Research Article

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A Multiparametric Serum Marker Panel as a Complementary Test to Mammography for the Diagnosis of Node-Negative Early-Stage Breast Cancer and DCIS in Young Women

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Abstract

Background: The sensitivity of mammography for the detection of small lesions, including node-negative early-stage (T1N0) primary breast cancer (PBC) and ductal carcinoma *in situ* (DCIS), is significantly decreased in young patients. From a clinical standpoint, an inconclusive mammogram reflects the inability of clinicians to confidently decide whether patients should be referred for biopsy or for follow-up with repeat imaging.

Methods: Specific ELISAs were developed for a panel of 13 well-recognized breast autoantigens (HSP60, FKBP52, PRDX2, PPIA, MUC1, GAL3, PAK2, P53, CCNB1, PHB2, RACK1, RUVBL1, and HER2). Circulating autoantibody levels were measured in a cohort of 396 serum samples from histologically confirmed DCIS ($n = 87$) or T1N0 PBC ($n = 153$) and healthy controls ($n = 156$).

Results: Individually, antibodies against CCNB1, FKBP52, GAL3, PAK2, PRDX2, PPIA, P53, and MUC1 demonstrated discriminatory power between breast cancer and healthy control groups. At 90% sensitivity, the overall combined specificity of the autoantibody serum screening test was 42%. Adjustment for higher sensitivities of 95% and 99% resulted in 30% and 21% specificities, respectively (33% and 18% in T1N0 PBC and 28% and 21% in DCIS). Finally, in patients with node-negative early-stage breast cancer younger than 50 years, the autoantibody assay exhibited 59% specificity with a fixed sensitivity at 90%.

Conclusions: Our autoantibody panel allows accurate detection of early breast cancer and DCIS, notably in younger patients.

Impact: Clinical assessment of this autoantibody panel displays a potential to facilitate clinical management of early-stage breast cancer detection in cases of inconclusive mammogram. *Cancer Epidemiol Biomarkers Prev; 23(9); 1834–42. ©2014 AACR.*

Introduction

Despite the fact that mammography is the only available screening method proven to reduce breast cancer mortality, the method is not void of limitations (1). For instance, in younger women (ages 40–49 years), mammography is less sensitive and is associated with overall less reduction of mortality compared with older women (age $\geq$50 years; refs. 2, 3). Moreover, it is now established that the method is not suited for the detection of small-size tumors, including node-negative early-stage (T1N0) primary breast cancer (PBC) and ductal carcinoma *in situ* (DCIS; ref. 4), mainly because of their high mammographic breast density, which results in X-ray attenuation properties similar to those of breast lesions (5). Occult mammography consequently delays cancer detection, resulting in an advanced disease at diagnosis, negatively impacting the prognostic outcome of patient (6). In addition, it has been postulated that tumors in younger women display a more aggressive nature, which further explains why they are often detected at a more advanced stage (7, 8).

In light of these limitations, a minimally invasive screening test administered at the time of mammography or before biopsy in the case of a suspicious mammogram could improve overall management of patients, especially in cases in which mammography results are inconclusive. To efficiently complement mammography, such a test should be powerful in distinguishing benign from malignant tumors, particularly in early-stage breast cancer and DCIS in women younger than 50 years.

Evidence for a specific humoral response against a number of intracellular and surface tumoral antigens is now well-established in patients with breast cancer (9).

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Quite unexpectedly though, very few studies have investigated autoantibodies as biological tools for the detection of breast cancer and especially in node-negative early-stage disease (T1N0), DCIS (10–15), and younger women population (10–14).

In this study, we explored the humoral response against several autoantibodies already identified in the literature (HSP60, FKBP52, PRDX2, PPIA, MUC1, GAL3, PAK2, P53, CCNB1, PHB2, RACK1, RUVBL1, and HER2) in a large prospective cohort of 240 patients with exclusively patients with T1N0 or DCIS and 156 healthy volunteers as controls. Results showed that our panel could accurately detect early-stage breast cancer and DCIS, particularly in women younger than 50 years.

