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Research article

Psychological suppressive profile and autoantibodies variability in women living with breast cancer: A prospective cross-sectional study

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ABSTRACT

Breast cancer (BC) is a leading cause of women's morbimortality worldwide. Unfortunately, attempts to predict women's susceptibility to developing BC well before it becomes symptomatic, based on their genetic, family, and reproductive background have proved unsatisfactory. Here we analyze the matching of personality traits and protein serum profiles to predict women's susceptibility to developing cancer. We conducted a prospective study among 150 women (aged 18–70 years), who were distributed into three groups (n = 50): women without breast pathology and women diagnosed with BC or benign breast pathology. Psychological data were obtained through standardized psychological tests and serum protein samples were analyzed through semiquantitative protein immunoblotting. The matching for psychological and immunological profiles was constructed from these data using a mathematical generalized linear model.

The model predicted that women who have stronger associations between high-intensity stress responses, emotional containment, and an increased number and reduced variability of serum proteins (detected by IgG autoantibodies) have the greatest susceptibility to develop BC before the disease has manifested clinically. Hence, the present study endorses the possibility of using psychological and biochemical tests in combination to increase the possibility of identifying women at risk of developing BC before the disease shows clinical manifestations. A longitudinal study must be instrumented to test the prediction ability of the instrument in real scenarios.

Trial registration: Committee of Ethical Research of the Hospital General de México "Dr. Eduardo Liceaga," Ministry of Health (DI/12/111/03/064).

1. Introduction

Early detection of breast cancer (BC) is the fundamental tool advised by international official regulations to reduce the number of recorded cases, which currently exceeds some two million new cases per year (Global Cancer Observatory, 2020). In Mexico, BC is the leading cause of cancer death and the most prevalent cancer. To bring these numbers down, the Official Mexican Standard indicates that women aged 20 and over should examine their breasts monthly, with prior instruction. After 25 years of age, they should go to their health center for a clinical examination of the breast, while the screening mammogram is only indicated for women aged 40–69 years (Norma Oficial Mexicana NOM041-SSA2-2011). However, in Mexico, we have a shortage of mastographers and trained radiologists for the interpretation of images. The genetic biomarkers BRCA1 and 2, or p53, are found to be present in only 15%–40% of tumors and, in Mexican women at least, the former mutation genes are only 9%.

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Although it is assumed that BC is a multicausal pathology, clinical parameters are most used to diagnose it, even though they only explain 40% of cases (Chávarri-Guerra et al., 2012). Other factors have recently been suggested as the cause of this pathology, such as lifestyle or psychological factors. For example, personality traits such as emotional suppression and repression, advanced as determining factors for the appearance and development of the disease, are even associated with poor prognosis (Fischer et al., 2018; Giese-Davis et al., 2006; Morris et al., 1981) and with adverse consequences for mental health (Fortin et al., 2021).

The relationship between physiological and psychological processes has a long scientific tradition (Dukes et al., 2021); however, to the best of our knowledge, this work is the first to verify the relationship between a unique psychological profile and an autoantibody profile with sensitivity to BC.

In this regard, we have identified in a sample of Mexican women, personality traits related to emotional suppression and low-stress symptoms reporting that can be useful in identifying susceptibility to BC. In particular, in BC cases, suppression of anxiety was the most important feature (Romo-González et al., 2018). Likewise, the analysis of networks of these characteristics showed that the groups with pathology (BC and benign breast pathology (BBP)) presented their own spatial rearrangement. This rearrangement included the presence of modules for the stress symptomatology and emotional suppression variables. The latter, notably, was found to be an indicator of susceptibility to BC when an analysis of the main components was made. It is noteworthy that the principal component analysis was performed, including the psychological and clinical variables, and it was found that the latter variables did not contribute to the variance (Montes-Nogueira et al., 2018).

Autoantibodies (AAbs) have been detected in the early stages of some types of cancer and may correlate with disease progression (Macdonald et al., 2017). For example, in the case of melanoma, endogenous antibodies against the TA90 antigen were found in patients with negative nodular melanoma, which is inversely correlated with the relapse rate. Other molecules studied are the oncofetal protein (CEA), oncoproteins such as Her2, c-myc, p53, and antigens (CA15-3 and CA27-29). In addition, survivin, livine, NY-ESO-1 annexin XI-A, endostatin, Hsp60, and p62 have also been documented as possible markers for cancer. In the case of BC, a humoral response has been detected against many tumor-associated autoantibodies such as the c-erbB-2/HER2/neu protein, RS/DJ-1, and mucin-related antigens. Specifically, the c-erbB-2/-HER2/neu proto-oncogene is overexpressed in 20%–30% of BC patients, and the presence of its AAbs has been observed in 11% of cases. Elevated protein levels correlated with a poor prognosis in 29% of BC patients (Qiu et al., 2018). However, some markers have low sensitivity in the early detection of cancer, such as the case of biomarkers such as NY-ESO-1 (4%), SCP-1 (6%), and SSX2. In addition, to increase the predictive value of tumor-associated AAbs, and to refine their use in immunodiagnostics, many groups are testing multiple antigens in parallel (Loke and Lee, 2018; Qiu et al., 2018).

Based on the foregoing, in this paper, we show the results of the matching of two profiles: protein bands obtained with unidimensional immunoblot analysis and the psychological profile, to determine their effectiveness as predictors of susceptibility to BC.

2. Methods

2.1. Study design

We conducted a prospective cross-sectional study with non-probabilistic sampling generated by convenience sampling. Our sample consisted of 150 women who were recruited. They signed an informed consent form after learning about the scientific goals of the project, its technical basis, the policies regarding the use of their blood for BC immunodiagnostic reasons, and the confidentiality terms.

