Original Article

Eur J Breast Health 2024; 20(3): 185-193
DOI: 10.4274/ejbh.galenos.2024.2024-3-6

High Levels of Superoxide Dismutase 2 Are Associated With Worse Prognosis in Patients With Breast Cancer

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ABSTRACT

Objective: Breast cancer is classified based on hormone receptor status and human epidermal growth factor receptor 2 (HER2) expression, including luminal, HER2+, or triple-negative (TNBC). The absence of a therapeutic target in TNBC and the resistance to treatment associated with other subtypes means that research for new biomarkers remains important. In this context, superoxide dismutase 2 (SOD2) has emerged as a potential therapeutic target due to its clinicopathological associations and its ability to predict responses in human tumors. To analyze SOD2 staining in samples obtained from individuals with breast cancer and explore its transcriptional pattern across tumor subtypes.

Materials and Methods: SOD2 staining was assessed using the immunohistochemistry (IHC) in 80 samples from breast cancer patients. To analyze the expression profile at the transcriptional level, international databases such as cBioPortal (1,980 patients) and PrognoScan were accessed.

Results: Significant differences were observed between SOD2 expression analyzed by IHC, and estrogen (p = 0.0008) and progesterone (p = 0.0003) receptors, as well as tumor subtypes (p<0.0001). These differences were found in conjunction with other associations, including clinical and pathological data, such as tumor stage (p = 0.0129), tumor size (p = 0.0296), and node metastasis (p = 0.0486). Moreover, elevated SOD2 expression correlated with an unfavorable prognosis. The *in silico* analysis revealed a similar pattern, despite operating at the transcriptional level. Moreover, notable correlations were identified between elevated SOD2 expression and worse survival.

Conclusion: These results highlight the importance of SOD2 in breast cancer, particularly in aggressive subtypes. Increased SOD2 staining correlates with poorer outcomes, suggesting it as a potential therapeutic target.

Keywords: Biomarkers; breast cancer; SOD2; superoxide dismutase 2; triple negative breast cancer

Cite this article as: Rodrigues de Bastos D, Longatto-Filho A, Conceição MPF, Termini L. High Levels of Superoxide Dismutase 2 Are Associated With Worse Prognosis in Patients With Breast Cancer. Eur J Breast Health 2024; 20(3): 185-193

Key Points

- Superoxide dismutase 2 (SOD2) is elevated in advanced stage breast tumors, lymph node metastases, negative hormone receptors, and molecular subtypes.
- SOD2 is consistently increased in triple-negative breast tumors.
- High levels of SOD2 are associated with worse clinical outcomes in patients with breast cancer.

Introduction

Breast cancer is the most incident cancer among women worldwide. The Globocan/2020 project estimated 4.4 million cancer-related deaths, with breast cancer ranking fifth in terms of mortality when considering both men and women, and first if considering only women (1).

Due to the heterogeneity of these tumors, classifications based on histopathological characteristics have emerged. The classification takes into account immunohistochemical (IHC) staining for estrogen hormone receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor type 2 (HER2). In addition, Ki67 proliferation marker analysis can be used to distinguish luminal tumors into A and B. As a result, tumors can be categorized into luminal A, luminal B, HER2-positive, and triple-negative (TNBC) (2, 3).

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Received: 12.03.2024
Accepted: 16.04.2024
Available Online Date: 01.07.2024

Patient survival varies according to the tumor subtype and a better prognosis is observed in patients with luminal tumors compared to those with HER2-positive or TNBC tumors (4). Several factors contribute to the survival of the patients, ranging from early diagnosis to the existence of targeted therapies capable of assisting in the pathological response. For instance, luminal and HER2-positive breast carcinomas are responsive to hormone therapy (e.g., tamoxifen, anastrozole) and targeted therapy (e.g., trastuzumab, lapatinib), respectively. In contrast, patients with TNBC do not have a specific therapeutic target, which contributes significantly to the frequent low survival observed in this population. Thus, the search for new biomarkers to assist in prognosis and a better understanding of these tumors remains the focus of much rsearch (2, 3).

Superoxide dismutase 2 (SOD2) is a mitochondrial enzyme with an important function in modulation of reactive oxygen species (ROS). Briefly, SOD2 reacts with superoxide (O2-) and hydrogen ions (H+) to generate oxygen (O_2) and hydrogen peroxide (H_2O_2) in a physiological process of cell detoxification (5). This balance is essential for cellular homeostasis. However, in cancer cells there is an impairment of this balance, leading to resistance to conventional treatments and worse survival (6). Despite its suppressive role in certain tumors, studies have shown that high levels of SOD2 can increase cell proliferation, as well as contribute to resistance to treatment and dissemination of metastases, especially in advanced-stage tumors (6-8).

Our group has investigated SOD2 since it was observed its differential expression in response to pro-inflammatory stimulus in human papilloma virus-immortalized keratinocytes (9). Complementary studies using IHC analyses were performed to detect SOD2 expression in samples from patients with cervical and penile cancer, revealing an important clinical and pathological association. High levels of SOD2 protein are associated with severity in these tumors (10-12). Moreover, it was shown that higher SOD2 expression was associated with a very poor prognosis for squamous cell carcinoma of the uterine cervix stage IIIB in terms of treatment with chemo- and radiotherapy (13).

