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Familial Breast/Ovarian Cancer and *BRCA1/2* Genetic Screening: The Role of Immunohistochemistry as an Additional Method in the Selection of Patients

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SUMMARY Only 20–25% of families screened for *BRCA1/2* mutations are found positive. Because only a positive result is informative, we studied the role of *BRCA1/2* immunohistochemistry as an additional method for patient selection. From 53 high-risk-affected probands, 18 (34%) had available paraffin blocks of their tumors and were selected for this study. Mutation screening was done by conformation-sensitive gel electrophoresis and multiplex ligation-dependent probe amplification. For immunohistochemistry, 21 neoplastic specimens (15 breast carcinomas, 5 ovary neoplasms, and 1 rectal adenocarcinoma) were analyzed with *BRCA1* (monoclonal antibody, Ab-1, oncogene) and *BRCA2* (polyclonal antibody, Ab-2, oncogene) antibodies. Absence of the *BRCA1* protein was confirmed in negative tumors by Western blotting. Seven patients were positive for *BRCA1/2* mutations: 5 for *BRCA1* and 2 for *BRCA2*. Four out of five positive patients had tumors negative for *BRCA1* immunostaining, and the remaining 13 *BRCA1*-negative patients had positive *BRCA1* immunostaining in all tumor samples. Sensitivity to predict for *BRCA1* mutation carriers was 80%, and specificity was 100%, with a positive predictive value of 100% and a negative predictive value of 93%. This correlation was statistically significant ($p=0.001$). No correlation was observed for *BRCA2*. If larger studies confirm these results, high-risk patients with *BRCA1*-negative tumors should be screened first for this gene. (J Histochem Cytochem 55:1105–1113, 2007)

KEY WORDS

BRCA1/2
genetic screening
hereditary breast cancer
immunohistochemistry

BREAST CANCER IS HEREDITARY in 10% of cases, the majority related to mutations in the *BRCA1* and *BRCA2* genes (Ford et al. 1998). *BRCA1* and *BRCA2* are tumor suppressor genes (Crook et al. 1997; Scully et al. 1997) that span over 100 kb of genomic DNA each and encode proteins of 1863 and 3418 amino acids, respectively. Mutations in these genes predispose carriers mainly to breast cancer but also to other cancers (Easton et al. 1995; Breast Cancer Linkage Consortium 1999; Liede et al. 2004).

BRCA1/2 mutation detection is complex because of the large size of both genes and the absence of hot

spots. Besides positive and negative tests, indeterminate results of this screening pose particular problems in the management of high-risk families. Selection of patients is then crucial and relies mainly on family history and phenotype ascertainment (Frank et al. 1998; Parmigiani et al. 1998; American Society of Clinical Oncology 2003), but only 20–25% of screened families have positive mutation results (Shih et al. 2002). This is due to the disparity of selection criteria used in different breast evaluation clinics, to the different molecular methodologies available, and also because other genes may be involved in hereditary breast cancer (Walsh et al. 2006). Screening is also complicated by the fact that mutation detection in one family must start in an affected relative, and in many families, all affected relatives are deceased.

More specific selection for genetic screening, taking into account characteristics of *BRCA1/2* tumors, is essential. Histopathology of *BRCA1/2* hereditary breast

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cancer may be useful, because a basal epithelial phenotype appears to be associated with germline *BRCA1* mutations (Foulkes et al. 2003) and other pathological features distinguish *BRCA1* tumors from *BRCA2* and sporadic breast cancers (Lakhani et al. 2002). Immunohistochemistry of *BRCA1/2* proteins in tumor cells could be useful as an additional method in patient selection for genetic screening. If the cancer tissue from the genetic proband of a high-risk breast/ovarian cancer family (the first person to be tested is a cancer-affected individual) shows an absence of labeling for either *BRCA1* or *BRCA2* protein in tumor cells, that could predict a *BRCA* mutation carrier. Tumorigenesis in individuals with germline *BRCA* mutations requires somatic inactivation of the wild-type allele, and *BRCA* deficiency is critical to the development of disease (Welch and King 2001). With a reliable immunohistochemical method, in most cases, only the mutated gene would be screened. This methodology has recently been demonstrated to be useful in the identification of carriers of mutations in the mismatch repair genes associated with hereditary non-polyposis colorectal cancer (Stormorken et al. 2005).