Materials and Methods

Patient selection

Sera from female patients with breast cancer were prospectively collected between 2005 and 2009 at the Centre Régional de Lutte contre Cancer Val d'Aurelle (Montpellier, France). The study was approved by the institutional review board and all participants signed an informed consent form. All samples were collected, processed, and stored in a similar fashion. Sera from patients with breast cancer were collected just prior surgery, and the healthy controls were collected during the same period. Blood samples were centrifuged at $1,250 \times g$ for 5 minutes, and sera were then stored at $-80°C$. The cohort of 396 subjects included 240 women who underwent surgery and had a histopathologic diagnosis of breast cancer (DCIS, $n = 87$; node-negative early-stage PBC, $n = 153$) and 156 healthy controls with negative mammograms, a history of at least 4 years negative physical breast examinations, and no history of prior malignancy, immunodeficiency, autoimmune disorder, hepatitis, or HIV infection. *In situ* carcinomas were classified according to the World Health Organization (WHO) classification of tumors and were graded according to Bloom and Richardson by both the pathologists. There were no significant differences between the breast cancer and control groups with regard to age ($P > 0.05$).

ELISA

Autoantibodies against HSP60, FKBP52, MUC1, PRDX2, PPIA, GAL3, PAK2, PHB2, RACK1, and RUVBL1 were detected as previously described (10, 12). Anti-p53, HER2, and CCNB1 autoantibodies were detected using the same procedure with the recombinant protein p53 (#H00007157), HER2 (#H00002064), and CCNB1 (#H0000891) from Abnova Corporation. Briefly, MaxiSorp High Protein-Binding plates (Nunc) were coated with 50 ng of recombinant protein in 100 μL PBS at 4°C overnight. The plates were washed twice with PBST and blocked with a buffer containing 50 mmol/L HEPES, 200 mmol/L NaCl, 0.08% Triton X-100, 25% glycerol, 1 mmol/L dithiothreitol (DTT), 40 mmol/L NaOH, and 1% BSA at pH 7.5 for 2 hours at room temperature. The plates were then washed twice and incubated with the serum samples (diluted 1:50) for 2 hours at room temperature. After 4 washes, the plates were incubated with a horseradish peroxidase–conjugated polyclonal antibody specific for human IgG (Jackson ImmunoResearch) for 1 hour at room temperature with agitation. After 4 washes, the plates were incubated with TMB substrate solution (Stressgen) for 15 minutes, and the absorbance values were monitored at 450 nm after the addition of H_2SO_4 to stop the reaction. Each serum sample was assayed in triplicate.

The intra- and interassay reproducibility of anti-HSP60, -FKBP52, -PRDX2, -PPIA, -MUC1, -GAL3, -PAK2, -RACK1, -PHB2, and -RUVBL1 autoantibody detection has been reported elsewhere (10, 12). Therefore, we only tested the intra-assay (5 sera tested 3 times in one assay) and interassay (5 sera tested 3 times in one assay at 3 different days) reproducibility of the anti-p53, -HER2, and -CCNB1 autoantibody ELISAs with 6 samples in triplicate. The cutoff value of positivity was defined as the mean optical density of normal samples plus $2\times$ SD of the mean. The assays had a linearity of $R^2 > 0.99$ for a serum dilution range from 1:100 to 1:2,000. The coefficient of variability in intra- and interassays was consistently <10%.