In breast cancer, most of the studies regarding SOD2 investigated the relation between the polymorphism of this gene, especially variant C, with treatment toxicity and efficacy. In addition, there are also studies evaluating different post-translational modifications of the enzyme (acetylated and methylated) in animal and cellular models. However, there are still few published studies that have investigated the expression of SOD2 in the context of breast cancer, particularly concerning molecular subtypes and other clinicopathological features, such as tumor stage, lymph node involvement, its relationship with hormone receptors, as well as its prognostic role (14-17). In the present study, we evaluated the expression of SOD2 in samples from patients with breast cancer and investigated its transcriptional pattern in the luminal, HER2-positive, and TNBC subtypes. Our results may contribute to assessing the value of SOD2 expression as a biomarker in breast cancer.

Materials and Methods

Ethical Approval

This study was approved by the Research Ethics Committee (REC) of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo (HCFMUSP) with approval number 797466 (date: 11.11.2022). Samples were collected between April 2010 and January 2012, and were analyzed for this study in March 2023. The waiver of the informed consent form was approved by the REC. The patients' data were anonymized, ensuring that no identifiable information was used. Additionally, the age of all patients was over 18 years.

Immunohistochemical Analysis

IHC reactions were performed on the automated VENTANA BenchMark GX System (Roche Diagnostics, Mannheim, Germany). The anti-SOD2 polyclonal antibody produced in rabbits (Abcam, ab13533) was used at a concentration of 1:1000 according to the manufacturer's instructions. Positive reactions were visualized with a cocktail containing peroxidase-conjugated secondary anti-rabbit antibodies using the UltraView Universal 3,3'-Diaminobenzidine Detection Kit (Ventana Medical Systems Inc, Roche, Tucson, Arizona, USA) according to the manufacturer's instructions. Each of the 80 samples from breast tumors was evaluated in triplicate. Fully standardized TNBC biopsies were employed as both positive and negative controls. The negative control underwent no application of the primary antibody. The IHC results were evaluated according to the extent (E) and intensity (I) of SOD2 staining. Values from 0 to 3 were assigned to both parameters. The value 0 (zero) was assigned to samples without detectable staining for SOD2, 1 when was less than 30% of tumor cells were stained, 2 when 30 to 60% of tumor cells were stained and 3 when SOD2 staining was detected in more than 60% of tumor cells. For the intensity, the value 0 corresponds to no staining reaction, 1 for weak staining intensity, 2 for mild staining intensity and 3 for strong staining intensity. For each sample, the average between the triplicates for the variables extension and intensity was calculated, then a staining score was established by multiplying the mean values of E and I for each sample [x-E×(x-I)], resulting in a last score ranging from 0 to 9 as described by Klein and colleagues (18). Finally, the average of all scores obtained was extracted. The samples were classified as "low" when the score was below the mean value or "high" when the score was above the mean. Based on this classification, a contingency table and survival curves were constructed.

In Silico Analysis

To complement the findings of the IHC investigation, we additionally conducted an *in silico* analysis to investigate the expression pattern of SOD2 at transcriptional levels and determine its reproducibility in other patient groups. Data from the Metabric project available on the cBioPortal for Cancer Genomics were accessed. The cBioPortal (https://cbioportal.org) is a database that provides access to genomic and transcriptomic data and is hosted by the Center for Molecular Oncology at MSK (19, 20). In the present study, we accessed the SOD2 expression of 1,980 cases of patients with breast cancer. The microarray mRNA data were downloaded and correlated with the clinicopathological information of the patients. Missing data was excluded. For the construction of the contingency table, the median was initially obtained from the SOD2 expression data. Values below the median were classified as low and values above the median were defined as high. Then, a chi-square test or Fisher's exact test was applied. The low/high classification was also used for survival analysis using the Kaplan-Meier method and the logRank test.

PrognoScan is a user-friendly tool for fast access to microarray data. Using this tool, it is possible to access different genes and observe prognostic associations (21). In this study, we utilized SOD2 as the input and downloaded the relevant studies (p<0.05) for the graphical construction of the forest plot using MedCalc software (https://www.medcalc.org/).

Statistical Analysis

The assessment of Gaussian distribution was conducted to determine the most appropriate statistical test. Qualitative variables were analyzed using the chi-square test or Fisher's exact test. The t-test or Mann-Whitney test were applied to assess possible differences between two groups. ANOVA or Kruskal-Wallis was applied to analyze differences between three or more groups. Kaplan-Meier method and the log-rank test were utilized for survival analysis. Employing Cox regression, both univariate and multivariate analyses were performed. To achieve this, data from patients who underwent IHC analysis were utilized, alongside the patient cohort sourced from the Metabric database. This combined approach allowed for a comprehensive exploration of the factors influencing the outcomes under investigation. Results were considered statistically significant when $p<0.05$. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 25 (IBM Inc., Armonk, NY, USA) or GraphPad Prism v. 7 (California, USA).

