidence for their safety (Kessler, 1992). The 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 by Sanchez Guerrero *et al.,* 1994). However, to date most large scale epidemiological studies (Gabriel *et al.,* 1994; Sanchez Guerrero *et al.,* 1995; Nyren *et al.,* 1998; Friis *et al.,* 1997; Edworthy *et al.,* 1998; Hochberg *et al.,* 1996) and systematic reviews of the scientific literature (Perkins *et al.,* 1995; Noone, 1997; Hochberg and Perlmutter, 1996) have found no evidence to support an association between SBI and connective tissue disease, although some studies could not rule out a small increased risk (Silverman *et al.,* 1996; Hennekens *et al.,* 1996). Furthermore, the Independent Review Group (IRG) in the UK (Independent Review Group, 1998), the National Science Panel in the US (National Science Panel, 1998), the Committee on the safety of silicone breast implants in the US (Bondurant *et al.,* 1999), and the Committee on Silicone Implants of the Health Council of the Netherlands (Health Counsil of the Netherlands, 1999) concluded that there is no scientific proof for a link between SBI and any connective tissue disease. A recent meta-analysis of 20 published studies revealed no evidence of an association between breast implants in general, or silicone gel filled implants specifically, and any of the individual connective tissue diseases, all definite connective tissue diseases combined, or other autoimmune or rheumatic conditions combined (Janowsky *et al.,* 2000). Also for neurological disorders no causal association between silicone breast implants and neurological disease was observed in a survey of Danish women (Winther *et al.,* 2001).

The study results mentioned above 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 (Todhunter and Farrow, 1998). 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 (Todhunter and Farrow, 1998; Noone, 1997). In a recent study women with breast implants reported a multitude of symptoms more often than women who underwent breast reduction surgery (Fryzek *et al.,* 2001a). However, a lack of specificity and the absence of dose response relationships suggested that the excess of reported symptoms was not causally related to the cosmetic implants (Fryzek *et al.,* 2001a).

Although there seems to be no major systemic risk involved in the use of SBI, for local complications the situation is quite different. Local complications include amongst others severe fibrosis and implant rupture, the latter was observed in more than 50% of the implants involving 77% of the study population with a rupture or intermediate (suspected) implant (Brown *et al.,* 2000). In another study local complications were noticed in 31% of the study population (Fryzek *et al.,* 2001b). Women with breast implants and local complications more frequently reported general symptoms compared to women in the breast reduction group or women with breast implants but no local complications (Fryzek *et al.,* 2001b). So, the presence of local complications may be an important factor in symptom reporting. The presence of extracapsular silicone after breast implant rupture was found to result in an increased risk for fibromyalgia compared to SBI women without extracapsular silicones, suggesting an association between extracapsular silicone and fibromyalgia (Brown *et al.,* 2001).

The presence of anti-silicone antibodies in SBI recipients was claimed by several authors (Vinuya *et al.,* 1998; Wolf *et al.,* 1993; Goldblum *et al.,* 1992; Vojdani *et al.,* 1994). Part of these responses were found to be non antigen specific (Goldblum *et al.,* 1995; White, Jr. and Klykken, 1998). The binding of human and animal antibodies to silicone was demonstrated to be most likely dependent on serum proteins and consistent with the interaction of hydrophobic molecules (IgG) with hydrophobic surfaces (silicones) (White, Jr. and Klykken, 1998). Recently false positive results for anti-silicone antibodies were reported probably caused by storage conditions, while no anti-silicone antibodies were detected in SBI women (Oliver *et al.,* 2000).

Besides antisilicone antibodies the presence of antipolymer antibodies (APA) has been observed in SBI recipients (Tenenbaum *et al.,* 1997a) and fibromyalgia patients (Wilson *et al.,* 1999). 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 (Tenenbaum *et al.,* 1997b). So, the antigen specificity of the immunoglobulin binding in the ‘APA’ assay in terms of ‘antipolymer antibody’ remains a question. Polymer binding immunoglobulins seems a more suited description for this activity in the serum.

The prevalence of the polymer binding immunoglobulins was highest in groups of patients with severe symptoms (Wilson *et al.,* 1999; Tenenbaum *et al.,* 1997). Although a diagnosis cannot be made on the presence of these polymer binding immunoglobulins alone, the value of such an assay would be the objectivity of a laboratory test. The APA assay was succesfully

introduced in our laboratory. The assay provided reproducible results and thus could be used for the evaluation of the presence of polymer binding immunoglobulins in the serum of women with a silicone breast implant (De Jong *et al.,* 1998).

In order to confirm or refute the results of Tenenbaum *et al.,* 1997 a fullblown epidemiological study would be necessary. The aim of such a study would be to assess whether the 'APA' assay shows higher values and prevalence among women with SBI and severe complaints/symptoms, as compared to women with SBI and mild or no complaints/symptoms, women without SBI and severe complaints/symptoms, women without SBI and with rheumatic or connective tissue diseases, and healthy women without SBI and without complaints/symptoms. However, beforehand it is wise to determine whether there exists in the Netherlands a population of women with severe complaints/symptoms and a high antipolymer titer. A first attempt to identify such a population was made by us in 1998 (De Jong *et al.,* 1999, 2002). A study population was selected based on a self reported questionnaire. Blood samples were obtained and the women were clinically investigated by an independent rheumatologist to estimate their disease status at the moment of the study. A rather low prevalence (7%) of antipolymer antibodies was detected in our study population, while non of the APA positive SBI recipients belonged to the severe symptomatic disease group (De Jong *et al.,* 1999, 2002). In addition most of our SBI recipients were catagorized in the mild and/or limited severity subgroups according to the functional capability and physicians estimation of disease activity. Conclusively we were not able to recruit SBI recipients with severe symptoms.

In this second study the evaluation of the general practitioner was used as selection criterium for the inclusion of participants in our study in order to optimise the selection of sympyomatic SBI recipients.

2. Materials and Methods

2.1 Study design

The study was conducted as a cross sectional study in a population of women with SBI and complaints. Clinicals examinations, blood sampling and general laboratory assays were performed from April 2000 to November 2000 for 38 women, and additionally in August 2001 for 4 women. The APA assay was performed from November 2000 to September 2001. Participants were selected with the cooperation of the 'Steunpunt voor Vrouwen met Siliconen-implantaten' (SVS, Lelystad Haven, the Netherlands) an organisation dedicated to the support of women with silicone breast implants.
Fivehundred and seventy-seven questionnaires were mailed by the SVS, of which 3 were returned as undeliverable. Sixty-five percent (n=375) were returned. Eighty percent (n=299) of the SBI recipients returning the questionnaires gave permission to contact their general practitioner or consulted specialist. The general practitioners (or consulted specialist) were approached for their opinion of the disease status of the women. Ninety-two percent (n=274) of the questionnaires send out to the practitioners were returned (Table 1)

The questionnaire for the SBI recipients 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.,* 1995. The Shortened Fatigue Questionnaire (Alberts *et al.,* 1997), a validated questionnaire, was included to determine the intensity of the patient's fatigue. The RAND 36 item Health Survey was included for evaluation of the physical and psychosocial well-being of the participants (Hays *et al.,* 1993, Van Der Zee and Sanderman 1994). Additional questions included SBI specific questions, such as date and reason for implant, the date and reason if the SBI was explanted and specific SBI related health problems. No information was obtained on the brand of implant.
The questionnaire sent to the physicians included questions on the history of complaints and symptoms of arthralgia and fatigue, and possible comorbidity. Physicians were asked whether these complaints and symptoms were open to objectification, and to assess the patients' functional capacity.

