Published in final edited form as:

Breast Cancer Res Treat. 2011 August ; 129(1): 211–219. doi:10.1007/s10549-011-1448-8.

Differential expression of prognostic biomarkers between interval and screen-detected breast cancers: does age or family history matter?

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Abstract

The aim of this study was to compare tumor expression of prognostic biomarkers between interval breast cancers and screen-detected breast cancers overall, and according to age at diagnosis and familial risk. Tissue micro-arrays were constructed from 98 breast cancers (47 interval and 51 screen-detected) diagnosed in women in the Cancer Genetics Network. Arrays were immunostained to compare protein expression of six biomarkers including estrogen and progesterone

receptor (ER/PR), Her2/neu, EGFR, cytokeratin 5/6, and Ki67. Fisher's Exact test was used to compare expression between interval and screen-detected cancers. Interval cancers were larger ($P = 0.04$), higher stage ($P < 0.001$), and more likely to have lobular histology ($P = 0.01$) than screen-detected cancers. Overall, interval cancers more often over-expressed EGFR ($P = 0.01$) and were somewhat more likely to be ER- (55% vs. 43%, $P = 0.3$), and triple negative (ER-/PR-/Her2-) (21 vs. 12%, $P = 0.26$). A greater difference in the proportion of interval versus screen-detected tumors that were ER- (53 vs. 35%; $P = 0.29$), PR- (35 vs. 21%; $P = 0.25$) and EGFR+ (17 vs. 0%; $P = 0.02$) was evident among women over 50. There was a trend toward differential expression among women with familial risk for PR- ($P = 0.005$) and triple negative status ($P = 0.02$). This study provides new data indicating that EGFR may be important in the etiology of interval cancer and be a possible therapeutic target. Our data also suggest that biological differences between interval and screen-detected cancers are more defined in older women. Future studies to confirm this finding and to elucidate novel markers for characterizing interval cancers may be more beneficial to this subgroup.

Keywords

Interval breast cancer; Biomarkers; Tissue micro-arrays

Background

Interval breast cancers that present in-between screening exams are larger and higher stage than breast cancers detected at screening [1–7]. Their rapid presentation and more advanced clinical features suggest that interval cancers may be biologically distinct from screen-detected cancers. This raises the question whether they are more likely to express tumor markers of aggressive behavior and poor prognosis, so that appropriate therapy can be recommended. Results from these studies have been mixed when only conventional markers such as ER/PR, Her2/neu, p53 and Ki-67 have been studied.

While several studies have shown that interval cancers are more likely to be ER-/PR-, overexpress p53, and to have higher cell proliferation rates (Ki67) than screen-detected cancers [1–3, 8–12], not all of them were able to confirm these findings [2, 8, 12]. At least two studies reported that interval cancers were more likely to overexpress Her2/neu [13, 14]; however, three others showed that they were *as* likely or even *less* likely than screen-detected cancers to overexpress Her2/neu [1, 8, 11]. A study by Collet [11] suggests that interval cancers are more likely to exhibit a basal-like phenotype (as determined by expression of Cytokeratin (CK) 5/6 and P-cadherin), which tend to be more aggressive tumors [15, 16]. To our knowledge, there have been no additional studies to confirm or refute this finding. Nor have there been studies of interval cancers specifically to examine expression of the epidermal growth factor receptor (EGFR), which has also been associated with poor prognosis for breast cancer [17].

Inconsistent findings across studies may be explained by differences in the sample population, the definition of interval cancer or the methodologies used for measuring tumor markers. An alternative explanation is that interval cancers may be biologically distinct from screen-detected cancers only in subgroups of women. In two previous studies, we found that

interval cancers more often occurred in younger women (<50) and in women with a family history of breast cancer [18], confirming previous reports [1, 2, 7]. Breast cancers in these subgroups tend to be more aggressive as evidenced by the propensity for these to be high grade, ER– and to overexpress Her2/*neu* [1, 19–23]. We also found higher mortality rates for interval versus screen-detected cancer, primarily among women over 50 (Lowery et al. unpublished). These findings led us to question whether biological differences between interval and screen-detected cancers may be more pronounced in older age groups and in women with sporadic breast cancers compared to women with familial risk.