Results

Immunohistochemistry

The tissue microarray (TMA) employed in this study comprised samples from 92 patients, with seven exclusions due to incomplete clinicopathological data, and an additional five exclusions resulting from sample loss during technical procedures. In this way, a total of 80 samples were used for statistical analyses. Illustrated in Figure 1 are representative images showing the SOD2 staining patterns within the analyzed samples. Notably, elevated SOD2 results manifest a distinctive punctate cytoplasmic staining pattern, while exhibiting an absence of observable membrane or nuclear staining. To facilitate subsequent analyses, samples scoring below the mean were categorized as low, while those surpassing the mean were denoted as high.

Significant associations were observed between IHC results for SOD2 and clinicopathological aspects of the study population. There were differences in ER ($p<0.0001$), PR ($p<0.0001$) levels, tumor stage ($p = 0.042$), size of the tumor ($p = 0.0296$) lymph node involvement ($p = 0.0486$) and molecular classification ($p<0.0001$). Regarding age, HER2 and metastasis status, no significant differences were found (Table 1).

In addition, regarding the TMA, we used the score to analyze the dispersion of data based on classical markers in breast cancer. We observed that ER-negative patients had a higher SOD2 score compared to those who were ER-positive ($p = 0.0008$) (Figure 2A). Similarly, PR-negative samples exhibited higher SOD2 scores ($p = 0.0003$) (Figure 2B). No differences were observed in relation to HER2 ($p = 0.083$), possibly due to the limited number of samples (Figure 2C). Regarding the tumor subtype, luminal A were found to have a lower SOD2 score, while TNBC showed significantly higher average SOD2 scores ($p<0.0001$) (Figure 2D).

In order to assess the prognostic value of SOD2 in the studied population, we conducted a survival analysis. It was observed that patients with higher SOD2 expression had a worse overall survival (hazard ratio = 2.10; $p = 0.0363$; Figure 3A). Regarding relapse-free survival (RFS), the analysis did not reveal significant differences (hazard ratio = 2.22; $p = 0.0748$; Figure 3B).

In Silico Contributions

To further confirm our observations in a larger number of samples from different centers, we conducted an *in silico* analysis. Significant differences were observed regarding patients' age and menopausal status ($p<0.0001$), cellularity ($p<0.0001$), tumor subtype ($p<0.0001$), ER ($p<0.0001$) and PR ($p<0.0001$) expression, HER2 ($p<0.0001$) expression, and according to the Neoplasm Histologic Grade ($p<0.0001$) (Table 2).

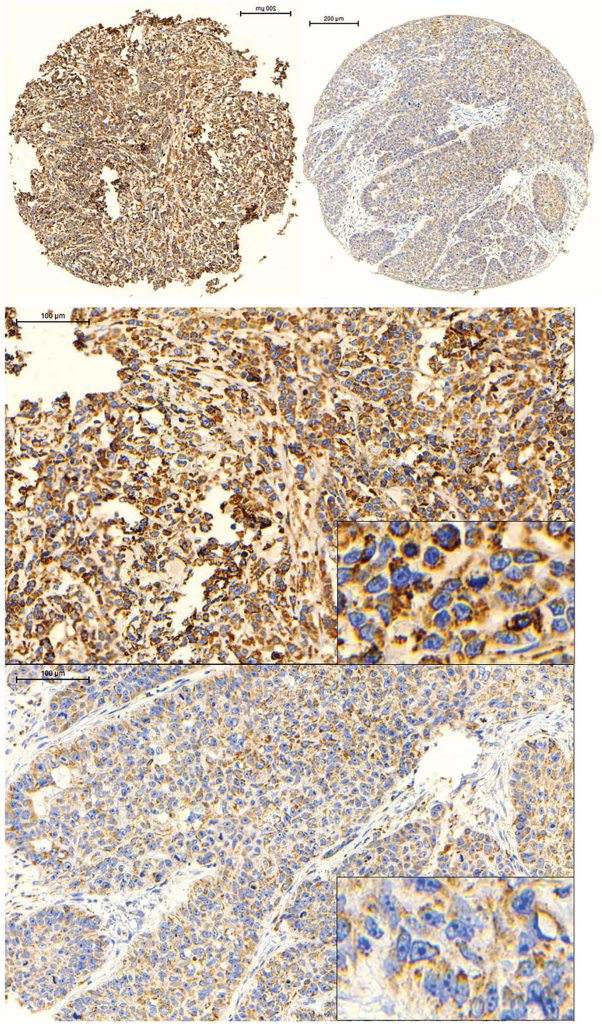

Figure 1. Representative images of SOD2 staining within breast cancer samples are presented. The images reveal **A, C.** High score and **B, D.** Low score. The upper images are captured at a 5X magnification, while the lower images are depicted at a 10X magnification. Additionally, an inset image is provided at a 20X magnification for detailed examination

SOD2: Superoxide dismutase 2

Furthermore, in relation to the patients in the Metabric study, we observed increased levels of SOD2 transcript in patients with negative staining for ER ($p<0.0001$; Figure 4A) and PR ($p<0.0001$; Figure 4B). Interestingly, with a larger number of cases, it was possible to observe a significant difference in terms of HER2 status ($p<0.0001$; Figure 4C). A similar profile was also observed regarding tumor subtypes, with lower SOD2 levels in luminal tumors and higher levels in basal tumors ($p<0.0001$; Figure 4D).

