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Potential of PSMA for breast cancer in nuclear medicine: digital quantitative immunohistochemical analysis and implications for a theranostic approach

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Abstract

Background Further research is still needed to fully understand the potential of prostate-specific membrane antigen (PSMA) in breast cancer (BC) and to develop and optimize targeted therapies and imaging modalities. The objective of this study was to present a comprehensive analysis of immunohistochemistry data on PSMA staining in BC and to discuss its potential value in a theranostic approach.

Methods Fifty-eight male and female patients were randomly selected from a retrospective database of patients who underwent surgery for breast cancer between January 2012 and December 2017 and for whom a specimen is available in our tumour library. Immunodetection of PSMA and CD31 was performed on serial slides. The digitized slides were reviewed and analysed by an experienced pathologist. Additionally, the corresponding TIFF images were processed to calculate the percentage of positive neovessels.

Results Eighteen patients (31.6%) had no expression, 29 (50.9%) had PSMA neovascular expression scored as "1", and 10 (17.5%) had neovascular expression scored as "2". Digital immunohistochemistry analysis for this last specific group of patients showed a median proportion of positive neovessels equal to 5% (range: 3–19). A multivariable logistic regression demonstrated that the odds of PSMA positivity were 4.55 times higher in non-luminal tumours and decreased by a factor of 0.12 in lobular subtypes. There was no association between sex or the presence of a germline BRCA1/2 mutation and PSMA expression in tumours.

Conclusions Our study highlights generally low neovascular expression of PSMA in specific histopathological subtypes of breast cancer, which will likely hamper the development of an adequate theranostic strategy.

Trial registration The procedure has been retrospectively registered to the French National Institute for Health Data (N° F20220615153900).

Keywords Breast cancer, Human FOLH1 protein, PSMA, Nuclear medicine

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Background

Prostate-specific membrane antigen (PSMA) is highly expressed in prostate cancer cells. Due to its limited expression in normal tissues and its promising potential as a biomarker and therapeutic target, PSMA has garnered significant attention in prostate cancer management. PSMA-targeted imaging and therapy have demonstrated remarkable success in this field [1]. Beyond its role in prostate cancer, PSMA-PET is currently being investigated for its potential in managing other types of cancers [2, 3]. The expression of PSMA in breast cancer, among many other targets, has recently attracted much interest, with researchers exploring its potential as both a therapeutic target and an imaging biomarker [4]. Notably, PSMA appears to be frequently expressed in the tumour neovasculature of breast cancer, which holds particular significance for triple-negative breast cancer [5]. The discovery of PSMA expression in breast cancer has raised expectations for developing targeted therapies and imaging techniques to offer more effective and personalized treatment options, especially for subtypes with poorer prognoses or fewer therapeutic options. Further research is necessary to fully comprehend the potential of PSMA in breast cancer and to develop and optimize PSMA-targeted therapies and imaging modalities for clinical use. The objective of our study was to present a comprehensive analysis of immunohistochemistry data on PSMA staining in breast cancer and to discuss its potential value in a theranostic approach. This analysis was conducted using a database from the Breast Cancer Unit of a Comprehensive Cancer Centre.

Methods

Patients were randomly selected from a retrospective database of male and female patients over 18 years of age who underwent surgery for breast cancer at our institution between January 2012 and December 2017 and for whom a specimen is available in our tumour library. Each patient received comprehensive information about the study's objectives, procedures, and potential implications, and their consent was obtained before their tissue samples were used for analysis. The procedure has been declared to the French National Institute for Health Data (N° F20220615153900).

Immunodetection of PSMA was performed to assess its cellular and neovascular expression. Immunohistochemistry was performed on paraffin embedded tumour tissues using a Ventana Discovery XT autostainer on 4 µm-thick sections. Slides were deparaffinised with EZPrep buffer at 75 °C for 8 min, and epitopes were unmasked at 95 °C for 56 min in CC1 buffer. Sections were incubated 40 min at 37 °C with PSMA antibody (ab133579, Abcam, 1/1000) or CD31 antibody (ab28364, Abcam, 1/50). Secondary antibody (Omnimap Rabbit) was incubated for 16 min at 37 °C. After washes, staining was performed with 3,3′-diaminobenzidine (DAB), and sections were counterstained with hematoxylin and bluing reagent. Whole slide images were digitized at 20×(0,5 µm/pixel) using the VS120 scanner (Olympus). The slides were controlled by a certified pathologist. They were recorded as tiled tiff images.

