

# Isolated Tumor Cells in Sentinel Lymph Nodes of Primary Invasive Breast Carcinoma: A Cohort Analysis

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## Abstract

The finding of isolated tumor cells (ITCs) in lymph nodes from resected early stage breast cancer has been found to have limited prognostic relevance. We examined 2 different strategies for ITC detection among cohorts of 250 patients over 2 different time intervals (2010-2011 and 2015-2016). There were significantly fewer cases of ITC observed over the latter time period during which selective, as opposed to reflex cytokeratin staining, was used ( $P < .02$ ). This observation resulted in cost savings to the pathology laboratory and did not affect adjuvant treatment decision-making.

**Background:** The practice of performing routine cytokeratin immunohistochemistry (CK-IHC) on sentinel lymph nodes in early stage invasive breast cancer leads to frequent identification of isolated tumor cells (ITCs), the clinical significance of which remains unclear. After emergence of guidelines that suggested limited clinical utility of ITC detection, routine CK-IHC (rCK-IHC) staining was discontinued at our institution. We studied the rate and clinical utility of ITC detection before and after the discontinuation of rCK-IHC. **Patients and Methods:** We retrospectively reviewed 2 cohorts of 250 consecutive early stage invasive breast cancer (IBC) patients with sentinel lymph node biopsies (SLNBs) in 2010 to 2011 (rCK-IHC) and 2015 to 2016 (selective CK-IHC [sCK-IHC]). Variables abstracted included: tumor histology, tumor size, grade, lymphatic-vascular invasion, hormone receptor expression, HER2 status, and nodal status including ITCs. All cases from the 2015 to 2016 cohort for which sCK-IHC was performed underwent pathology review. A clinical review of treatment decision effect and cost analysis was undertaken. Data were analyzed using descriptive statistics and Fisher exact test. **Results:** In the rCK-IHC cohort, all 250 cases underwent CK-IHC staining versus 57 cases in the sCK-IHC cohort. There were 23 ITC cases observed in the rCK-IHC cohort compared with 11 in the sCK-IHC cohort ( $P = .049$ ). Excluding lobular carcinomas, 19 ITC cases were observed with rCK-IHC versus 7 with sCK-IHC ( $P = .02$ ). ITC detection did not affect adjuvant treatment decision-making and resulted in savings of at least Can\$8000. **Conclusion:** Selective rather than routine use of CK-IHC staining for SLNB evaluation in early-stage IBC results in decreased ITC detection without affecting treatment decisions and leads to cost savings.

*Clinical Breast Cancer*, Vol. 19, No. 4, 286-91 © 2019 Elsevier Inc. All rights reserved.

**Keywords:** Adjuvant therapy decision, Cost savings, Cytokeratin immunohistochemistry, Isolated tumor cells, Sentinel lymph node biopsy

## Introduction

Sentinel lymph node (SLN) biopsy (B) has been the standard of care in the surgical management of clinically node-negative invasive breast cancer for at least 2 decades.<sup>1,2</sup> In attempts to reliably exclude

metastases in sentinel nodes and accurately determine the pathologic nodal (pN) stage, pathologists might examine multiple levels and perform cytokeratin immunohistochemistry (CK-IHC).<sup>3</sup> Such practices lead to increased detection of small-volume occult

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Submitted: Jan 25, 2019; Revised: Feb 27, 2019; Accepted: Mar 1, 2019; Epub: Mar 11, 2019

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**Table 1** Pathologic Characteristics of Routine CK-IHC and Selective CK-IHC Cohorts

Tumor Cohort	2010-2011 (n = 250)	2015-2016 (n = 250)	Combined (n = 500)
<b>Tumor Type</b>			
ILC, n (%)	27 (10.8)	27 (10.8)	54 (10.8)
IDC and mixed IDC/ILC, n (%)	216 (86.4)	214 (85.6)	430 (86)
Other, n (%)	7 (2.8)	9 (3.6)	16 (3.2)
Median Tumor Size (Range), mm	15 (1.2-70)	15 (1.0-80)	15 (1.0-80)
<b>Tumor Grade, n (%)</b>			
1	50 (20)	65 (26)	115 (23)
2	134 (53.6)	107 (42.8)	241 (48.2)
3	66 (26.4)	78 (31.2)	144 (28.8)
LVI Positivity, n (%)	72 (28.8)	88 (35.2)	160 (32)
ER Positivity, n (%)	224 (89.6)	214 (85.6)	438 (87.6)
PR Positivity, n (%)	223 (88.8)	187 (74.8)	410 (82)
HER2 Positivity, n (%)	19 (7.6)	26 (10.4)	45 (9)