Several antibodies for *BRCA* proteins are available, but conflicting results have been published in the literature concerning their diagnostic usefulness. Concerning *BRCA1* protein, different types of staining have been described: nuclear, cytoplasmic, or both (Chen et al. 1995; Scully et al. 1996; Coene et al. 1997; Perez-Valles et al. 2001). Although these different observations could be explained by differences in the specificity of the antibodies (Perez-Valles et al. 2001), different fixation methods (Scully et al. 1996; Coene et al. 1997), or by the existence of splice variant isoforms of the *BRCA1* protein (Wilson et al. 1997), the use of monoclonal antibodies and fixation in neutral buffered formalin after antigen exposure in a microwave demonstrates a predominantly nuclear labeling (Scully et al. 1996). This localization is consistent with the role of *BRCA1* in the maintenance of genome integrity: cell cycle control, apoptosis, and DNA repair. *BRCA2* is also found in the nucleus, as expected for a key component of cell cycle control and DNA repair pathways (Marmorstein et al. 1998), although truncated forms have been observed in the cytoplasm (Spain et al. 1999). Because nuclear entry for both *BRCA1* and *BRCA2* is dependent on nuclear localization signals (Spain et al. 1999; Henderson 2005), it is expected that mutations in these domains may affect subcellular localization of *BRCA* proteins (Henderson 2005).

The use of *BRCA1* and *BRCA2* immunohistochemistry in the prediction of *BRCA* mutation carriers has been more extensively studied for *BRCA1*, but with contradictory results. The absence of *BRCA1* in 4% of 50 tissue sections was proposed to represent familial cases (Chen et al. 1995), and more recent studies have

considered the use of *BRCA1* staining to be a good indicator for the presence of mutations in this gene (Kashima et al. 2000; Schofield et al. 2000). However, the opposite has also been reported (Perez-Valles et al. 2001). In this study, we analyzed the correlation between the immunoexpression for *BRCA1/2* and the results of genetic screening for *BRCA1/2* mutations in a group of high-risk women with available tumor paraffin blocks. We also established the sensitivity, specificity, and predictive values of the method.

Materials and Methods

Case Selection

Between July 2000 and July 2002, we identified 18 women selected for *BRCA1/2* mutation screening with available paraffin-embedded tissue blocks of their tumors. These women belonged to a group of 53 affected individuals at high risk for *BRCA* mutations based on personal and family history [combined probability of *BRCA1/2* mutation over 25% (Frank et al. 1998; Parmigiani et al. 1998) or female breast cancer under 30 years of age] included in a research study for the analysis of these genetic events in the Portuguese population. All patients were counseled and consented to genetic screening, according to procedures approved by the ethics committee of our center. In one case, *BRCA1* genetic screening results were correlated with immunochemistry data from an obligate carrier in the same family.

Clinicopathological Data

Information about age and other neoplasias whose specimens were not available for review was obtained from patients' medical records. A pathologist blinded to the results of *BRCA1/2* mutation testing reviewed the cases. Histological grade of differentiation was estimated using the Elston and Ellis system (Elston and Ellis 1991).

BRCA1/2 Mutation Screening

DNA was extracted from whole blood using the QIAmp blood Midi Kit (Qiagen; Hilden, Germany) and aliquots of patients' DNA were each subjected to PCR amplification using primers designed to amplify whole exonic sequences and intronic/exonic boundaries of *BRCA1* and *BRCA2* (The Breast Cancer Information Core Database 1999; <http://research.nhgri.nih.gov/bic/>). The amplified products were analyzed by conformation-sensitive gel electrophoresis (CSGE) (Ganguly et al. 1993) and positive samples were sequenced using an automated fluorescence-based cycle sequencer (ABI Prism 310; Applied Biosystems, Foster City, CA). Mutations were classified according to current guidelines (den Dunnen and Antonarakis 2000).

Detection of *BRCA1* Rearrangements

All samples negative for *BRCA1/2* mutations by CSGE analysis were tested for the presence of large rearrangements in the *BRCA1* gene with the use of the multiplex ligation-dependent probe amplification (MLPA) (MRC Holland; Amsterdam, The Netherlands), according to the instructions

provided by the manufacturer. Separation and relative quantification of the amplification products were obtained with an ABI Prism 310 automatic sequencer (Applied Biosystems) using the Genescan software.