2.2. Participants

The women recruited (see supplementary material) were patients of the Hospital General de México "Dr. Eduardo Liceaga", who were presented for a gynecology appointment during the first semester of the year 2012. The age range for our sample was 16–79 years because BC diagnosis among Mexicans is not limited to women over 50 years of age (Chávarri-Guerra et al., 2012; Norma Oficial Mexicana NOM041-SSA2-2011). Women with no breast pathology formed the healthy (H) group (n = 50). BBP group (n = 50) was integrated with women diagnosed with fibrocystic breast disease, mammary fibroadenomas, or mastitis. Finally, BC group (n = 50) was composed by women who had untreated BC.

A blood sample from each woman was drawn by trained personnel at the Hospital General de México and the Institute for Biomedical Research of the National Autonomous University of Mexico. This procedure was performed before knowing the final diagnosis (by biopsy) of the patients. The protocol was reviewed and approved by the Committee of Ethical Research of the Hospital General de México "Dr. Eduardo Liceaga," Secretaría de Salud (DI/12/111/03/064).

2.3. Test methods

2.3.1. Immune profile

2.3.1.1. Blood samples. From each participant, 10 ml of venous blood were extracted, to later obtain the serum by centrifugation at 1500 rpm. Aliquots of 500 μl were prepared from each sample, which were frozen until use at -80C.

2.3.1.2. Cell culture and antigen preparation. The human breast cancer cell line T47D was cultured and harvested as previously described (Larralde et al., 1989). Briefly, cells were grown on plastic tissue culture plates under conditions of 95% humidity and 5% CO_2 at 37 °C. Once confluence was reached, the cells were harvested following treatment with phosphate-buffered saline (PBS) supplemented with EDTA, pelleted, and frozen at −80 °C. On the day of the experiment, the pellets were lysed in CHAPS buffer supplemented with M urea (4% w/v), DTT (65 mM), and Halt protease inhibitor cocktail (Thermo Fischer Scientific) as recommended by the supplier. Samples were then centrifuged, and the supernatants were collected; protein concentration was estimated through Bradford assays. Samples were aliquoted and kept at −80 °C for further use.

2.3.1.3. Unidimensional electrophoresis and immunoblotting of T47d protein extracts. Protein extracts (100 μg) from the T47D cell line were electrophoresed through polyacrylamide gels (4%–20% TGX Bio-Rad, Hercules, California, USA) at 80 V for 2 h at 4 °C. Proteins were transferred onto nitrocellulose membranes (High Bond, Amersham Biosciences) at 100 V for 1.15 h using a Mini Trans-Blot cell (Bio-Rad, Hercules, CA, USA).

At the end of this procedure, the membranes were stained with copper phthalocyanine-tetra sulphonic acid diluted in 12 mM HCl, imaged, and de-stained. Then, the membranes were blocked with 5% skimmed Nestlé Svelty milk diluted in PBS supplemented with 0.3% Tween 20 (PBS-T) for 16 h at 4 °C. The uppermost and lowermost fronts of the membrane were lined out with a pencil and then cut vertically into 17 or 18 4 mm-wide strips. The strips were individually incubated with the serum of each participant, diluted 1:300 in 2 mL PBS-T, for 5 h at room temperature. After a thorough wash, primary autoantibodies were detected by incubating the membrane's secondary polyclonal antibodies conjugated with horseradish peroxidase, raised in goat antihuman H/L IgGs diluted 1:2500 (Thermo Fischer Scientific) in PBS-T for 1 h at room temperature. Peroxidase activity was revealed by incubating the membranes with 2, 2-diaminobenzidine (0.1 mg/ml; SIGMA, San Luis Missouri, USA) and 0.015% hydrogen peroxide in PBS-T for 5 min at room

temperature. The peroxidase reaction was stopped by gently washing the strips five times with deionized water.

2.3.1.4. Image analysis. Air-dried, immune-stained strips were scanned (resolution 300 dpi; Hewlett-Packard Scanjet G4050) and digitized at a resolution level of 300 dpi in TIF format. Brightness, contrast, and gain were kept constant during image acquisition. Once aligned (Adobe Photoshop), the digital images were analyzed by using Quantity-One (Bio-Rad, Hercules, CA, USA) software. Banding patterns of all strips from different gels and membranes were compared based on the control strips (H111) to identify the total number of different bands and create a binary database with the presence (1) or absence (0) of each band in each strip.

2.3.2. Psychological profiling

2.3.2.1. The Courtauld Emotional Control Scale. The Courtauld Emotional Control Scale (CECS) is a 21-item questionnaire which evaluates emotional suppression. It was developed in 1983 by Watson and Greer, and adapted to native Spanish-speaking (Durá et al., 2010). The CECS includes subscales for anger suppression (A), depression suppression (D) and anxiety suppression (ANX), which have Cronbach's alpha coefficients of 0.86, 0.88, 0.88 respectively, and 0.95 for the total scale. Items are rated from 1 to 4 ("rarely" to "almost always"), so higher scores indicate greater suppression. In this study we use cut-off points low [0–15 points], medium [16–18 points] and high ≥19 points, for the subscales. For the total scale the cut-off points were: low [0–50 points], medium [51–55] and high ≥56. The scores were categorized using the 95% confidence interval of the response variable. After scores were categorized we made a binomial transformation (1 to presence of the feature; 0 to absence to the feature).