Abbreviations: CK-IHC = cytokeratin immunohistochemistry; ER = estrogen receptor; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; LVI = lymphovascular invasion; PR = progesterone receptor.

metastases, including isolated tumor cells (ITCs)/cell clusters, which are currently staged as pN0(i+).<sup>4-8</sup>

Although it has become clear that patients with limited nodal disease do not benefit from axillary lymph node dissection (ALND), there remains some uncertainty regarding the long-term prognostic significance of ITCs, their effect on clinical decision-making, and the best approach for handling SLNB in the pathology laboratory.<sup>6,7,9-16</sup> In 2010, the American Joint Committee on Cancer (AJCC) recommended histopathologic examination of 2-mm sections of SLNs without routine use of immunohistochemistry (IHC).<sup>2</sup> At our center, routine CK-IHC (rCK-IHC) was used in the examination of SLNBs until 2013, at which time it was discontinued in accordance with revised guidelines and used only selectively (selective CK-IHC [sCK-IHC]), at the discretion of the pathologist.

We evaluated the effect of eliminating rCK-IHC on the incidence of ITCs in early stage breast cancer at our institution. A laboratory budget analysis of the practice change was undertaken and a review of the effect of ITC detection on adjuvant treatment decision-making was performed.

## Patients and Methods

The study was conducted at the Nova Scotia Health Authority, Halifax, Nova Scotia, and approval was obtained from the institutional research ethics board. The study population (n = 500)

included 2 consecutive cohorts (2010-2011 and 2015-2016) of 250 patients who had breast surgery and SLNB for a primary invasive breast cancer and whose pathology information was available. Those who underwent completion ALND and those who received neoadjuvant chemotherapy were excluded.

Sentinel lymph node biopsies were processed as follows: lymph nodes were fixed in 10% buffered formalin for a minimum of 24 hours and sectioned at 2-mm intervals or less. The tissue was routinely processed, paraffin embedded, and 5 spaced step levels were cut on each tissue block (4- $\mu$ m sections) at intervals of 150  $\mu$ m. Three hematoxylin and eosin (H&E) stains were performed (levels 1, 3, and 5). One blank slide (level 2) on each block was routinely stained with CK-IHC (Dako AE1/AE3; Santa Clara, CA) as per manufacturer's protocol, in 2010 to 2011 (rCK-IHC cohort) only.<sup>17</sup> In 2015 to 2016 (sCK-IHC cohort), CK-IHC staining was performed using the same method but included cases at the pathologists' discretion and all classic lobular carcinomas.

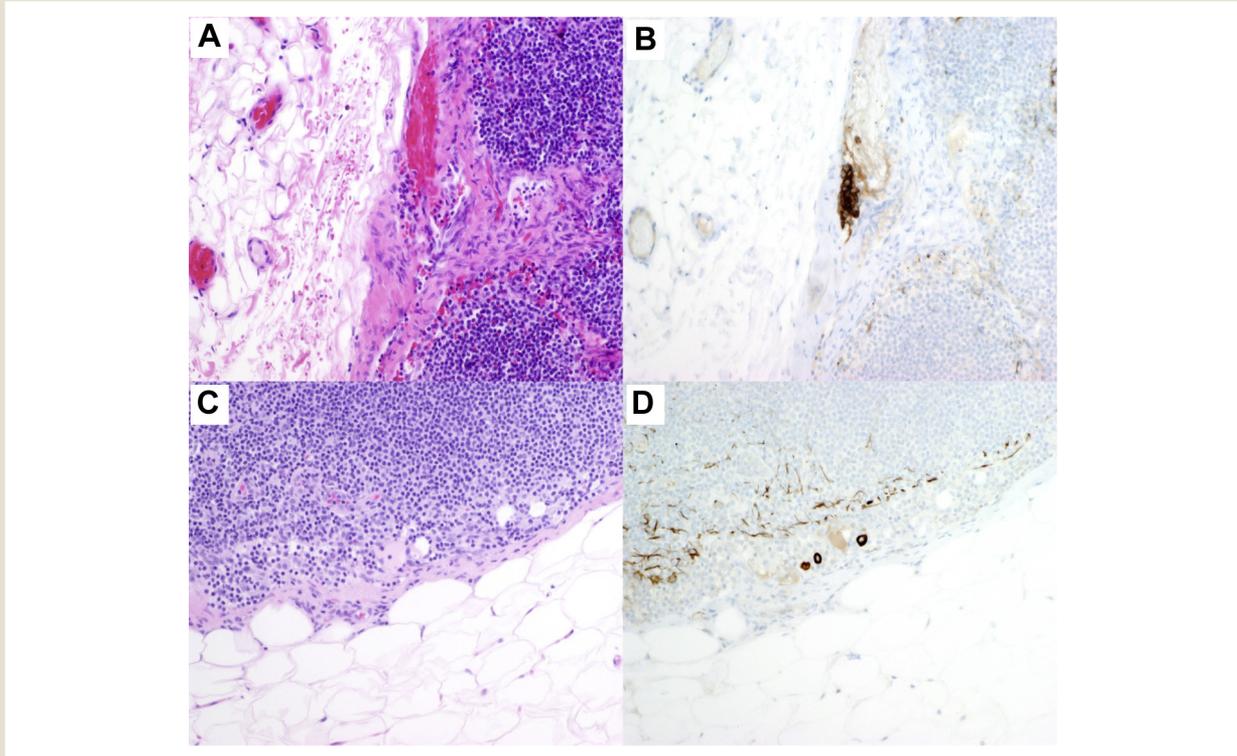
The following information was retrieved from the pathology database for each case: tumor type, tumor size, Nottingham grade, presence of lymphatic-vascular invasion (LVI), estrogen receptor (ER), progesterone receptor (PR), and HER2 status, number of SLNs examined, and nodal status. In the second cohort, any sCK-IHC staining performed on SLNs was recorded. The slides and pathology reports from this subset were retrospectively reviewed by

