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Original article

Reliability of core needle biopsy in the breast cancer diagnosis

Ahlem Bdioui[1,2,3] Zineb Lajmi[1], Ons Kaabia[4], Soufiene Braiek[2], Sihem Hmissa[1,3], Moncef Mokni[2], Nabiha Missaoui[3]

[1] *Pathology Department, Sahloul University Hospital of Sousse, Medicine Faculty of Sousse, University of Sousse, Tunisia*
[2] *Pathology Department, Farhet Hached University Hospital of Medicine Faculty of Sousse, University of Sousse, Tunisia*
[3] *LR21ES03 Oncogenesis and Tumor Progression, Medicine Faculty of Sousse, University of Sousse, Tunisia*
[4] *Gynecology Obstetric Department, Farhet Hached University Hospital of Sousse, Medicine Faculty of Sousse, University of Sousse, Tunisia*

Abstract

We investigated the concordance between the histopathological and immunohistochemical results of core needle biopsy (CNB) and surgical specimens to assess the CNB reliability in the diagnosis and management of breast carcinoma. We studied breast carcinomas collected retrospectively between 2015 and 2017. Immunostaining for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2(HER2) and Ki-67 was conducted on archived tissue. We analyzed the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CNB compared with surgical samples. The kappa test (κ) was used for concordance analysis. A total of 81 cases were selected. The concordance of histological diagnosis between CNB and surgical specimens was 98.8% (κ=0.903). The sensitivity, specificity, PPV and NPV of the diagnosis of invasive carcinoma of no special type were 100%, 83.3%, 98.7%, and 100%, respectively. ER expression displayed a total concordance (κ=1). For PR expression, sensitivity, specificity, PPV and NPV were 100%, 87%, 95.1%, and 100%, respectively, with a concordance of 96.3% (κ=0.905). The concordance of the HER2 status was 87.6% as there were 10 discordant findings (κ=0.722). The concordance of the Ki-67 index was only 55.6% (κ=0.193). The overall concordance of molecular subtypes was only 58% (κ=0.424). In conclusion, CNB is reliable in determining histological subtypes, hormone receptor expression, and HER2 status. However, its reliability is limited in evaluating Ki-67 index and the subsequent diagnosis of molecular subtypes.

Keywords: Breast carcinoma; Core needle biopsy; Surgical specimen; Reliability; Immunohistochemistry.

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1. Introduction

Breast cancer remains the commonest malignant tumor in women, with almost 2,261,419 new cases diagnosed in 2020, accounting for roughly 11.7% of all cancers [1]. It constitutes the fourth prominent cause of cancer deaths, with 684,996 deaths worldwide [1]. In Tunisia, breast cancer is still the most frequent female cancer, with an annual female incidence rate of 41.4 per 100,000 [1,2]. Improvements in radiation tools have led to the discovery of many neoplastic lesions at an earlier stage, thus increasing the number of core needle biopsies (CNB). The emergence of new technologies has also improved histological diagnosis, thereby optimizing the treatment management of breast tumors [3]. Currently, CNB is an essential step in breast cancer diagnosis and treatment management strategies. However, the reliability of CNB still mainly involves morphological parameters and immunohistochemical characteristics.

In this study, we described the experience of The Department of Pathology of Farhat Hached University Hospital of Sousse, Tunisia, in the diagnosis of breast cancer. We investigated the concordance between the histopathological and immunohistochemical results of CNB and surgical samples to evaluate the reliability of CNB in the diagnosis of breast cancer.

***Correspondence:** Dr Ahlem Bdioui, Pathology Department, Sahloul University Hospital of Sousse, Medicine Faculty of Sousse, University of Sousse, Tunisia. Email: ahlembdioui@hotmail.com

2. Material and methods

Tissue samples

We conducted a retrospective study of breast cancer collected from the files of the Pathology Department of Farhat Hached University Hospital of Sousse (Tunisia) from January 2015 to July 2017. The local Human Ethics Committee at our University Hospital approved this study in accordance with the Declaration of Helsinki.

