Evaluation of cancer-associated myositis and scleroderma autoantibodies in breast cancer patients without rheumatic disease

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ABSTRACT

Objective. *Systemic sclerosis (scleroderma) and dermatomyositis are two prototypic autoimmune diseases that are strongly associated with malignancy. While specific autoantibodies in these diseases are markers of an increased risk of cancer at scleroderma and dermatomyositis onset, it is not known whether these autoantibodies are biomarkers of cancer risk in patients without rheumatic disease.*

Methods. *In a matched case-control study of women without rheumatic disease, identified from a familial breast cancer cohort, 50 breast cancer cases and 50 controls were assayed for 3 autoantibodies that are known markers of cancer-associated scleroderma and dermatomyositis: anti-RNA polymerase III, anti-NXP2, and anti-TIF1γ.*

Results. *No subject had moderate or strong autoantibody positivity. Eleven women were borderline positive for at least one autoantibody. The prevalence of borderline autoantibody positivity did not differ between cases and controls.*

Conclusion. *Our results suggest that scleroderma and dermatomyositis autoantibodies are cancer biomarkers only in patients with clinical manifestations of specific rheumatic diseases and are unlikely to improve risk stratification for cancer in the general population. However, prospective studies are needed to examine whether scleroderma and dermatomyositis autoantibodies are markers of malignancy in other cancer types.*

Introduction

Intriguing and complex connections between cancer and autoimmune rheumatic diseases have emerged recently, suggesting that cancer drives the development of autoimmunity in some patients (1). The data suggesting a model of cancer-induced autoimmunity are most compelling in systemic sclerosis (scleroderma) and dermatomyositis (DM). In these diseases, patients have an increased age- and gender-adjusted risk of cancer compared to the general population, and clustering of cancer diagnosis around symptomatic onset of scleroderma and DM. The temporal relationship between cancer diagnosis and rheumatic disease onset is particularly striking in scleroderma patients with breast cancer (2). Reports of cancer therapy improving scleroderma and DM outcomes further support a possible mechanistic relationship (3, 4).

In both scleroderma and DM, specific autoantibodies are present that (i) associate with distinct clinical phenotypes, (ii) play an important role in risk stratification and prognosis, and (iii) may provide insight into the pathogenesis of disease (1, 5). For instance, scleroderma patients with RNA polymerase III (RNAP) autoantibodies have a higher risk of rapidly progressive diffuse cutaneous disease and scleroderma renal crisis than scleroderma patients without these autoantibodies. Recent studies have also demonstrated that patients with RNAP autoantibodies have a >5-fold increased risk of developing cancer within 2 years of scleroderma onset (6), suggesting that heightened cancer surveillance at the time of disease onset may be warranted. Genetic alterations (somatic mutations and/or loss of heterozygosity) of the *POLR3A* locus, which encodes RNAP, have been detected in cancer tissues from scleroderma patients with RNAP autoantibodies, and these patients have evidence of mutation-specific T cell immune responses and cross-reactive autoantibodies that recognise both mutated and wild type RNAP proteins (7).

In aggregate, these data strongly suggest that some scleroderma patients with RNAP autoantibodies have cancer-induced autoimmunity (1). Similar data are evident in dermatomyositis, where NXP-2 or TIF-1γ autoantibodies are found in more than 80% of patients who have cancer within 3 years of myositis onset (8). It is also noteworthy that these scleroderma- and DM-specific autoantibodies may themselves have a functional role in mediating the disease process.

The data among scleroderma and DM patients raise the question of whether certain autoantibodies may have utility as an early detection biomarker in cancer patients without symptomatic rheumatic diseases. In this case control study, we sought to address whether RNAP, NXP-2 and TIF-1γ autoantibodies were associated with breast cancer compared to cancer free controls in women without rheumatic disease.

Materials and methods

Cases and controls were identified from the Breast and Ovarian Surveillance Service (BOSS) Cohort, an ongoing prospective study of women and men with familial breast cancer (9). Study participants were recruited between 2005 and 2013 primarily from the cancer genetics clinic at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center. After informed consent was obtained, participants were asked to complete an extensive baseline questionnaire on demographic characteristics, behavioural and lifestyle factors, cancer history and treatments, medication use, and breast cancer risk factors including body mass index (BMI), menopausal status, parity, oral contraceptive use, hormone replacement therapy, breastfeeding, hormone receptor status, and family history. All cancer diagnoses and information on tumour characteristics were confirmed by pathology records. At enrolment, serum and plasma was collected, processed within 4 hours and stored at -80°C. The research was approved by the Johns Hopkins Bloomberg School of Public Health (H.34.04.08.17.A2, H.34.04.08.12.AR2) and conducted in accordance with the Helsinki Agreement.