Elevated SOD2 expression demonstrated a correlation with a less favorable 120-month overall survival rate (hazard ratio = 1.40; $p<0.0001$; Figure 5A) Furthermore, it exhibited a similar association with an adverse relapse-free survival rate (hazard ratio = 1.31; $p<0.0001$; Figure 5B).

We then conducted an analysis using ROC plotter and plotter. We observed that non-responders had high SOD2 expression (p<0.0001; Figure 6A, B). The data were analyzed, and the hazard ratio along with the confidence interval observed in each study were used to construct the forest plot. Using this approach, we observed that high SOD2 expression was associated with different overall survival, relapse-free survival, metastasis-free survival, and disease-specific survival of patients with breast cancer (Figure 6C).

Finally, we conducted a univariate and multivariate analysis with the aim of exploring the factors influencing the clinical outcome of the patients. For this purpose, classical variables were employed for both the group of patients analyzed through IHC and the group

Table 1. Clinicopathological parameters and association with immunohistochemical staining for SOD2

Clinical parameters	Low (score ≤1.2)		High (score >1.2)		
	n	%	n	%	*p*-value
Age					
≤50	9	17.00	9	33.30	0.0980
>50	44	83.00	18	66.70	
ER					
Negative	6	11.50	14	56.00	*p*<0.0001
Positive	46	88.50	11	44.00	
PR					
Negative	8	15.40	18	66.70	*p*<0.0001
Positive	44	84.60	9	33.30	
HER2					
Negative	46	90.20	19	73.10	0.0930
Positive	5	9.80	7	26.90	
Tumor stage					
1	13	24.50	1	3.70	0.0129
2	27	50.90	11	40.70	
3	9	17.00	12	44.40	
4	4	7.50	3	11.10	
T					
1	16	30.20	2	7.40	0.0296
2	26	49.10	12	44.40	
3	6	11.30	7	25.90	
4	5	9.40	6	22.20	
N					
Negative	28	52.80	8	29.60	0.0486
Positive	25	47.20	19	70.40	
M					
Negative	49	92.50	24	88.90	0.6829
Positive	4	7.50	3	11.10	
Molecular classification					
Luminal A	12	26.10	2	8.00	*p*<0.0001
Luminal B	29	63.00	7	28.00	
HER2	2	4.30	5	20.00	
TNBC	3	6.50	11	44.00	

The low or high category was defined based on the average score obtained. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor type 2; T: Tumor; N: Node; M: Metastasis; TNBC: Triple-negative breast cancer; SOD2: Superoxide dismutase 2

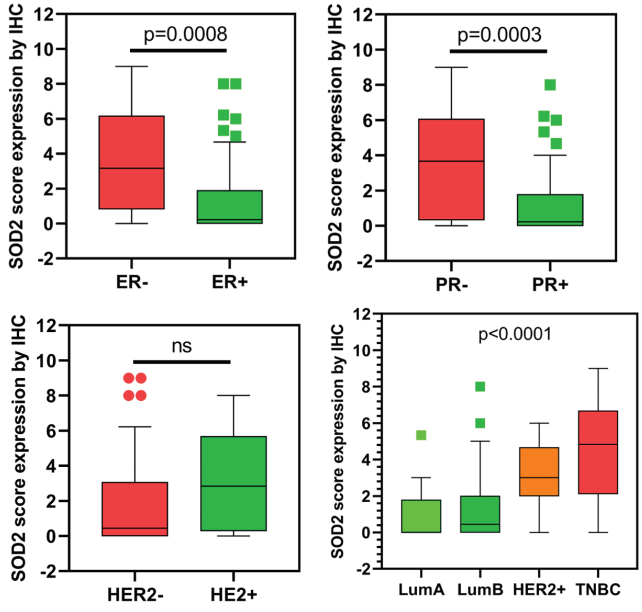

Figure 2. Scoring SOD2 after immunohistochemical technique. SOD2 score based on **A.** Estrogen receptor status; **B.** Progesterone receptor status; **C.** Human epidermal growth factor receptor type 2 status; and **D.** Molecular subtype of patients with breast cancer

SOD2: Superoxide dismutase 2

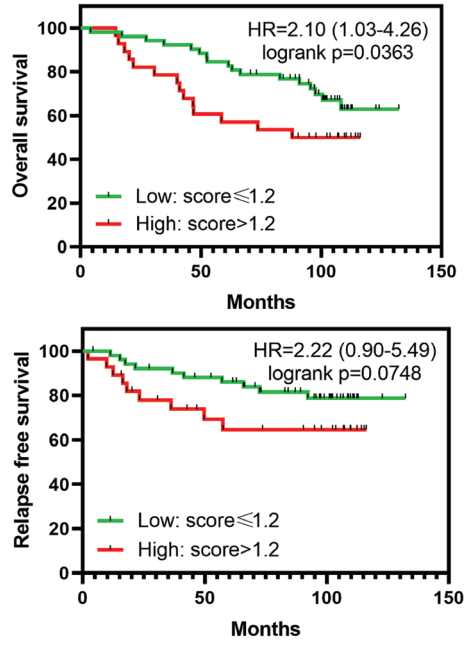

Figure 3. Survival analysis as a function of SOD2 staining. **A.** Overall survival and **B.** Relapse-free survival of patients with breast cancer based on SOD2 score