Immunohistochemistry

Formalin-fixed, paraffin-embedded blocks of the 21 specimens available were analyzed using a monoclonal antibody for the BRCA1 protein [anti-BRCA1 (Ab-1), human (mouse); epitope: 1–304 N-terminal amino acids of *BRCA1*] and a polyclonal antibody for the BRCA2 protein [anti-BRCA2 (Ab-2), rabbit polyclonal IgG; epitope: 3245–3418 of human *BRCA2*] (Oncogene Research Products/Calbiochem; Darmstadt, Germany) at a dilution of 1:300 and 1:100, respectively. Antigen retrieval was performed by heating the specimens in a pressure cooker for 6 min in citrate buffer (pH 6). The Envision system (Dako; Copenhagen, Denmark) was used for detection. Sections from positive breast invasive ductal carcinoma were used as positive controls, and negative controls were obtained by omitting the primary antibodies. The reaction was considered positive if more than 10% of the cells showed distinctive nuclear staining. Specific staining was evaluated by two investigators who had no knowledge of the patients' family histories or the results of mutation analysis.

Protein Extraction

Total protein extracts were obtained from formalin-fixed paraffin-embedded tissue sections. Three 50- μ m-thick sections were deparaffinized, and exclusively cancer tissues were collected and further cut into small pieces. Proteins were extracted from tissue sections with 50 μ l of radioimmunoprecipitation (RIPA) buffer, pH 7.6 [50 mM Tris, 5 mM EDTA, 1% Triton X-100, 0.4% sodium deoxycholate, 150 mM NaCl, and protease inhibitors (Complete Mini, EDTA-free; Roche, Mannheim, Germany)], followed by incubation at 100C for 20 min and incubation at 60C for 2 hr. Next, the tissue lysates were centrifuged at 14,000 \times g for 20 min at 4C, and the supernatants were collected [Ikeda et al. 1998]. Protein concentration of the lysates was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories; Munich, Germany). This reaction is similar to the well-documented Bradford assay (Bradford 1976).

Immunoprecipitation and Western Blot Analysis

Total protein extracts were immunoprecipitated with the Ab-1 monoclonal antibody. Samples were precleared with protein G-Sepharose beads (Sigma-Aldrich Chemie; Steinheim, Germany) for 1 hr at 4C in RIPA buffer, and supernatants were recovered and incubated overnight at 4C with the antibody at 2 μ g/ml. The binding reactions were incubated for 1 hr 30 min at 4C with protein G-Sepharose beads. The immunoprecipitated proteins were obtained after three washes in RIPA buffer and boiling at 100C for 5 min in sample loading buffer (2-fold concentrate: 125 mM Tris, 20% glycerol, 4% SDS, 2% 2-mercaptoethanol, and 0.001% bromophenol blue). Proteins were analyzed by 8% SDS-PAGE gels and transferred onto nitrocellulose membranes (Hybond-C extra; Amersham Life Sciences, Buckinghamshire, UK). After incubation with the primary antibody (Ab-1) overnight at

2 μ g/ml, the membranes were incubated for 2 hr with a goat anti-mouse IgG-horseradish peroxidase (Santa Cruz Biotechnology; Santa Cruz, CA) at a dilution of 1:5000. An enhanced chemiluminescence detection system and Kodak film (Amersham Pharmacia Biotech; Buckinghamshire, UK) were used to visualize the presence of proteins on the nitrocellulose blots.

Statistical Analysis

Sensitivity, specificity, and positive and negative predictive values were calculated for the results of immunohistochemistry to predict for *BRCA1/2* mutations. The association between negative immunohistochemistry and mutation carrier status was analyzed using the two-tailed Fisher's exact test.

Results

Clinicopathological Data

Nine out of 18 women had been diagnosed with unilateral breast cancer, 2 with bilateral breast cancer, 3 with breast and ovarian cancer, 2 with ovarian cancer, 1 with breast and rectal cancer, and 1 with breast and lung cancer. Twenty-one specimens were available: 15 with breast cancer tissue, 5 with ovarian cancer, and 1 with rectal cancer. Two of the five ovarian cancer specimens were of surgically excised metastasis (epiploon and hepatic metastasis). The other three specimens included the primary tumor. Clinicopathological characteristics are described in Table 1.

Genetic Screening

Of the 18 women with available paraffin-embedded tumor blocks, 7 were found to be positive for *BRCA1/2* mutations: 5 with *BRCA1* mutations (c.536delA, g.Ex13ins6Kb, c.211A>G, and g.Ex11_Ex15del) and 2 with *BRCA2* mutations (c.1369_1370ins2 and c.7208_7211del4). The remaining 11 women were negative for *BRCA1/2* mutations, by CSGE and MLPA screening of *BRCA1* rearrangements (Table 2).