**Table 2** Rates of Lymph Node Metastases in Routine CK-IHC and Selective CK-IHC Cohorts<sup>a</sup>

	2010-2011 (n = 250)	2015-2016 (n = 250)	P
CK-IHC Staining, n (%)	250 (100)	57 (22.8)	<.0001
ITC Detection Rate, n (%)	23 (9.2)	11 (4.4)	.049
ITC Detection Rate Excluding ILC Cases, n (%)	19 (8.5)	7 (3.1)	.02
Macro-/Micrometastases, n (%)	52 (20.8)	65 (26.0)	.2

Abbreviations: CK-IHC = cytokeratin immunohistochemistry; ILC = invasive lobular carcinoma.  
<sup>a</sup>With routine CK-IHC staining in 2010 and without routine CK-IHC staining in 2015.

**Figure 1** Detection of Breast Cancer Isolated Tumor Cell Clusters in Sentinel Lymph Nodes for Invasive Ductal Carcinoma (A and B) and Invasive Lobular Carcinoma (C and D). Cytokeratin Immunohistochemistry (B and D) Highlights Isolated Tumor Cells That Are Not Readily Identifiable on (A and C) Routine Hematoxylin and Eosin-Stained Sections (Magnification ×200)



2 of the authors (G.C.B., R.D.) to determine reason for ordering CK-IHC. Reasons were classified into 3 groups: lobular carcinoma/lobular features, tumor characteristics (extensive LVI, inconspicuous cells), and nodal characteristics (atypical cells noted on H&E, crush artifact).

A chart review for all ITC-positive cases was performed by a medical oncologist (D.R.) to assess the clinical significance and/or potential effect of ITC detection on adjuvant treatment decision-making in the context of all other standard clinical-pathologic variables.

A cost analysis using 2015 costs for the cytokeratin antibody, slides, reagents, and technologist time for each CK-IHC ordered was performed, assuming only 1 CK-IHC assessment per sentinel node. The cost per node was then extrapolated to the total number of sentinel nodes evaluated to determine annual cost in 2010 to 2011 and 2015 to 2016. Descriptive statistics and Fisher exact test were used to describe differences between the 2 cohorts.

**Results**

The pathologic features of the 2 cohorts are shown in Table 1. There was an expected distribution of tumor types, grade, and size in both cohorts. Each group had 27 cases (10.8%) of invasive lobular carcinoma (ILC). In 2010, there were 4 mucinous, 1 tubular, and 2 medullary carcinomas. In 2015, there were 6 tubular, 1 secretory, 1 metaplastic, and 1 mucinous carcinoma. The

remainder were invasive ductal carcinoma (IDC), no special type or mixed invasive ductal and lobular carcinoma.