SOD2: Superoxide dismutase 2

of patients sourced from the Metabric database. In the univariate analysis, significant differences were observed in terms of ER (p = 0.002; p<0.0001) and PR (p = 0.02; p<0.0001), triple-negative and non-triple-negative status (p = 0.01; p = 0.0008), and SOD2 (p = 0.04; p<0.0001) in both patient groups (Table 3). With a larger patient sample and considering the distinct SOD2 evaluation technique, significant differences were also observed in terms of patient age (p = 0.01) and HER2 status (p<0.0001). In the multivariate analysis, no significant differences were observed concerning the group of

Table 2. Clinicopathological parameters and association with SOD2 expression of patients from the Metabric study

Clinical parameters	Low (mRNA ≤8.26)		High (mRNA >8.26)		*p*-value
	n	%	n	%	
Age					
≤50	175	17.70	249	25.20	<0.0001
>50	815	82.30	741	74.80	
Menopausal state					
Pre	175	17.70	249	25.20	<0.0001
Post	815	82.30	741	74.80	
Cellularity					
Low	112	11.70	103	10.70	0,002
Moderate	400	41.90	337	35.00	
High	443	46.40	522	54.30	
PAM50 with claudin-low subtype					
Basal	24	2.40	185	18.70	<0.0001
Claudin-low	27	2.70	191	19.30	
HER2	65	6.60	159	16.10	
Luminal A	512	52.00	188	19.00	
Luminal B	260	26.40	215	21.70	
Normal-like	97	9.80	51	5.20	
ER					
Negative	74	7.60	365	37.70	<0.0001
Positive	894	92.40	604	62.30	
PR					
Negative	335	33.80	605	61.10	<0.0001
Positive	655	66.20	385	38.90	
HER2					
Negative	918	92.70	815	82.30	<0.0001
Positive	72	7.30	175	17.70	
Neoplasm histologic grade					
1	114	12.20	55	5.80	<0.0001
2	489	52.20	282	29.50	
3	333	35.60	619	64.70	

Low or high expression according to median value. Analysis using the chi-square technique. Microarray data of breast cancer patients from the Metabric study; HER2: Human epidermal growth factor receptor type 2; SOD2: Superoxide dismutase 2

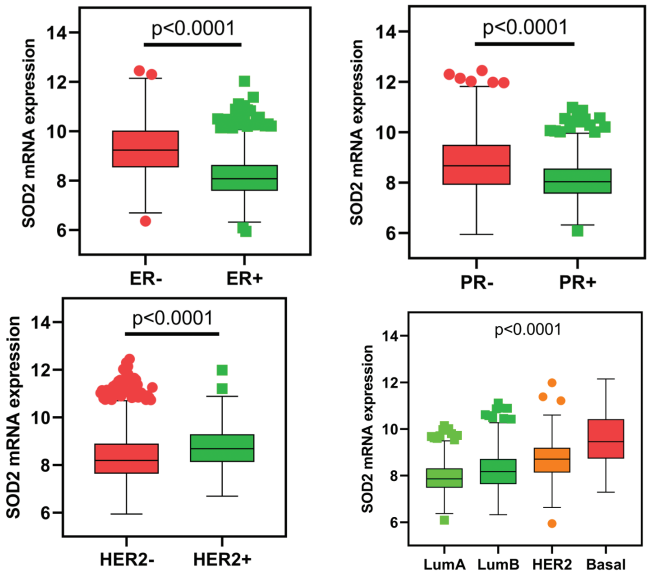

Figure 4. Transcriptional expression of SOD2. SOD2 mRNA expression based on **A.** Estrogen receptor; **B.** Progesterone receptor; **C.** Human epidermal growth factor receptor type 2; and **D.** Molecular subtype. As for the molecular subtype, clowdin-low and normal-like were excluded. Data analyzed from the Metabric study

LumA: Luminal A; LumB: Luminal B; HER2: Human epidermal growth factor receptor type 2; ER: Estrogen receptor; PR: Progesterone receptor; SOD2: Superoxide dismutase 2. Patients from the Metabric study and mRNA expression data accessed through cBioPortal