To increase the possibility of selecting a severely symptomatic subgroup, the assessment of womans' health status by the general practitioner or consulted specialist formed the basis of our selection criteria. Four subgroups of women were defined, the first group being the most likeliest to be classified in the advanced severity subgroup after clinical examination later on. Group 1 included women whose complaints were open to objectification according to their general practitioner or consulted specialist, suffered pain in more than 3 joints, and had a

Steinbrocker classification for functional disability grade 3 or 4 (functional capacity either adequate to perform few or none of the tasks of usual occupation or self care, or largely or wholly incapacitated to perform little or no self care) (Steinbrocker *et al.,* 1949). Women in the second group (Group 2) were comparable to women in the first group except for a Steinbrocker classification grade 1 or 2 (functional capacity either complete or adequate to perform normal activities, despite handicap or discomfort). Group 3 included women that were comparable to those in Group 1 but had pain in less than 3 joints. Finally, the fourth group (Group 4) included women who did not fulfill the criteria of Groups 1- 3. Women of Group 4 had self-reported pain in 3 or more joints, scores on subscales for physical functioning and pain in the RAND 36 item Health Survey lower than reported mean scores minus 2 standard deviations in the aged matched general population (Van Der Zee and Sanderman 1994), and a score of 26, the average score for patients suffering from the chronic fatique syndrome, or higher on the Shortened Fatique Questionnaire (Alberts *et al.*, 1997). We aimed at 50 participants in the study preferably recruited from Group 1. If we would not identify 50 women in Group 1, we would extend our invitation to Group 2 women and so on. Exclusion criteria were co-morbidity explaining the complaints (i.e. osteoarthritis, trauma), psychological diseases and classic systemic diseases diagnosed before SBI implantation. Women who received a SBI after breast reconstruction because of breast cancer were not included, as were women who participated in our previous study.

Fourteen Group 1 women, 7 Group 2 women, 7 Group 3 women, and 32 Group 4 women were identified, and selected for inclusion in the study. The medical history, physical examination and the presence of polymer binding immunoglobulins was examined in 42 SBI recipients, 12 Group 1 women, 4 Group 2 Women, 5 Group 3 women, and 21 Group 4 women. Eighteen of the 60 selected women did not participate. In order to increase the participation rate, initially non-participating women were approached by telephone and asked for their reason not to participate in the study. Transportation was offered when it was impossible to visit the hospital for the clinical examination. Two of the approached women made use of the facility offered. Reasons mentioned for not participating in the study were: disease or handicap disabling participation (n=4); not reached (n=3); not willing to participate (n=11).
The selection process is presented in Table 1. Evaluation of the returned questionnaires during the selection, revealed a compromised perception of well-being, with RAND scores on all subscales below that of women from the general Dutch population (Figure 1). The number of missing values in the RAND scores (1.9%-7.7%) was similar to previously reported data (Van Der Zee *et al.,* 1996).

The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht.

For control on the presence of polymerbinding immunoglobulins in the serum of the SBI recipients, serum samples were used from a healthy female population. The sera were collected in a pilot study of the 'REGENBOOG' project, which is the Dutch acronym for 'Risk Factors and Health in the Netherlands, a Survey by Municipal Public Health Services'. The main aim of this project is to monitor risk factors or determinants of chronic and infectious diseases in the general population. Nationally representative health data are collected by means of personal interviews, questionnaires and physical examinations. These data are also used to build up a biological plasma and DNA database for studies of chronic and infectious diseases. The RIVM carries out the population based health surveys in cooperation with Statistics Netherlands and the National Association of Municipal Public Health Services. The control serum samples, n=80, were age (plus or minus 5 years) and gender (females) matched to the study population. The age range of the study population was 31-73 years, and 26-69 years for the controls. Most control serum samples were obtained from october 1996 to december 1996, 2 samples were obtained in April 1997.

2.2 Clinical examination of participants

The medical history was taken and a complete physical examination was performed on the 42 included SBI recipients by a registered rheumatologist (C.M.V.). Information on prosthesis explant and local breast complications like pain, capsular contraction, migration of the SBI, etcetera were recorded. The systemic symptoms evaluated included fatigue, sleep disturbance, painful joints and muscles, muscle-weakness, swollen joints, sicca symptoms, 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). Total tender joint count (53 joints), total swollen joint count (44 joints) (Prevoo *et al.,* 1993) and tender points according to the American College of Rheumatology (ACR) criteria for fibromyalgia were included (Wolfe *et al.,* 1990). A Schirmer test for detection of Sjögrens syndrome was performed (Bijsterveld, 1969). 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) (Steinbrocker *et al.,* 1949). General assessment of pain and disease activity of the SBI recipients was recorded on a visual analogue scale (VAS) of 100 mm, while the physicians general assessment of disease activity was recorded on a 5 point scale ranging from asymptomatic to very severe.

To determine severity subgroups, SBI recipients were further classified according to 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).

Auto-immune diseases were diagnosed according to the classification criteria for the specific disease; criteria for systemic lupus erythematodes (SLE), rheumatoid arthritis (RA) and scleroderma and fibromyalgia according to the ACR criteria (Wolfe *et al.,* 1990; Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee, 1980; Arnett *et al.,* 1988; Tan *et al.,* 1982), and Sjögren's syndrome according to the criteria of Fox *et al.,* 1986.

The clinical examination of the SBI recipients was performed at the Department of Rheumatology, University Hospital Utrecht, Utrecht, the Netherlands.

2.3 Clinical chemistry

2.3.1 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 (Utrecht Diagnostics, University Hospital Utrecht, the Netherlands). 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 of 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 western-blot technique were performed according to standard hospital routine procedures (University Hospital Utrecht, Utrecht, the Netherlands). TSH, thyroid-stimulating hormone, in serum was determined according to hospital routine procedures to exclude hypo- or hyperthyroidism as explanation for the participants complaints.

2.3.2 'APA' assay

2.3.2.1 Materials

Nitro-cellulose strips containing partially polymerised acrylamide (Wilson *et al.,* 1999), 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 (Wilson *et al.,* 1999; Tenenbaum *et al.,* 1997).

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), distillated water (SVM), milk powder (Campina Melkunie B.V., Eindhoven, the Netherlands).

2.3.2.2 Methods

The nitrocellulose strips were placed in a 20 wells tray and washed in 2 ml of washing buffer (0.1 M NaCl, 0.3% Tween, pH 7.4) for 5 min 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 µl serum sample was added (1:400 serum sample dilution). Four reference samples, a strongly positive control, a positive control, a weakly positive control and a negative control sample 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). Detection buffer was decanted and strips were washed several times with distilled water. Strips were dried on a filter paper and placed on a record sheet. The colour of the bands on the strips was quantified using a CCD camera and the Molecular Analyst software (BioRad Laboratories, Hercules, CA, USA) and expressed as Optical Units (O.U.). The mean value in O.U. of all three bands was determined for each serum sample.

