ORIGINAL ARTICLE

Agreement between incisional and excisional biopsies for hormone receptors and her2 in breast cancer

Concordancia de biopsia incisional y escisional en receptores hormonales y her2 en cáncer de mama

Fernando Candanedo-Gonzalez[1], Juan C. Cordova-Ramón[2], Jessica Smeke-Smeke[3], Wilfredo Valenzuela-Gonzalez[4], and Armando Gamboa-Dominguez[5]*

[1]Departmento de Patología. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México; [2]Departmento de Patología. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México; [3]Facultad de Medicina, Universidad Anahuac, Ciudad de México; [4]Facultad de Medicina, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa; [5]Departamento de Patología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México. Mexico

Abstract

Introduction: *Breast cancer is the leading cause of cancer mortality in Mexican women.* **Objective:** *The objective of the study was to identify concordances among core needle biopsy (CNB) and excisional biopsies (EB) regarding diagnosis, hormonal receptors (HR), and human epidermal growth factor receptor 2 (Her2).* **Materials and methods:** *Core number, demographic data, histological type, and treatment were documented for each sample. Reported HR and Her2 score from both samples were compiled.* **Results:** *70 women with both CNB/EB were included. Median age was 58 (36-87) years; initial diagnosis in CNB were invasive ductal 56 (80%), lobular 10 (14%), and mixed 4 (6%) carcinomas. Diagnostic agreement among CNB and EB was of 97%, k = 0.65. A concordance of 92% (k = 0.75), 75% (k = 0.26), and 67% (k = 0.46) was observed for estrogen receptors, progesterone receptors, and Her2 determinations, and positive predictive values in CNB were 0.96, 0.89, and 0.44, respectively.* **Conclusion:** *HR and Her2 concordances using manual-immunohistochemistry (IHC) were found within the range of values obtained using automatized-IHC. When compared to tumor heterogeneity, technical/reading errors contribute more to discordances.*

Keywords: *Concordance. Core needle biopsy. Breast cancer. Hormonal receptors. Her2.*

Resumen

Introduction: *El cáncer de mama es la principal causa de mortalidad por cáncer en mujeres mexicanas.* **Objetivo:** *Identificar la concordancia entre la biopsia con aguja de corte (BAC) y la biopsia escisional (BE) con respecto al diagnóstico, receptores hormonales (RH) y Her2.* **Material y Métodos:** *Se registró el número de fragmentos cilíndricos, datos demográficos, tipo histológico y tratamiento. Se recopilaron resultados de RH y Her2.* **Resultados:** *Se incluyeron 70 mujeres con mediana de edad de 58 años. El diagnóstico inicial en BAC fue carcinoma ductal invasivo 56 (80%), lobular 10 (14%) y mixtos 4 (6%). El acuerdo de diagnóstico entre BAC y BE fue del 97%, k = 0.65. Se observó una concordancia de 92% (k = 0.75), 75% (k = 0.26) y 67% (k = 0.46) para las determinaciones de receptor de estrógenos (RE), receptor de progesterona (RP) y Her2, y los valores predictivos positivos en BAC fueron 0.96, 0.89 y 0.44, respectivamente.* **Conclusión:** *Los RH y la concordancia de Her2 mediante inmunohistoquímica (IHC) manual se encuentran dentro del rango de valores obtenidos mediante el uso de IHC automatizada. Los errores técnicos/de lectura contribuyeron más a discordancia que la heterogeneidad tumoral.*

Palabras clave: *Concordancia. Biopsia con aguja gruesa. Cáncer de mama. Receptores hormonales. Her2.*

***Correspondence:**
Fernando Candanedo-Gonzalez
E-mail: fa_candanedo@yahoo.com.mx

Date of reception: 28-03-2022
Date of acceptance: 24-08-2022
DOI: 10.24875/CIRU.22000197

Cir Cir. 2023;91(4):451-456
Contents available at PubMed
www.cirugiaycirujanos.com

Introduction

Breast cancer is the leading cause of cancer mortality in Mexican women since 2006, accounting for 14% of cancer-related deaths[1], and yet, scarce information is available on tumor sampling and subcellular determinations[2]. Estrogen receptors (ER) and progesterone receptors (PR) along with c-erbB2 human epidermal growth factor receptor 2 (Her2) and Ki-67 expression are currently used to classify invasive epithelial breast tumors into four clinically relevant categories[3]. Immunohistochemistry (IHC) is the current method to accomplish such measurements, quantifying its nuclear or membrane signals[2,3]. Concern about IHC results compelled its automatization and nowadays to "next generation IHC"[4]. However, for economic reasons in developing countries most laboratories are still doing them manually. How much confidence should be given to measurements in these settings?