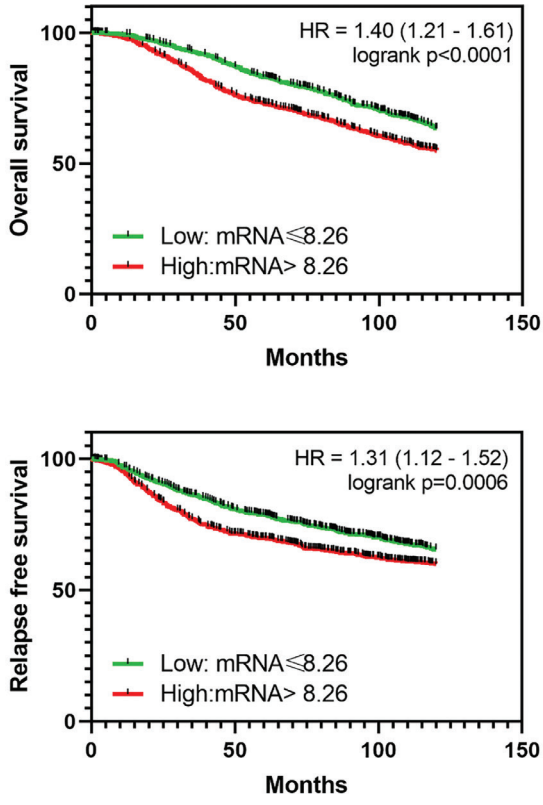

Figure 5. 120-month survival of patients with breast cancer from the Metabric study. **A.** Overall survival as a function of increased or reduced SOD2 expression. **B.** Recurrence-free survival as a function of increased or reduced SOD2 expression

SOD2: Superoxide dismutase 2

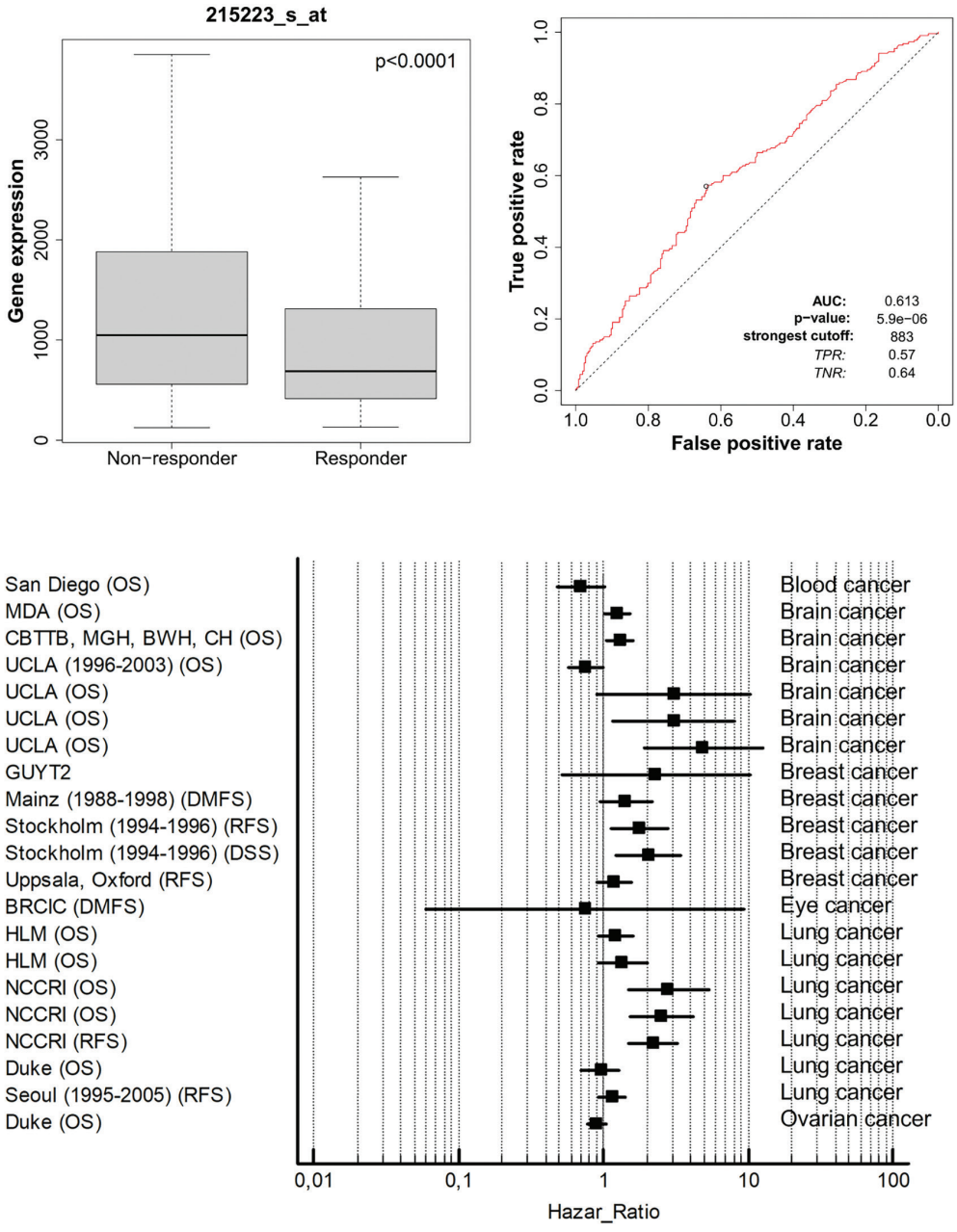

Figure 6. ROC plotter analysis and forest plot. **A.** SOD2 expression profile and **B.** ROC curve of responders and non-responders to chemotherapy treatments. **C.** Forest plot generated from the studies obtained through the PrognoScan search. The central square represents the hazard ratio (HR) of each study and the bold horizontal lines represents the confidence interval (lower and upper bounds). Studies with HR less than 1 indicate a protective factor, while HR greater than 1 indicates risk

OS: Overall survival; RFS: Relapse free survival; DMFS: Distant metastasis free survival; ROC: Receiver operating characteristic; The forest plot was constructed in MedCalc software

patients evaluated through IHC. Conversely, among patients from the Metabric cohort, significant differences were observed in terms of age (p<0.0001), ER (p<0.0001), PR (p<0.0001), HER2 (p<0.0001), and SOD2 (p<0.0001).