2.3.2.2. The Weinberger Adjustment Inventory. The Weinberger Adjustment Inventory (WAI) is a 44-item questionnaire which evaluates repression, defensiveness, and restraint (Weinberger, 1990). Romo et al. (2014) translated into Spanish, adapted and validated for the Mexican population, with *Cronbach's alpha* coefficients upper 0.69. The WAI is composed into three sub-scales: the subjective experience of distress scale (DSS); the restraint (RST) scale; and the defensiveness (RD) scale. The scores reached in this subescales (DSS, RST) and the RD/RST composite allows the identification of adjustment styles: (1) reactive, (2) sensitized, (3) over-socialized, (4) under-socialized, (5) self-assured, and (6) repressive. Items are rated 1 to 5 ("false" to "true") in the first part of the instrument, and 1 to 5 ("rarely or never" to "always or almost always") in the second part. The cut-off points for the subscales were 1) DSS: low 0–47 points, and high ≥47 points; 2) RST: low ≤94, medium 95–107 points, high ≥108; 3) RD/RST composite: low 0–57 points, and high ≥58 points. The scores were categorized using the 95% confidence interval of the response variable. After scores were categorized we made a binomial transformation (1 to presence of the feature; 0 to absence to the feature).

2.3.2.3. Symptoms of Stress Inventory. The Symptoms of Stress Inventory (SSI) is a 30- item questionnaire which asses the frequency of stress symptoms under three subescales: physical (SPhys), psychological (SPsych), and social (SSoc) (Benavides et al., 2002). It has a *Cronbach's alpha* coefficient of 0.93. Items are rated from (0) "never" to (4) "always." In our work, the cut-off points for the subscales SPhys and SSoc were: low 0–5, medium 6–8, and high ≥56. Meanwhile, for the subscale SPsych the cut off points were: low 0–11, medium 12–19, and high ≥20 points. Finally, for the total scale (SGlob) the cut-off points were: low 0–22, medium 23–32, and high ≥33. After scores were categorized we made a binomial transformation (1 to presence of the feature; 0 to absence to the feature).

2.3.3. Data analysis

2.3.3.1. Immune profile. To analyze if there is an immune profile that discriminates BC patients, the means, standard deviations, and variances of the total number of bands in all strips belonging to the same group of participants were calculated using Microsoft Excel (supplementary material). The odds ratios for each band present in all groups of participants were calculated as the ratio of the given band being positive in a given group over the ratio of the same band being positive in a different group (i.e., the band's frequency in BC/band$_i$'s frequency in H). To visualize the similarities and differences by group, a Venn diagram was constructed with the aid of an online program (http://bioinformatics.psb.ugent.be/software/details/Venn-Diagrams). To identify the set of bands that best differentiate the group of participants, we used the method of immune-plotting. This method consists of plotting each band frequency in a given group against the same band's frequency in a contrasting group (Larralde et al., 1989). Further information on the procedure and the characteristics of the immune profiles for Mexican women may be revisited in the study by Romo-González et al. (2015).

2.3.3.2. Psychological profile. To analyze whether there is a psychological profile or features on the three psychometric instruments used that discriminate BC patients, a unifactorial general linear model (GLM) was used to test the statistical significance of the scores recorded for each psychological test completed by BC and BBP patients and H women. Means, standard errors and variances of the total number of psychosocial traits belonging to the same group of participants were also calculated using Microsoft Excel (supplementary material). The odds ratios for each psychological trait present in all groups of participants were calculated as the ratio of the given psychological trait being positive in a given group over the ratio of the same trait being positive in a different group (i.e., psychological features' frequency in BC/psychological features' frequency in H). To visualize the similarities and differences by group, a Venn diagram was constructed with the aid of an online program. For protein band analyses, we identified the set of psychological traits that most differentiated the group of participants by using the plotting method. Further information on the procedure and the characteristics of the psychological profiles of Mexican women may be obtained in the study by Romo-González et al. (2018).

2.3.4. Prospective mathematical modeling

To reveal the pattern of interactions between personality traits and protein bands, we estimated the probability of profile matching between both groups of variables in H women and BBP and BC patients. For the analysis, we defined BC susceptible profiles as those having at least one of the seven high-risk bands (164, 85, 129, 158, 193, 180, and 46) and/or one of the five high-risk personality traits (low Rl, low SGblol, low SPhysl, low RDl, and high DSh). A match was established regardless of whether the interactions between immune and psychological profiles were positive (i.e., profiles with risk bands and traits) or negative (i.e., profiles without risk bands and traits).

As a first step toward calculating the probability of profile matching for BC and BBP patients and H women, we first asked whether profile matching occurred by chance in the population of 150 women, assuming that matching events might be positive–positive, positive–negative, negative–positive, or negative–negative. The formula used to estimate the random probability (P) was:

$$P = \frac{f}{n}, \tag{1}$$

where (f) represents the number of ways events may occur divided by the number of ways any outcome may actually occur. We also estimated P for each of the groups evaluated using the same procedure. The random

probability estimated for the entire sample was 0.027 and 0.08 for each group.

Once profile matching was shown to occur in all groups at statistical levels above chance, we modeled the interactions mathematically between personality traits and protein bands. The model accounted for 13 quantitative variables (i.e., number of serum protein bands recognized by autoantibodies; PBN) and 12 categorical or independent variables which included type of patient (TP), anger suppression (A), suppression of depression (D), anxiety suppression (AXN), global suppression (S), subjective experience of distress (DSS), restraint (R), restraint/defensiveness (RD) composite, physical stress symptoms (SPhys), psychic stress symptoms (SPsych), social stress symptoms (SSoc) and global stress symptoms (SGlob). Descriptive statistical techniques were used as conditional diagrams and cross-frequency tables.