The trend for less radical surgeries as a treatment for breast cancer brings the opportunity to use core needle biopsies (CNB) and excisional biopsies (EB) in the same patient and, it offers the chance to bring about concordance studies regarding IHC analyses. Few studies addressing concordance of ER, PR, and Her2 have been published[5-17]. Such studies have shown a good proficiency of CNB for morphologic diagnosis and subcellular studies. Most of the published series are based on automatized protocols of IHC excluding patients submitted to neoadjuvant therapy and showed a concordance rate for ER, PR, and Her2 in core needle biopsies and excisional resections of 62-98%, 69-89%, and 60-98%, respectively[5-17].

Our aim was to calculate concordances among core needle biopsies and EB regarding histological type, ER, PR, and Her2 in consecutive patients, including those receiving neoadjuvant treatments without a complete pathologic response.

Materials and methods

Study population

A search of medical records at the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran was performed and patients with suspicious breast lesions submitted to CNB with subsequent EB, being lump excision, radical mastectomy, or cuadrantectomy due to *in situ* or invasive breast carcinoma were retrieved since January 2008 through December 2012. Medical charts of each patient were reviewed, and clinical information on gender, presentation, and age at diagnosis was recorded. The pathology reports from the diagnostic CNB and EB were reviewed. The data recorded from the CNB were core number, histological type of lesion, and IHC results. Data recorded from the excision were type of excision, tumor type according to the WHO classification, size, and ER, PR, and Her2 results. In both samples, the reported ER, PR, and Her2 score was compiled. The study followed the guidelines of the Declaration of Helsinki and was approved by the institutional review board. Patient identity and its pathological specimens remained anonymous in the context of this study. Cases were grouped as tumors as *in situ* carcinoma (either ductal or lobular), invasive carcinoma (either ductal or lobular), mixed, or other types of invasive carcinoma.

IHC analyses

In our institution, IHC is done manually and is interpreted according to the college of the American pathologists guidelines. Briefly, deparaffinized 1.5 μm tissue sections on charged slides were submitted to antigen retrieval for 20´ in an electric pressure cooker and mounted in cover plates in a plastic rack (Thermo Scientific, Cashire UK). Protein and endogen peroxidase was blocked for 5´ and the primary antibody incubated for 45´. After washing with Tris-buffered saline (TBS), two drops of mouse/rabbit immunodetector biotin link was applied for 10´ and rinsed with TBS. Finally, two drops of mouse/rabbit immunodetector HRP were used and slides developed with DAB under microscopic (Olympus CH2) observation. Extension of the stain was evaluated in neoplastic cells and classified as 0% or negative, 1-25%, 26-50%, 51-75%, and > 75% as positive and, nuclear staining intensity in a scale from 0 to 3+. Her2 score was obtained according to the American society of clinical oncology guidelines, considering 0/1+ as negative, 3+ as positive, and 2+ equivocal. ER detection was performed with 6F11, RBT11, or 1D5 clones, for PR either 16, RBT22, or PgR636 clones were used. All Her2 assessments were made using HercepTest™, Dako (Glostrup, Denmark), and the manufacturer protocol. Equivocal Her2 results were considered in the analysis only if gene amplification was confirmed or discarded by either chromogenic (CISH) or fluorescent (FISH) *in situ* hybridization.

Disagreement among CNB and EB was considered when a change from noninvasive to invasive carcinoma or vice versa was observed in hematoxylin and eosin sampling or when a change from positive/negative in incisional biopsy turned on negative/positive in excisional resection for hormonal receptors (HR) and Her2 determinations.

Statistical analyses

Descriptive statistics were employed to summarize the characteristics of the study population. Discordant reports led us to review all samples from that patient to ascertain pre analytical, analytical, or post-analytical reasons. Concordance analysis by means of a kappa coefficient test was performed using SPSS 15.0 for Windows. Sensibility, specificity, and predictive values were calculated considering excisional resections as the gold standard. Significance test was two sided.

Results

Clinicopathologic characteristics of the study population

We recorded 424 surgical specimens from 321 patients, and 70 women with both CNB and surgical excision turn out to be the body of this study. When a patient had more than two breast biopsies, the one obtained closer to CNB sampling was preferred, if complete pathologic response was observed in the final excision, the case was discarded. Median age was 58 (36-87) years. Initial diagnosis of the patients in CNB was invasive ductal 56 (80%), lobular 10 (14%), and mixed 4 (6%) carcinomas; four were *in situ* lesions. Half of the patients had stage I/II disease, and neoadjuvant chemotherapy was received by 38 (54%).