The inclusion criteria were as follows: all patients with a CNB, primary breast carcinoma and sufficient pathological samples. Non-inclusion criteria comprise all samples of *in situ* ductal carcinoma, *in situ* lobular carcinoma, breast carcinoma treated with neoadjuvant chemotherapy, mesenchymal tumors, or uninterruptable specimens (totally necrotic, crushed, electrocoagulated, and/or poorly preserved).

Clinicopathological data were collected using clinical records of patients from the Departments of Pathology and Gynecology and Obstetrics at Farhat Hached University Hospital, Sousse (Tunisia). Clinical parameters, including the age at diagnosis, family history of breast cancer, circumstances of discovery, radiological classification of biopsy lesions, epidemiological features, and pathological manifestations of CNB and final surgical specimens were recorded.

All tissues of CNB and surgical specimen had been routinely formalin-fixed and paraffin-embedded (FFPE). Two pathologists (AB and MM) examined the hematoxylin and eosin-stained sections of selected cases. One or two

tissue blocks containing representative tumor samples from CNB and surgical specimen, were selected for histopathological and immunohistochemical analyses. The tumor grade was determined by the Scarff-Bloom and Richardson scores (SBR) revised by Ellis and Elston [4,5].

Immunohistochemistry

All FFPE tissues were tested for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 immunostaining. Briefly, 4µm thick sections of FFPE tissues were deparaffinized and rehydrated. Afterwards, the antigen was retrieved with an appropriate buffer (Table 1) for 40 minutes at 98°C, and then the endogenous peroxidase activity was blocked with a 5% H2O2 solution for 5 minutes. The sections were incubated with the appropriate primary antibody (Table 1). Immunostaining was displayed by using Envision + Dual Link System-HRP kit (K4063, Dako, Carpinteria, CA, USA), and visualized by diaminobenzidine. To finish, the slides were counterstained with hematoxylin and mounted.

For each immunohistochemistry, positive and negative controls were included. A negative control was obtained by replacing the primary antibodies with phosphate buffered saline. For the ER and PR expression, the staining control was internal, the selected block included tumoral and healthy tissue and the non-tumor mammary glands were considered as internal control. The expression of ER and PR was scored according to Allred's score [6]. For HER2 expression, the positive control was external and consisted of a breast carcinoma previously classified as HER2 score 3+.

According to the clinical guidelines for assessing the HER2 status [7], the HER2 immunostaining score was 0-3. Score 0 was defined as no or incomplete membrane staining in less than 10% of tumor cells. A score 1+ comprised faint or partially stained membrane in more than10% of tumor cells. The staining was scored 2+ when weak to moderate complete membrane staining was present in more than 10% of tumor cells. A score of 3+ corresponded to a strong and complete membrane staining in more than 10% of the tumor. Cases with score 0 and 1+ were considered negative, cases with a score 3+ were regarded positive, and cases with a score 2+ were considered as equivocal [7].

Ki-67 was estimated at high magnification, only invasive specific components were considered, and it was recorded as the average percentage of positive cells among the 500 to 2000 cells evaluated. There are two possibilities, one is uniform staining: 500–2000 cells are selected from different microscope views; the other is heterogeneous staining: 2000 cells should be selected from hot spots and negative regions as well [8]. Using 20% as the cut-off value, samples were classified as revealing low or high expression [9].

According to the immunohistochemistry findings, breast cancer cases were recognized on the following molecular subtypes: luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC) [10].

Statistical analysis

The data were analyzed using Social Science Statistical Software Package (SPSS) version 21. For concordance assessment, we used kappa test (κ). The concordance of 0-0.2 was defined as negligible, 0.21-0.40 as weak, 0.40-0.60 as moderate, 0.60-0.80 as good, and 0.80-1.00 as perfect. Compared to surgical specimens, the CNB study was a diagnostic test to be estimated by determining its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). All statistical tests were bi-directional, with a significance level of 0.05.