For this pilot study, we sought to examine and compare expression of six prognostic markers, ER, PR, Her2/*neu*, C5/6, Ki67, and EGFR between interval and screen-detected cancers in a sample of women of varying familial risk matched according to age at diagnosis.

Materials and methods

Study population

Our population consisted of women diagnosed with breast cancer in Colorado 1999–2000 who enrolled in the Cancer Genetics Network, a population-based family cancer registry funded by the National Cancer Institute [24]. The University of Colorado enrolled over 4,800 participants into CGN including 1,200 breast cancer cases. Cases were ascertained through hospital registries and the Colorado Central Cancer Registry. Among the 1,250 breast cancer cases enrolled, approximately 60% had a first-degree relative with breast cancer.

For this study, 700 breast cancer cases in CGN were randomly selected for participation. Women were contacted by mail to seek permission to obtain paraffin-embedded tumor tissue from their diagnosis. Women were also asked to complete a brief questionnaire to determine *how* their cancer was detected, either via routine mammography or as a result of symptoms, such as presentation of a lump or breast discharge, and *when* they had their last normal mammogram before their diagnosis. Of 700 women contacted, 398 (57%) completed the questionnaire and consented to tissue procurement.

Definitions of Interval and Screen-Detected Cases

Women were categorized as “interval” or “screen-detected cases as follows: interval cases included women whose cancer was self-detected (by lump or other symptom; $n = 36$ of 47), or detected by their provider during a routine exam ($n = 6$) or by mammogram ($n = 3$) done less than 12 months following a normal screening. Screen-detected cases comprised women whose cancer was detected by screening mammography and who had a normal mammogram 12 months before their diagnosis. In total, 64 women were determined to be interval cases and included in this study. A comparable number of screen-detected cases ($n = 65$) was randomly selected from the 334 identified, frequency matched to interval cases according to age (<50 vs. 50+). The sample size for this study was based on resources available but determined adequate to detect a 20% difference in protein expression.

Tissue blocks, hematoxylin and eosin (H&E) slides and pathology reports were obtained for 98 of 129 cases requested (76%); blocks were not available for 27 cases and available but deemed insufficient for analysis for three cases. H&E slides were reviewed by a pathologist to confirm presence of tumor and identify optimal location for sampling cores for the arrays.

Tissue Micro Arrays (TMAs)

The TMAs were assembled using a tissue-array instrument (Beecher Instruments, Silver Springs, MD). Use of TMAs for immunophenotyping of malignant tumors has been described and validated by others [25]. Taking tumor heterogeneity into account, we used a stylet with 1.0-mm diameter and when possible, retrieved three replicate core samples of tumor tissue for each case. Three array blocks consisting of up to 44 cases each were constructed. Multiple consecutive 4- μ m sections were cut from the arrays. One section from each tissue array block was stained with H&E and examined to confirm the presence of tumor. For 10 cases, the amount of tumor obtained was insufficient for evaluation. For these cases, additional whole sections of the original tumor block were cut, immunohistochemically stained and examined.

Immunohistochemistry (IHC)

The following primary antibody clones were utilized: ER (clone 6F-11), PR (clone 16), and Ki67 (MIB1). Immunostaining was performed using the Ventana, automated staining system (Ventana Medical Systems, Tuscon AZ). Immunostaining for Her2/*neu* (DAKO) was performed manually using the HercepTest method^(TM). For EGFR and cytokeratin (CK) 5/6, the primary antibodies (EGFR, Zymed Laboratories #28-0005 at 1:10 dilution; CK 5/6, DAKO #M 7237 at 1:50 dilution) were used. Positive and negative controls of validated tissue for each marker were included for quality control purposes with each staining run. Negative controls were performed on all sections by substitution of the primary antibodies with a subclass-matched IgG of the same dilution and directed against unrelated antigens.