Immunohistochemistry

Tumor samples from four of five women with *BRCA1* mutations were *BRCA1* negative, with absence of nuclear or cytoplasmic staining (Figure 1B). These cases included two breast cancer specimens (from cases 3 and 14), three ovarian cancer specimens (two from case 6 and one from case 18), and one rectal cancer specimen (case 14). In contrast, the other patient with a *BRCA1* mutation (g.Ex11_Ex15del) had her ovarian cancer specimen stain positive for *BRCA1* immunohistochemistry, with a clear nuclear immunoreactivity in tumor cells. All other tumor samples, from the 2 *BRCA2*-positive women and from the 11 patients negative for mutations in both genes, were positive for *BRCA1* immunostaining (Table 2).

Table 1 Clinopathological data

Case	Age (years)	Tumor specimen	Histological diagnosis	Histological grade	Pathological stage	Estrogen receptors
1	28	Breast	Invasive ductal carcinoma with intraductal component (<10%)	G2	IIB	+
2	69	Breast	Invasive ductal carcinoma, NOS	G2	IIA	+
3	28	Breast	Invasive ductal carcinoma, NOS	G1	IIA	+
4	46	Breast	Invasive ductal carcinoma, NOS	G2	IIB	-
5	35	Breast	(1) Invasive ductal carcinoma with predominant intraductal component (2) Invasive ductal carcinoma, NOS	(1) G2 (2) G2	(1) IIB (2) IIB	+
6	41	Ovary	Serous adenocarcinoma*	G2	IIIB	NA
7	43	Breast	Invasive ductal carcinoma, NOS	G3	IIA	+
8	49	Breast	Osseous metastasis of invasive ductal carcinoma	G3	IV	+
9	52	Breast	Invasive ductal carcinoma	G3	I	-
10	42	Breast	Intraductal carcinoma	G2	0	+
11	58	Ovary	Serous (60%) and endometrioid (40%) carcinoma	G3	IB	NA
12	34	Breast	Invasive ductal carcinoma with intraductal component (<20%)	G2	IIB	+
13	67	Breast	Invasive ductal carcinoma with predominant intraductal component	G3	I	+
14	37	(1) Breast (2) Rectum	(1) Invasive ductal carcinoma (2) Adenocarcinoma	(1) G3 (2) G2	(1) IIB (2) IIIB	(1)- (2) NA
15	32	Breast	Invasive ductal carcinoma	G3	IIA	+
16	33	Breast	Invasive ductal carcinoma with predominant intraductal component	G2	I	+
17	62	Ovary	Liver metastasis of ovary serous carcinoma	G3	IV	NA
18	55	Ovary	Clear cell carcinoma	G3	IIIC	NA

*Two samples reviewed, one of a surgically excised metastasis and the other including the primitive tumor.

Only specimens available for review are shown. In case 3, the patient had contralateral breast cancer at age 35, and in case 5 a first diagnosis of invasive lobular carcinoma of the other breast had been made at age 33; Cases 9,17, and 18 had both breast and ovarian cancer, and case 13 was treated for lung cancer before her breast cancer diagnosis. NA, not applicable; NOS, not otherwise specified.

BRCA2 staining was also optimized and nuclear staining observed in tumor samples. Cytoplasmic staining was not observed. One *BRCA2*-positive woman had her invasive breast cancer sample stain negative for

BRCA2, whereas the opposite was observed in a sample of invasive ductal carcinoma with large areas of intraductal carcinoma of the other *BRCA2* mutation carrier. In this last case, a relapse in the same location

Table 2 Correlation between mutation testing and immunohistochemistry

Case	<i>BRCA1/2</i> mutation (designation in BIC database)	Codon	Exon	Coding effect	<i>BRCA1</i> nuclear immunostaining	<i>BRCA2</i> nuclear immunostaining
1	Negative	-	-	-	+	+
2	Negative	-	-	-	+	+
3	c.536delA	179	8/1	STOP 233	-	-
4	Negative	-	-	-	+	-
5	c.1369_1370ins2	457	10/2	STOP 460	+	+
6	g.Ex13ins6Kb (exon13ins6Kb)	NA	13/1	STOP 1460	-	-
7	c.7208_7211del4 (7436del4)	2403	14/2	STOP 2467	+	-
8	Negative	-	-	-	+	-
9	Negative	-	-	-	+	-
10	Negative	-	-	-	+	+
11	g.Ex11_Ex15del	NA	11-15/1	ID	+	-
12	Negative	-	-	-	+	-
13	Negative	-	-	-	+	-
14	c.211A>G (R71G)	71	5/1	STOP 64	-	-
15	Negative	-	-	-	+	-
16	Negative	-	-	-	+	+
17	Negative	-	-	-	+	+
18	c.211A>G (R71G)	71	5/1	STOP 64	-	+

*This patient relapsed in the breast 3 years later and the specimen of invasive ductal carcinoma was negative for *BRCA2* immunohistochemistry. BIC, Breast Cancer Information Core; ID, indeterminate; NA, not applicable.