Table I. Baseline characteristics of cases and controls identified from BOSS cohort.

Characteristics	Controls (n=50[1])	Cases (n=50)	*p*-value
Age (years, SD)	50.3 (11.7)	50.2 (11.7)	0.60
Race, no. (%)			0.48
White	41 (85.4)	40 (80.0)	
Non-White	7 (14.6)	10 (20.0)	
Education, no. (%)			0.36
HS graduate	19 (39.6)	13 (26.0)	
College graduate	14 (29.2)	18 (36.0)	
Post-college graduate	15 (31.2)	19 (38.0)	
Marital Status, no. (%)			0.32
Not Married	18 (37.5)	14 (28.0)	
Married at Baseline	30 (62.5)	36 (72.0)	
Family History, no. (%)			0.025
None	1 (2.0)	9 (18.0)	
1st degree only	20 (40.0)	19 (38.0)	
2nd degree only	29 (58.0)	22 (44.0)	
BRCA Status, no. (%)			0.63
Negative	17 (68.0)	35 (76.1)	
BRCA 1	4 (16.0)	4 (8.7)	
BRCA 2	4 (16.0)	7 (15.2)	
Age at Menarche (years), no. (%)			0.27
<12	11 (22.9)	7 (14.3)	
12-13	11 (22.9)	18 (36.7)	
>13	26 (54.2)	24 (49.0)	
Oral Contraceptive Use, no. (%)			0.55
Never	11 (22.9)	9 (18.0)	
Ever	37 (77.1)	41 (82.0)	
Parity, no. (%)			0.78
Never Pregnant	7 (14.6)	7 (14.6)	
Age at First Birth <25	15 (31.2)	12 (25.0)	
Age at First Birth ≥25	26 (54.2)	29 (60.4)	
Breastfeeding[2], no. (%)			0.86
Never	13 (36.1)	14 (34.1)	
Ever	23 (63.9)	27 (65.9)	
Menopausal Status, no. (%)			0.69
Pre-menopausal	25 (52.1)	24 (48.0)	
Post-menopausal	23 (47.9)	26 (52.0)	
Hormone Replacement Therapy, no. (%)			0.15
Never	37 (77.1)	44 (88.0)	
Ever	11 (22.9)	6 (12.0)	
Cigarette Smoking, no. (%)			0.43
Never	25 (52.1)	30 (60.0)	
Ever	23 (47.9)	20 (40.0)	
Alcohol Use, no. (%)			0.85
Never	7 (14.6)	8 (16.0)	
Ever	41 (85.4)	42 (84.0)	
BMI[3] (kg/m^2), no. (%)			0.51
<25	18 (37.5)	22 (44.0)	
≥25	30 (62.5)	28 (56.0)	
Tumour Characteristics			
Estrogen Receptor (ER), no. (%)			
Negative		9 (18.0)	
Positive		39 (78.0)	
Unknown/Missing		2 (4.0)	
Progesterone Receptor (PR), no. (%)			
Negative		15 (30.0)	
Positive		33 (66.0)	
Unknown/Missing		2 (4.0)	
HER-2 Status, no. (%)			
Negative		20 (40.0)	
Positive		18 (36.0)	
Unknown/Missing		12 (24.0)	
Triple Negative, no. (%)			
No		33 (66.0)	
Yes		5 (10.0)	
Unknown/Missing		12 (24.0)	
Stage, no. (%)			
0		11 (22.0)	
1		5 (10.0)	
2		18 (36.0)	
3		14 (28.0)	
Missing/Unknown		2 (4.0)	

*some percentages are over 100 due to rounding error.
[1]No baseline data for 2 controls; [2]Among parous women; [3]BMI: Body Mass Index.

In this study, 50 women with stage 0-III pathologically confirmed breast cancer who donated plasma within 2 years of diagnosis were matched to 50 controls on age and year of cohort enrolment. A history of a preexisting connective tissue disease was an exclusion criterion. The sample size was based on data reporting that 28% of US SSc patients are positive for anti-RNAP compared to 2% of blood bank controls (10). Additionally, anti-NXP2 and TIF-1γ antibodies were previously detected in 17% and 38% of DM patients, respectively, compared to 0% in normal controls (8). The plan was to increase the sample size if moderate-strong autoantibody positivity was detected in at least 2% of the cancer patients.