Immunohistochemistry Scoring

Light microscopy evaluation of immunostained TMAs was performed for ER, PR, and Her2/*neu*. For ER and PR, both the percentage of cells stained and the intensity of staining (on a scale of 0–3+) were scored. Consistent with clinically established cutoff points for positivity used at the time of study, tumors with >10% positively stained nuclei were considered positive for ER or PR. Only one interval case with 5% staining was scored as ER –, and therefore, the 1% cut-off proposed currently would not have made a noticeable difference. Results from cell membrane staining for Her2/*neu* were scored using the manufacturer's guidelines. Cases were placed into one of four categories: scores of 2+ and 3+ were considered positive; scores of 0 and 1+ were considered negative. Tumors with initial scores of 2+ or 3+ were evaluated further using image analysis (IA) performed via the ChromaVision Automated Cellular Imaging System (ChromaVision Medical Systems, CA) that utilizes color detection and pattern-recognition technology to score immunostained sections. The validity of using IA for scoring Her2/*neu* has been previously reported in our laboratory [26]. Scores <2.0 from IA were interpreted as negative; scores $\geq$ 2.0 were interpreted as positive. Using IA, 13 of 22 tumors that originally scored as 2+ positive by

microscopy analysis were downgraded to negative for Her2/*neu*. NOTE: This study was performed before the ASCO/CAP Her2/*neu* guidelines were established that recommend additional testing by FISH for 2+ IHC cases. Confirmation by FISH was not feasible for this study, as we were unable to retain tissue blocks. EGFR and CK 5/6 expression was considered positive if any membranous or cytoplasm staining was observed in tumor cells, respectively.

The percentage of cells staining positive for Ki67 was also calculated using IA technology. With the guidance of a board-certified pathologist with sub-specialty expertise in breast pathology (MS) and image analysis technology, the appropriate areas on the array sections for evaluation were selected. When available, six fields of a 40× magnification objective from areas with tumor from each of the three cores collected for each case were evaluated. The average of these scores was used as the final score. Tumors were categorized as having low (<14% positive cells) or high (>14% positive cells) proliferative activity, as determined to be the optimal cut-point to distinguish low or high proliferation breast cancer sub-types [27].

Patient and Tumor Characteristics

Women included were diagnosed with breast cancer from 1999 to 2000 and thus had survived their disease for a minimum of 5 years. Demographic information including age at diagnosis and family history status were provided by women upon enrollment into CGN. Women with at least one first-degree relative with breast cancer were considered “positive” for family history. Height and weight at diagnosis was ascertained from the supplemental questionnaire along with mode of diagnosis. Clinical data such as tumor size, stage, grade, and histology were abstracted from the pathology report or provided by the cancer registry.

Analysis

Personal and tumor characteristics for interval and screen-detected cancers were compared using the Chi-square test. The Fisher’s Exact test was used to compare protein expression for all six markers overall and stratified by age (<50 vs. ≥50 years) and family history (positive vs. negative) [28]. The relationships between protein expression and tumor and host characteristics were assessed using the Chi-square and Fishers Exact Test as appropriate. This study was approved by the Colorado Multiple Institutional Review Board.

Results

We analyzed tumor markers for 47 interval and 51 screen-detected cancers. Approximately one-third of interval cases occurred in women <50 years of age (Table 1). Owing to a priori frequency matching by age category, there was little difference in age between interval and screen-detected cancers. Nearly 40% of all patients had a first-degree relative with breast cancer and this percentage was slightly higher for interval cases. There was no difference in body mass index between cancer types. A larger portion of interval cancers were greater than 2 cm (26 vs. 8%; $P = 0.04$) and had spread to regional nodes at the time of diagnosis as compared to screen-detected cancers (35 vs. 14%; $P < 0.0001$). Interval cancers were also less likely to be in situ carcinomas alone and more likely to have an invasive lobular

histology as compared to a ductal histologic subtype ($P = 0.01$). Differences in tumor grade and nodal involvement were not statistically significant.