The influence of categorical variables on PBN was then tested by using a generalized linear model for which dummy variables were created for each of the independent variables under the k-1 dummy variables rule, where k represented the number of levels observed in a specific categorical variable (Table 1). The data were then analyzed through Poisson's regression using the zero truncated model (Jackman et al., 2008; Long, 1997; Tango, 1994; Zou, 2004) after simulating two possible scenarios. One of the scenarios included all independent variables on the assumption that all psychological traits influence PBN. The other scenario considered only the independent variables that showed a significant statistical difference in their behavior in the density polygon at all levels of TP according to the first scenario. It is worth mentioning that if psychological profiles had shown the same patterns of interactions in all groups, such profiles would have affected TP similarly. This circumstance, in turn, would have indicated that those psychological profiles affect PBN even if the patients have no pathological conditions. Furthermore, Iteratively Reweighted Least Squares and Fisher Scoring optimization methods were used to estimate the parameters built in each scenario. The two could then be compared based on the differences between the values of $-2log\widehat{L}$ for each model, where $\widehat{L}(1)$ represents the maximized log-likelihood value of model 1 (M1) and $\widehat{L}(2)$ shows the same value for model 2 (M2). The large difference between $-2log\widehat{L}(1)$ and $-2log\widehat{L}(2)$ can be interpreted that the q variables in model 2, that are additional in model 1, do improve the adequacy of the model. Hence, to compare the value $-2log\widehat{L}$ for model 1 and model 2, we use the fact that the statistic $-2log\widehat{L}(1) + 2log\widehat{L}(2)$ has a chi-squared distribution with q degrees of freedom, under the null hypothesis that all the extra parameters in model 2 that are not included in model 1 are all zero. The value q represents the number of extra variables included in model 2 that are not present in model 1 (Collett, 2014).

$$M1: \ln(PBN) = TP + A + D + AXN + S + DSS + R + RD + SPhys + SPsych + SSoc + SGlob \quad (2)$$

$$M2: \ln(PBN) = TP + S + R + SPsych + SSoc \quad (3)$$

3. Results

3.1. Demographic and clinical features of the participants

In our sample, breast tumors were classified as infiltrating canalicular (94%), lobular (2%), mucinous (2%), and tubular (2%). Fifty-eight percent of them were positive for estradiol receptors, 30% were HER2 positive and, 12% were triple-negative. In the case of women suspected of having breast pathology after clinical evaluation, the personality type was inquired about before a definitive diagnosis was made. To have a homogeneous sample size, the recruitment was suspended after reaching n = 50 for each subgroup (H, BBP, and BC) [Figure 1]. All patients recruited for the study were "mestizo" and born in Mexico from middle-class families. Age, educational level, marital status, family history of cancer, nutritional and reproductive backgrounds were taken into consideration when interpreting the results (see supplementary material). Although the age of the subjects was heterogeneous in the three groups, there were no statistical differences in most of the psychological variables when a linear regression model was carried out in respect of age. Only in the case of DSS and ANX was there a statistical difference, but the explained variance was pretty low (6.4% and 3.3%, respectively). This result allows us to safely discard age as a confounding variable. Pregnant women, as well as women exhibiting signs of autoimmune disease or having non-diagnosed breast abnormalities at the time of the recruitment process, were excluded from the study. Women who failed to answer the questionnaire were also excluded.

3.2. Prospective mathematical modeling

3.2.1. The psychological traits and immunological markers have the greatest probability of interacting in the group of women with BC

We assess the probability of interaction between variables of the immune and psychological profiles both within and across groups. The profile matching probability across groups was 0.77, a much higher value than that expected by chance (0.027). Thus, psychological traits and PBN did indeed interact across the population of the women sampled. Interestingly, the probability of such interactions occurring was greatest in BC patients (Figure 1). In addition, the probability of interaction between psychological traits and immunological markers was also estimated when the profile matching for BC was either risk positive or negative. In these alternative scenarios, the probability of psycho-immune interactions occurring for BC patients was only significant when the risk was positive (100%). A similar result was obtained for BBP patients (78%), but not for the H group (22%) (Figure 2).

3.2.2. Overall, women with BC display high-stress responses, likely due to poor emotional containment; a psychological profile associated with increased PBN and reduced banding pattern variability

To begin revealing the associations between PBN and personality traits, we first estimated mean PBN values (±standard deviation) per TP group. BC patients show the highest PBN values (33.7 ± 5.3), followed by BBP patients (27.6 ± 9.8) and H women (25 ± 7.1). Hence, women with BC consistently have more protein bands than BBP and H women. In addition, the banding pattern was remarkably similar among BC patients. In contrast, this feature varied more among BBP patients or H women. Further analyses suggest that psychological traits influence PBN values (Table 1). For instance, higher scores of restraint, restraint-defensiveness composite, and stress physical symptoms were associated with decreased PBN values. Similarly, the greater the intensity of psychological traits such as suppression, restraint, psychic stress symptoms, and social stress symptoms, the higher the PBN values and the lower the variability of the banding patterns except for the social stress symptoms (Figure 3). The model derived from these observations (Table 2: Model 1) predicts that women who display high-stress responses as a result of their poor emotional containment will increase PBN and reduce banding pattern variability, all of which are conditions that either make them susceptible to developing BC or that favor BC progression. Model 1 seems the most likely since the adjusted one (Table 2: Model 2) supports the fact that model I accounts for the variability associated with the response variable ($\chi^2 = -12.13$, p-value = 0.999). This contention is strengthened because, when simulated under model 2 presumptions, high suppression, low restraint, and high

Table 1. Descriptive Statistic by type of patient (TP) and Psychological Features.

