Association of anti-RNA polymerase III antibody with silicone breast implants rupture in a multicentre series of Italian patients with systemic sclerosis

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Received on April 9, 2020; accepted in revised form on August 31, 2020.

Clin Exp Rheumatol 2021; 39 (Suppl. 131): S25-S28.

Key words: systemic sclerosis, silicone breast implants, breast cancer, anti-RNA polymerase III, autoantibodies

Funding: this project was funded by a grant from GILS (Gruppo Italiano Lotta Sclerodermia), Italy.

Competing interests: none declared.

ABSTRACT

Objective. *Systemic sclerosis (SSc) is a heterogeneous systemic autoimmune disease with distinct subsets identified by specific autoantibodies. Some environmental agents might play a role in SSc pathogenesis, including silicone breast implants (SBI). This association has been controversial in previous literature and only few studies reported the autoantibody status in these SSc women.*
The objective of this study was to evaluate the association of SBI with SSc in a large cohort of Italian patients, classified according to their SSc-related autoantibodies and to their history of breast cancer.

Methods. *Three Italian referral centres retrospectively collected clinical and laboratory data of consecutive SSc women, that were included when fulfilling the 2013 ACR/EULAR criteria and when SSc specific auto-antibodies status was available (anti-centromere (ACA), anti-Topoisomerase I (anti-Topo I) and anti-RNA Polymerase III antibodies (anti-RNAP3)). Data regarding history of SBI, SBI rupture and breast cancer were recorded.*

Results. *Among 742 SSc women, a history of SBI was recorded in 12 patients (1.6%); in only 1 case the implantation occurred after SSc diagnosis. In SSc patients with anti- RNAP3+ a significantly higher frequency of SBI rupture and SBI rupture without breast cancer were observed, as compared to anti-RNAP3-negative patients. No association was noted for SBI without rupture.*

Conclusion. *In this study we demonstrated a link between SBI rupture and induction of anti-RNAP3+ SSc; further studies are needed to better define the characteristics of this syndrome and the possible effects of SBI removal and immunosuppressive treatment.*

Introduction

Systemic sclerosis (SSc) is a heterogeneous systemic autoimmune disease, in which distinct disease subsets can be identified on the basis of disease-specific autoantibodies (1, 2).

In the peculiar scenario of patients with cancer diagnosed in close temporal relationship with SSc onset, a possible model with the elicitation of the autoimmune response has recently been suggested (3). In this setting, an association with the presence of anti-RNA polymerase III antibodies (anti-RNAP3) was described by several studies (4-6). The hypothesis that SSc could represent a para-neoplastic disorder in these patients was supported by the presence in their cancer tissues of genetic abnormalities determining the synthesis of RNAP3 mutated protein and mutation-specific T- and B-cells, cross-reacting with the wild-type RNAP3 protein (3).

Some environmental agents might play a role in the elicitation of an autoimmune response and the development of SSc. Among them, silica dust appears to be a risk factor for developing SSc in men (7). Several epidemiological studies in the last decades have also investigated the link between silicone breast implants (SBI) and SSc, concluding that there was no support for a causal association (8,9). However, a recent analysis by United States Food and Drug Administration post approval studies, including nearly 100,000 individuals with SBI, described an increased rate of SSc and other autoimmune diseases, as compared to normative data (Standardised incidence ratio 7.00) (10).

The analysis of the clinical associations in SSc is particularly difficult considering the heterogeneity of the disease, both in immunological and clinical terms. Interestingly, a specific associa-

tion of SBI and anti-RNAP3+ SSc was described in a Japanese single-centre SSc cohort (10). However, an increased risk of breast cancer diagnosis close to SSc onset was demonstrated in anti-RNAP3+ SSc patients (5, 6), possibly confounding the association with SBI.
The objective of this study was to evaluate the association of SBI with SSc in a large cohort of Italian patients, classified according to their SSc-related autoantibodies and to their history of breast cancer.

Methods

Patients

Women with SSc from 3 Italian referral centres were included in this study, when fulfilling the 2013 ACR/EULAR classification criteria for SSc (12), and data regarding the 3 SSc-specific autoantibodies (anti-Topoisomerase-I (anti-Topo-I), anticentromere (ACA), and anti-RNAP3 were available. Patients were excluded from the analysis when more than one SSc-specific antibody was positive.
To collect clinical and laboratory data, a dedicated form was created including history of breast cancer, SBI and SBI rupture (4-56). SBI rupture was confirmed by CT or MRI scan, that were performed when clinically indicated.
The study was performed according to the principles of the Declaration of Helsinki and was approved by all the Local Ethic Committees. All patients signed a written informed consent.