The digitized slides were reviewed and analysed by an experienced pathologist. The number of positive cells per field at 20-fold magnification was used to evaluate PSMA expression. The quantification of expression was performed on the entire slide. This measurement represents an average value, reflecting the overall expression across the entire tissue sample. CD31 immunodetection was used to specifically stain endothelial cells. This marker allowed the pathologist to differentiate between tumoural PSMA expression and PSMA expression in the neovasculature. By highlighting the blood vessels, CD31 staining enabled the identification and quantification of vessels expressing PSMA, thereby ensuring assessment of neovascular PSMA expression. Tumours with no detectable PSMA expression were scored as "0," tumours with 1 to 5 positive cells per field were scored as "1," and tumours with more than 5 positive cells per field were scored as "2". For tumours scored as "2," the corresponding TIFF images were processed to calculate the percentage of positive neo-vessels: $\frac{\%\,PSMA\,DAB}{\%\,CD31\,DAB} \times 100$.

Genetic status was determined by germline sequencing. These analyses were conducted using DNA extracted from a blood sample at the Biology and Genetics Laboratory of the François Baclesse Centre. Next generation sequencing (NGS) was performed using specific genetic analyses on the Illumina platform (mainly by Nextseq500-MiSeq or GAIIx). The bioinformatics pipeline included CASAVA, NextGENe, CNVseq and Alamut-HT. Patients received genetic counselling both before and after genetic testing during a face-to-face consultation.

Graph analysis and statistical analysis were performed on XLSTAT software (XLSTAT: Data analysis and statistical solutions for Microsoft Excel. Addinsoft (2017)). Clinical characteristics were compared according to PSMA expression ("0" vs. "1 or 2") using the Fisher's exact test. Variables associated with PSMA expression at an alpha level of 10% were entered into a logistic regression model for stepwise selection. The model maximizing Akaike's criterion was retained. For all statistical tests, a two-tailed p value of less than 0.05 was considered statistically significant.

Results

Fifty-eight patients were included (Table 1). The median age of the population was 51 years (range: 31–91).

Only one patient, a 40-year-old woman with stage III and grade 2 HER2-positive non-specific carcinoma,

Table 1 Clinical and histological characteristics of the population

Variable\Statistic	Categories	All patients (n = 58)		PSMA score 1 or 2 (n = 39) ‡		PSMA score 0 (n = 18)		P value *
		Frequency	Rel. frequency (%)	Frequency	Rel. frequency (%)	Frequency	Rel. frequency (%)	
Sex	Female	52	89.7	34	87.2	17	94.4	0.653
	Male	6	10.3	5	12.8	1	5.6	
AJCC Stage	I/II	26	44.8	19	48.7	7	38.9	0.574
	III	32	55.2	20	51.3	11	61.1	
Histological Subtype	Non-special type	47	81.0	35	89.7	11	61.1	0.026
	Lobular	11	19.0	4	10.3	7	38.9	
Histological Grade	G3	29	50.0	23	59.0	6	33.3	0.092
	G1 or G2	29	50.0	16	41.0	12	66.7	
HER2 status	Positive	26	44.8	20	51.3	5	27.8	0.151
	Negative / Low	32	55.2	19	48.7	10	72.2	
Molecular Classification	Non-luminal	27	46.6	21	53.8	5	27.8	0.089
	Luminal	31	53.4	18	46.2	13	72.2	

‡ the only patient with tumoural expression of PSMA was not considered in this analysis. * Fischer exact tests were used. Score 0: no detectable PSMA expression, score 1: 1–5 positive cells per field; 2: > 5 positive cells per field

exhibited tumoural PSMA expression, which was scored as "1". All other patients presented PSMA expression exclusively in the neovasculature. Figure 1 displays representative immunohistochemical images. Among the remaining 57 patients, 18 (31.6%) had no expression, 29 (50.9%) had PSMA neovascular expression scored as "1," and 10 (17.5%) had neovascular expression scored as "2". Digital immunohistochemistry computation for this last specific group of patients showed a median proportion of positive neovessels of 5%, ranging from 3 to 19%.