Variable	Levels	N	PBC-Mean	PBC-Std
TP	BBP	50	27.58	9.841789
	BC	50	33.74	5.28305
	H	50	25	7.119963
A	Ah	56	30.76786	6.946526
	Al	53	26.18868	8.764124
	Am	41	29.39024	9.183894
D	Dh	64	30.46875	7.309417
	Dl	54	27.03704	8.402522
	Dm	32	28.3125	10.084922
AXN	ANXh	60	30.08333	8.776493
	ANl	50	26.56	8.310701
	ANm	40	29.575	7.699109
S	Sh	53	31.62264	7.14473
	Sl	67	26.92537	8.494764
	Sm	30	27.86667	9.30974
DSS	DSh	70	31.01429	7.942717
	DSl	80	26.8125	8.421031
R	Rh	72	26.94444	9.027172
	Rl	27	33	6.101702
	Rm	51	29.11765	7.913652
RD	RDh	82	27.57317	9.024015
	RDl	68	30.22059	7.488988
SPhys	SFh	51	28.15686	8.251964
	SFl	61	29.21311	7.821157
	SFm	38	28.89474	9.7365
SPsych	SPh	30	31.6	6.891274
	SPl	60	28.1	8.987185
	SPm	60	28.03333	8.408968
SSoc	SSh	44	31.38636	7.260008
	SSl	77	28.62338	7.723919
	SSm	29	25.2069	10.594403
SGlob	SGh	49	30.06122	7.853895
	SGl	60	29.65	8.299122
	SGm	41	25.95122	8.85424
Total		150	28.77333	8.440194

Variable: Codification of variables' names. Levels: Codification of variables' levels. N: number of individuals by variables' level. PBC-Mean: Average protein band count by the variables' levels. PBC-Std: Standard deviation of protein band count by variables' levels (Notice that the codification of variables' levels is constructed as "variable's name"+[h = high, m = medium, l = low], no space between).

PBN: Protein Band Number: 228. TP: Type of Patient. Levels: H, BBP, BC. A: Anger Suppression. Levels: high (h), medium (m), low (l). D: Depression Suppression. Levels: h, m, l. ANX: Anxiety Suppression. Levels: h, m, l. S.: Global Suppression. Levels: h, m, l. DSS: Subjective Experience of Distress. Levels: h, m, l. R: Restraint. Levels: h, m, l. RD: Restraint/Defensiveness composite. Levels: h, m, l. SPhys: Physical Symptoms of Stress. Levels: h, m, l. SPsych: Psychic Symptoms of Stress. Levels: h, m, l. SSoc: Social Symptoms of Stress. Levels: h, m, l. SGlob: Global Symptomatology of Stress. Levels: h, m, l.

psychosocial stress symptoms precisely predict shifts in PBN values, and thus, the likely susceptibility of women to develop BC. Lastly, as a final step, we evaluated the prediction power of model 1. As seen in Figs. 4a and b, the model fits best the phenomenon modeled as the intensity of the psychological risk traits increases. This suggests that model 1 has greater predictability for BC patients.

4. Discussion

BC is one of the leading causes of morbimortality in women worldwide. Unfortunately, we do not have robust prognostic tools capable of identifying BC susceptible women long before BC becomes symptomatic. Predictions based on each woman's family and reproductive histories are imprecise and BC gene-base prognosis accounts for approximately only 10% of potential patients (Macdonald et al., 2017).

Bearing this scenario in mind, we present in this article two mathematical models that show the association between the abundance and low protein variability of autoantibodies, and the presence of personality traits oriented toward emotional suppression and low reporting of symptoms of depression and stress with the diagnosis of BC.

Indeed, even though the approach was constructed only after investigating the personality traits and serum protein profiles of patients with a diagnosis of BC and BBP, mathematical modeling allowed us to predict that women with higher scores of restraint, the restraint-defensiveness composite, psychic/physical/social stress symptoms and an increased number of serum proteins detected by autoantibodies would have the highest susceptibility to developing BC before the disease manifests clinically.

Our data support the above contention since the combination of the positive and negative psychological and immunological profiles observed in the BBP and H groups suggests that there is a path in BC development which could guide prevention and treatment policies. For example, in the H group, 22% were positive on the two profiles, but they did not yet have a positive mammogram. Thus, the fact that these women both had high-risk profiles, even though the disease is not detected by traditional tools, could enhance the life expectancy of those women if they are treated properly or at least monitored. It is also worth noting that only 28% of the H women and 4% of BBP patients present both profiles as negative. In addition, the H women have a higher percentage of the positive psychological profile vs. negative immune profile (46%) than the positive immune profile vs. negative psychological profile (4%), while in women with BBP, these percentages are pretty similar (Figure 2). Therefore, it seems that in H women, the positive psychological profile is more frequent and could be the first sign of BC as it has been reported (Montes-Nogueira et al., 2018; Romo-González et al., 2018).

The present study, therefore, endorses the possibility that the combined use of psychological and immune-serological tests would enable us to identify women at risk of developing BC before the disease manifests clinically. The present results and the ensuing suppositions fully agree

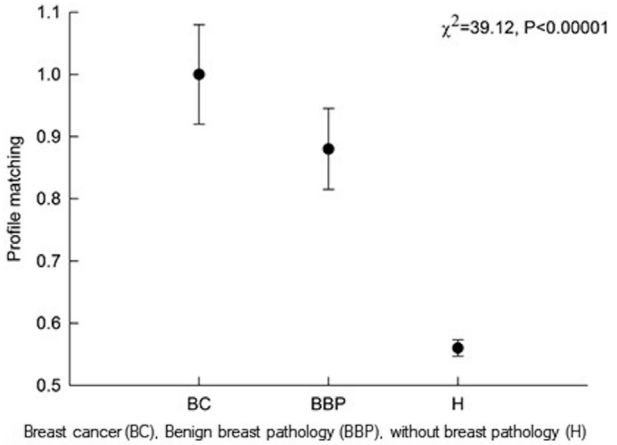

Figure 1. Probability of profiles matching by group.