All buffers were prepared freshly and preservative (thiomersal) was not added.

3. Results

3.1 Participant characteristics

Characteristics of the 42 SBI recipients are described in Table 2. Most SBI recipients were older than 40 years of age. Most women (n=33) received their SBI before 1986, of which 16 before 1977. Cosmetic surgery was the reason for the SBI in the majority of the women (76%). Thirty-two (76%) of the SBI recipients had had their SBI explanted. Most explantations (n=29) were performed after 1992, the year of the FDA ban on the use of SBI for cosmetic surgery (Kessler, 1992). The duration of the SBI exposure was 16.4 years for the total group when explantation was considered as the end of the exposure time, and 20.4 years when the examination by the study physician was considered the end point.

3.2 Clinical features

Fatigue and arthralgia was reported by almost all of the women and myalgias by 91%. (Table 3). Arthralgia of various joints including neck, shoulders, and upper and lower extremities ranged from 62% to 91% of the participants. Myalgias and muscle weakness were reported by 91% and 74% of the participents, respectively. Twenty-nine percent reported morning stiffness. Sleep disturbances were reported by 81% (Table 3).

On physical examination (Table 4) tender points were present in the majority of women (n=38), while 26 had more than 7 tenderpoints. The distribution according to the Steinbrocker classification indicated that the majority of our study group belonged to a group with no or mild disability, Steinbrocker score I and II. Only 9 SBI recipients had a Steinbrocker score of III, while none had a score of IV. The physicians general assessment of disease activity showed that most SBI recipients (n=37) belonged to a group with mild disease activity (Table 4). For 39 of the 42 women a primary diagnosis could be established, the majority being fibromyalgia (n=16), tendinomyalgia (n=9), and osteoarthritis (n=8), (Table 4). Severity subgroups were determined combining the Steinbrocker score and the physicians general asssessment of disease activity. Only 5 SBI recipients were categorized as moderate (n=2) or advanced (n=3) (Table 5). There were no differences in age, year of SBI, duration of SBI exposure, and local complications between the separate severity subgroups (Table 5).

Capsular contraction and prosthesis rupture or leakage, being local complications of SBI, were reported by 31 women (74%) and 18 (43%) women, respectively (Table 2). On physical examination capsular contraction was diagnosed by the study physician in 2 women. The

discrepancy between the reported and diagnosed number of capsular contractions can be explained by the high percentage (76%) of the participants that had their prostheses removed.

3.3 Clinical chemistry

The results of the blood parameters are presented in Table 6. No indications were found for a specific parameter, expressed as mean of the study group, being out of the normal range when compared to either the control group or reference values. In some SBI recipients blood values were observed outside the reference ranges (Table 7). These deviations are generally only marginally outside the reference range, and are likely to belong to the normal individual variation. In one participant the increased absolute number of WBC and neutrophilic granulocytes may have been indicative for the presence of an inflammatory reaction.

TSH values (data not shown) were with the exception of one of the participants within normal ranges (0.35-5.0 mU/l). For one women the TSH value was above the normal range (TSH 13 mU/l), and was therefore suspected for hypothyroidism.

Table 8 and Figure 2 show 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. The cut-off point for positivity was determined at 20% of the positive reference sample. Below 20% a sample was considered negative. Values between 20% and 50% were designated weakly positive, and values above 50% positive. The cut-off points of 20% and 50%, respectively for weakly positive and positive samples, were determined at the upper level of the negative and weakly positive reference samples, being the mean plus three times the standard deviation. In this study similar values were observed for the negative and weakly positive reference samples compared to the previous study, 6 ± 8 versus 6 ± 5, and 16 ± 9 versus 18 ± 10, respectively (Table 9) (De Jong *et al.,* 1999, 2002). At the 1:400 dilution five (11.9%) samples show a clear positive response and four samples a weakly positive response.

Four of the five participants with a positive APA responses were diagnosed with fibromyalgia and one with tendinomyalgia (Table 8). For the four women with a weakly positive APA response the diagnosis were, one fibromyalgia, one tendinomyalgia, one osteoarthritis and one osteomalacia.

Nine of the SBI recipients showed a positive ANA response (Table 10).

In the control group of 80 healthy females 5 (6%) positive samples, and one weakly positive sample were observed, the remainig 74 samples were negative (Table 9). The study group of SBI recipients showed 11.9% (5 out of 42) “APA” positivity, while the control group showed

6.3% (5 out of 80) APA positivity, respectively. The binomial 95%-confidence interval was for SBI recipients 4.0% - 25.6%, and for the controls 2.1% – 14.0% (not significantly different).

3.4 Laboratory tests and disease severity

Table 10 shows the results of the 'APA' and ANA assay in relation to the severity subgroups (disease activity and functional disability). Most SBI recipients were classified as belonging to the limited severity subgroups, 31 of 42 (71%). Only five SBI women were classified in the moderate and advanced subgroup, 2 in the moderate and 3 in the advanced subgroup, respectively. The negative serum samples of SBI recipients for the presence of polymer binding immunoglobulins are equally distributed over the separate severity subgroups. All five positive serum samples belonged to the limited severity subgroup. Similar results are obtained with regard to the ANA score. There was no difference in age, the reason of SBI, number of reports of local complications, and duration of SBI exposure between the positive and negative women (Table 11).

4. Discussion

The results of this study are similar to the ones reported previously (De Jong *et al.,* 1999, 2002). Again we demonstrated that the 'APA' assay detected the presence of polymer binding immunoglobulins both in a population of women exposed to SBI and a control population. When the SBI recipients were classified in severity subgroups based on functional disability and the study physician's estimation of disease activity, only 3 of the 42 participants were classified in the advanced severity subgroup, and 2 in the moderate severity subgroup. Therefore, again we were unable to select the advanced severity subgroup we opted for, the subgroup expressing a high prevalence of APA reactivity. (Tenenbaum *et al.,* 1997). Furthermore, high values of polymer binding immunoglobulins were found in the sera of 5 of the 42 SBI recipients, all classified to belong to the limited severity subgroup. However, as a result of our inability to select the subgroup of interest we were not able to reproduce the results of Tenenbaum who observed a high prevalence of polymer binding immunoglobulins (APA reactivity) in a group of severely symptomatic SBI recipients. (Tenenbaum *et al.,* 1997)

In our previous study, the failure to select severely symptomatic SBI could be attributed to the fact that women were selected on basis of the severity of self-reported complaints. At physical examination by a rheumatologist these complaints could not be objectified. (De Jong *et al.*, 1999, 2002) To optimise the selection of severely symptomatic SBI recipients in this study the selection of the participants was performed in cooperation with the SVS, 'Steunpunt voor Vrouwen met Siliconen-implantaten', an organisation dedicated to the support of women with silicone implants. SBI recipients contacted were subscribers to the journal of the SVS. It is likely that in this group of women SBI recipients with complaints are overrepresented. We further opted for the clinical evaluation by the treating physician of the SBI recipient's disease status. As it turned out our strategy was once again unsuccesful. Far less than the 50 SBI recipients we opted to invite had objective complaints of pain in more than 3 joints and a Steinbrocker classification for functional disability (Steinbrocker *et al.,* 1949) grade III or IV according to their physician. So, we had to mitigate our inclusion criteria to fill up the 50 SBI recipients we intended to examine.
Is the fact that we now were twice unable to recruit a group of severely symptomatic SBI recipients attributable to the methods employed by us or is there an alternative explanation? Considering the efforts we put in the selection of our study population, we feel that it is very difficult or even impossible to recruit within the Netherlands, even among SBI recipients with severe complaints, a symptomatic study population which could be classified as belonging to the advanced severity subgroup.