Results from IHC and tumor analysis revealed that as a group, a larger proportion of interval cancers than screen-detected cancers overexpressed EGFR (13 vs. 0%, $P = 0.01$); none of the 51 screen-detected cancers analyzed overexpressed EGFR. Interval cancers were also slightly more likely than screen-detected cancers to be ER- (55 vs. 43%) though this difference was not significant ($P = 0.3$). With respect to Her2/*neu*, we found that a lower percentage of interval cancers were Her2/*neu* positive; 5% compared to 14% of screen-detected cancers ($P = 0.16$). There was little difference with respect to expression of PR, CK 5/6 or Ki67 (Table 2).

Triple negative (TN) cancers (ER-/PR-/Her2/*neu*-) comprised 16% of all breast cancers studied and were slightly more common in interval compared to screen-detected cancers (21 vs. 12%; $P = 0.26$). Of the 15 TN breast cancers, six were + either for CK 5/6 or EGFR or both. Of note, of the nine total TN interval cancers, six were + for either CK 5/6, or EGFR, or both whereas in the six TN-screen-detected cancers, none were+ for CK 5/6 or EGFR.

When we stratified by age, we found that differences in marker expression between interval and screen-detected cancers, though most not significant, were more evident among women age 50 and older. For example, in older women, interval cancers were more likely than screen-detected cancers to be ER- (52 vs. 35%, $P = 0.29$) and PR- (35 vs. 21%, $P = 0.25$) whereas in the younger group (<50) there was no difference in ER expression and there was an opposite trend for PR expression (Table 3). Interval cancers in older women were also significantly more likely than screen-detected cancers to be EGFR+ ($P = 0.02$), which was not true in younger women. The percent of interval cancers that were Her2/*neu* positive was similar across age groups. With respect to familial risk, we saw a trend toward differential expression of markers among women with positive histories. Interval cancers in women with familial risk were significantly more likely than screen-detected cancers to be PR- ($P = 0.005$) and TN ($P = 0.02$) (Table 4).

Examination of the relationship between host and tumor characteristics and protein expression revealed that tumor size was associated with stage ($P < 0.001$) and ER+ status was strongly related to PR+ status ($P < 0.001$)(data not shown). ER negativity was associated with higher tumor grade ($P = 0.01$) but was less prevalent in tumors among women with a family history ($P = 0.01$). TN status was correlated with larger tumor size ($P = 0.09$) and higher grade ($P = 0.02$). Cell proliferation (Ki67) and Her2/*neu* overexpression were not significantly associated with any tumor or host-related factors.

Discussion

This study compared expression of several prognostic tumor markers between interval and screen-detected breast cancers and explored whether differential expression might be related to age at diagnosis or family history. Though limited in size, our study provides new information to suggest that EGFR may be an important pathway in the etiology of interval cancers, and some evidence that interval cancers are more likely to have triple-negative

phenotypes, which may explain the rapid onset and poor prognosis of these cancers. Moreover, our findings suggest that differential expression of tumor markers between interval and screen-detected cancers may be more evident in older women.