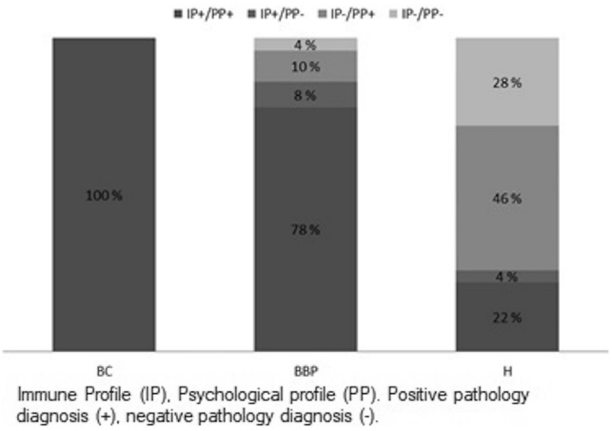

Figure 2. Positive or negative matching probability of the two profiles between groups.

with the vast body of literature published, which hypothesizes that emotional states lead to chronic stress response conditions, likely through epigenetic means, for women's bodies to develop BC at later stages of life (Johnstone and Baylin, 2010; Khan et al., 2010; Montes-Nogueira et al., 2017).

In a previous study, we showed, on the one hand, that the number of serum proteins recognized by autoantibodies increases in BC patients as compared with BBP patients or H women (Romo-González et al., 2015). On the other hand, the inter-individuals variability of these protein profiles was significantly less in BC patients than BBP patients or H women.

Both sets of observations, confirmed in the present study, suggest that even though BC patients seem to release a higher number of antigenic proteins and/or a greater number of autoimmune B-lymphocyte clones, the immune-serological response is much more homogeneous in BC patients than that demonstrated by BBP patients and healthy women. From a network analysis standpoint, this would mean that the network in BC patients is weak, as compared with the BBP patient or H women's networks, by decreasing the connectivity established toward a handful of hubs, represented here by the identifying high-risk protein bands, even though the number of nodes increases (Albert and Barabasi, 2000; Romo-González et al., 2015). In support of these contentions stands the fact that the number of nodes receiving between 19 and 24 connections (hubs) was significantly lower in women diagnosed with BC than in those with BBP or considered to be healthy (Romo-González et al., 2015).

Thus, the loss of connectivity in BC patients' serum protein network as identified by autoantibodies is characterized by a decrease in the number of hubs and much less inter-individuals variability (Romo-González et al., 2015). This feature, in a population screening, could also be used to identify women with middle to high-risk of developing BC in population screenings.

Finally, our model also shows the mind–body connection, since there was an association between the count of IgG protein bands and some psychological traits, associations that are distinctive of the disease. The model proposed here is more robust if it considers some psychological traits, namely, suppression, restraint, and psychic and social stress symptoms. Thus, if we identify these psychological traits and treat them promptly, we might be able to avoid conditions that promote BC development. This presumption is supported by the fact that in the H group, the occurrence of risk factors impacted the psychological profile first (Figure 2).

We consider this study the first approach to integrate the analysis of psychological data with the analysis of autoantibodies. However, we accept the limitations of the study, because only 150 women's data were analyzed, and the identification of the proteins included in each band is necessary. Thus, in the future, further, longitudinal studies are required to evaluate the same parameters, plus the traditional risk factors (Andò et al., 2019), and psychosocial factors (such as mental, dietary, and