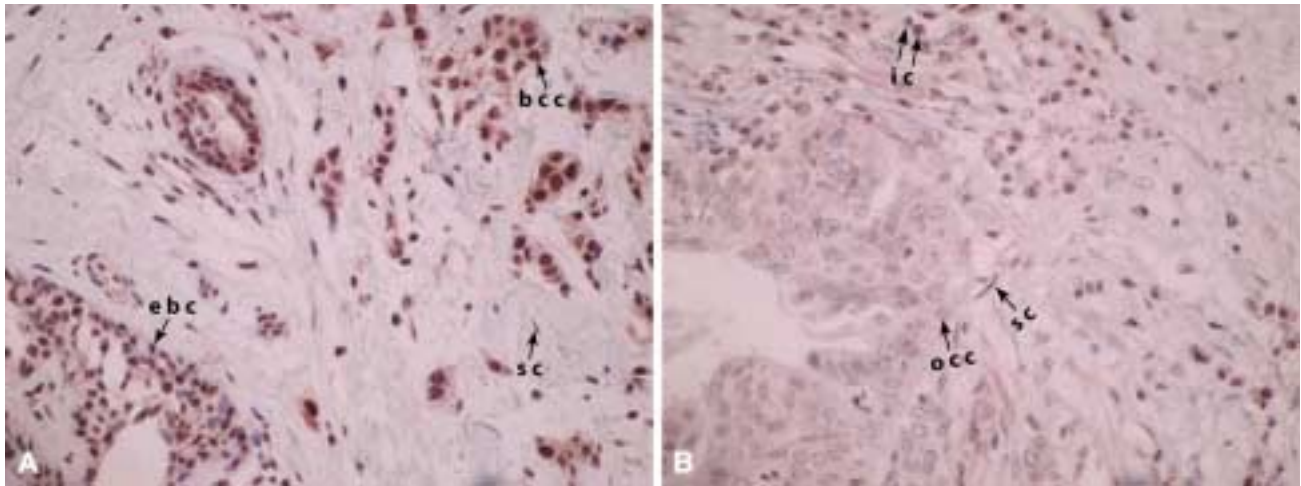


Figure 1 BRCA1 immunohistochemistry in cancer specimens. (A) Breast cancer specimen with clear nuclear BRCA1 labeling in cancer (bcc), epithelial (ebc), and stromal (sc) cells contrasts with an ovarian cancer sample (B), where cancer cells (occ) are negative for BRCA1, even if inflammatory (ic), and stromal cells (sc) keep the expected nuclear labeling.

of the right breast was observed 3 years later, and the corresponding tumor sample, of invasive ductal carcinoma, was negative for *BRCA2* staining. Most of the tumor samples from the 18 women were negative for *BRCA2* labeling (Table 2).

Protein Analysis

For *BRCA1*, the absence of the 220-kDa *BRCA1* protein in negative immunohistochemistry tumor samples was further confirmed with total protein extracts from paraffin tumor specimens, by Western blot. Normal breast tissue was used as control, and adequate total protein lysates were obtained from seven of our tissue-embedded samples. Total proteins were concentrated by immunoprecipitation, and in the experiment shown in Figure 2, the band corresponding to the *BRCA1* protein was observed by Western blotting. *BRCA1* protein was observed in normal breast tissue (positive control) and in a *BRCA1*-negative mutation case with positive-

stained *BRCA1* specimens (case 1) but not in two *BRCA1* mutation-positive cases with negative *BRCA1* immunostaining (cases 6 and 14) and in the negative control (protein G-Sepharose beads with Ab-1 monoclonal antibody).

Correlation Between Genetic Screening and Immunohistochemistry

Sensitivity of immunohistochemistry to detect *BRCA1* mutation carriers was 80% and specificity 100%. Negativity for *BRCA1* labeling in a tumor paraffin specimen was significantly correlated with *BRCA1* carrier status ($p=0.001$); the positive predictive value of this test was 100%, and the negative predictive value 93%. For *BRCA2*, sensitivity of immunohistochemistry was 50% and specificity 38%. The results of immunohistochemistry for *BRCA2* were not significantly correlated with *BRCA2* carrier status ($p=1$).