The results of SLNB evaluation for the 2 cohorts are summarized in Table 2. In the rCK-IHC cohort all 250 cases had CK-IHC performed with ITCs detected in 23 (9.2%) cases. In the sCK-IHC cohort, CK-IHC was performed on 57 cases, with ITC detection in 11 (4.4%) cases. Upon excluding cases of lobular carcinoma for which CK-IHC staining continues to be routine practice, ITCs were detected in 8.5% compared with 3.1% of cases in the routine and sCK-IHC cohorts, respectively ( $P = .02$ ). Only 1 case in the later cohort had ITC detected and reported on H&E slides alone. Figure 1 shows H&E and CK-IHC-stained SLN

**Table 3** Pathology Review of Cases With Selective CK-IHC in 2015/2016

Reason for IHC	Number	Number With ITC
ILC	22	4
Other Tumor Characteristics	12	3
Nodal Characteristics	22	3
Total	56	10

Abbreviations: CK-IHC = cytokeratin immunohistochemistry; IHC = immunohistochemistry; ILC = invasive lobular carcinoma; ITC = isolated tumor cells.

**Table 4** Summary of Cases With ITCs

Tumor Characteristics	2010-2011 (n = 23)	2015-2016 (n = 11)	Combined (n = 34)
Average Tumor Size, mm	24.8	26.9	25.9
<b>Tumor Grade, n (%)</b>			
1	3 (13.0)	2 (18.2)	5 (14.7)
2	14 (60.8)	5 (45.5)	19 (55.9)
3	6 (26.1)	4 (36.4)	10 (29.4)
LVI Positivity, n (%)	10 (43.5)	5 (45.5)	15 (44.1)
ER Positivity, n (%)	20 (87.0)	11 (100)	31 (91.2)
PR Positivity, n (%)	19 (82.6)	10 (90.9)	29 (85.3)
HER2 Positivity, n (%)	4 (17.4)	1 (9.1)	5 (14.7)

Abbreviations: ER = estrogen receptor; ITCs = isolated tumor cells; LVI = lymphovascular invasion; PR = progesterone receptor.

sections with ITC in representative cases of IDC and ILC, respectively, neither of which were identified on H&E alone.

The 57 cases from the sCK-IHC cohort underwent pathology review (Table 3). Of the 11 cases positive for ITC in this cohort, CK-IHC was performed on 10 for reasons of lobular morphology (n = 4), extensive LVI (n = 3), and cells indicative of cancer in the node on H&E (n = 3). One case reported ITC without the adjunctive use of CK-IHC. Upon review, ITCs could not be seen in H&E sections in 4 of the 11 cases (1 ILC, 3 IDC) and it was the selective use of CK-IHC that allowed the detection of these occult tumor cells (Figure 1).

Table 4 shows a summary the tumor characteristics of the 34 total ITC cases across both cohorts. In our sample, the presence of ITCs appeared to be associated with larger tumor size (26 mm vs. 15 mm for all cases) and higher rates of LVI (44% vs. 32%). A chart review was performed on all cases with ITC to assess the effect of ITC detection on adjuvant treatment decision-making with results presented in Table 5. Two cases had no information available (1 treated at an outside institution and 1 lost to follow-up). Among the 32 cases with available information, 10 received adjuvant endocrine therapy only, and 20 received adjuvant chemotherapy with or without subsequent endocrine therapy. Factors influencing treatment decisions included tumor size, tumor grade, patient age,

biomarker status, and presence of micro-/ macro-/ distant metastasis. In 2 cases treatment decisions were influenced by patient preference. All patients with follow-up information, except for the one case with synchronous distant metastases, received either local or local-regional adjuvant radiation therapy depending on whether the nodes were negative or positive for microscopic disease as per American Society of Clinical Oncology breast radiation therapy guidelines.

The cost of a single CK-IHC test in 2015 was Can\$19.16, which includes slides, reagents, and technologist time. In 2010 to 2011, 583 CK-IHC tests were performed on SLNs whereas in 2015 to 2016, 169 CK-IHC tests were performed. Assuming that 1 test was ordered per SLN, the cost to the laboratory was \$11,170 in 2010 to 2011 and \$3,238 in 2015 to 2016, resulting in an estimated savings of \$7,932 in 2015 to 2016.