Discussion

Despite significant advances in cancer treatment, a considerable number of patients suffer from relapses and/or therapeutic resistance, resulting in high mortality rates. Therefore, the search for new therapeutic and prognostic targets is important. SOD2 has been investigated in several studies, including those conducted by our research group. In a previous study, using cervical tumor cell lines, our group identified alterations in the SOD2 expression pattern after treatment with tumor necrosis factor (9). In the literature, other authors have explored approaches involving drugs capable of modulating of SOD2 (22). Thus, understanding the expression pattern of SOD2 in human tumors, including its subtypes in the case of breast cancer, is essential to identify and characterize tumor profiles that are candidates for therapies that may directly or indirectly affect their transcriptional and protein levels.

In the present study, we observed elevated SOD2 staining in hormone receptor-negative breast cancer patients. These results agree with the

Table 3. Univariate and multivariate analysis of patients with breast cancer assessed by IHC and patients from the Metabric study

Variable HR (95% CI)		IHC staining		Metabric mRNA	
		p	HR (95% CI)	*p*	
Univariable					
Age	≤50	1	0.5082	1	0.0103
	>50	0.76 (0.34–1.68)		1.28 (1.06–1.54)	
ER	Positive	1	0.0025	1	<0.0001
	Negative	3.03 (1.48–6.20)		1.57 (1.34–1.85)	
PR	Positive	1	0.0181	1	<0.0001
	Negative	2.32 (1.15–4.66)		1.63 (1.41–1.88)	
HER2	Negative	1	0.2609	1	<0.0001
	Positive	1.62 (0.70–3.73)		1.75 (1.44–2.11)	
TN nTN	nTN	1	0.0115	1	0,0008
	TN	2.78 (1.26–6.13)		1.39 (1.15–1.69)	
SOD2	Negative	1	0.0407	1	
	Positive	2.10 (1.03–4.26)		1.40 (1.21–1.61)	<0.0001
Multivariable					
Age	≤50	1	0.3881	1	<0.0001
	>50	1.45 (0.62–3.41)		1.45 (1.19–1.76)	
ER	Positive	1	0.4670	1	0,0293
	Negative	2.17 (0.27–17.60)		1.24 (1.02–1.51)	
PR	Positive	1	0.9288	1	<0.0001
	Negative	0.91 (0.11–7.50)		1.37 (1.16–1.61)	
HER2	Negative	1	0.6625	1	<0.0001
	Positive	1.23 (0.48–3.15)		1.47 (1.20–1.80)	
SOD2	Negative	1	0.4268	1	0,0299
	Positive	1.43 (0.59–3.49)		1.19 (1.02–1.39)	

TN: triple negative; nTN: non triple negative; HR: Hazard ratio; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor type 2; SOD2: Superoxide dismutase 2; IHC: Immunohistochemistry; CI: Confidence interval

study conducted by Li and colleagues, who identified an elevated SOD2 transcriptional expression pattern in patients with breast cancer, compared to adjacent tissue (23). In addition, Li's group analyzed a population of 60 patients and observed higher SOD2 staining in cancer samples compared to non-tumor tissue (23). Many studies have reported that the SOD2 enzyme in the context of tumors acts as an oncogene, promoting signaling for malignant transformation, cell survival and ability to generate tumor stem cells (24). It is well known that SOD2 function cannot be replaced by others SOD family member such as SOD1. SOD2 knockout in mice model, for example, resulted in neonatal lethality revealing its importance for normal cell functionality. However, SOD2 plays a dichotomous role and is regulated differently during tumor progression depending on the tumor type. Interestingly, some cancers exhibit increased SOD2 expression compared to healthy tissues (25). Therefore, considering our data and other studies regarding SOD2 role in the context of breast cancer, we can infer that increased levels of SOD2 in tumor tissue may indicate an oncogenic role in breast tissue.

We found that increased expression of SOD2 was associated with advanced tumor stages and lymph node metastases, as well as worse overall survival (Figure 3A). These findings are supported by the study conducted by Li et al. (23), although they performed survival analysis on a different population than the one assessed by IHC. Our distinct approach included survival analysis on the same population subjected to IHC for SOD2 detection. In addition, we observed a clear differentiation between the "low" and "high" SOD2 expression curves for RFS (Figure 3B). Although there was no significant difference by the logRank test, with the *p*-value close to 0.05, a larger sample size may provide additional confirmation of this trend. Following a 60-month follow-up, only two events were observed (Figure 3B). Given that most recurrences occur within the first 60 months (26), and few events were observed in our population after this period, we decided to analyze the data adjusted for 60 months, with subsequent events censored. In this context, we observed a significant association between high SOD2 expression and increased likelihood of tumor recurrence (Supplementary Figure 1). These findings not only reinforce the importance of our initial discoveries but also underscore

the clinical potential of SOD2 as a predictive marker for identifying individuals at higher risk of tumor recurrence.