Statistical analyses

The seroreactivities of the autoantibodies were compared using the Mann–Whitney test. Differences were considered statistically significant when $P < 0.05$. Individual and combined autoantibody performances were based on the ROC curves, support vector machine (SVM), and linear discriminant analysis (LDA), as previously described (10, 12). The generalized ROC criterion finds the best linear combination (virtual marker) of tumor markers such that the area under the ROC curve (AUC) is maximized. The associations of 2 or more autoantibodies were randomly permuted, and the corresponding AUC was estimated for each linear combination. The accuracy, sensitivity, and specificity of the combined antibody performance were evaluated using the optimal threshold value calculated to maximize the Youden index. This index is defined as the sum of the sensitivity and specificity minus 1. SVM analysis identified the maximum margin hyperplane, which was the hyperplane separating the 2 classes of samples in an n-dimensional space while maximizing the distance between the hyperplane and the closest training point. LDA was used to find a linear combination of features, which characterized or separated 2 or more classes of objects or events. The resulting combination was then used as a linear classifier. To determine how accurately learning algorithms were able to predict the data, we used a 10-fold cross-validation in which the data were divided into 10 subsets of approximately equal size and performed 10 iterations of training and validation. The 10-fold cross-validation procedure was replicated 100 times. Statistical analyses were performed using TANAGRA (v1.4.41), InStat (v3.06, GraphPad), and mROC.

Results

We first quantified the average levels of autoantibodies by targeting HSP60, FKBP52, PRDX2, PPIA, MUC1, GAL3, PAK2, P53, CCNB1, PHB2, RACK1, RUVBL1, and HER2 autoantigens using specific ELISAs, in a newly diagnosed cohort of 396 patients. The demographics of the patients with cancer are detailed in Table 1. The results of the measurements of autoantibodies to the 13 antigens are shown in the scatter plots in Fig. 1. Using univariate analysis, 8 of 13 autoantibodies (FKBP52, PRDX2, PPIA, MUC1, GAL3, PAK2, P53, and CCNB1) showed significant discrimination between the patient with breast cancer and healthy control groups as single diagnostic assays (Fig. 1). As individual markers, these autoantibodies displayed relatively weak performance in discriminating breast cancer from healthy control groups, with areas under the ROC curves (AUC) ranging from 0.52 to 0.65 (Table 2).

We next tested a multiparametric panel that combined these 13 autoantibodies with the goal of differentiating patients with node-negative early-stage breast cancer from healthy controls. We used 3 statistical classification methods to evaluate the performance of our panel, as previously described (10, 12). With a linear combination identified using an mROC multiparametric analysis, the best antigen combination was obtained with the association of all the 13 autoantibodies. The discrimination power of our panel displayed an AUC of 0.82 with a 95% confidence interval (CI) of 0.74–0.86, in distinguishing breast cancer from healthy control groups (Table 2). In agreement with this, the linear combination, SVM, and LDA classification methods also achieved similar overall classification accuracy, sensitivity, and specificity values (Table 3). For instance, the estimated overall accuracies were identical (71%, 73%, and 72% for the cancer group compared with the healthy control group) using either of the 3 classification methods (linear combination, SVM, and LDA, respectively). We then confirmed the robustness of our panel using a 10-fold cross-validation, which showed stable estimate accuracies for all the classification methods used at 69%, 70%, and 70% for the breast cancer group compared with healthy controls using the linear combination, SVM, and LDA classification methods, respectively (Table 3).

To further assess the potential use of this multiparametric assay as a surrogate rule-out diagnostic test (a test which could be used to decrease the false-negative results of mammography), we evaluated its performance in maximum sensitivity values. As shown in Table 4, at 90% sensitivity, each individual autoantibody achieved a weak specificity in the range of 18% to 29%, whereas the full autoantibody panel displayed a significantly improved specificity of 42% in discriminating healthy controls from all patients with breast cancer (Table 4). Regarding the different subtypes, the specificity of the panel in discriminating T1N0 patients and patients with DCIS from healthy control was 51% and 32%, respectively (at 90% sensitivity). Expectedly, at higher sensitivity values, the overall specificity of the panel dropped to 30% (at 95% sensitivity) and to 21% (at 99% sensitivity). A summary of all the statistical values for each subgroup is depicted in Table 4.