As was true for our study, interval breast cancers tend to be larger and more advanced than screen-detected cancers [1–3]. They are also more likely to be ER–/PR– tumors, conferring non-response to endocrine therapy and poor prognosis [1, 8, 10, 12, 14, 29, 30]. Our data confirmed a greater tendency for interval cancers to be ER–/PR– though only among women aged 50 and older. Few studies have compared biomarker expression between interval and screen-detected cancers stratified by age. Our finding is contrary to that from Porter et al. [1] citing a greater difference in ER/PR expression among younger women (<50). It should be noted however, that their definition of interval cancers was different than ours (24 vs. 12 months post screening). We did not find any difference in ER expression between interval and screen-detected cancers in our younger group. Furthermore, we saw fewer PR– interval cancers in this group compared to screen-detected cancers. Of interest is that among younger women, screen-detected cancers were more likely to be ER–/PR–, and Her2/neu+, compared to screen-detected cancers in older women. This is consistent with the fact that breast cancers overall in younger women (regardless of mode of detection), have more aggressive phenotypes [1, 19, 20, 22, 23, 31]. Conversely, screen-detected cancers in older women tend to be slow growing, less aggressive tumors (ER+/PR+), which our data confirmed. Our findings suggest that interval cancers may be biologically distinct and potentially more aggressive than screen-detected cancers only in older women. For this subgroup, knowledge of mode of cancer detection may be clinically important for assessing prognosis and treatment.

This study is the first that we know of to examine expression of EGFR specifically in interval breast cancers. We found that interval cancers were significantly more likely to over-express EGFR, indicating that this may be an important pathway in the etiology of these cancers. This is relevant in current context, as EGFR dysregulations are known to be more frequent in basal-like cancers, which tend to be aggressive and to more commonly present as interval cancers [32, 33]. Furthermore, EGFR expression independently predicts worse disease-free survival for breast cancer [17, 32]. Although the number of EGFR+ tumors in our sample was relatively small, we found that EGFR+ tumors were also more likely to be ER– (83 vs. 47%) and PR– (67 vs. 24%), consistent with results from Rimawi et al. [17] that evaluated EGFR expression for 2,500 breast tumors. Of interest is that we did not also see a higher frequency of CK5/6 expression among interval cancers, also a marker of basal-like phenotype and previously shown to correlate with interval cancer status [15]. Additional studies in larger samples would be needed to confirm our findings and to further examine the potential role of EGFR in the development of interval cancers.

In recent years, triple negative (TN) breast cancers have become a special focus of clinical research [34–37]. Similar to basal-like cancers, TN cancers tend to be aggressive, and to occur more frequently in younger women, and in African American and Hispanic women [32, 36, 37]. Furthermore, current regimens are ineffective in treating these cancers [32]. Because of their aggressive behavior, it seems plausible that a greater proportion of TN cancers would present as interval cancers, which is supported by our study. We found that a

TN phenotype was 70% more common among interval cancers. Although few comparative studies exist, our finding supports that of Domingo et al. [38] citing that interval cancers were nearly nine times more likely to be TN cancers than screen-detected cancers. These findings provide added evidence that interval cancers are biologically distinct from screen-detected cancers that renders them less sensitive to screening and less responsive to treatment.

In contrast to previous studies [1, 2, 15], we did not find higher proliferative activity (Ki67) among interval cancers when evaluated overall or by subgroup. One possible explanation is that we had a higher percentage of lobular cancers relative to other studies [2, 11]. Owing to the difficulty in detecting these cancers, lobular cancers tend to be over-represented among interval cancers and to demonstrate low proliferation activity [1, 5, 39, 40]. We also did not find greater Her2/*neu* overexpression for interval cancers, confirming some previous studies [1, 8, 11]. It is notable that <10% of cancers evaluated were Her2/*neu*+, much less than expected based on current estimates [41]. Her2/*neu* overexpression is highly correlated with tumor grade and ER negativity [14, 42, 43], both of which predict survival. In our sample, a lower proportion of high grade tumors were interval (21%) versus screen-detected cancers (33%), which was not expected given other advanced clinical features of these cancers. This may in part be explained by survival bias. Women included must have survived their cancer at least 5 years. If a greater percentage of women with interval cancers had high grade tumors, then they may not have survived long enough to be represented in this study.