3. Results

Throughout the study period, a total of 521 new cases of malignant breast tumors were recorded. However, only 81 cases met our inclusion criteria and were selected for research. The remaining 440 documents were excluded for the following reasons: 204 CNBs were invalid, 112 surgical specimens were unavailable, 91 patients underwent neoadjuvant chemotherapy, 20 patients with *in situ* carcinoma only on surgical specimens, 13 patients with recurrent tumors, and 3 cases of CNB stenosis.

CNB results

The median age of patients was 50.3 years (24-88 years). A family history of breast cancer was described in 10 patients (12.3% of cases). The consultation reasons were mainly the discovery of a breast nodule (70.4%), mastodynia (13.6%) and nipple discharge (9.9%). Routine screening by mammography, requested during a consultation in gynecology, concerned only 6.2% of patients. CNB was reported in lesions of American College of Radiology (ACR) 5 (55.6%), ACR 4 (10.7%), and ACR 3 (3.7%) in the presence of a family history of breast neoplasia.

The histological diagnosis of the biopsy lesion showed that invasive carcinoma of no special type was found in 93.8% of the samples. The remaining patients had invasive lobular carcinoma of the pleomorphic type (n = 2), cystic adenoid carcinoma, invasive micropapillary carcinoma, and neuro-endocrine carcinoma. The presence of an intraductal carcinoma was reported in seven breast tumors (8.6%). According to SBR grading system, tumors were classified into SBR I (27.2%), SBR II (51.9%) and SBR III (21%).

Immunohistochemistry showed ER and PR expression in 84% and 75.3% of breast cancers, respectively.27.2% of biopsy lesions exhibited HER2 overexpression (score 3). The remaining tumors yielded score 2 (3.7%) or score 1-0 (69.1%). Ki-67 was higher than 20% of tumor cells in 47 samples (58% of cases).

According to molecular classification, Luminal B type was the most common subtype (58%), followed by Luminal A (19.8%), while HER2 subtype accounted for 13.6% and the TNBC subtype accounted for 8.6%.

Analytical study

Table 2 detailed the findings of the concordance, sensitivity, specificity, PPV, and NPV. Overall, the concordance of histological types between CNB and surgical samples was 98.8% (κ=0.903). Only one case was reclassified as invasive papillary carcinoma. The sensitivity, specificity, PPV, and NPV of the diagnosis of non-specific invasive carcinoma were 100%, 83.3%, 98.7%, and 100%, respectively (Table 2). However, for the remaining histological subtypes, the sensitivity, specificity, PPV, and NPV were 100%, respectively (κ=1). For the presence of intraductal component, the sensitivity was 18.9% and the specificity was 100% (κ=0.202).

Table 1. Immunohistochemistry conditions and evaluation.

Protein	Clone	Provenance	Dilution	Retrieval solution	Positive immunostaining
RE	1D5	Dako	1/35	Citrate pH9	Cytoplasmic staining
RP	PgR636	Dako	1/50	Citrate pH7	Nuclear staining
HER2	Cerb2	Dako	1/1000	Citrate pH6	Cytoplasmic staining
Ki-67	MIB1	Dako	1/50	Citrate pH6	Nuclear staining

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2.

Table 2. Concordance between the core needle biopsy and surgical specimen.