Some discussion is needed on the non-participants in this study. After all 18 of the selected women (30%) were not willing to participate or cancelled their appointment for physical examination. For 14 of these women the reason for refusal is unknown, but might be due to serious illness resulting in inability to come to the clinic. Four women were suffering of various other diseases which resulted in exclusion from the study. To overcome this problem door-to-door transportation was offered. However, had all these women been included and had all of them been designated to the advanced severity subgroup this subgroup would still have remained a small subset of the SBI recipients with severe complaints. Moreover, only in combination with a positive APA response it would have added to the validity of the APA assay for SBI recipients. We feel that this would be rather unlikely.

Whether an immune reaction against silicone in terms of an antigen specific response can occur, is not clear. Both for the humoral and cellular responses positive and negative results were reported (Goldblum *et al.,* 1995; White, Jr. and Klykken, 1998; Vinuya *et al.,* 1998; Wolf *et al.,* 1993; Goldblum *et al.,* 1992; Vojdani *et al.,* 1994; Shanklin *et al.,* 1996; Smalley *et al.,* 1995; *Ellis et al.,* 1997). In a recent study also anti-silicone antibodies were not found in SBI women, but false positive responses were observed in controls due to an unknown but human specific immunoglobulin binding phenomenon (Oliver *et al.,* 2000).

The majority (76%) of our study population had their implants removed at the time of the study. As severity of the complaints was the primary reason for selection this explains why women in which the SBI was explanted were included in the study. Similar to our previous study the study group had a rather long exposure time to silicones with a mean of 16 years till explantation and even 20 years until the study in the year 2000. Comparing women with and without SBI we found that there were no differences in the presence of polymer binding immunoglobulins. 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. Tenenbaum mainly found positive APA reactivity in symptomatic SBI recipients (Tenenbaum *et al.*, 1997). Compared to our population control an increased number of weakly positive values was observed in the SBI recipients. As these weakly positive reacting women were mainly categorized in the limited severity subgroup, we feel this is of no significance.

Like in our previous study, a substantial number of participants (38%) were diagnosed with fibromyalgia. Since our selection criteria were based on complaints of arthralgia open to objectivication by the SBI recipient's treating physician or severity of pain as expressed by the SBI recipient on the subscale pain of the RAND-36, this explains the high prevalence of fibromyalgia among the participants in this study. Four of the five women positive for APA were diagnosed with fibromyalgia and the remaining one was diagnosed with tendinomyalgia.

In the fibromyalgia diagnosed SBI recipients the percentage APA reactivity (25%) is increased when compared to our control group with an APA reactivity of 6%. No indications were obtained for a relation with severity of disease as all APA positive individuals were classified to belong to the limited severity subgroup. In this respect our results do not provide information whether the APA reactivity might be indicative for severe fibromyalgia as reported by Wilson *et al.,* 1999.

In conclusion, although we cannot confirm nor negate the results of Tenenbaum on the presence of antipolymer antibodies in symptomatic SBI recipients, our failure in both studies to recruit symptomatic SBI recipients suggests that the population of severely symptomatic SBI recipients in the Netherlands is rather small. In addition, also in the normal population a substantial number of positive reacting women were observed. Hence, we cannot recommend the use of the ‘APA’ assay for diagnostic purposes in the clinical evaluation of SBI recipients with severe complaints/symptoms

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Tables and Figures

Table 1 Selection of women with silicone breast implants (SBI)

Questionnaires and invitation for participation	n=577
Returned for incorrect address	n= 3
Total invitations	n=574
Total returned	n=375 (65%)
Permission to contact physician	n=299 (80%)
Empty questionnaires	n= 10
Late responses	n= 2
Evaluated	n=363 (63%)
Questionnaires send to physicians	n=299
Returned by physicians	n=274 (92%)
Selected for inclusion	n=60
Physicians statement	n=28
Self reported complaints	n=32
Final inclusion in study	n=42
Physicians statement	n=21
Self reported complaints	n=21
Non-participation	n=18
4x other disease diagnosis	
3x not reached for appointment	
11x not willing to participate	

Table 2. Characteristics of study population.

Characteristics		Participants n=42 N (%)
Age	< 40	7 (17)
	40-50	15 (36)
	> 50	20 (48)
Year of first SBI	< 1977	16 (38)
	1977 - 1985	17 (41)
	> 1985	9 (21)
Reason for SBI	Cosmetic	32 (76)
	Fibrocystic	10 (24)
Explantation	Yes	32 (76)
	No	10 (24)
Year of explantation (n=32)	< 1992	3 (9)
	> 1991	29 (91)
Encapsulation	Reported by participants (present during SBI)	31 (74)
	Diagnosed by study physician (still present)	2 (5)
Rupture/leakage	Reported by participants	18 (43)
Duration of SBI exposure		16.4 ± 6.6 (until explantation) 20.4 ± 5.9 (until examination by study physician)

Table 3. Characteristics of study population. Inventarisation of complaints.

Complaints		Participants n=42 N (%)
Fatigue	Present	41 (98)
Arthralgia	None	0 (0)
	1-2 joints	2 (5)
	3-8 joints	18 (43)
	> 8 joints	22 (52)
	Neck	38 (91)
	Shoulder	37 (88)
	Elbow	26 (62)
	Wrist	30 (71)
	Hand	36 (86)
	Hip	30 (71)
	Knee	38 (91)
	Ankle	30 (71)
	Foot	31 (74)
	Spine	36 (86)
Morning stiffness	> 1 hour	12 (29)
Myalgias		38 (91)
Sleep disturbances		34 (81)
Rashes		13 (31)
Dry eyes/mouth		28 (67)
Mouth ulcers		20 (48)
Muscle weakness		31 (74)
Fevers		12 (29)

Table 4. Observations at physical examination.

Diagnosis	Classification	Incidence N (%)
Number of SBI recipients		n=42(100)
Tenderpoints	None	4 (10)[a]
	1-6	12 (29)
	7-10	9 (21)
	11-18	17 (41)
Functional disability (Steinbrocker)	None/Limited (I)	6 (14)
	Mild (II)	27 (64)
	Moderate (III)	9 (21)
	Severe (IV)	-
Physicians general assessment of disease activity	Asymptomatic	0
	Mild	37 (88)
	Moderate	5 (12)
	Severe	-
	Very severe	-
Primary diagnosis	Rheumatoid arthritis	1 (3)
	Osteoarthritis	8 (21)
	Fibromyalgia	16 (38)
	Tendinomyalgia	9 (21)
	Chronic pain syndrome	1 (3)[b]
	M. Sjögren	2 (5)
	Reflex Sympathetic Dystrophy	1 (3)
	Osteomalacie	1 (3)
	None	3 (8)

a) Within brackets percentage
b) Despite the fact that this woman had 12 tenderpoints the rheumatologist classified her as suffering from chronic pain syndrome rather than from fibromyalgia

Table 5. Characterisation of study population in relation to severity subgroups as determined by functional dsiability and disease activity.