We did not find as hypothesized, that differential expression of tumor markers would be greater among women with sporadic cancer. In our sample, interval cancers were slightly more common among women with positive histories. However, breast cancers overall in these women did not have poorer prognostic factors than those in women without family history, which was not expected. This may in part be due to survival bias as mentioned above. It is plausible that our sample therefore may not adequately reflect “familial” breast cancers that in general tend to have adverse prognostic indicators. Future studies with more rapid case ascertainment could better assess this question.

This study was limited by its sample size, which may have hindered our ability to detect true (significant) differences in marker expression. In addition, because interval-cancer status was determined by self-report, misclassification of cancer type was possible. Up to 30% of interval cancers are thought to be present at screening but missed because of technological or reader error [39, 44]. We could not review mammography reports to validate the radiological classification of cancers nor the screening date. Misclassification would have minimized the differences in marker expression between groups. However, it is noteworthy that we were able to confirm in our sample that interval cancers were significantly larger, and more advanced stage than screen-detected cancers, a difference we would likely have not seen if misclassification were a considerable factor.

Conclusion

This study provides new information to suggest that EGFR may be an important pathway for interval cancers, and that differential expression of prognostic markers may be more

pronounced in older women. We also found that triple negative breast cancers appeared to be more common among interval cancers than screen-detected cancers. Future studies with larger sample size to assess this potential association are needed. Because we are able to identify interval cancers in screened populations, interval cancers provide a unique opportunity for the development and identification of novel etiological and prognostic biomarkers, beyond those currently in clinical use.

Acknowledgements

The authors would like to acknowledge the Cancer Genetics Network, funding for which was provided by the National Cancer Institute (Grant No. CA078174), and the Tissue Procurement Core of the University of Colorado Cancer Center Support Grant #P30 CA046934.

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Table 1

Characteristics of interval and screen-detected breast cancers

Characteristic	Interval cancer (<i>n</i> = 47)		Screen-detected cancer (<i>n</i> = 51)		<i>P</i> -value*
	<i>N</i>	%	<i>N</i>	%	
Age					0.91
<50	17	36%	17	33%	
50–59	14	30%	16	31%	
60–69	8	17%	11	22%	
70+	8	17%	7	14%	
Family history					0.25
Positive	21	45%	17	33%	
Negative	26	55%	34	67%	
Body mass index mean (kg/m ²)	25.0	–	25.6	–	0.70
Tumor Size (cm)					0.04
<1	9	21%	16	42%	
1–2	23	53%	19	50%	
>2	11	26%	3	8%	
Unknown	4		13		
Tumor stage					<0.0001
In situ ^a	1	2%	16	31%	
Local	26	57%	27	53%	
Regional spread	16	35%	7	14%	
Distant spread	3	7%	1	2%	
Unknown	1		0		
Tumor grade ^b					0.36
1	12	28%	12	29%	
2	22	51%	16	38%	
3	9	21%	14	33%	
Unknown	4		9		
Histology					

Characteristic	Interval cancer (<i>n</i> = 47)		Screen-detected cancer (<i>n</i> = 51)		<i>P</i> -value*
	<i>N</i>	%	<i>N</i>	%	
DCIS ^a	1	2%	12	24%	0.01
Invasive ductal	30	64%	25	49%	
Invasive lobular	11	23%	7	14%	
Other	5	11%	7	11%	

* Significance level for chi-square test for proportions

^a Ductal carcinoma in situ

^b Modified Bloom-Richardson grading system

Results from immunohistochemical staining for ER, PR, Her2/*neu*, EGFR, CK 5/6, and Ki67 for interval and screen-detected cancers