## Discussion

The AJCC guidelines for breast cancer staging, along with others, state that the priority for the histologic evaluation of SLNBs is to identify all macrometastases (metastases larger than 2.0 mm). An H&E-stained section of a thinly sliced SLN (no thicker than 2-mm slices), which has been submitted completely is sufficient for the routine evaluation of SLNBs.<sup>1,6,9,18</sup> At our institution, routine use

**Table 5** Clinical Review of All Cases From Both Cohorts in Which ITCs Were Detected

Adjuvant Treatment Modality	Major Factor Driving Treatment Decision	Number of Cases (n = 34) <sup>a</sup>
Hormonal Therapy Only		10
Additional Chemotherapy		22
	Tumor size	8
	Grade/LVI	2
	HER2 <sup>+</sup>	4
	Triple negative	2
	Micro-/macrometastases	2
	Synchronous distant metastases	1
	Patient age	1
	Patient preference	2

All patients received local or local-regional radiation therapy according to American Society of Clinical Oncology guidelines. Abbreviations: ITCs = isolated tumor cells; LVI = lymphovascular invasion.

<sup>a</sup>One patient did not receive treatment at Queen Elizabeth II Health Sciences Centre and 1 patient chart was not available.

## Isolated Tumor Cells and Breast Cancer

of CK-IHC in the evaluation of SLNB was discontinued in 2013, but it remains current practice to obtain 3 spaced levels on each 2-mm section of node. We found that the discontinuation of rCK-IHC resulted in a 5% reduction in the detection of ITCs (9.2% ITC rate in 2010-2011 vs. 4.4% in 2015-2016;  $P = .049$ ).

Our current practice (and that of 2015-2016) of sCK-IHC in early stage breast cancer includes all cases of classic lobular carcinoma, and any tumor or nodal characteristics that are believed by the pathologists to warrant further investigation with IHC. These include extensive LVI, tumor cells that are small and non-gland-forming, crush artifact in the node, and cells of uncertain origin identified in the lymph node on H&E. This selective use of CK-IHC resulted in 57 tests in 2015 to 2016, 10 of which had ITCs (detection rate of 17.5%). Because the SLNs from all classic lobular carcinomas from both cohorts were routinely subjected to CK-IHC, an analysis excluding these cases resulted in a more significant decrease in ITC detection rate between the rCK-IHC and sCK-IHC cohorts (8.5% vs. 3.1%;  $P = .02$ ). Only 1 case with ITCs from the sCK-IHC cohort was reported on the basis of H&E alone. This suggests that in certain circumstances, including cases with lobular morphology, selective use of CK-IHC is useful in identifying patients with SLNs more likely to harbor occult metastases. It is well known that metastatic classic lobular carcinoma is notoriously difficult to identify on H&E sections, even in the setting of macrometastasis in which the tumor cells might exhibit minimal nuclear atypia and a dispersed pattern of growth.<sup>1</sup> For this reason, others also advocate for routine use of CK-IHC in these tumors.<sup>1,16,19</sup> Of note, locally advanced breast cancers in which neoadjuvant chemotherapy was administered were excluded from this study. In our laboratory, CK-IHC is used liberally in such cases, because the detection of even a single ITC might have management and prognostic implications for these patients.

There are a plethora of studies contributing to the debate about the prognostic significance of ITCs, and others that have established ALND as overtreatment in the setting of ITCs.<sup>6,7,20,21</sup> We have taken a unique approach by comparing different pathologic methods of evaluating SLNBs over 2 time periods. Our study confirms previous reports and the intuitive notion that the use of CK-IHC increases the detection of ITCs.<sup>6,7,16</sup> Retrospective analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-32 trial data showed a 15.9% increase in detection of occult metastases (ITCs and micrometastases) with the use of IHC and 2 deeper levels in the pathologic assessment of SLNs with 72% of these occult findings being ITCs.<sup>6</sup> The prevalence of occult metastases identified by the American College of Surgeons Oncology Group (ACOSOG) Z0010 investigators in their prospective observational study was 10.5% with the use of keratin IHC on one deeper level although size of the metastases was not reported.<sup>7</sup> Occult metastases were defined as ITCs and micrometastases not identified on the initial histologic assessment of 1 routinely stained section of a thinly sliced SLN.<sup>6</sup>

In attempts to provide meaningful information to treating clinicians and patients, pathologists strive for accuracy and clinical relevance in their reports. The eighth edition of the AJCC Cancer Staging Manual and recently updated the College of American Pathologists invasive breast cancer checklist both maintain the pN0(i+) category in the nodal staging of invasive breast cancers.<sup>8,22</sup>