In the following assessment, it becomes apparent that elevated SOD2 expression is associated with cancer cases that are unresponsive to conventional chemotherapy agents. Moreover, by utilizing the Metabric cohort, both OS and RFS have demonstrated significance, thus solidifying our initial hypothesis. Finally, we employed Cox regression to conduct both univariate and multivariate analyses, uncovering a range of significant associations, including the classical hormonal receptors of breast cancer, triple-negative and non-triple-negative statuses, and SOD2. In summary, the present study's results indicate that various factors, including receptor status (ER, PR), triple-negative status, SOD2 levels, age, and HER2 status, can influence the clinical outcomes of patients with the analyzed condition. The impact of these factors might differ depending on the patient cohort and the evaluation techniques used (IHC or Metabric database). It is likely that with a larger sample size and the use of the IHC technique, we may observe similar results to those found in the Metabric dataset. Furthermore, the multivariate analysis suggests that some of these factors might have more pronounced effects when considered together rather than individually.

In our analysis, TNBC showcased the highest mean SOD2 scores among molecular subtypes (p<0.0001). This subgroup of tumors represents a remarkably diverse cohort, comprising approximately 12-17% of all breast cancer cases (27). Due to the absence of classical markers, such as ER, PR and HER-2, this tumor subtype does not benefit from endocrine or target-directed therapies. Although the patients initially respond to a therapeutic strategy including surgery, radiotherapy and chemotherapy, they eventually develop therapeutic resistance, leading to tumor recurrence. Therefore, our data are of paramount importance to encourage research studies of potential therapeutic targets for this population (28-30). So far, there are no specific therapies targeted at SOD2. However, its potential as a biomarker in the current context could be valuable, especially regarding its role in therapeutic resistance in patients, particularly those with TNBC. Nonetheless, additional investigations are imperative to unravel the mechanisms that underlie the elevated SOD2 expression, thereby facilitating a more comprehensive exploration of its therapeutic potential.

Conclusion

Our analyses revealed significant correlations between increased SOD2 expression and hormone receptor negativity. Moreover, consistently elevated levels of SOD2 were observed in TNBC compared to luminal and HER2 subtypes. This observation is particularly intriguing as it suggests a specific association between SOD2 and TNBC, a subtype known for its aggressiveness and treatment resistance. The observation of high SOD2 levels in these tumors may have important implications for understanding the underlying mechanisms of TNBC's aggressive biology and for developing more effective targeted therapies.

Lastly, analysis of IHC and transcriptomic data unveiled a clear association between high SOD2 levels and poorer prognosis. This link between elevated SOD2 and adverse clinical outcomes strongly suggests that this antioxidant enzyme plays a crucial role in breast cancer progression and disease course determination. The consistency of these results across different analytical platforms further confirms the clinical and prognostic importance of SOD2 in the context of breast cancer, highlighting its potential as a promising therapeutic target and valuable prognostic marker.

Acknowledgment

We would like to express our heartfelt gratitude to Prof. Dr. Maria Aparecida Nagai, Dr. Evandro Sobroza de Melo, and the Pathological Anatomy service of the Faculty of Medicine of the University of São Paulo for generously providing us with the tissue microarray samples.

Ethics Committee Approval: This study was approved by the Research Ethics Committee (REC) of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo (HCFMUSP) with approval number 797466 (date: 11.11.2022).

Informed Consent: The waiver of the informed consent form was approved by the REC.

Authorship Contributions

Surgical and Medical Practices: D.R.d.B., A.L-F., M.P.F.C., L.T.; Concept: D.R.d.B., A.L-F., M.P.F.C., L.T.; Design: D.R.d.B., A.L-F., M.P.F.C., L.T.; Data Collection and/or Processing: D.R.d.B., A.L-F., M.P.F.C., L.T.; Analysis and/or Interpretation: D.R.d.B., A.L-F., M.P.F.C., L.T.; Literature Search: D.R.d.B., A.L-F., M.P.F.C., L.T.; Writing: D.R.d.B., A.L-F., M.P.F.C., L.T.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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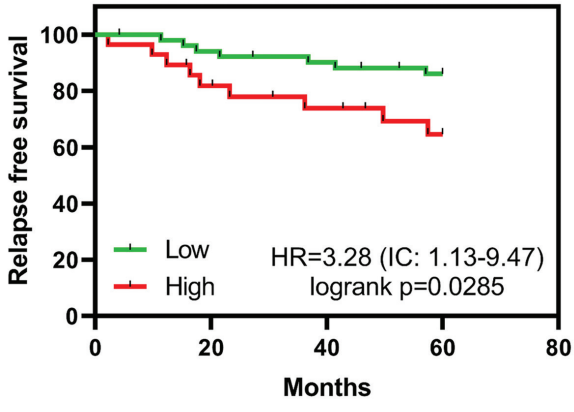

Supplementary Figure 1. Overall survival analysis as a function of adjusted SOD2 staining over a 60-month period

SOD2: Superoxide dismutase 2