Table 2

Biomarker	Interval cancers (<i>n</i> = 47) ^a		Screen-detected cancers (<i>n</i> = 51) ^a		<i>P</i> -value [*]
	<i>N</i>	%	<i>N</i>	%	
Estrogen receptor					0.30
Positive	19	45%	29	57%	
Negative	23	55%	22	43%	
Progesterone receptor					0.81
Positive	31	72%	38	76%	
Negative	12	28%	12	24%	
Her2/ <i>neu</i>					0.16
Positive	2	5%	7	14%	
Negative	42	95%	42	86%	
EGFR					0.01
Positive	6	13%	0	0	
Negative	39	87%	48	100%	
Cytokeratin 5/6					0.90
Positive	4	9%	4	8%	
Negative	41	81%	45	91%	
Ki67 (% of positive cells)					0.67
<14% of cells	18	42%	19	39%	
14% of cells	25	58%	30	61%	
Triple negative (ER−/PR−/Her2−)	9	21%	6	12%	0.26

^{*} Fishers Exact test

^a Numbers do not always add up to total because few samples were not evaluable by IHC

Results from immunohistochemical staining for ER, PR, Her2/*neu*, EGFR, and Ki67 for interval and screen-detected cancers stratified by age at diagnosis (<50 vs. 50+)

Table 3

Biomarker	Age<50 years (n = 34) ^a			Age 50 years (n = 64) ^a		
	Interval (17)		Screen- detected (17)	Interval (30)		Screen- detected (34)
	N (%)			N (%)		
ER						
Positive	7 (41)		7 (43)	12 (48)		22 (65)
Negative	10 (59)		10 (57)	13 (52)		12 (35)
PR						
Positive	14 (82)		11 (69)	17 (65)		27 (79)
Negative	3 (18)		5 (31)	9 (35)		7 (21)
Her2/ <i>neu</i>						
Positive	1 (6)		4 (25)	1 (4)		3 (9)
Negative	16 (94)		12 (75)	26 (96)		30 (91)
EGFR						
Positive	1 (6)		0	5 (17)		0
Negative	15 (94)		16 (100)	24 (83)		32 (100)
CK 5/6						
Positive	3 (19)		1 (6)	1 (3)		3 (9)
Negative	13 (81)		15 (94)	28 (97)		30 (91)
Ki67						
<14%	7 (41)		7 (44)	12 (46)		12 (36)
14%	10 (59)		9 (56)	14 (54)		21 (64)
Triple Negative (ER-PR-/Her2-)	3 (18)		1 (6)	6 (24)		5 (15)
						0.50

* Fishers Exact test

^a Numbers do not always add up to total because few samples were not evaluable by IHC

Results from immunohistochemical staining for ER, PR, Her2/*neu*, EGFR, and Ki67 for interval and screen-detected cancers stratified by family history status

Table 4

Biomarker	Family history positive (<i>n</i> = 38) ^a			Family history negative (<i>n</i> = 60) ^a		
	Interval N (%)	Screen- detected (17)	<i>P</i> -value [*]	Interval N (%)	Screen- detected (34)	<i>P</i> -value [*]
ER			0.16			0.59
Positive	11 (58)	14 (82)		8 (35)	15 (44)	
Negative	8 (42)	3 (18)		15 (65)	19 (56)	
PR			0.005			0.23
Positive	12 (60)	16 (100)		19 (83)	22 (65)	
Negative	8 (40)	0		4 (17)	12 (35)	
Her2/ <i>neu</i>			0.57			0.38
Positive	1 (5)	2 (13)		1 (4)	5 (15)	
Negative	19 (95)	14 (87)		23 (96)	28 (85)	
EGFR			0.24			0.08
Positive	3 (15)	0		3 (12)	0	
Negative	17 (85)	16 (100)		22 (88)	32 (100)	
CK 5/6			0.67			0.43
Positive	3 (15)	4 (25)		1 (4)	0	
Negative	17 (85)	12 (75)		24 (96)	33 (100)	
Ki67			0.74			0.79
<14%	9 (45)	6 (37)		10 (43)	13 (39)	
14%	11 (55)	10 (63)		13 (57)	20 (61)	
Triple Negative (ER−/PR−/Her2−)	6 (32)	0	0.02	3 (13)	6 (18)	0.73

^{*} Fishers Exact test

^a Numbers do not always add up to total because a few samples were not evaluable by IHC