As such, pathologists continue to feel the need to stage the nodes as precisely as possible, particularly in the setting of uncertain prognostic significance represented by ITCs. Laboratory protocols for handling SLNBs might vary as a result of confusion in interpreting guidelines, that on the one hand suggest reporting ITCs if found, and on the other state that IHC and multiple levels are not recommended. Although we missed some nodes with occult ITCs, our cost-saving and practical approach of sCK-IHC enables recognition of many more cases harboring ITC than H&E alone. The conservative estimate of \$8000 savings per year is on the basis of the assumption that 1 CK-IHC was ordered per SLN. In reality, a single sentinel node sectioned at 2 mm often requires more than 1 tissue block, resulting in 2 or more slides on which to perform IHC and a greater cost saving when eliminated.<sup>23</sup>

We observed numerically more adverse prognostic factors in the ITC compared with the non-ITC cases such as larger median tumor size, higher tumor grade, and more frequent presence of LVI, each of which would have factored into treatment decisions. Moreover, these cases showed higher rates of ER, PR, and HER2 positivity compared with the non-ITC cases. The immunophenotype of each case plays a dominant role in adjuvant therapy decision-making and, although not all adverse parameters, would trigger the use of endocrine or biologic therapy irrespective of ITC status further diluting any independent contribution of ITC detection to adjuvant therapy decision-making. Importantly, Oncotype DX testing was not yet funded over the time frame of our study and would be expected to further weaken the clinical utility of ITC detection.

Our observation that ITC detection did not affect adjuvant treatment decision-making is similar to that reported in other studies. The NSABP B32 trial showed that identification of minimal nodal disease (MND) correlated significantly with tumor size, younger patient age, higher tumor grade, and adjuvant systemic therapy.<sup>6</sup> The NSABP B32 trial as well as the ACOSOG Z0010 trial have reported that adjuvant treatment was recommended irrespective of the detection of MND, implying that MND is associated with other factors that more strongly influence adjuvant treatment approaches and therefore has limited clinical utility.<sup>6,7</sup> Although these studies showed no prognostic relevance of MND with respect to overall and disease-free survival, it would be difficult to tease out the effect of untreated MND because all patients would have received adjuvant treatment. It has been suggested that with effective adjuvant therapies in routine clinical practice, there is limited utility in exploring the potential independent contribution of ITCs to survival outcomes.<sup>7</sup>

Our study is limited by its retrospective design and the use of convenience cohorts.<sup>24</sup> Although the cases were not selected randomly, all consecutive cases that met inclusion criteria were assessed and therefore were representative of the time frames selected. We recognize that the protocol used during the study period of obtaining 3 H&E levels on each SLNB is not a standard practice, but continues to be used by several laboratories. This protocol did not change between the 2 cohorts and therefore did not contribute to the differences noted in our study. Although not specifically addressed by our data, we do not believe that obtaining additional levels significantly increases the detection of metastases and have recently changed our protocol to 1 full-face H&E level per SLNB tissue block.

## Conclusion

We have shown that the discontinuation of routine use of CK-IHC in the evaluation of SLNB results in a decrease in ITC detection rate. Until consensus is reached regarding the prognostic significance, clinical implications, and reporting requirements for staging MND, we believe that selective use of CK-IHC in cases more likely to harbor occult metastases is a reasonable approach in the pathologic evaluation of SLNBs for early stage invasive breast carcinoma. Adoption of this approach would not be expected to adversely affect adjuvant therapy decision-making and would lead to cost savings for laboratories.

## Clinical Practice Points

- Performing rCK-IHC on sentinel lymph nodes in early stage invasive breast cancer leads to frequent identification of ITCs, the clinical significance of which remains unclear.
- We reviewed 2 cohorts (n = 250 each) of consecutive early stage breast cancer specimens with sentinel node biopsies with one cohort having rCK-IHC performed and the other having only sCK-IHC performed.
- Excluding lobular carcinomas, we observed a significant reduction in ITC detection rate with 19 ITC cases observed with rCK-IHC vs. 7 with sCK-IHC ( $P = .02$ ).
- Isolated tumor cell detection did not affect adjuvant therapy decision-making by the medical oncology department and the use of selective rather than routine testing resulted in a cost savings for the pathology laboratory.
- Future research should replicate our findings to further support the elimination of reflex CK-IHC testing of sentinel nodes, consistent with the lack of prognostic and treatment decision information it provides.
- Our budget impact analysis was very conservative. Future work in other jurisdictions might lead to more cost savings leading to more effective use of resources.

## Disclosure

The authors have stated that they have no conflicts of interest.

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