Characteristics N=42		Limited n=31	Mild n=6	Moderate n=2	Advanced n=3
Age	< 40	7 (23)	-	-	-
	40 - 50	10 (32)	3 (50)	2 (100)	-
	> 50	14 (45)	3 (50)	-	3 (100)
Year of first SBI	< 1977	10 (32)	3 (50)	-	3 (100)
	1977 - 1985	14 (45)	2 (33)	1 (50)	-
	> 1985	7 (23)	1 (17)	1 (50)	-
Reason for SBI	Cosmetic	21 (68)	6 (100)	2 (100)	3 (100)
	Fibrocystic	10 (32)	-	-	-
Leakage reported		15 (48)	3 (50)	-	-
Encapsulation reported		22 (71)	5 (83)	1 (50)	3 (100)
Explantation		23 (72)	5 (83)	2 (100)	2 (67)

a) Within parentheses percentage (%) of number in severity subgroup

Table 6. Blood parameters of study population.

Group	WBC[a]	Lympho's	Mono's	Neutro's	Eo's	Baso's
SBI recipients n=42						
Absolute (x10^9/L)	7.87 ± 2.2[b]	2.10 ± 0.57	0.41 ± 0.12	5.02 ± 1.88	0.18 ± 0.14	0.06 ± 0.03
Percentage (%)	n.a [c].	28 ± 6.6	5.3 ± 1.6	62 ± 7.8	2.3 ± 1.7	0.8 ± 0.3
Controls n=12[d]						
Absolute (x10^9/L)	6.08 ±1.17	1.95 ± 0.63	0.36 ± 0.09	3.52 ± 0.91	0.12 ± 0.06	0.03 ± 0.02
Percentage (%)	n.a.	32 ± 8.7	6.0 ± 1.1	57 ± 9.2	1.9 ± 1.1	0.5 ± 0.3
Reference[e]						
Absolute (x10^9/L)	4.00 - 10.0	0.8 – 4.5[f]	0.08 – 0.95[f]	1.6 – 7.5[f]	0.04 – 0.5[f]	0.0 – 0.15[f]
Percentage(%)	n.a.	20 - 45	2.0 - 9.5	40 - 75	1.0 - 5.0	0.0 - 1.5

	RBC X10^{12}/L	HGB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	RDW %	HDW mmol/L	PLT x10^9/L	MPV fL
SBI	4.46 ± 0.38	8.4 ± 0.8	0.41 ± 0.04	93 ± 6	1.88 ± 0.11	20.2 ± 0.4	13.5 ± 0.8	1.29 ± 0.11	277 ± 65	8.3 ± 0.8
Controls	4.43 ± 0.27	8.3 ± 0.5	0.390 ± 0.02	88 ± 3	1.78 ± 0.07	21.2 ± 0.6	12.4 ± 0.4	1.45 ± 0.11	254 ± 37	8.5 ± 0.6
Reference	3.90 - 5.30	7.5 - 9.8	0.360 - 0.46	80 - 100	1.70 - 2.10	19.5 - 22.5	n.a.	n.a.	150 - 400	n.a.

a) WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; HDW, hemoglobin distribution width; PLT, platelets; MPV, mean platelet volume.
b) Mean ± standard deviation
c) n.a. not available or not applicable.
d) Controls selected at random of samples of female laboratory workers used for equipment validation during the last two years.
e) Reference values (upper and lower limits) see Materials and methods.
f) Calculated values

Table 7 Blood parameters outside reference values

Parameter	Reference value [a]	Above (n)	Below (n)	Total (n)[b]
RBC ($x10^{12}$/L)	3.90 – 5.30	1 (5.32)[c]	3 (3.54)	4
HGB (mmol/L)	7.5 – 9.8	2 (10.5)	5 (6.73)	7
HCT (L/L)	0.36 – 0.46	2 (0.525)	4 (0.331)	6
MCV (fL)	80 – 100	4 (104.6)	1 (73.4)	5
MCH (fmol)	1.70 – 2.10	-	2 (1.47)	2
PLT ($x10^9$/L)	150 – 400	2 (425)	1 (108)	3
WBC ($x10^9$/L)	4.0 – 10.0	6 (13.84)	1 (3.0)	7
Neutroph. abs. ($x10^9$/L)	1.6 – 7.5	4 (11.44)	1 (1.4)	5
Neutroph. %	40 – 75	1 (83)	-	1
Eosinoph. Abs. ($x10^9$/L)	0.04 – 0.5	2 (0.76)	2 (0.03)	4
Eosinoph. %	1 – 5	3 (7.0)	7 (0.55)	10
Lymphocytes %	20 – 45	-	4 (11)	4
Monocytes %	2 – 9.5	1 (9.77)	-	1

a) Reference values (upper and lower limits) see Materials and methods.
b) Total number of SBI women investigated n=42.
c) Within brackets highest or lowest value observed.

Table 8. Presence of polymer binding immunoglobulins in serum of study population.

Sample number	1 : 400 dilution	Primary diagnosis
R101	39 ± 18[a]	Osteoarthritis
R103	51 ± 18	Tendinomyalgia
R104	11 ± 12	Osteoarthritis
R105	16 ± 10	Osteoarthritis
R106	16 ± 8	M. Sjögren
R107	7 ± 10	Rheumatoid arthritis
R108	6 ± 5	Chronic pain syndrome
R109	59 ± 22	Fibromyalgia
R110	7 ± 3	Osteoarthritis
R111	7 ± 7	Reflex Sympathetic Dystrophy
R112	13 ± 11	None
R113	36 ± 19	Tendinomyalgia
R114	1± 2	Tendinomyalgia
R115	4 ± 4	Fibromyalgia
R116	113 ± 45	Fibromyalgia
R117	9 ± 6	Osteoarthritis
R118	4 ± 5	Fibromyalgia
R119	32 ± 17	Osteomalacia
R120	7 ± 7	Tendinomyalgia
R121	1 ± 2	Tendinomyalgia
R122	74 ± 40	Fibromyalgia
R123	0 ± 0	Tendinomyalgia
R124	23 ± 27	Fibromyalgia
R125	0 ± 0	Fibromyalgia
R126	5 ± 5	None
R127	3 ± 5	Fibromyalgia
R128	13 ± 4	None
R129	2 ± 3	Osteoarthritis
R130	0 ± 0	Fibromyalgia
R131	0 ± 0	Fibromyalgia
R132	3 ± 3	Osteoarthritis
R133	4 ± 5	M. Sjögren
R134	7 ± 11	Osteoarthritis
R135	7 ± 1	Fibromyalgia
R136	8 ± 9	Tendinomyalgia
R137	231 ± 86	Fibromyalgia
R138	2 ± 3	Fibromyalgia
R139	9 ± 6	Tendinomyalgia
R141	8 ± 7	Tendinomyalgia
R143	13 ± 12	Fibromyalgia
R144	1 ± 0	Fibromyalgia
R146	10 ± 6	Fibromyalgia

a) Mean ± s.d.,expressed as percentage of positive (reference) sample, n=3.