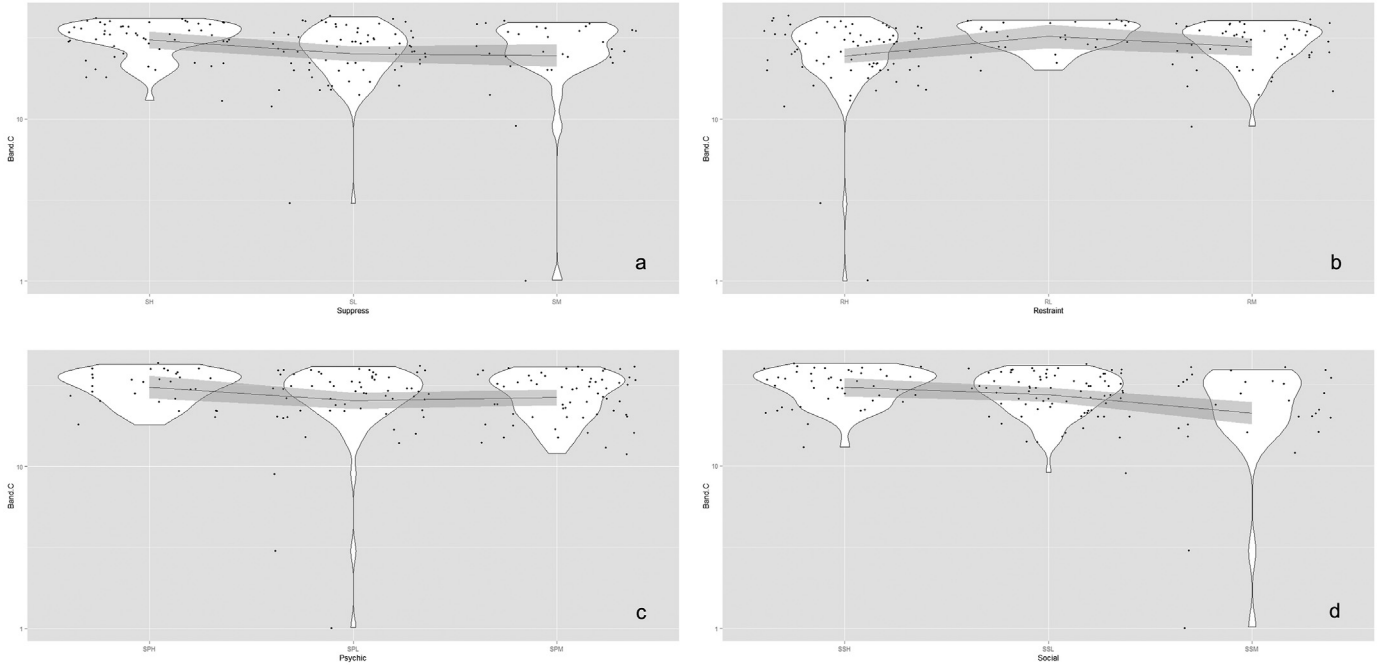

Figure 3. Conditional density diagram. A polygon with a wide head indicates more variability, a polygon with spreads indicates that influence features are presented, and irregular polygons shapes indicate feature concentrations.

Table 2. Model summary (M1-M2).

Model 1			Pearson residuals			
	Min	1Q	Median	3Q	Max	
loge (lambda)	-4.413	-0.8276	0.0003454	0.8025	3.08	
Coefficients						
	Estimate	Std. Error	z value	Pr (>|z|)		
(Intercept)	3.435822	0.05759	59.66	<2.00E-16	***	95%
TP-BC	0.17644	0.039895	4.423	9.75E-06	***	95%
TP-H	-0.053027	0.043624	-1.216	0.22415		
A-l	-0.029744	0.053118	-0.56	0.57551		
A-m	0.069028	0.049228	1.402	0.16085		
D-l	-0.016263	0.05977	-0.272	0.78555		
D-m	-0.015357	0.059143	-0.26	0.79512		
AXN-l	-0.013163	0.052125	-0.253	0.80064		
AXN-m	0.007714	0.043004	0.179	0.85765		
S-l	-0.089316	0.083298	-1.072	0.28361		
S-m	-0.161323	0.064496	-2.501	0.01237	*	95%
DSS-l	-0.008782	0.040352	-0.218	0.82771		
R-l	0.161084	0.051856	3.106	0.00189	**	95%
R-m	0.078288	0.039908	1.962	0.0498	*	95%
RD-l	-0.040337	0.042572	-0.947	0.34339		
SPhys-l	0.023218	0.062197	0.373	0.70893		
SPhys-m	0.048332	0.052059	0.928	0.35319		
SPsych-l	-0.191694	0.076803	-2.496	0.01256	*	95%
SPsych-m	-0.074628	0.05722	-1.304	0.19216		
SSoc-l	-0.027694	0.06889	-0.402	0.68767		
SSoc-m	-0.104414	0.060552	-1.724	0.08464	.	90%
SGlob-l	0.091925	0.107203	0.857	0.39118		
Sglob-m	-0.025672	0.073519	-0.349	0.72695		
Log-Likelihood	-532.4033		Df	127		
Iterations	4					
Model 2			Pearson residuals			
	Min	1Q	Median	3Q	Max	
loge (lambda)	-4.339	-0.8613	-0.04524	1.052	2.731	
Coefficients:						
	Estimate	Std. Error	z value	Pr (>|z|)		
(Intercept)	3.4354	0.04648	73.905	<2e-16	***	95%
TP-BC	0.18159	0.03708	4.897	9.72E-07	***	95%
TP-H	-0.08052	0.0411	-1.959	0.050079	.	90%
S-l	-0.11072	0.03517	-3.148	0.001644	**	95%
S-m	-0.14478	0.04378	-3.307	0.000942	***	95%
R-l	0.13873	0.04337	3.198	0.001382	**	95%
R-m	0.05106	0.03547	1.439	0.150039		
SPsych-l	-0.14085	0.06168	-2.283	0.022406	*	95%
SPsych-m	-0.05213	0.04865	-1.072	0.283926		
SSoc-l	0.02503	0.05187	0.483	0.629363		
SSoc-m	-0.11587	0.05249	-2.208	0.027275	*	95%
Log-Likelihood	-538.4679		Df	139		
Iterations	4					

All results are based on lambda's natural logarithm. *Pearson residual* refers to order statistics (minimum, first quartile, median, third quartile, and maximum) of the model residuals. *Coefficients* refer to the coefficient estimators by the model with standard error metrics and the corresponding values of the z test. Symbols ***, **, * and • suggest that the z test can be validated at the 99.9%, 99%, 95%, and 90% levels of significance. Finally, log-Likelihood with its respective degrees of freedom is presented as a model error measure and the number of iterations needed to optimize the estimation.

physical activity habits). In this regard, it has been suggested that the presence of habits or activities to reduce psychological stress, together with support during diagnosis and treatment, promote a better prognosis. Therefore, the identification of a psychological profile that predisposes the subject to stress and the application of evidence-based psychological interventions would be very useful to avoid a negative prognosis (i.e., the appearance of biomarkers for BC) (Cerezo et al., 2020; Witek Janusek et al., 2007, 2019; Yang et al., 2022). Additionally, this model could be corroborated in animal models that help us demonstrate the changes and connections that occur in the hypothalamus–adrenal–pituitary axis,