Table 1. Clinicopathologic characteristics of patients with breast carcinoma

Patient characteristic	CIS *N* = 87 (%)	Early-stage breast cancer *N* = 153 (%)
Age median (min–max), y	56 (38–79)	60 (40–86)
<50	27 (31.0)	22 (14.3)
≥50	60 (69.0)	131 (86.7)
Histotype		
Ductal	78 (89.7)	144 (94.1)
Lobular	9 (10.3)	9 (5.9)
Tumor size		
<20 mm	32 (45.1)	153 (100)
≥20 mm	39 (54.9)	—
Missing	16	—
Histologic grade		
CIS		
Low or intermediate	34 (44.2)	NA
High	43 (55.8)	NA
Missing	10	NA
T1		
1	NA	45 (29.4)
2	NA	84 (54.9)
3	NA	24 (15.6)
Lymph node status		
Negative	87 (100)	153 (100)
Positive	0 (0)	0 (0)
Estrogen receptor		
Negative	9 (27.3)	19 (12.4)
Positive	24 (72.7)	134 (87.6)
Missing	54	—
Progesterone receptor		
Negative	18 (54.5)	34 (22.2)
Positive	15 (45.5)	119 (77.7)
Missing	54	—
HER2 overexpression		
Negative	6 (40.0)	133 (86.9)
Positive	9 (60.0)	20 (13.1)
Missing	72	—
Architecture		
Comedocarcinoma	19 (31.7)	NA
Cribriform	26 (43.3)	NA
Mixed or other	15 (25.0)	NA
Missing	27	NA
Necrosis		
Absent	56 (64.4)	NA
Present	31 (35.6)	NA

Abbreviations: HER2, human epidermal growth factor receptor 2; NA, not applicable.

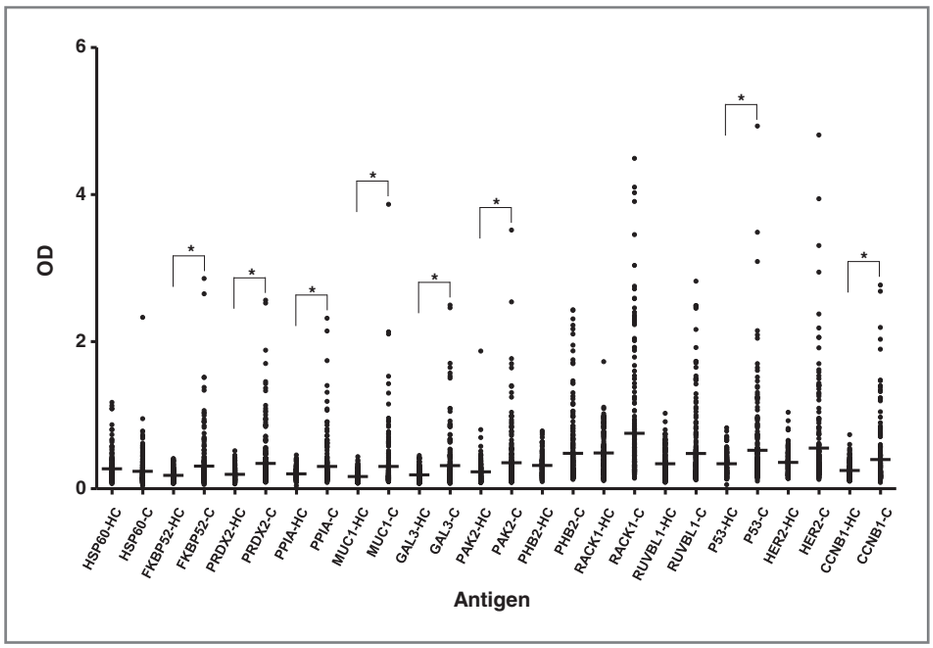

Figure 1. ELISA antibody titers of individual patients with breast cancer and normal controls. Scatter plots of the OD values of autoantibodies against HSP60, FKBP52, PRDX2, PPIA, MUC1, GAL3, PAK2, P53, CCNB1, PHB2, RACK1, RUVBL1, and HER2 in breast cancer sera ($n = 240$) and matched normal sera ($n = 156$). The mean values are indicated. BC, breast cancer; HC, healthy controls. *, $P < 0.05$.