The concordance rate of primary diagnosis of *in situ* or invasive cancer between the core biopsy and the excision specimens was 97%. Nine discordances in morphologic diagnosis were explained by mixed phenotypes of the invasive carcinoma in four EB (two ductal in CNB become lobular in EB; two lobular in CNB become ductal in EB), and because only two *in situ* lesions were observed in CNB, whereas infiltrative carcinoma admixed with the *in situ* lesion was identified in EB or because in 2 CNB of invasive ductal carcinoma, only *in situ* carcinoma was observed in EB. The one left was a poorly sampled CNB (one tissue fragment) with crushing artifact diagnosed as ductal invasive, which turned out to be invasive lobular carcinoma in the final excision.

IHC findings

Six pairs of samples were excluded from the IHC analysis because the surgical pathology report of CNB (2) and EB (4) did not include ER, PR, or Her2 determinations. In addition, four Her2 misleading samples lacking CISH/FISH amplification procedures were excluded. Thus, concordance analysis was based on 64 patients for HR and in 60 pairs of samples for Her2. A concordance of 92% (kappa 0.75), 75% (kappa 0.26), and 67% (kappa 0.47) was observed for ER, PR, and its two determinations between CNB and EB, and the positive predictive values were 0.96, 0.89, and 0.44, respectively.

ER discordances between CNB and EB in six patients were due to tumor heterogeneity with weak nuclear staining (2 cases), associated with neoadjuvant treatment (1 case), as well as with technical variations in IHC, leading to slides without adequate external controls (2 cases) and misinterpretation of the slide by the original observer (1 case). Discordances with PR were identified in 18 patients and its source attributed to technical and reading mistakes (7 each), neoadjuvant chemotherapy (4 cases), and signal heterogeneity (3 cases); some pairs having more than one factor. Her2 variations were observed in seven patients among CNB and EB. Variations were associated with tumor heterogeneity (3 cases), technical (3 cases), and reading errors (3 cases). Any discordance in diagnosis and HR/Her2 interpretation was more frequent if the number of tissue fragments by CNB sampling was less than three 29/31 (93%) versus 8/39 (21%), ($p < 0.0001$).

Discordances in HR and Her2 determinations were observed in 27 patients; the consequences of these variations were clinically irrelevant in 26 because hormonal treatment was granted using ER or PR results in CNB and Her2 treatment based on a positive result in either core biopsy or excision specimen. Serious implications were observed in one patient (Fig. 1).

Discussion

A concordance series of CNB and excisional resections of malignant breast tumors, -without excluding those submitted to the current neoadjuvant

Table 1. Concordance of core needle and excisional biopsy interpretation of immunohistochemistry performed on infiltrative lesions

Author	Countryn	n	IHC method	ER%	PR%	HER2%
Mann GB, et al., 2005[6]	Australia	100	A	86	83	80
Cahill RA, et al., 2006[7]	Ireland	95	U	70	72	64
Burge Ch, et al., 2006[8]	USA	87	A	95	89	96
Usami S, et al. 2007[9]	Japan	111	A	95	88	88
Wood B, et al., 2007[10]	Australia	100	U	96	90	87
Sutela A, et al., 2008[11]	Finland	41	A	83	88	93
Arnedos M, et al., 2009[12]	UK	336	U	98	85	98
Park SY, et al. ,2009[13]	Korea	104	U	99	97	86
Richer C, et al., 2009[14]	Germany	164	U	75	75	54
Tamaki K, et al., 2010[15]	Japan	353	A	93	78	89
Lorgis V, et al., 2011[16]	France	175	A	84	78	98
Ricci MD, et al., 2012[17]	Brazil	69	U	95	87	78
Present study	Mexico	64	M	92	75	67

IHC: immunohistochemistry; A: automatic; M: manual; U: unknown.

therapies, accurately reveal the day-to-day surgical pathology practice. None of the published articles[5-16] (Table 1) is based on manual IHC and frequently do not mention the type of clones used. The present series include consecutive cases, even those receiving neoadjuvant therapies without complete pathologic response and, performing the IHC procedures manually. This is a common practice in countries with limited resources.