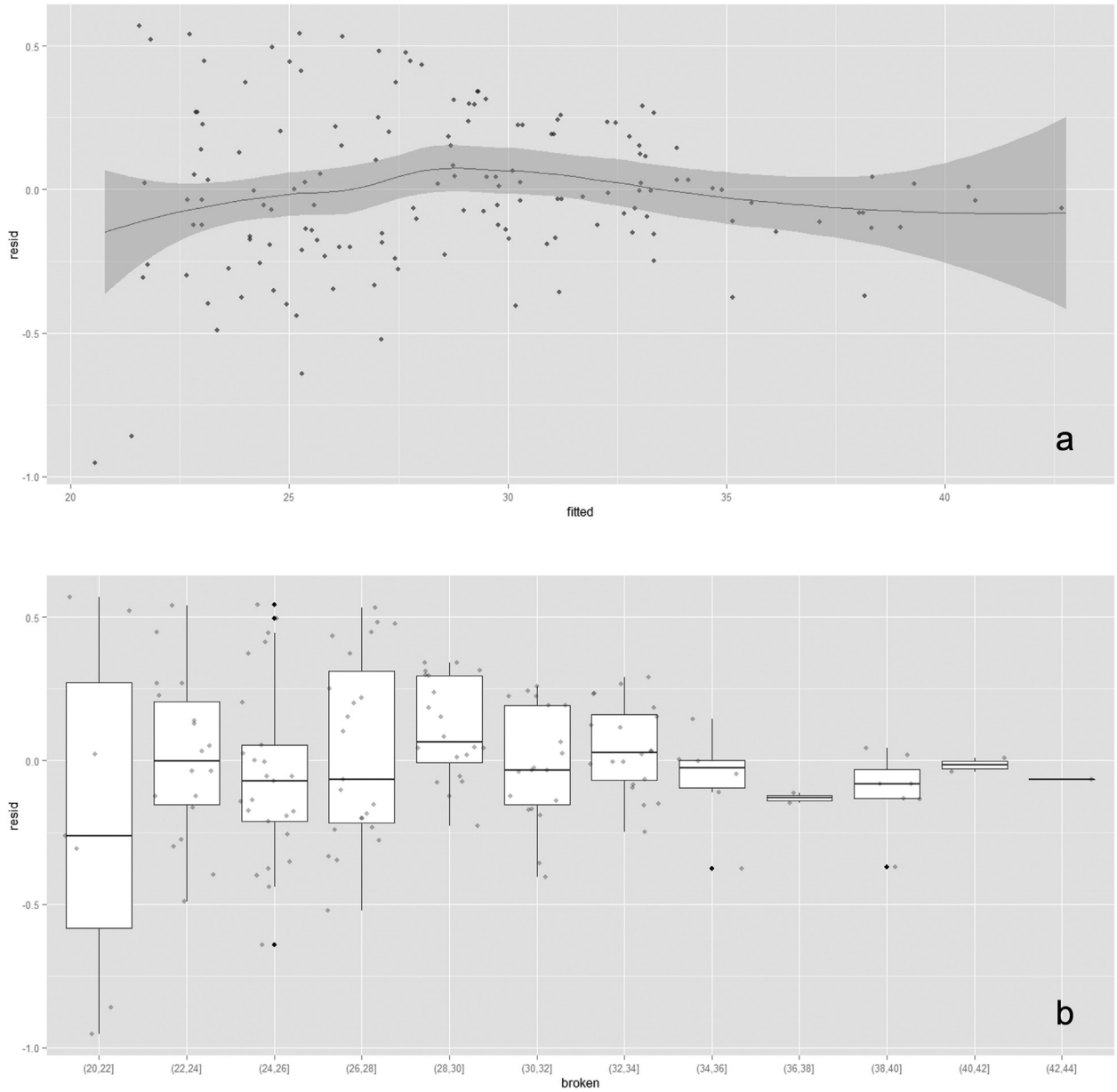

Figure 4. Fitted values vs. residuals. Notice that the estimation's error shows less variability at greater values of protein band count.

under conditions of emotional suppression, stress and cancer (Devlin and Roberts, 2022).

5. Conclusion

The mind–body connection has been described since the time of Hippocrates and more than 30 years of scientific evidence have shown that the psychological state influences the immune system (Blume et al., 2011; Segerstrom and Miller, 2004). Also, autoantibodies are excellent candidates for early detection of BC (Qiu et al., 2018) well beyond traditional methods. In this paper, with the analysis of the data under the GLM, we found that women with BC expressed less autoantibody variability than the groups without BC, and this feature matches a suppressive psychological profile.

Ethics approval

This work was carried out following the Code of Ethics of the Declaration of Helsinki and approved by the Committee of Ethical Research of the Hospital General de México "Dr. Eduardo Liceaga," Ministry of Health (DI/12/111/03/064) retrospectively registered.

Declarations

Author contribution statement

Tania Romo-González: Conceived and designed the experiments, Analyzed and interpreted the data; Wrote the paper.

Antonia Barranca-Enríquez; Rosalba León-Díaz; Gabriel Gutiérrez-Ospina: Analyzed and interpreted the data; Wrote the paper.

Enrique Del Callejo-Canal: Performed the experiments; Analyzed and interpreted the data.

Angela María Jimenez Urrego; Cristina Bolaños; Alejandro Botero Carvajal: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Competing interest statement

The authors declare no conflict of interest.

Additional information

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