		CNB	Surgery	Se	Sp	PPV	NPV	Concordance	κ
Histological type	IC(NST)	76	75	100%	83.3%	98.7%	100%	98.8%	0.903
	ILC	2	2	100%	100%	100%	100%	100%	1
	ACC	1	1	100%	100%	100%	100%	100%	1
	MPC	1	1	100%	100%	100%	100%	100%	1
	NEC	1	1	100%	100%	100%	100%	100%	1
SBR grade	SBRI	22	18	61.1%	82.5%	50%	88.1%	77.8%	0.404
	SBRII	42	36	66.7%	60%	57.1%	69.2%	63%	0.262
	SBR III	17	27	44.4%	90.7%	70.6%	76.6%	75.3%	0.388
Hormonal receptors	ER	68	68	100%	100%	100%	100%	100%	1.0
	PR	61	58	100%	87%	95.1%	100%	96.3%	0.905
HER2 status	Negative (0/1+)	56	57	96.5%	95.8%	98.2%	92%	96.3%	0.912
	Score (2+)	3	4	0	96.1%	0	94.9%	91.4%	0.044
	Positive (3+)	22	20	80%	90.2%	72.7%	93.2%	87.6%	0.679
Ki-67	>20%	47	15	86.7%	48.5%	27.6%	94.1%	55.6%	0.193
Molecular subtype	Luminal A	16	34	41.2%	95.7%	87.5%	69.2%	72.8%	0.398
	Luminal B	47	22	95.4%	55.9%	44.7%	97.1%	66.7%	0.379
	TNBC	7	18	38.9%	100%	100%	85.1%	86.4%	0.497
	HER2	11	7	71.4%	91.9%	45.4%	97.1%	90.1%	0.503

Se: sensibility, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, SBR: Scarff-Bloom and Richardson score modified by Ellis and Elston, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2, IC (NST): invasive carcinoma with no special type, ILC: invasive lobular carcinoma, ACC: adenoid cystic carcinoma, MPC: micropapillary carcinoma, NEC: neuroendocrine carcinoma, TNBC: Triple-negative breast cancer.

For the SBR grade, the specificity was 82.5%, 60% and 90.7% for the grade I, II and III, respectively. The concordance was 77.8%, 63%, 75.3% for grade I, II, and III, respectively (κ = 0.343). Analysis of the histological criteria of the SBR grade showed that the agreement between CNB and surgical specimens for the architecture score, nuclear pleomorphism and the mitotic index was 59.2% (κ = 0.341), 63% (κ = 0.406) and 51.8% (κ = 0.298), respectively.

In all cases, the ER status identified in the CNB was validated by pathological examination of the surgical specimen (κ = 1). For PR expression, the sensitivity, specificity, PPV, and NPV were 100%, 87%, 95.1%, and 100%, respectively, with a concordance of 96.3% (κ = 0.905). Due to 10 inconsistent findings between CNB and surgical specimens, the agreement of HER2 status was 87.6% (κ = 0.722). When considering tumor of score 3 alone, the sensitivity, specificity, PPV and PNV were 80%, 90.2%, 72.7% and 93% (κ = 0.679), respectively. For HER2-negative tumors, κ = 0.912, while for cases of score 2, κ = -0.044. The concordance of the Ki-67 index between CNB and surgical specimen was 55.6% (κ = 0.193). Therefore, sensitivity, specificity, PPV and NPV were 86.7%, 48.5%, 27.6% and 94.1%, respectively (Fig. 1 and Fig. 2).

The diagnosis of molecular subtypes was concordant between CNB and surgical specimen in 47 cases, while the molecular classification was discordant in 34 cases. Accordingly, the overall concordance was 58% (κ = 0.424). When each molecular subtype was analyzed separately, the specificity of the identification of luminal A, luminal B, HER2 and TNBC was 95.7%, 55.9%, 91.9%, and 100%, respectively, whereas the sensitivity was 41.2%, 96.4%, 38.9% and, 71.4%, respectively (Table 2).