Table 9. Presence of polymer binding immunoglobulins in serum of a healthy female population[a]

Total number	80
Value <20	74[b]
Value 20-50	1
Value >50	5
Reference samples	
Strong positive	155 ± 58[c]
Positive	100 ± 0
Weak positive	16 ± 9
Negative	6 ± 8

a) Serum samples of healthy females were obtained from a serum bank of our Institute. Samples were age matched with our SBI study population (age ± 5 years). For each study sample two control samples were investigated. Age range study population 31-73 years, age range controls 26-69 years.
b) Samples were investigated two times on two separate days.
c) Mean ± s.d. n=6, determined on 3 separate days in 3 separate assays.

Table 10. Laboratory results in relation to functional disability and physicians assessment of severity of disease activity.

Severity classification	N	Assay polymer binding immunoglobulins			Antinuclear antibodies (ANA)[a]	
		< 20 [b]	20 - 50	> 50	Negative	Positive
Total	42	33 (79)[c]	4 (10)	5 (12)	33 (79)	9 (21)
Severity subgroups (functional disability and disease activity) [d]						
Limited	31	23 (74)	3 (10)	5 (16)	23 (74)	8 (26)
Mild	6	6 (100)	-	-	6 (100)	-
Moderate	2	2 (100)	-	-	1 (50)	1 (50)
Advanced	3	2 (67)	1 (33)	-	3 (100)	-

a) ANA positive, positive reaction in 1:40 serum dilution.
b) Value expressed as percentage of positive control reference, sample dilution 1:400 (=100%).
c) Within parentheses percentage (%).
d) 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

Table 11. Characteristics of study population in relation to level of polymer binding immunoglobulins.

Characteristics		Assay polymer binding immunoglobulins			
			< 20	20 - 50	> 50
n=42		n=42	n= 33	n=4	n=5
Age	< 40	7	5 (15)[a]	1 (25)	1 (20)
	40 - 50	15	12 (36)	1 (25)	2 (40)
	> 50	20	16 (48)	2 (50)	2 (40)
Year of SBI	< 1977	16	14 (42)	1 (25)	1 (20)
	1977 - 1985	17	13 (39)	1 (25)	3 (60)
	> 1985	9	6 (18)	2 (40)	1 (20)
Reason for SBI	Cosmetic	32	26 (79)	4 (100)	2 (40)
	Fibrocystic	10	7 (21)	-	3 (60)
Complications	Leakage	18	14 (42)	1 (25)	3 (60)
	Encapsulation	31	24 (73)	3 (75)	4 (80)
	Explantation	32	25 (76)	2 (50)	5 (100)
Exposure time (yrs)			17.1 ± 6.1	16.3 ± 10.4	12.0 ± 5.4

a) Within parentheses percentage (%) of number of women.

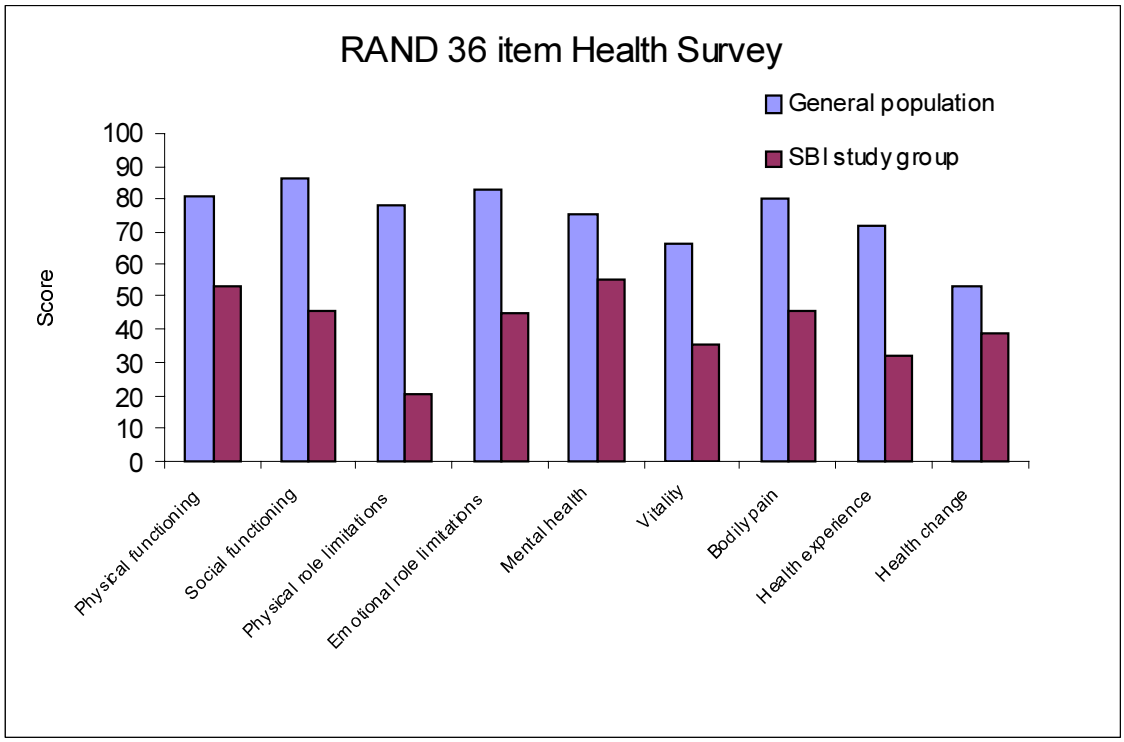

Figure 1. Rand 36 item health survey of SBI recipients (n= 363). The number of missing values for each subscale ranges from 1.9% - 7.7%.

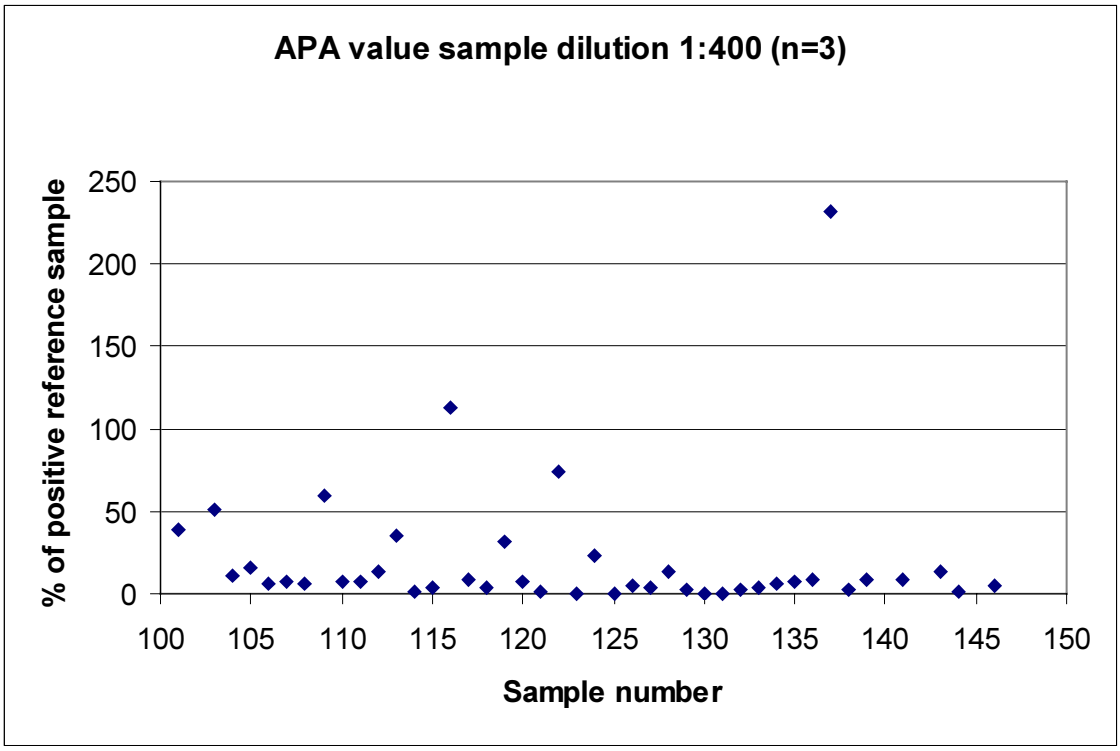

Figure 2. Polymer binding immunoglobulins in serum of SBI recipients.