Discussion

In this study, we observed that immunostaining with a monoclonal antibody against the N-terminal amino acids of the *BRCA1* protein has a high specificity for the prediction of *BRCA1* mutation carriers. These results suggest that *BRCA1* immunohistochemistry, a rapid and easy test, can be used before the expensive mutation screening, to select which high-risk cases should be submitted to analysis of this gene; when *BRCA1* staining shows integrity of the protein, *BRCA2* screening should be done first. This methodology is helpful in case selection for subsequent mutation analysis and it can also be the only method to demonstrate inherited breast or ovarian cancer in deceased individuals belong-

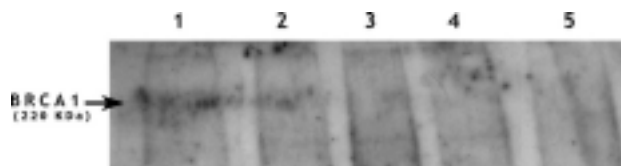


Figure 2 Absence of *BRCA1* protein in negative breast cancer specimens. After Western blotting of tumor protein extracts with the Ab-1 antibody, *BRCA1* protein was identified in normal breast, positive control (1) and in breast cancer from a *BRCA1* mutation-negative woman but with positive Ab-1 immunostaining (2). In *BRCA1* mutation-positive cases with negative immunostaining, the 220-kDa band corresponding to the *BRCA1* protein was not observed (3 and 4). Negative control, protein G-Sepharose beads with Ab-1 monoclonal antibody (5).

ing to high-risk breast/ovarian cancer families. However, the inclusion of *BRCA1* immunohistochemistry in algorithms concerning patient selection for *BRCA1/2* mutation screening is not recommended at this time, because the results of our study need confirmation in a larger, preferentially prospective sample. Confirmations of familiar cancers are not easy to obtain in breast cancer risk evaluation clinics, and cancer specimens are even more difficult to obtain. Collaborative studies are needed to overcome these difficulties.

Specificity of *BRCA1* immunohistochemistry to predict for *BRCA1* mutations was 100% and sensitivity 80%. In only one case was *BRCA1* immunohistochemistry not concordant with the final mutation results: a woman negative for *BRCA1/2* screening by CSGE was found to test positive for a large *BRCA1* deletion after MLPA screening. This rearrangement included the deletion of exons 11–15 of *BRCA1*, and immunohistochemistry of this patient's ovarian tumor sample showed positive labeling of the *BRCA1* protein. The most reasonable explanation for this observation is that besides this large deletion, the N-terminal portion of the *BRCA1* protein is transcribed and is accessible to the Ab-1 antibody. A previous study had already shown that *BRCA1* exon 11 mutations may not affect the immunostaining of both C-terminal and N-terminal antibodies (Kashima et al. 2000) (Table 3). In the present study, most of the *BRCA1* mutations occurred downstream of exon 11; the only two exceptions were the large deletion encompassing exons 11–15 and Ex13ins6Kb, another large rearrangement occurring upstream of exon 11. In this last case, complete negativity of *BRCA1* was observed, as in all other cases with mutations downstream of exon 11. These observations reinforce the notion that *BRCA1* immunohistochemistry can be used as a prescreen for mutation testing but, because sensitivity is 80%, it cannot replace mutation testing. However, its inclusion in a decision process also considering phenotypic and pathological factors can rationalize the approach and the cost of *BRCA1/2* mutations.

In cases negative for immunostaining, this finding was striking in that almost all tumor cells were negative. Although we indicated a cutoff at less than 10% of labeled tumor cells to consider the labeling negative, in only one of the tumors analyzed (one of the ovarian cancer specimens) very few cells (much less than 10%) were scarcely positive for *BRCA1*. This is in contrast with the results of another study in which only "islands" of negativity for *BRCA1* immunohistochemistry were observed in the presence of *BRCA1* mutations (Schofield et al. 2000) (Table 3). The results we present here, however, were confirmed by Western blotting: negative samples after *BRCA1* immunohistochemistry were negative for the detection of the *BRCA1* protein, and the opposite was observed for positive samples. No other study had previously done this type of assay, probably

because of the technical difficulties in obtaining protein lysates from paraffin-embedded specimens. A recent study whose aim was to assess the specificity and sensitivity of immunohistochemistry as a screening method for demonstrating *BRCA1* expression concluded that Ab-1 was the only *BRCA1* antibody whose staining correlated significantly with loss of *BRCA1* expression as determined by RT-PCR (Al-Mulla et al. 2005). Table 3 summarizes prior studies attempting to analyze the sensitivity and specificity of several *BRCA1* antibodies and the prediction of *BRCA1* mutation carrier status with immunohistochemistry.