4. Discussion

This study investigated the concordance of histopathological and immunohistochemical results between CNB and surgical specimens of the breast to evaluate its reliability in the diagnosis of malignant breast tumors. Previously, several studies have been conducted to establish the utility of ultrasound-guided biopsies in breast pathology. Compared with the pathological findings of the surgical specimens, it showed very encouraging results in the prediction of malignancy, with a specificity of 100% and high sensitivity [11,12]. In fact, Hao et al. [13] reported a concordance of 92.4%. Even for small breast lesions, the reliability of CNB has been confirmed [14]. In a meta-analysis of 16287 ultrasound-guided biopsies from 27 series, the sensitivity and specificity were 99% and 97%, respectively [15]. In our study, the concordance between the CNB and the surgical specimens for the diagnosis of histological subtypes of breast cancer was 98.8%. Our results are consistent with previous surveys reporting a high concordance on histological subtypes [16-18]. However, in the study of Greer et al. [19], there was only moderate agreement with a concordance rate of 81% (κ = 0.55).

According to the SBR grade, the overall agreement between the CNB and the surgical specimen was low (k =

Knuttel's meta-analysis [20], which analyzed 6029 patients. CNB was consistent with the surgical specimen for tumor

grade in 71.1% of cases (κ = 0.54). The disagreement mainly concerned the assessment of the mitotic index, and the difference was explained by inter-observer variability and tumor heterogeneity.

Although the importance of CNB in the histological diagnosis of breast tumors has been well-established, its reliability in determining the immunohistochemical characteristics of breast carcinoma is still controversial [21-37]. In our study, the concordance between CNB and surgical specimen was relatively better on ER (100%) than on RP (96.3%), and the agreement was excellent for both hormone receptors (κ = 1 and κ = 0.905, respectively). Several authors considered CNB as a relevant method in the evaluation of ER immunostaining [22,24,29]. Indeed, in a recent meta-analysis of 21 studies involving 2,450 patients, Li et al. [24] reported sensitivity and specificity of 97.3% and 82%, respectively. Likewise, other authors reported a high concordance [25-27]. However, in the recent study of Chen et al. [20] including 1003 patients, the CNB correctly assessed the ER status in 78.8% of cases (κ = 0.522).

Similarly, this disparity was perceived for PR results. In the meta-analysis of Li et al. [24], the CNB sensitivity and specificity in determining these receptors were 92.3% and 76.5%, respectively. Chen et al. [25] confirmed these results. However, other studies reported lower concordance