Immunological methods

The method for auto-antibodies detection was at discretion of the participating centre. Regarding anti-RNAP3 antibodies, the method was Line Immunoblot or ELISA in all three centres.

Statistical methods

Continuous variables were reported as median (25^{th}-75^{th} percentile), whereas categorical variables as proportion and/or percentage. Fisher's exact test or Chi-square test for categorical variables were applied as appropriate.

Results

Among 742 women with SSc enrolled in this study, a history of SBI was recorded in 12 patients (1.6%); indication for SBI was breast cancer in 8 cases and cosmetic reasons in 4 cases.
In 11 out of 12 cases, SSc onset occurred after SBI implantation; only 1 patient had SBI implantation many years after SSc diagnosis and was excluded from further analysis. Main demographic and clinical characteristics of the other 741 patients are reported in Table I.
The associations of SBI and SBI rupture with SSc-autoantibodies are reported in Table II. The total number of patients with SBI was higher in the anti-RNAP3+ subset than in the other groups of SSc patients (p=0.0002). It should be noted that significantly higher frequencies of SBI rupture (p<0.0001), and SBI rupture in the absence of a history of breast cancer (p=0.006) were recorded among SSc women with anti-RNAP3+ than in anti-RNAP3- patients, whereas no association with specific autoantibodies was found in patients with SBI without signs of rupture (p=0.27). Only one patient received immunosuppressant therapy before demonstration of the SBI rupture.

Table I. Main demographic and clinical characteristics of 741 women with SSc enrolled in the study.

Characteristics	
Age at the last visit, years;	68 (57-77)
Follow-up, years	12 (6-18)
Cutaneous subset, n (%)	-diffuse: 157 (21.1%) -limited: 561 (75.7%) -unknown: 24 (3.2%)
Autoantibodies, n (%)	-anti-RNAP3+: 38 (5.1%) -anti-Topo1+: 176 (23.8%) -ACA: 414 (55.9%) -others: 113 (15.2%)
Main clinical features, n (%)	- interstitial lung disease: 213/740 (28.8%) - pulmonary arterial hypertension: 66/740 (8.9%) - scleroderma renal crisis: 11/741 (1.5%) - oesophageal involvement: 576/740 (77.8%) - digital ulcers: 301/740 (40.7%) - mRSS at the last visit: 5 (2-8)
Nailfold capillaroscopy n (%) (at the last available evaluation)	- aspecific/normal: 49/514 (9.5%) - early scleroderma pattern: 191/514 (37.2%) - active scleroderma pattern: 199/514 (38.7%) - late scleroderma pattern: 75/514 (14.6%)
History of breast cancer, n (%)	34 (4.6%)
History of SBI, n (%)	12 (1.6%)
Indication for SBI, n	-Breast cancer: 8/12 -Cosmetic reasons: 4/12
History of SBI rupture, n (%)	6 (0.8%)

Data are expressed as the median (25^{th}-75^{th} percentile).
SBI: silicone breast implants; n: number; anti-RNAP3+: anti-RNA polymerase 3; anti-Topo1: anti-topoisomerase 1; ACA: anti-centromere antibodies; mRSS: modified Rodnan skin score.

Discussion

SSc is a heterogeneous disease in which the role of autoantibodies is essential for the distinction of different subsets (1). In particular, anti-RNAP3 are less frequent than other SSc-specific autoantibodies (ACA and anti-Topo 1) and for many years their identification was based on cumbersome and not easily accessible methods. Although probably sub-optimal, the recent diffusion of commercial kits allowed a routine testing of anti-RNAP3 in SSc patients (13), leading to a better definition of the related clinical profile. Besides the clinical associations originally described, including diffuse cutaneous involvement and scleroderma renal crisis, previously unnoticed clinical manifestations were identified. Notably, an increased frequency of gastric antral vascular ectasia (13) and malignancies

Table II. Frequency of SBI, SBI rupture, breast cancer and breast cancer synchronous to SSc onset in the 741 SSc patients, classified according to auto-antibodies status.