Appendix 1 Raw data hematology

Sample id	RBC x 10^{12}/l	HGB Mmol/l	HCT L/l	MCV Fl	MCH fmol	MCHC mmol/l	RDW %	HDW Mmol/l	PLT x 10^{9}/l	MPV fl
101	4,75	8,47	0,402	84,6	1,79	21,10	13,93	1,44	207	9,5
103	4,96	9,30	0,453	91,2	1,87	20,52	12,93	1,28	296	9,2
104	4,58	**6,73**	**0,336**	**73,4**	**1,47**	20,04	15,70	1,61	307	10,3
105	4,06	7,70	0,381	93,8	1,89	20,21	12,97	1,32	325	8,0
106	**3,80**	**6,90**	**0,334**	87,9	1,81	20,61	13,20	1,41	268	7,5
107	4,17	8,40	0,403	96,6	2,01	20,82	12,90	1,26	217	7,0
108	4,52	8,07	0,397	87,9	1,79	20,35	13,90	1,34	**421**	8,1
109	4,38	8,17	0,400	91,4	1,86	20,36	13,00	1,25	224	9,8
110	4,36	8,10	0,412	94,6	1,86	19,70	14,30	1,25	222	7,7
111	4,44	8,20	0,404	91,2	1,85	20,30	13,50	1,26	204	8,2
112	4,52	8,70	0,435	96,3	1,93	19,97	14,33	1,15	241	8,9
113	4,21	**6,90**	**0,348**	82,7	**1,64**	19,82	15,13	1,47	365	6,9
114	4,77	8,53	0,424	89,0	1,80	20,17	12,88	1,17	306	8,0
115	4,47	8,15	0,394	88,0	1,83	20,78	13,00	1,37	218	9,3
116	4,50	8,20	0,400	88,9	1,83	20,56	13,20	1,32	307	7,6
117	4,86	8,98	0,440	90,5	1,85	20,40	14,33	1,38	339	7,6
118	4,39	7,80	0,380	86,4	1,79	20,67	12,85	1,27	235	10,1
119	4,38	7,80	0,390	89,2	1,79	19,99	12,78	1,25	205	9,1
120	4,82	9,47	0,471	97,8	1,97	20,13	13,77	1,28	324	7,9
121	4,26	8,35	0,421	98,8	1,96	19,83	12,65	1,06	267	8,4
122	**3,85**	**7,27**	0,373	96,9	1,90	19,54	12,77	1,18	376	7,8
123	4,92	9,00	0,455	92,4	1,83	19,80	13,70	1,32	392	9,1
124	4,41	8,27	0,410	93,0	1,88	20,18	12,53	1,30	299	9,1
125	4,18	8,53	0,419	**100,3**	2,05	20,40	12,58	1,13	275	8,3
126	4,73	8,60	0,434	91,6	1,82	19,88	15,30	1,23	313	8,0
127	4,47	8,40	0,405	90,6	1,88	20,71	12,60	1,40	250	8,1
128	3,94	8,20	0,412	**104,6**	2,08	19,89	13,30	1,07	329	7,8
129	4,00	8,10	0,406	**101,5**	2,02	19,90	13,43	1,24	231	7,8
130	4,39	8,33	0,417	95,1	1,91	20,03	13,43	1,30	240	8,5
131	4,94	9,22	0,456	92,3	1,87	20,28	13,32	1,37	**425**	7,8
132	4,56	8,54	0,435	95,3	1,87	19,66	13,90	1,36	248	8,2
133	4,81	9,10	0,463	96,2	1,90	19,74	13,80	1,33	291	7,9
134	5,06	**9,90**	**0,486**	96,2	1,95	20,32	12,80	1,31	230	9,2
135	4,99	9,36	0,460	92,1	1,88	20,39	14,70	1,39	**108**	7,8
136	**5,32**	**10,50**	**0,525**	98,5	1,97	20,03	14,93	1,32	300	7,6
137	4,53	8,10	0,401	88,6	1,78	20,13	14,28	1,42	267	8,3
138	4,37	8,83	0,442	**101,1**	2,03	20,02	12,65	1,28	295	8,4
139	4,21	8,27	0,412	97,9	1,96	20,02	12,53	1,18	316	8,2
141	4,21	7,77	0,384	91,3	1,84	20,18	13,77	1,43	278	8,9
143	**3,54**	**6,90**	**0,331**	93,5	1,95	20,83	12,50	1,18	177	7,2
144	3,99	8,10	0,390	97,9	2,03	20,75	13,45	1,24	238	8,0
146	4,78	9,25	0,434	90,8	1,94	21,35	12,75	1,25	278	8,3
Mean	4,46	8,37	0,41	92,80	1,88	20,25	13,48	1,29	277	8,31
SD	0,38	0,79	0,04	5,66	0,11	0,40	0,83	0,11	64	0,80
Count	42	42	42	42	42	42	42	42	42	42