Finally, when we evaluated the correlation of all the autoantibodies with the classical biologic, histologic, and clinical parameters for each group (see Table 1 for list of these parameters), no significant correlations were observed. Interestingly, in the subgroup of patients younger than 50 years (in which the false negativity of current mammography is maximized), the performance of our panel remained significantly high, with specificity values of 59% and 45%, at 90% and 95% sensitivity values, respectively.

Discussion

Despite its widespread use, cancer screening via mammography has limitations. A meta-analysis of 7 population-based community screening programs in the United States, including 463,372 screening mammograms, revealed an overall sensitivity of 75.0% and a specificity of 92.3% for breast cancer (16). Even with these high performance values, the overall reduction of mortality in women ages 40 to 49 years due to mammography screening is limited to 15% to 16% (compared with 25%–35% reduction observed for those ages 50 to 69 years), demonstrating the inability of current mammographic modalities to detect early-age cancer (17). The incidence of mammographically occult primary tumors (e.g., false-negative results) is particularly significant in smaller tumors (T1N0 or DCIS), particularly in women with dense breasts and women younger than 50 years (3, 18–21). In addition, a meta-analysis of the proportional incidence of interval breast cancers revealed rates between 19% and 27% (22). False reassurance causes delays in diagnosis and leads to carcinoma presenting at a more advanced stage, requiring more invasive treatment, which contributes to poor long-term outcomes (6, 23). In such cases, the

Table 2. Diagnostic values of individual markers in patients with breast cancer and controls

TAA	AUC (95% CI)
HSP60	0.57 (0.51–0.63)
FKBP52	0.60 (0.55–0.66)
PRDX2	0.62 (0.56–0.68)
PPIA	0.57 (0.51–0.62)
MUC1	0.65 (0.59–0.71)
GAL3	0.58 (0.52–0.64)
PAK2	0.59 (0.53–0.65)
PHB2	0.55 (0.49–0.61)
RACK1	0.55 (0.50–0.61)
RUVBL1	0.52 (0.46–0.58)
p53	0.57 (0.51–0.63)
HER2	0.55 (0.49–0.61)
CCNB1	0.58 (0.52–0.64)
Combination	0.82 (0.74–0.86)

Table 3. Diagnostic accuracies of the autoantibody panel calculated using AUC linear combination, SVM, and LDA classification

Statistical model	Accuracy (%)	Sensitivity (%)	Specificity (%)
AUC linear combination	71	70	79
SVM classification	73	77	67
LDA classification	72	76	68

Table 4. Estimated specificities of individual autoantibodies and the panel autoantibody assay at fixed sensitivities of 90%, 95%, and 99%