Factors more frequently associated with discordances among both measurements were sampling and IHC omissions (pre-analytical) and interpretation of the slides (analytical). In contrast with previous studies[5-17], tumor heterogeneity and neoadjuvant treatments were less related to discrepancies. Some information is available on the impact of tumor sampling in diagnosis and IHC interpretation in CNB. Patients with less than four tissue cores have higher probabilities of inadequate diagnosis/subcellular measurements, than those with more than four[9,13,15,18]. This finding move us to qualify the sampling we receive as adequate (≥ 4 cores) or inadequate (< 4 cores) in our surgical pathology report, to prospectively share possible disagreements in excisional specimens. As we did not find relevant clinical disagreements with HR in both measurements, only a warning was made to our technical staff to select and use in every run adequate positive/

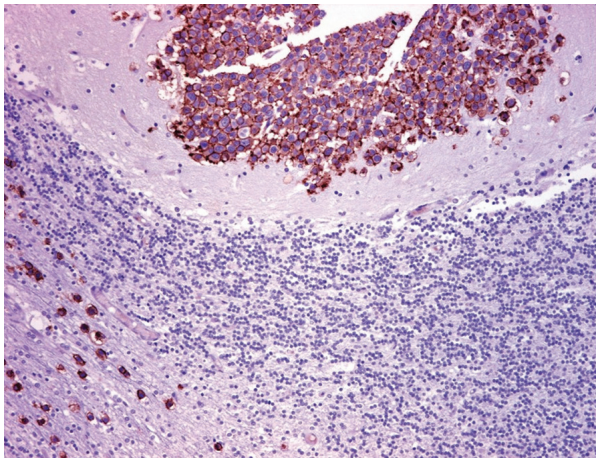

Figure 1. *A discordant her2 evaluation with cerebellar metastases. Epithelial cells had strong membrane signal and a CNB of the primary lesion read negative. The breast cancer was evaluated before the introduction of the "Seguro Popular."*

negative controls; a frequent omission observed in this review. Since hormonal blocking agents are prescribed using data from ER and PR, none of our tested patients with discordances was excluded of receiving this therapy.

A different picture was observed regarding Her2 with 33% of discordant evaluations in CNB and EB. A reading mistake excluded of specific blocking

agent therapy to a patient in whom we observed the natural history of a Her2 type invasive ductal carcinoma. Reading mistakes, technical omissions, and heterogeneity in IHC expression contribute to Her2 discordances. Although within previous series concordance rates (Table 1)[5-16], the observed Her2 discordances were too high for current pathology practice. As a consequence, all 1+, 2+, and 3+ Her2 IHC results will be submitted to chromogenic amplification assessments in this facility for the next 12 months, and a blind double check of the slides was advised for all breast samples. Mexico lacks of a national proficiency assessment programs of IHC and pathologist should contrast the IHC results with the morphology of the infiltrative lesion, i.e., consider features suggestive of Her2 discordances the presence of a positive result in a Grade 1 tumor. Recommendations sustained and shared in some previous reports[14,19,20].

IHC automatization brings more interlaboratory consistency but without statistically significant differences in the estimation of nuclear HR percentage/intensity signal when compared with manual procedures[21]. Besides, the percentage of HR-positive cells assessment is more reliable than intensity signal interpretation, suggesting that combining percentage of positive cells and signal intensity as a score, lack of a practical advantage[22]. Budget availability is considered the main reason for IHC automation, our current cost of a manual slide is less than US 20 dollars, and it will rise more than the double with the marketed closed IHC platforms. We wager here for a model of morphological tests in sound with our pockets, in contrast with other scenarios[23].

In conclusion, acceptable concordances were observed for diagnosis and HR, but worrisome results were identified for Her2 determination using handmade IHC protocols. The main sources of disagreements were analytical and poor sampling of suspicious breast lesions with fewer than three core needle biopsies.

Funding

The authors declare that they have not received funding.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical responsibilities

Protection of humans and animals. The authors declare that no experiments on humans or animals have been performed for this research.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained approval from the Ethics Committee for analysis and publication of routinely acquired clinical data and informed consent was not required for this retrospective observational study.