Appendix 2 continued Raw data hematology

sample id	WBC X 10^9/l	Neut abs	lymph abs	Mono abs	eos abs	baso abs	luc abs	Neut%	lymph%	mono%	eos%	baso%	luc%
101	7,07	4,45	1,80	0,33	0,35	0,05	0,08	62,93	25,47	4,70	4,97	0,70	1,20
103	5,30	2,88	1,72	0,52	0,06	0,03	0,11	54,27	32,33	**9,77**	1,10	0,53	2,00
104	6,77	3,43	2,75	0,31	0,09	0,03	0,16	50,67	40,63	4,63	1,27	0,50	2,33
105	6,36	3,77	1,68	0,40	0,31	0,09	0,10	59,23	26,33	6,33	5,00	1,43	1,63
106	4,76	2,85	1,35	0,45	**0,03**	0,01	0,07	59,95	28,30	9,45	**0,55**	0,30	1,50
107	5,92	4,19	1,17	0,35	0,12	0,03	0,07	70,75	**19,70**	5,95	1,95	0,55	1,05
108	**10,90**	**8,14**	1,20	0,58	**0,76**	0,10	0,12	74,63	**10,97**	5,30	**7,00**	0,93	1,10
109	5,15	2,41	1,85	0,42	0,30	0,04	0,13	46,80	36,03	8,13	**5,73**	0,83	2,53
110	**13,84**	**11,44**	1,65	0,47	0,13	0,04	0,12	**82,65**	**11,95**	3,35	**0,95**	0,30	0,80
111	7,62	4,32	2,57	0,40	0,14	0,11	0,08	56,77	33,73	5,27	1,83	1,40	1,10
112	6,50	4,11	1,86	0,29	0,10	0,07	0,07	63,27	28,53	4,47	1,60	1,07	1,03
113	5,43	3,11	1,65	0,25	0,27	0,07	0,07	57,30	30,43	4,63	4,97	1,40	1,27
114	**10,27**	**7,72**	1,95	0,37	0,08	0,06	0,09	75,23	**18,98**	3,63	**0,78**	0,53	0,88
115	5,86	3,81	1,73	0,17	0,07	0,03	0,05	65,05	29,60	2,90	1,20	0,55	0,80
116	6,10	3,51	1,61	0,35	0,42	0,09	0,12	57,57	26,43	5,73	6,90	1,50	1,93
117	9,85	6,72	2,40	0,39	0,20	0,06	0,08	68,20	24,35	3,95	2,00	0,63	0,85
118	9,34	6,40	2,19	0,45	0,11	0,09	0,13	68,50	23,35	4,80	1,15	0,85	1,35
119	4,75	2,82	1,53	0,27	**0,03**	0,04	0,08	59,33	32,15	5,60	**0,65**	0,70	1,60
120	8,57	4,89	2,83	0,54	0,14	0,08	0,08	57,07	33,00	6,33	1,70	0,90	1,00
121	7,47	4,61	2,23	0,35	0,10	0,06	0,13	61,75	29,85	4,65	1,35	0,75	1,65
122	7,39	4,45	2,06	0,43	0,27	0,07	0,10	60,23	27,93	5,87	3,63	0,90	1,33
123	**11,22**	6,89	3,20	0,62	0,24	0,12	0,16	61,43	28,50	5,50	2,13	1,03	1,43
124	9,28	6,47	1,92	0,51	0,22	0,03	0,12	69,77	20,73	5,53	2,33	0,33	1,33
125	**12,42**	**8,37**	3,14	0,60	0,10	0,06	0,16	67,33	25,28	4,83	**0,78**	0,50	1,28
126	8,15	5,15	1,89	0,56	0,28	0,11	0,16	63,20	23,20	6,80	3,50	1,25	1,95
127	6,83	3,51	2,56	0,35	0,21	0,06	0,13	51,50	37,57	5,13	3,10	0,80	1,87
128	9,52	6,08	2,65	0,47	0,13	0,08	0,12	63,85	27,80	5,00	1,30	0,80	1,25
129	7,53	4,37	2,56	0,32	0,16	0,05	0,09	57,95	34,00	4,20	2,08	0,60	1,20
130	5,94	3,29	2,12	0,27	0,12	0,05	0,09	55,38	35,68	4,60	1,98	0,85	1,45
131	8,30	5,76	1,87	0,43	0,09	0,07	0,09	69,37	22,55	5,13	1,10	0,82	1,05

132	7,17	5,15	1,60	0,24	0,04	0,05	0,08	71,80	22,36	3,30	**0,64**	0,68	1,14
133	7,40	4,37	2,54	0,21	0,10	0,10	0,09	59,00	34,25	2,75	1,35	1,25	1,25
134	7,90	5,35	1,83	0,39	0,13	0,12	0,09	67,77	23,20	4,90	1,63	1,47	1,07
135	8,31	5,16	2,03	0,71	0,12	0,10	0,19	62,06	24,46	8,54	1,42	1,26	2,22
136	**10,79**	7,24	2,74	0,49	0,11	0,07	0,15	67,15	25,38	4,50	**0,95**	0,60	1,38
137	9,64	4,65	3,65	0,47	**0,55**	0,10	0,22	48,20	37,90	4,84	**5,70**	1,06	2,30
138	7,99	5,46	1,89	0,35	0,10	0,10	0,08	68,38	23,70	4,35	1,32	1,23	1,02
139	9,53	5,92	2,86	0,42	0,18	0,03	0,11	62,07	30,03	4,50	1,87	0,37	1,20
141	7,61	5,01	1,87	0,44	0,14	0,04	0,11	65,80	24,57	5,77	1,87	0,43	1,50
143	**3,04**	**1,39**	1,23	0,24	0,09	0,03	0,06	45,95	40,50	7,95	2,75	0,85	2,15
144	10,00	6,61	2,38	0,56	0,28	0,07	0,10	66,15	23,75	5,60	2,80	0,70	0,95
146	6,98	4,49	1,95	0,34	0,09	0,04	0,08	64,30	27,95	4,85	1,20	0,55	1,10
Mean	7,87	5,02	2,10	0,41	0,18	0,06	0,11	62,39	27,70	5,33	2,33	0,83	1,41
SD	2,21	1,88	0,57	0,12	0,14	0,03	0,04	7,76	6,61	1,57	1,74	0,34	0,45
Count	42	42	42	42	42	42	42	42	42	42	42	42	42

Bold face data outside reference values (see table 5)

Appendix 2 TSH values of SBI recipients

Participant Number	TSH value[a]
101	2.2
103	2.7
104	1.2
105	0.5
106	1.3
107	1.3
108	2.3
109	1.8
110	1.0
111	0.72
112	2.4
113	1.8
114	2.7
115	2.9
116	1.4
117	1.8
118	0.49
119	2.3
120	2.1
121	1.1
122	1.4
123	1.3
124	1.8
125	0.87
126	1.2
127	2.1
128	2.6
129	n.d.[b]
130	3.4
131	1.5
132	2.1
133	1.1
134	0.78
135	**13**
136	1.4
137	1.1
138	0.76
139	0.91
141	n.d.
143	n.d.
144	n.d.
146	n.d.

a) Normal values of TSH 0.35 – 5.00 mU/l.
b) n.d. not done

Appendix 3. Combined results of two studies (H98002 and H99001) to the presence of antipolymer antibodies (APA) in serum of SBI recipients

Laboratory results in relation to functional disability and physicians assessment of severity of disease activity.

Severity Classification		Assay polymer binding immunoglobulins H98002 and H99001			Assay polymer binding immunoglobulins H98002 and H99001 combined			
	N	< 20 [a]	20 - 50	> 50	N	< 20 [a]	20 - 50	> 50
Total	42-42 [b]	30-33	9-4	3-5	84	63 (75)	13 (15)	8 (10)
Severity subgroups (functional disability and disease activity) [c]								
Limited	34-31	24-23	8-3	2-5	65	47 (72)	11 (17)	7 (11)
Mild	2-6	1-6	-	1-0	8	7 (88)	-	1 (12)
Moderate	2-2	2-2	-	-	4	4 (100)	-	-
Advanced	4-3	3-2	1-1	-	7	5 (71)	2 (29)	-
Non SBI Laboratory personnel					12	9 (75)	1 (8)	2 (17)
Non SBI control Population					80	74 (93)	1 (1)	5 (6)

a) Value expressed as percentage of positive control reference, sample dilution 1:400 (=100%). Within parentheses percentage (%).
b) Numbers in study H98002 – H99001 (n= 42- 42)
c) 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

Appendix 4 Mailing list

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