	Specificity (95% CI) at 90% sensitivity			Specificity (95% CI) at 95% sensitivity			Specificity (95% CI) at 99% sensitivity		
	Cancer vs. HC	Early-stage PBC vs. HC	DCIS vs. HC	Cancer vs. HC	Early-stage PBC vs. HC	DCIS vs. HC	Cancer vs. HC	Early-stage PBC vs. HC	DCIS vs. HC
HSP60	18 (13–24)	16 (10–24)	20 (12–31)	11 (7–16)	9 (5–16)	13 (7–23)	2 (1–5)	2 (0–5)	3 (0–9)
FKBP52	25 (19–32)	27 (20–36)	21 (13–32)	21 (16–27)	24 (17–32)	17 (10–28)	16 (11–22)	19 (12–26)	12 (6–22)
PRDX2	29 (23–36)	31 (23–40)	27 (17–38)	23 (17–29)	24 (17–32)	21 (13–32)	19 (14–25)	21 (14–29)	16 (9–26)
PPIA	23 (17–29)	25 (18–33)	20 (12–31)	17 (12–23)	19 (13–27)	13 (7–23)	16 (11–21)	18 (12–26)	12 (6–22)
MUC1	26 (20–33)	27 (20–36)	19 (11–29)	20 (15–26)	22 (16–31)	16 (9–26)	16 (11–22)	19 (13–27)	11 (5–20)
GAL3	25 (20–32)	26 (19–35)	24 (15–35)	21 (15–27)	22 (16–31)	17 (10–28)	19 (14–25)	21 (14–29)	16 (9–26)
PAK2	25 (19–31)	26 (18–34)	23 (14–34)	20 (14–26)	22 (15–30)	16 (9–26)	9 (6–14)	10 (5–17)	8 (3–17)
PHB2	26 (20–33)	24 (17–32)	25 (16–37)	21 (16–27)	20 (14–28)	23 (14–34)	16 (11–22)	17 (11–25)	15 (8–25)
RACK1	21 (15–27)	22 (16–31)	17 (10–28)	19 (14–25)	21 (14–29)	17 (10–28)	18 (13–24)	19 (13–27)	16 (9–26)
RUVBL1	19 (14–25)	19 (13–27)	19 (11–29)	18 (13–24)	18 (12–26)	19 (11–29)	13 (8–18)	15 (9–22)	9 (4–18)
p53	25 (19–32)	26 (19–35)	23 (14–34)	21 (15–27)	22 (16–31)	17 (10–28)	16 (11–22)	18 (12–26)	13 (7–23)
HER2	23 (17–29)	22 (16–31)	24 (15–35)	20 (15–26)	20 (14–28)	20 (12–31)	15 (10–20)	15 (10–23)	13 (7–23)
CCNB1	26 (21–33)	27 (20–36)	25 (16–37)	19 (14–25)	19 (13–27)	19 (11–29)	15 (10–20)	15 (10–23)	13 (7–23)
Panel[a]	42 (34–50)	51 (43–59)	32 (25–40)	30 (23–38)	33 (26–41)	28 (21–35)	21 (15–28)	18 (12–25)	21 (5–28)

Abbreviation: HC, healthy controls.

[a]The virtual marker (panel of the 13 autoantibodies) is a linear combination based on mROC analysis. The contribution of each autoantibody (after logarithmic transformation) in the statistical model is: Panel $= -0.812 \times$ (HSP60) $- 0.147 \times$ (FKBP52) $+ 2.716 \times$ (PRDX2) $- 2.016 \times$ (PPIA) $+ 4.402 \times$ (MUC1) $+ 0.168 \times$ (GAL3) $- 0.311 \times$ (PAK2) $+ 0.339 \times$ (PHB2) $+ 0.638 \times$ (RACK1) $- 3.853 \times$ (RUVBL1) $- 1.363 \times$ (p53) $- 1.497 \times$ (HER2) $+ 2.099 \times$ (CCNB1).

treatment may also be more costly, and the patient may seek legal redress for the distress that has been caused.

The main cause of false-negative results is high breast density often observed among younger women (24). Several studies have emphasized the need for screening techniques with higher sensitivity than conventional mammography in women with dense breasts (25). In this direction, several alternative imaging technologies have been investigated as complementary technologies to mammography. For instance, ultrasound examination has been shown to increase the sensitivity of imaging in early-stage breast carcinoma (26, 27); however, its use was also associated with higher false-negative rates (28, 29). These results have reinforced the debate of whether there is a need to biopsy palpable abnormalities in patients with normal mammography and ultrasound results, or not (30). MRI displays high sensitivity for invasive breast cancer (including mammographically occult invasive breast cancer; ref. 31–33); however, its use is limited, by its relatively low sensitivity in the detection of DCIS and low specificity for both invasive carcinoma and DCIS (34–37).