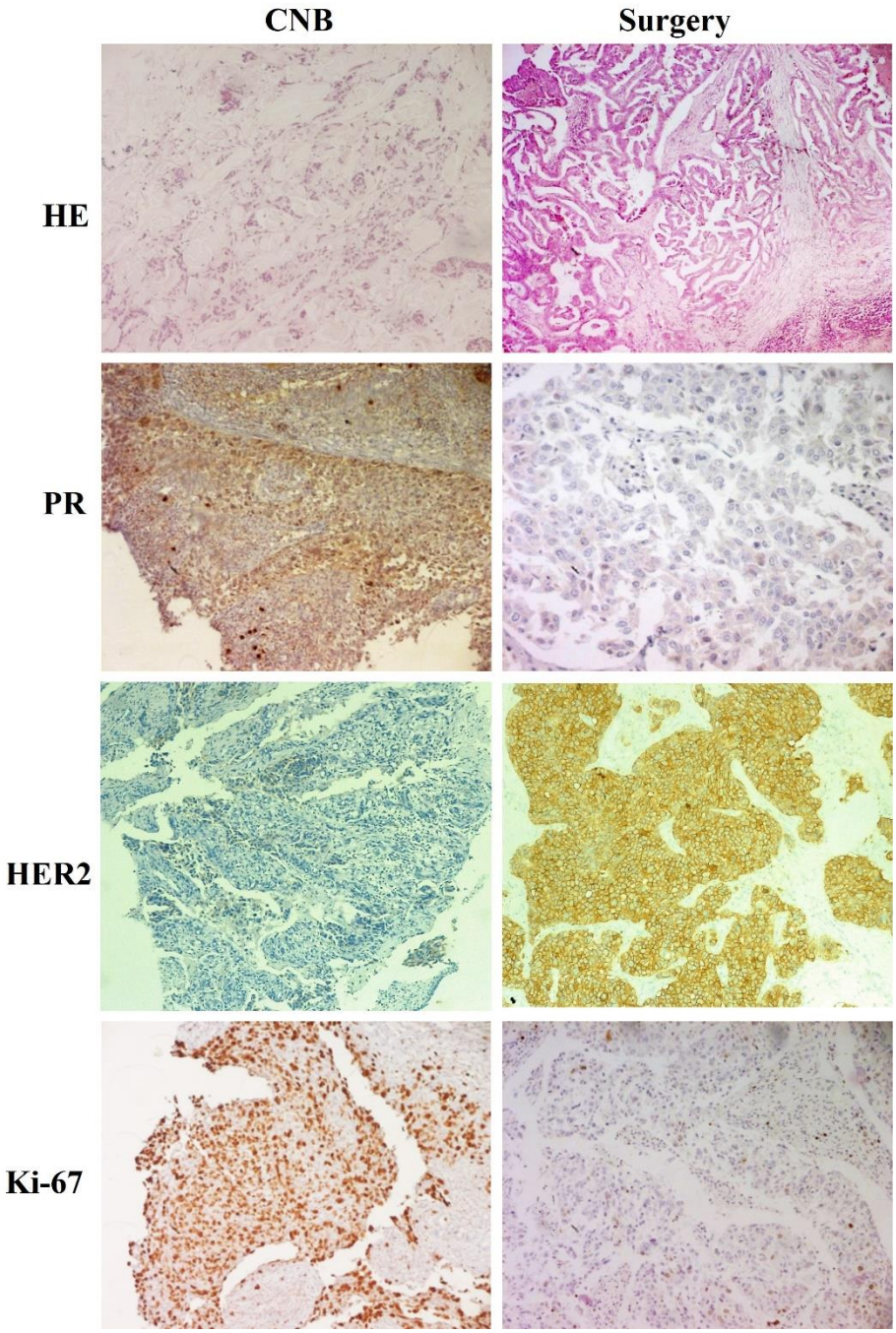

Fig.1 Discordant results between CNB and surgical specimen. Invasive ductal carcinoma of no special type in CNB (A) was rectified in invasive papillary carcinoma in surgical specimen B) (x100). Positive PR expression in CNB (C) and negative status for PR in surgical specimen (D). Negative testing for HER2 in CNB (E) and HER2 score 3 in surgical specimen (F). High expression of Ki-67 in CNB (G) while low expression in surgical specimen (H).