The median age of patients with tumours scored as 1 or 2 for neovascular PSMA expression was not different from those with a score of 0: 51 years (range: 31–91) *versus* 51 years (range: 33–79), $p=0.283$. A comparison of other clinical and histopathological characteristics is detailed in Table 1. Only histological subtypes showed a difference between groups, with a higher proportion of non-special subtypes in tumours with neovessel PSMA expression scored as 1 or 2. Although statistical significance was not reached, there was a trend toward a higher rate of positivity in G3 and non-luminal tumours.

Genetic panel analysis was available for 24 patients. Among them, 9 had a mutation: 6 *BRCA2* (66.7%), 2 *BRCA1* (22.2%), and 1 VUS *BRCA2* (11.1%). There was no association between the presence of a germline BRCA1/2 mutation and PSMA expression in tumours: 22.2% of patients with tumours scored 0 carried a germline *BRCA1/2* mutation, compared to 12.8% for patients with scored 1 or 2 tumours ($p=0.442$).

Among histological subtype, histological grade and molecular classification, the stepwise regression procedure retained histological grade and molecular classification as factors independently associated with positive PSMA expression, with OR = 0.12 [95% CI: 0.024–0.596], $p=0.009$ and OR = 4.55 [1.10-18.76], $p=0.036$, respectively.

Discussion

Our study's findings align with previous literature, which has reported higher PSMA expression in triple-negative breast cancer and HER2-positive subtypes compared to estrogen receptor-positive (ER+) subtypes [6]. The strengths of our study include its complementary digital quantification approach that ensures accurate and reproducible measurements, the inclusion of male patients providing insights in a subset of patients often underrepresented in breast cancer studies, and comprehensive genetic characterization that contribute to the knowledge base regarding the association between PSMA expression and specific genetic alteration in breast cancer. In this regard, PSMA expression does not appear to depend on the patient's sex or the presence of a germline BRCA1/2 mutation. PSMA radioligand uptake in PET imaging has been shown to be heterogeneous across different histopathological subtypes, with better detection rates in luminal B and triple-negative histologies [7, 8]. However, non-specific uptakes (not related to PSMA expression) have been reported in PSMA-PET scans for prostate cancer [9], emphasizing the need for further investigations to ensure that the uptakes observed in these studies are indeed specific to tumoural and/or neovascular PSMA expressions. Targeted radiotherapy using PSMA as a specific target has shown promising results in prostate cancer. However, in breast cancer, the potential of PSMA vectorized internal radiotherapy remains understudied. As presented above, the generally low or absent expression of PSMA in breast cancer cells and neovasculature implies a lack of specific targets for delivering the radioactive substance, which will clearly hamper the