In light of all these, increasing interest has been brought in the discovery of novel serum biomarkers as adjunct screening tools for the detection of early-stage breast cancer. Among these, autoantibodies have attracted considerable attention based on their innate characteristics. First, autoantibodies are secreted and therefore easily accessible. Moreover, autoantibodies are present in the serum at very early stages of cancer development, sometimes before any observation of clinical signs (38). They are biochemically well-characterized, and many reagents and techniques are available for their detection, simplifying assay development. Until very recently, several specific autoantibodies have been detected in the sera of patients with advanced-stage breast cancer (14, 39–58). However, very few studies have attempted to identify and/or evaluate autoantibodies in node-negative early-stage PBC ($\leq$2 cm) or, more importantly, in preinvasive breast cancer, for which mammography's sensitivity is decreased. Using SEREX technology, Fernandez-Madrid and colleagues identified 12 phages, including that harboring Annexin XI-A, that were significantly associated with DCIS in 15 patients (59). Regele and colleagues first detected anti-p53 autoantibodies in the sera of 5 of 43 patients with DCIS (11.6%; ref. 60). Autoantibodies against p53 and 6 other proteins (c-Myc, HER2, NY-ESO-1, BRCA1, BRCA2, and MUC1) were also assessed in a DCIS population of 40 patients by Chapman and colleagues. This panel achieved 45% sensitivity and 85% specificity (13). Previously, our group identified using SERPA, HSP60, MUC1, FKBP52, PPIA, and PRDX2 as tumor antigens associated with early-stage breast cancer and DCIS (10). More recently, using fractionated MCF10CA1 breast cancer cells to facilitate the identification of low-abundance antigenic sources, we identified

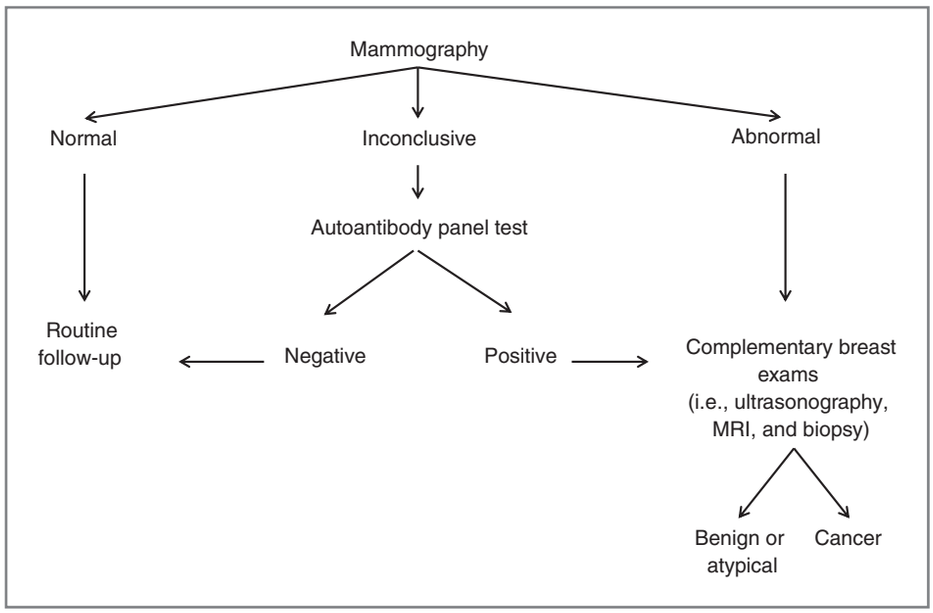

Figure 2. Flow chart shows a decision tree for autoantibody panel assay in complement to mammography screening.

GAL3, PAK2, PHB2, RACK1, and RUVBL1 as highly immunogenic antigens in early-stage breast cancer and DCIS (12). Finally, Lu and colleagues recently observed high titers of anti-p53, -HER2, and -CCNB1 autoantibodies in 78 prediagnostic samples from the Women's Health Initiative (WHI) cohort that were collected, on average, more than 150 days (range, 21–262 days) before breast cancer diagnosis, suggesting that these autoantibodies could be produced during the earliest developmental stages of the disease (14). Because Annexin XI-A, BRCA1, BRCA2, NY-ESO-1, and c-Myc were not confirmed as associated with early-stage breast cancer detection by other groups in large cohorts or were identified in very small patient cohorts, they were not selected for inclusion in the panel of autoantibodies to be assessed in the present study.