Small sample size, one of the limitations of our study, is due to the difficulty in assembling tissue specimens from all patients tested for *BRCA1/2* mutations. In spite of this, one of the strengths of our assay is that *BRCA1* results were concordant not only in tumors of different origins (breast, ovarian, and one case of rectal cancer) but also in primitive and metastatic lesions. Rectal cancer has been an interesting although complicated issue since it was first associated with *BRCA1* (Brose et al. 2002; Thompson and Easton 2002), but more recent studies did not confirm that association (Niell et al. 2004). *BRCA1* loss is expected in a subgroup of colorectal cancers (Grabsch et al. 2006), and it may be that our specimen was negative because it was a sporadic rectal cancer in a confirmed *BRCA1* carrier or because the specific splicing *BRCA1* mutation that this patient inherited is truly associated with colorectal and other digestive tumors (Vega et al. 2001).

Exclusive nuclear staining could be considered to represent the normal phenotype and was observed for both *BRCA1* and *BRCA2* antibodies in all tumor specimens analyzed in our study. Some studies (Chen et al. 1995; Coene et al. 1997; Perez-Valles et al. 2001; Al-Mulla et al. 2005) also reported a cytoplasmic staining. Several reasons may account for the observation of *BRCA1/2* in the cytoplasm: cross-reaction between the target epitopes; the presence of *BRCA1* splice variants (Wilson et al. 1997); *BRCA2* truncated forms (Spain et al. 1999), or loss of the nuclear localization signals of the *BRCA* proteins. In fact, the ability to enter the nucleus may be compromised by germline mutations in the *BRCT* (Rodriguez et al. 2004) domain of *BRCA1* and in the carboxyl terminus of *BRCA2* (Marmorstein et al. 1998).

Prediction of *BRCA2* mutation carriers remains a problem. We could not find a correlation with immunohistochemistry in our study, and several studies show that pathological or microarray tumor analysis of *BRCA1* and *BRCA2* tumors is more distinctive for *BRCA1* than for *BRCA2*: *BRCA1* tumors tend to be of higher grade and are also more frequently negative for hormone receptors and more p53-positive than are their *BRCA2* counterparts (Lakhani et al. 2002); type D cyclins and their associated *CDK4* and *CDK1s* (p16, p21, and p27) were found to be downregulated in *BRCA1* in respect to

Table 3 Prediction of BRCA1 carrier status with immunohistochemistry for the BRCA1 protein