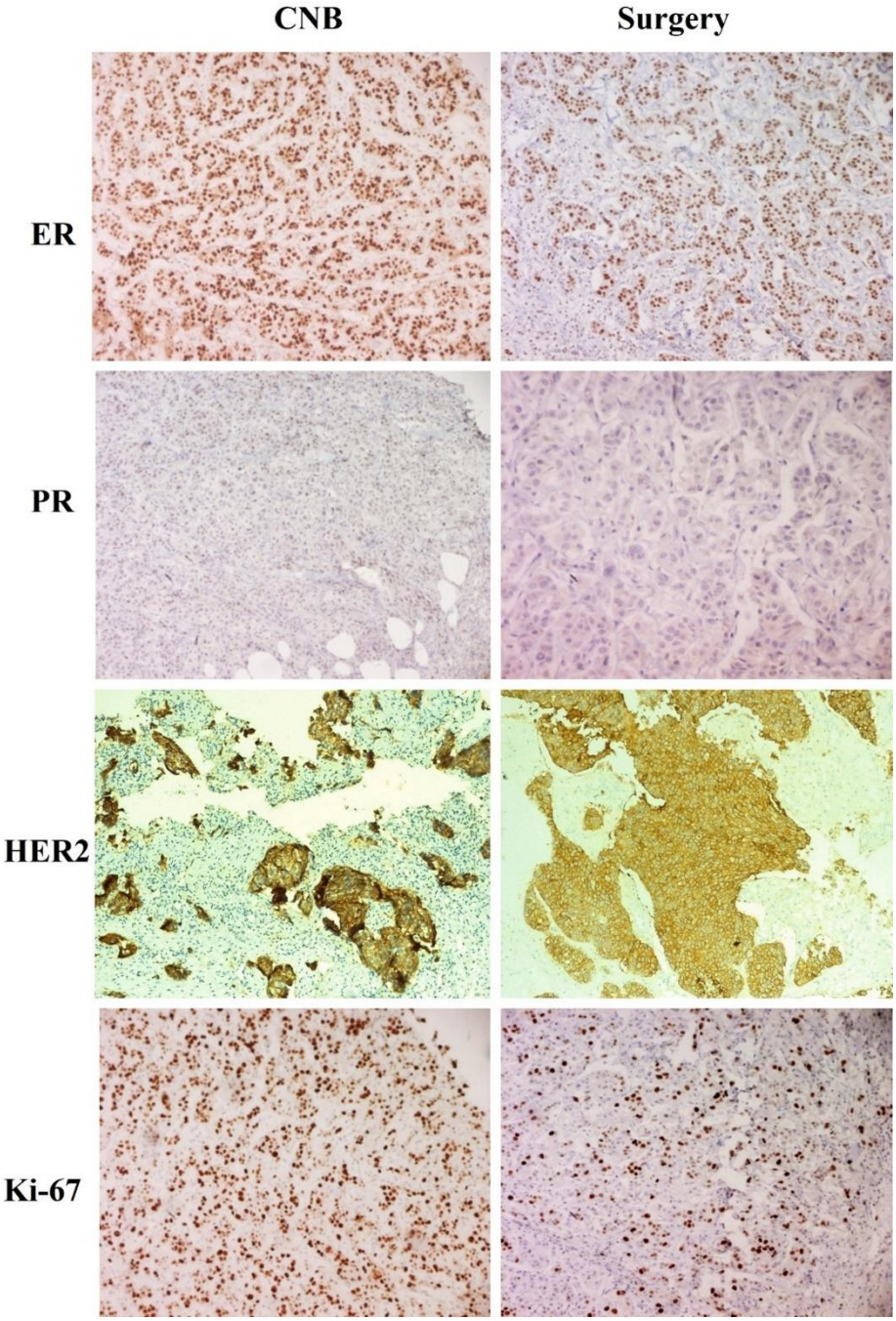

Fig.2. Concordant results between CNB and surgical specimen for ER expression (A-B), PR expression (C-D), HER2 score 3 (E-F) and Ki-67 expression (G-H) (x200).

[23,27]. Seferina et al. [27] found a false negative rate of 29.6%. Consequently, these authors considered that the therapeutic decision should not be based on the hormonal receptor status determined by the CNB, but the re-evaluation of hormonal receptors at the surgical specimen should be performed [27].

Several factors could explain the discrepancy between CNB and surgical specimen findings, such as the difference in the conditioning and fixation of samples, intra and inter-observer variability, and above all, the intra-tumor heterogeneity [27,30]. In this regard, the study by Douglas-Jones et al. [30] showed a significant decrease in ER positivity with the passage of tissue samples from the periphery to the center of the tumor [30]. Moreover, the literature results showed that the reliability of CNB for ER was higher than for PR One explanation for this difference is that the distribution of PR in the tumor is more heterogeneous when compared to ER [25].