Here, we report the largest study assessing a large number of autoantibodies in the sera of women with early-stage breast cancer, including DCIS. As a panel, our autoantibody assay exhibited 70% to 77% sensitivity and 67% to 79% specificity, depending on the statistical test used. Interestingly, when the assay sensitivity was fixed at 90% or 95%, the autoantibody assay showed specificities of 42% and 30%, respectively, and the specificities remained high in women younger than 50 years (59% and 45%, respectively). The increased sensitivity of our multiparametric autoantibody panel should be considered with respect to the corresponding risks of a potential increase in false-positive findings. In light of the importance of early diagnosis for overall survival, it seems reasonable that some extra false positivity (erroneous recalls for further investigation) could be afforded provided that a significant gain in the overall test's sensitivity is warranted. The other potential harms of our autoantibody screening include the overdiagnosis (i.e., finding a breast cancer that would not have clinically surfaced in the absence of screening). For clinicians and patients, overdiagnosis adds complexity to informed decision making. All overdiagnosed patients are needlessly exposed to the anxieties associated with the diagnosis, with obtaining treatment, and with the financial implications of the diagnosis. To address overdiagnosis, it is then important to ensure that patients are informed of the nature and the magnitude of the trade-off involved with its use. In particular, patients must clearly understand that while early diagnosis may offer the possibility of reducing the risk of death from cancer, it also can lead one to be diagnosed and treated for a "cancer" that is not intended to cause problems (61). Finally, the strongest evidence for overdiagnosis associated with the use of an autoantibody panel would come from reliable long-term data for direct comparison of the nonscreening population with patients randomly assigned to undergo autoantibody screening.

Mammography often leads to identification of a "probably benign" lesion or uninformative images. In such cases, clinicians may be reluctant to refer patient for a biopsy; however, they may also be reluctant to decide that no further action is needed. Therefore, these patients are referred for frequent repeat mammography examinations. As an alternative option, autoantibody panel assay could be proposed as a simple, noninvasive, and no-expensive test to help clinician in their decision to carry on with additional investigations rather than waiting for the next mammography (Fig. 2). Because we could not determine the detection performance of our autoantibody panel in the subgroup of women with inconclusive mammograms (e.g., "probably benign" lesion or uninformative images) and/or with mammograms that produced a false-negative result in our present study, it would be valuable to conduct a large-scale randomized controlled

trial of autoantibody assays as an adjunct to mammography with a 2- to 3-year follow-up. We could therefore assess the increase in cancer detection via mammography and serum testing versus mammography alone. Ultimately, a reduction in interval cancers in women who receive adjunct screening (relative to women who have mammography screening alone) would be assessed providing a surrogate indicator of the long-term benefits of this screening (62).

To reach safer conclusions, it would be valuable to conduct a large-scale randomized controlled trial of autoantibody assays as an adjunct to mammography with a 2- to 3-year follow-up to measure its impact on interval cancer rates. Ultimately, a reduction in interval cancers in women who receive adjunct screening (relative to women who have mammography screening alone) would provide a surrogate indicator of the long-term benefits of this screening (62).

In summary, the very early detection of breast cancer remains challenging, notably in younger women with dense breast. Complementary tools are needed to reduce false-negative results obtained through mammography screening and to timely and appropriate decision making and action by the patient and the health care professional. Serum autoantibody dosage could be proposed as an interesting and easy to do procedure to help clinician and increase early-stage breast cancer detection. Because of the large number of prospectively collected early-stage breast cancers and the use of calibrated assays, and the absence of available diagnostic biomarkers in this early phase of the disease, we believe that the data reported in this article pave the wave of the validation of a future usable seroreactivity assays for early breast cancer detection in young women with dense breasts.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Mangé, J. Solassol
Development of methodology: J. Lacombe, A. Mangé, J. Solassol
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Lacombe, A.-C. Bougnoux
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Lacombe, A. Mangé, J. Solassol
Writing, review, and/or revision of the manuscript: J. Lacombe, A. Mangé, I. Prassas, J. Solassol
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Mangé, J. Solassol
Study supervision: A. Mangé, J. Solassol

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