RNAP antibodies were assayed in duplicate using a commercially available ELISA kit (Inova Diagnostics), per the manufacturer's protocol. NXP2 antibodies were assayed by immunoprecipitation using ^{35}S-methionine-labeled protein generated from cDNA by *in vitro* transcription/translation (Promega kit) as described (11). Immunoprecipitates were separated by electrophoresis on SDS-polyacrylamide gels and visualised by fluorography. TIF-1γ antibodies were detected by immunoprecipitation from TIF-1γ transfected cell lysates using patient sera, followed by immunoblot analysis using an anti-TIF-1γ antibody (Novus) (8). Bands in the immunoprecipitation assays were independently reviewed by two experienced investigators and graded as negative, borderline (very faint band), or positive. Differences in breast cancer risk factors by case-control status were calculated using Chi-squared test for categorical variables and paired *t*-test for continuous variables. Statistical significance was defined as a 2-sided *p*-value ≤0.05.

Results

Characteristics of the breast cancer cases and controls are provided in Table I. The study was well matched. The mean age of the study participants was 50 years. There were no significant differences in race, education, marital status, BRCA status, age at menarche, oral contraceptive or hormone replacement therapy use, parity, history of breastfeeding, menopausal status, cigarette smoking, alcohol use, or BMI between the two study groups. Controls were more likely to have a family history of breast cancer than breast cancer cases given the inclusion criteria of the cohort. Seventy eight percent of breast cancer cases were ER positive.

Table II. Prevalence of RNA polymerase III, TIF-1γ, and NXP-2 autoantibodies among breast cancer cases and controls.

	RNA Polymerase III		TIF-1γ		NXP-2	
	Negative	Borderline	Negative	Borderline	Negative	Borderline
Case	50	0	47	3	46	4
Control	49	1	47	3	48	2
Total	99	1	94	6	94	6

None of the cases or controls had moderate or strong autoantibody positivity (Table II). Eleven individuals were borderline positive for at least one autoantibody: 1 control for RNAP (23 units, normal <20); 3 cases and 3 controls for TIF-1γ, and 4 cases and 2 controls for NXP2. One case and one control were borderline positive for both anti-NXP2 and anti-TIF1γ. No autoantibodies were detected in the one control who subsequently became a case during a follow-up of 5 years. There were no statistically significant differences in the frequency of borderline autoantibody positivity between cases and controls. Medical history based on baseline and ongoing follow-up questionnaires of the 11 borderline positive patients was reviewed, confirming that these patients had no features suggestive of autoimmune rheumatic disease.

Discussion

Among patients with scleroderma and DM, there is compelling evidence that specific autoantibodies are markers of underlying cancer and anti-tumour immune responses that become cross-reactive (1). Patients with these autoantibody markers are at increased risk of having cancer at the time of rheumatic disease onset, further supporting the development of cancer-induced autoimmunity. In this investigation, we examined whether 3 cancer-associated rheumatic disease autoantibodies are detectable in breast cancer patients and at-risk controls, all within the same cohort and without clinical manifestations of autoimmunity. We focused on a longitudinal cohort with breast cancer cases because the close temporal relationship between scleroderma onset and breast cancer is particularly notable in patients with RNAP antibodies (2), and these two rheumatic diseases predominantly affect women. While borderline autoantibody positivity was detected, the significance of this low-level positivity is unknown, and there were no significant differences in the prevalence of borderline positivity between cases and controls. These data suggest that scleroderma and DM autoantibodies are cancer biomarkers only in patients with clinical manifestations of rheumatic disease, and that these autoantibodies are unlikely to improve risk stratification for breast cancer in the general population. We recognise that a limitation of our study is the small sample size of patients; we had defined a priori that additional breast cancer patients would be studied if at least 2% of patients exhibited moderate-strong autoantibody positivity based on existing data on the prevalence of these antibodies in normal individuals, yet this threshold was not met. We cannot exclude the possibility of a very modest association. Additionally, while the majority of patients had a first or second degree relative with a history of breast cancer, 18% of patients had a sporadic breast cancer. Although this study population may limit the generalisability of our findings, we do not expect the relationship between these antibodies and breast cancer risk to necessarily be different. Future prospective studies should examine whether scleroderma and DM autoantibodies are markers of malignancy in other cancer types.

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