References

1. Palacio-Mejia LS, Lazcano-Ponce E, Allen-Leigh B, Hernández-Avila M. Regional differences in breast and cervical cancer mortality in Mexico between 1979-2006. Salud Publica Mex. 2009;51 Suppl 2:s208-19.
2. Chávarri-Guerra Y, Villareal-Garza C, Liedke PE, Knaulm F, Mohar A, Finkelsteinm DM, et al. Breast cancer in Mexico: a challenge to health and the health system. Lancet Oncol. 2012;13:e335-43.
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869-74.
4. Taylor CR, Levenson RM. Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II. Histopathology. 2006;49:411-24.
5. Di Loreto C, Puglisi F, Rimondi G, Zuiani C, Anania G, Della Mea V, et al. Large core biopsy for diagnostic and prognostic evaluation of invasive breast carcinomas. Eur J Cancer. 1996;32A:1693-700.
6. Mann GB, Fahey VD, Feleppa F, Buchanan MR. Reliance on hormone receptor assay of surgical specimens may compromise outcome in patients with breast cancer. J Clin Oncol. 2005;23:5148-54.
7. Cahill RA, Walsh D, Landers RJ, Watson RG. Preoperative profiling of symptomatic breast cancer by diagnostic core biopsy. Ann Surg Oncol. 2006;13:45-51.
8. Burge CH, Chang HR, Apple SK. Do the histologic features and results of breast cancer biomarker studies differ between core biopsy and surgical excision specimens? Breast. 2006;15:167-72.
9. Usami S, Moriya T, Amari M, Suzuki A, Ishida T, Sasano H, et al. Reliability of prognostic factors in breast carcinoma determined by core needle biopsy. Jpn J Clin Oncol. 2007;37:250-5.
10. Wood B, Junckerstorff R, Sterrett G, Frost F, Harvey J, Robbins P. A comparison of immunohistochemical staining for oestrogen receptor, progesterone receptor and her2 in breast core biopsies and subsequent excisions. Pathology. 2007;39:391-5.
11. Sutela A, Vanninen R, Sudah M, Berg M, Kiviniemi V, Rummukainen J, et al. Surgical specimen can be replaced by core samples in assessement of ER, PR and Her2 for invasive breast cáncer. Acta Oncol. 2008;47:38-46.
12. Arnedos M, Nerurkar A, Osin P, A´Hern R, Smith IE, Dowsett M. Discordance between core needle biopsy (CNB) and excisional biopsy (EB) for estrogen receptor (ER), progesterone receptor (PgR) and Her2 status in early breast cancer (EBC). Ann Oncol. 2009;20: 1948-52.
13. Park SY, Kim KS, Lee TG, Park SS, Kim SM, Han W, et al. The accuracy of preoperative core biopsy in determining histologic grade, hormone receptors, and human epidermal growth factor receptor 2 status in invasive breast cancer. Am J Surg. 2009;197:266-9.
14. Richter-Ehrenstein C, Muller S, Noske A, Schneider A. Diagnostic accuracy and prognostic value of core biopsy in the management of breast cancer: a series of 542 patients. Int J Surg Pathol. 2009;17: 323-6.
15. Tamaki K, Sasano H, Ishida T, Miyashita M, Takeda M, Amari M, et al. Comparison of core needle biopsy (CNB) and surgical specimens for accurate preoperative evaluation of ER, PgR and Her2 status of breast cancer patients. Cancer Sci. 2010:101:2074-9.

16. Lorgis V, Algros MP, Villanueva C, Chaigneau L, Thierry-Vuillemin A, Nguyen T, et al. Discordance in early breast cancer for tumor grade, estrogen receptor, progesterone receptors and her2 status between core needle biopsy and surgical excisional primary tumor. Breast. 2011;20:284-7.
17. Ricci MD, Filho CM, Filho HR, Filassi JR, Pinotti JA, Baracat EC. Analysis of the concordance rates between core needle biopsy and surgical excision in patients with breast cancer. Rev Assoc Med Bras. 2012;58:532-6.
18. Brenner RJ, Fajardo L, Fisher PR, Dershaw DD, Evans WP, Bassett L, et al. Percutaneous core biopsy of the breast: effect of operator experience and number of samples on diagnostic accuracy. AJR Am J Roentgenol. 1996;166:341-6.
19. Bilous M, Dowsett M, Hanna W, Isola J, Lebeau A, Moreno A, et al. Current perspectives on HER2 testing. A review of national testing guidelines. Mod Pathol. 2003;16:173-82.
20. Wolff AC, Hammond EH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer. American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Arch Pathol Lab Med. 2014;138:241-56.
21. Regitnig P, Reiner A, Dinges HP, Höfler G, Müller-Holzner E, Lax SF, et al. Quality assurance for detection of estrogen and progesterone receptors by immunohistochemistry in Austrian pathology laboratories. Virchows Arch. 2002;441:328-34.
22. Arihiro K, Umemura S, Kurosumi M, Moriya T, Oyama T, Yamashita H, et al. Comparison of evaluations for hormone receptors in breast carcinoma using two manual and three automated immunohistochemical assays. Am J Clin Pathol. 2007;127:356-65.
23. Bettigole C. The thousand-dollar Pap smear. N Engl J Med. 2013; 369:1486-7.