Autoantibody	Anti-RNAP3+	Anti-Topo 1+	ACA+	others	*p*-value*
SBI	5/38 (13.2%)	1/176 (0.6%)	4/414 (1.0%)	2/113 (1.8%)	0.0002
SBI rupture	4/38 (10.5%)	1/176 (0.6%)	1/414 (0.2%)	0/113 (0%)	<0.0001
SBI without rupture	1/38 (2.6%)	0/176 (0)	3/414 (0.7%)	2/113 (1.8%)	0.27
Breast cancer	4/38 (10.5%)	2/176 (1.1%)	23/414 (5.6%)	5/113 (4.4%)	0.09
SBI rupture in absence of breast cancer history	3/38 (7.9%)	1/176 (0.6%)	0/414 (0%)	0/113 (0%)	0.006

*Calculated comparing anti-RNAP3+ vs. anti-RNAP3-.
anti-RNAP3+: anti-RNA polymerase 3; SBI: silicone breast implants; anti-Topo1: anti-topoisomerase 1; ACA: anti-centromere antibodies.

synchronous to SSc onset was reported (4-6). Specifically, anti-RNAP3+ patients were found to have an odd risk of 20 of being diagnosed with breast cancer synchronous to SSc onset, as compared to other SSc subjects (6).

This suggested a possible role of malignancies, and particularly breast cancer, as triggers for anti-RNAP3 immune response in SSc patients (3). In fact, genetic abnormalities (missense mutations or loss of heterozygosity) in the *POLR3A* gene were demonstrated in cancers from anti-RNAP3+ (most commonly breast cancers), but not from other SSc patients. Moreover, mutation-specific T-cell and B-cell immune responses cross-reacting with both mutated and wild-type RNAP3 protein were demonstrated (3).

Although the association between anti-RNAP3 positivity and synchronous cancer was confirmed in different cohorts, it is noteworthy that most of these patients do not have a detectable synchronous malignancy. This led to the hypothesis that other mechanisms could have a role in the induction of these autoantibodies. The observation of a higher prevalence of SBI in anti-RNAP3+, as compared to other SSc patients, may provide a clue for these mechanisms. This was firstly reported by a small single-centre Japanese study evaluating 262 women with SSc: 6 had SBI and 4 of them were anti-RNAP3+ (11). Moreover, in some cases SBI had to be removed, suggesting a possible rupture of the implants (11).

We recently reviewed the literature concerning SSc cases associated with SBI, with focus on their autoantibody status (14). We identified 18 reports including 74 patients, with data on ACA and anti-Topo I available in only 39 cases. The majority of them (59%) were negative for both these autoantibodies (14), which is unusual in SSc series, whereas data on anti-RNAP3 status were only occasionally reported, and frequently found to be positive (14, 15).

In the present study, providing data from a large multicentre Italian cohort, we confirmed that anti-RNAP3+ patients had a higher prevalence of SBI compared to other SSc patients, and that this association specifically concerns cases with SBI rupture and was independent from the presence of breast cancer history.

A possible role of SBI as causal agents in the generation of anti-RNAP3 immune response may therefore be suggested (11). Since silicone may increase the innate immune response, binding to Toll-like receptors, its potential adjuvant action if leaked outside the foreign body capsule formed around the ruptured implants may be hypothesised (11). Since RNAP3 enzyme may be actively expressed in the process of innate immune response, it has been proposed that this might explain the link between the adjuvant role of silicone and the induction of specific anti-RNAP3 autoimmune response (11). Although this hypothesis remains speculative, it should be remarked that in several cases the removal of SBI was associated with clinical improvement of SSc (11, 14, 16), thus supporting the hypothesis that SBI might act not only in the induction of autoimmunity, but also as a continuous stimulation in the ongoing autoimmune response.

In conclusion, we herein provided further demonstration of a link between SBI rupture and induction of anti-RNAP3+ SSc, but future studies are warranted to confirm this association in large multicentre international cohorts. Many relevant issues, with clinical and pathophysiological relevance, should be addressed: a better definition of the features of this syndrome, the effect of SBI removal and immunosuppressive treatments (14), the possible differences between different types of SBI (10) and molecular mechanisms of autoimmunity induction by SBI.

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