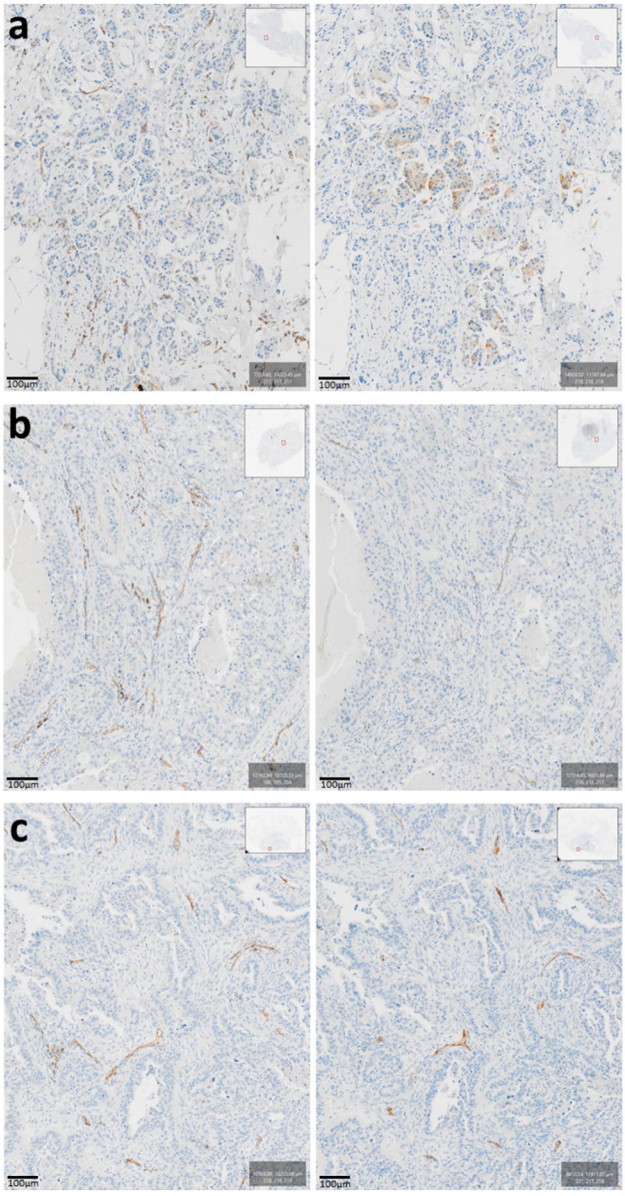

Fig. 1 Representative serial slides of CD31 (left panels) and PSMA (right panels) immunostaining in the only patient with tumoural expression of PSMA (**a**) and in patients with neovascular expression of PSMA scored 1 (**b**) and 2 (**c**). All images were captured at 5.0-fold magnification

effectiveness of this approach in breast cancer treatment [6]. Despite this, targeting neovascular PSMA expression may hold potential in specific clinical settings, such as monitoring anti-angiogenic therapies, assuming it can be demonstrated that the decrease in receptor density correlates with treatment efficacy, or conducting antiangiogenic internal vectorized radiotherapy [10, 11]. However, anti-angiogenic therapies have shown variable efficacy in breast cancer, with clinical trials demonstrating benefits in only specific subsets of patients. The complex and redundant nature of angiogenesis pathways, as well as the heterogeneity of breast cancer subtypes, contribute to limited overall efficacy of anti-angiogenic treatments in this setting [12]. Therefore, targeting neovascular PSMA expression alone may not provide significant benefits compared to existing anti-angiogenic treatments especially as the proportion of neovessels expressing the target is low, not exceeding 20%. However, without experimental validation, the theoretical potential for PSMA-targeted therapies in breast cancer remains speculative. Preclinical and clinical trials are necessary to assess the feasibility and effectiveness of PSMA-targeted treatments in breast cancer, particularly in subtypes with high neovascular PSMA expression. Although our study provides valuable data on PSMA expression in breast cancer and its potential implications for a theranostic approach, certain limitations should be considered when interpreting these findings. While the inclusion of male patients provides valuable insights, the small sample size, particularly the small number of male patients, may limit the ability to draw definitive conclusions. Future studies with larger, more diverse cohorts are needed to validate these findings and explore sex-specific differences in PSMA expression in breast cancer. The use of a simple scoring system provides a straightforward method for assessing PSMA expression, but it may not reflect the full spectrum of expression levels within and between tumours. Advanced imaging and quantitative techniques could offer more detailed insights into PSMA distribution and its clinical relevance. Additionally, it does not address whether PSMA expression has functional significance in this context. Future research should aim to correlate PSMA expression with clinical outcomes and explore the potential for PSMA-targeted therapies in breast cancer. Finally, longitudinal studies could provide valuable information on how PSMA expression evolves during disease progression or in response to therapies.

Conclusions

Our study highlights the importance of considering the tumour histopathological characteristics when considering PSMA expression in breast cancers. While PSMA-targeted therapies and imaging hold promise in prostate cancer, their effectiveness in breast cancer seems hampered by their generally low levels of neovascular PSMA expression.

Abbreviations

PSMA	Prostate Specific Membrane Antigen
HER2	Human epidermal growth factor receptor 2
BRCA	BReast CAncer gene
VUS	Variant of Uncertain Significance
PET	Positron Emission Tomography
DAB	3,3′-Diaminobenzidine

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki and compliance with the French Research Standard MR-004. Approval was granted by the Ethics Committee of Centre François Baclesse and registered (15/6/2022/ N° F20220615153900 on the French Health Data Hub). Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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