In our survey, the overall agreement in determining HER2 status was strong ($\kappa = 0.722$). Analysis of each score alone showed that CNB and surgical specimens were highly concordant in the diagnosis of HER2 tumors ($\kappa = 0.679$). Moreover, the agreement was excellent for the detection of tumors HER2-negative ($\kappa = 0.912$), while it was low for tumors with intermediate score ($\kappa = 0.044$). Previous studies reported an excellent concordance of HER2 status, showing that the tissue was well preserved and without artifact of crushing on biopsy samples [18,26,29]. Rare studies have found no encouraging results. In the study of Ough et al. [31] using immunohistochemical technique, only 56% agreement was found ($\kappa = 0.392$). However, it is recommended to use *in situ* hybridization techniques for the assessment of ambiguous immunohistochemistry cases [7]. In addition, studies using both immunohistochemistry and *in situ* hybridization methods for the investigation of HER2 expression reported higher concordance rates

[18,22,25,29]. Nevertheless, other surveys described less reliable results even after performing *in situ* hybridization (κ = 0.451), suggesting the role of other factors influencing the effectiveness of the biopsy such as the heterogeneity of HER2 expression within the tumor [21,27]. More recently, Slostad et al. [37] suggested that reexamination of ER and HER2 profiles was more clinically valuable than PR reinvestigating. To improve management and reduce healthcare charges, these authors proposed patient-centered recommendations on reevaluation biomarker status. In our study, we found a concordance with the surgical specimen for the determination of Ki-67 status of 59.3% (κ = 0.193). Only few studies reported a strong agreement [34], most previous reports described weak agreement between CNB and surgical specimen for this marker [22,23,31-35]. Compared to the remaining immunohistochemical markers, CNB appears to be less reliable in assessing the Ki-67 index. In Sohn's recent series [22], the sensitivity and specificity of CNB among 179 patients were 80.6% and 88.7%, respectively. This discrepancy is attributable to the lack of standardization and the lack of a recognized threshold for this antigen [19,21]. Ki-67 is useful to differentiate luminal subtypes of breast carcinoma and its evaluation may influence the therapeutic decision accordingly. This marker also has a prognostic value, as the high expression has been related to an increased risk of relapse and death. Therefore, the normalization of this inexpensive technique is necessary to guarantee a better reliability [20]. Recently, Romero et al. [38] recommended automated digital image analysis system for the Ki-67 labelling, as a better concordance rate was found with this technique (κ = 0.639) than with manual counting (κ = 0.534).

In the present study, the overall agreement in determining the molecular subtypes of breast carcinoma between CNB and specimen was moderate (κ = 0.424). The concordance was better in distinguishing HER2 (90.1%) and TNBC (86.4%) tumors compared to luminal A (72.8%) and B (66.7%) tumors, with a moderate agreement for the first two types (κ = 0.503 and κ = 0.497, respectively) and weak for the last two types (κ = 0.398 and κ = 0.379 respectively). Previously, few studies have evaluated the reliability of CNB in determining the different phenotypes of breast cancer [23,25,34-36]. In the Meattini et al. survey [34], the concordance rate for molecular subtypes was 87.1% (κ = 0.78). In another study [21], the concordance rate for the molecular subtypes evaluated in 590 patients was only 49.2% (κ = 0.195). In view of this rather important discrepancy, most authors recommend that the molecular subtypes must be redefined in the surgical specimen to allow a better management [21,34-36].

In conclusion, CNB is reliable in determining histological subtypes, hormone receptor expression, and HER2 status. However, its reliability is limited in the estimation of Ki-67 index and the subsequent diagnosis of molecular subtypes. Tumor heterogeneity, inter-observer variability and the lack of consensus for the Ki-67 assessment are the main factors reducing the reliability of CNB.A standardization of the recognized cut-off for Ki-67 and a homogenization of the technique are necessary to guarantee a better reliability.

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Conflict of Interest Disclosures

All authors declare that they have no conflict of interest.

Authors' contributions

Study concept and design were done by AB, SB and MM. Data were acquired by AB, OK and SB. The data were analyzed and interpreted by AB, SB, SH, MM, and NM. Drafting of the manuscript was done by NM. All authors read and approved the final manuscript.

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