Study	n / Risk	Antibody used	Results			Sensitivity (%)	Specificity (%)	Positive Predictive value (%)	Comments	
Perez-Valles et al. 2001	23 / Familial BC cases: 17 high-risk, 6 low-risk		7 BRCA1-positive			16 BRCA1-negative			<ul style="list-style-type: none"> • Heterogeneity sample risk and mutation screening methods (PTT, CSGE, DS) • High cut off for scoring, 25%, 6/7 BRCA1 mutations in exon 11 	
		Ab-1	3 IHC neg	10 IHC neg		ND	ND	ND		
			4 IHC posit*	6 IHC posit						
		D-20	7 IHC neg	16 IHC neg**		ND	ND	ND		
		I-20	2 IHC neg	5 IHC neg		ND	ND	ND		
5 IHC posit*	11 IHC posit									
K18	7 IHC neg	16 IHC neg**		ND	ND	ND				
Kashima et al. 2000	44 / Familial OC cases		24 BRCA1-positive			20 BRCA1-negative			<ul style="list-style-type: none"> • Screening methods, SSCP • Figures apply if not considering cytoplasmic staining with GLK-2 antibody as positive 	
		Ab-2	7 IHC neg: 5/5 with mutation upstream of exon 11 and 2/16 with mutation in exon 11		0 IHC neg		ND	ND		ND
			17 IHC posit: 3/3 with mutation downstream of exon 11 and 14/16 with mutation in exon 11		20 IHC posit					
GLK-2	24 IHC neg: 8/8 with mutation other than exon 11 and 16/16 with mutation in exon 11		2 IHC neg		100	90	92			
Schofield et al. 2000	4 and 96 / 4 Related BC cases from a BRCA1 family; 96 early-onset BC women (BRCA1 unknown status)		3 BRCA1-positive	1 BRCA1-negative	96 BRCA unknown			<ul style="list-style-type: none"> • Mutation screening methodology not specified • Only focal negativity in BRCA1-positive cases but complete negativity for the other 9 cases (these 9 cases had unknown BRCA1 status, but 2 were of medullar histology) 		
		Ab-1 and I-20	3 IHC neg	1 IHC posit	9 IHC neg		ND		ND	ND
					87 IHC posit					
Al-Mulla et al. 2005	48 / Early-onset BC women (BRCA1 unknown status)	Ab-1	23 mRNA expression-negative	6 mRNA expression-positive	19 tissue not sufficient for RNA extraction			<ul style="list-style-type: none"> • Genetic background unknown; only mRNA expression as detected by real-time PCR • Sensitivity and specificity for the various antibodies not for predictive of BRCA1 status • Labelling heterogeneity 		
			22 IHC neg	6 IHC neg	19 IHC neg		66.6		91.3	ND
		1 IHC posit	6 IHC posit	0 IHC posit						
		Ab-8F7	17 IHC neg	3 IHC neg	12 IHC neg		100		30.4	ND
			6 IHC posit	3 IHC posit	7 IHC posit					
D-20	23 IHC neg	6 IHC neg	19 IHC neg		66	13	(ND)			
	0 IHC posit	0 IHC posit	0 IHC posit							
hBRCA1	20 IHC neg	5 IHC neg	18 IHC neg		33	62	(ND)			
	3 IHC posit	1 IHC posit	1 IHC posit							
Vaz et al. in press	18 / High-risk (familial BC)		5 BRCA1-positive			13 BRCA1-negative			<ul style="list-style-type: none"> • High-sensitive mutation screening (CSGE+MLPA) • Cut off 10% • Negative IHC for BRCA1 predicted all but one mutation in exon 11 	
		Ab-1	4 IHC neg		13 IHC posit		80	100		100
			1 IHC posit							

*Cutoff $\leq 25\%$ for IHC-negative.

**Contradiction between Table and text presented in paper.

n, number of cases; BC, breast cancer; OC, ovarian cancer; ND, not done; NA, not applicable; IHC neg and IHC posit, negative and positive detection of BRCA1 protein, respectively; Ab-1 (see text); D-20 and K18, polyclonal antibodies targeting N-terminus of BRCA1; I-20, polyclonal antibody targeting C-terminus (exon 24) of BRCA1; Ab-2, description and origin as for Ab-1; GLK-2, monoclonal antibody against C-terminal amino acids 1839–1863; Ab-8F7, monoclonal antibody against exon 11; hBRCA1 (Ab-C-terminus), polyclonal antibody against C-terminus (exons 12–24); PTT, protein truncation test; CSGE, conformation-sensitive gel electrophoresis; DS, double sequencing; SSCP, single-stranded conformation polymorphism; MLPA, multiplex ligation-dependent probe amplification.

BRCA2 carcinomas. Also, over 80% of triple-negative breast tumors (negative for estrogen, progesterone, and *HER2* receptors) are “basal-like,” and basal markers have been shown to be specific to a subset of *BRCA1* carcinomas (Palacios et al. 2004). The apparent relative excess of *BRCA1* germinal mutations in association with triple negative tumors is under study (Kandel et al. unpublished data). The characterization of *BRCA2* breast tumors and their distinction from sporadic breast cancers is an area of active research, and one recent study proposed that *BRCA2* tumors could be identified through the use of an array of markers that included *CHEK2* and *RAD51*: *CHEK2* was found to be more frequently expressed in *BRCA1* and *BRCA2* tumors than in non-*BRCA1/2*, and *BRCA2* was found to be necessary for the translocation of *RAD51* (Honrado et al. 2005).

In conclusion, we observed a high specificity for the prediction of *BRCA1* carriers with immunohistochemistry using a monoclonal *BRCA1* antibody. Validation of this assay, using a larger sample, will allow the use of immunohistochemistry for deciding which high-risk patients should be screened first for the *BRCA1* gene. This recommendation does not exclude the relevance of other known risk factors for mutations in this gene (family history, age at cancer diagnosis, histological characteristics of the tumors, triple negativity) but is intended to contribute to a more specific patient selection. Because only positive results are informative for probands of these families, better patient selection is likely to increase the possibilities of obtaining informative genetic results.

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