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Pathological confirmation of primary lung cancer following breast cancer

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ABSTRACT

Purpose: Studies have shown that women who survive breast cancer have an increased risk of a future primary lung cancer, though many are based only on data recorded in tumor registries and none have conducted pathological confirmation. Previous studies and future use of large registries may be affected by misdiagnosis.
Methods: Pathological analysis was conducted on tumors from 110 women with breast cancer followed by lung cancer using morphology, Estrogen Receptor-alpha (ER), and Thyroid Transcription Factor-1 (TTF1). We developed an algorithm to classify lung tumors as unlikely lung cancer (score = 1) to likely lung cancer (score = 5).
Results: Mean time to diagnosis of lung cancer after breast cancer was 13 years. 76% of breast tumors and 20% of lung tumors were positive for ER and 51% of lung tumors were positive for TTF-1. 86% of the lung tumors were probable primaries, 7% were probable metastases from the breast, and 7% were of undetermined status. 70% of probable metastases had a latency of longer than 10 years.
Conclusion: Prior studies identifying the association of breast cancer and breast cancer treatments with lung cancer are likely to reflect true associations not confounded by misdiagnosis, as evidenced by the low rate of misclassification detected in this study. Analysis of the years of diagnosis suggests that latency may not be an accurate criterion for assignment of primary status, which could be significant in a clinical setting. These data may also benefit future retrospective studies using large registries.

Through earlier detection and improved treatments, the number of women surviving breast cancer is increasing, as is survival time [1,2]. Mortality rates among women with breast cancer have declined over the last decades and 5-year relative survival has risen from 75% to 90% [3]. This has led to an increasing population of women at risk for a second cancer [4–7]. As women live longer, there is an increased risk of developing a new primary cancer related to therapy, shared risk factors, genetic predisposition, or a combination of these [8,9]. Many studies have shown that women who have had breast cancer are subsequently at increased risk for a new primary lung cancer, with risks estimates ranging from 1.2 to 4.5 [10–17]. This risk is increased in women who have been treated with radiotherapy and there is an interaction for increased risk from smoking and radiotherapy [18–21]. Using the Swedish Cancer Registry, established in 1958, our group has previously reported that the standardized incidence ratio (SIR) overall for developing lung cancer following breast cancer is 1.27 within 10 years, 1.66 for 10–14 years, 2.0 for 15–19 years, and 2.53 for greater than 20 years after breast cancer diagnosis. Further, concordance of breast and lung cancer on the same side (ipsilateral; inferring a radiotherapy-related secondary lung cancer) had a relative risk of 2.0, while the SIR for contralateral cancers was 0.8 [16].

Most studies of breast and lung cancer within the same woman are conducted using tumor registries and these preclude pathological confirmation of the cancers. None to date have examined pathological tissue for confirmation. Development of lung metastases has been reported to occur in 10–20% of female breast cancer patients, making confirmation of primary lung cancer status important in order to provide appropriate treatment for the specific tumor. Several studies have included a review of medical records but not actual tumors [12,22]. Even with access to current complete medical records, there were still cases that could not be definitively categorized, emphasizing the challenge of verifying second primary lung tumors in registry studies. If the lung tumors were misclassified as primary lung cancer, rather than metastatic breast cancer, then an overestimation of secondary lung cancer risk could result.

As part of a study investigating biomarkers of susceptibility to lung cancer in women with breast cancer, we assessed the pathologic incidence for secondary lung cancers by conducting an in-depth, joint review with pathologists from Georgetown

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University (GU) and the Karolinska Institutet (KI) in Stockholm, Sweden. This review used patient records, morphological review, and immunohistochemistry (IHC). Use of morphology and patient information alone may not correctly assign tumors, but the use of IHC improves classification [23–28]. Comparisons of breast and lung cancer IHC staining also are helpful as primary tumors and their metastases share similar marker profiles [23,29–32]. We conducted a review using tumor tissue and TTF-1 and ER immunohistochemistry stains to determine whether there is misclassification of lung tumors following breast cancer in a population of Swedish women.

1. Methods

1.1. Study design

Cases from Stockholm were identified from the Swedish Cancer Registry (SCR), where a unique identification number assigned to all residents of Sweden identifies individuals in national registries. The SCR began in 1958 and records the mandatory reports of cancer from pathologists and physicians, so it includes 96% of all cancers in Sweden [16]. Data in this registry includes diagnosis, date of diagnosis, mode of detection of tumor, department and hospital where the tumor was diagnosed, carcinoma *in situ*, date of death, and underlying cause of death. Cases were women with an initial diagnosis of breast cancer of any histological type (including DCIS) and a later primary lung cancer. Also, cases had to have sufficient archived tumor tissue for analysis. Lung cancer diagnosis had to be at least 1 year after the breast cancer diagnosis. Cases were excluded if they were diagnosed only by autopsy or X-ray or if they had been previously diagnosed with any cancer. Of the 180 cases identified through the Swedish Cancer Registry, 70 were excluded for lack of tissue, resulting in a total of 110 cases analyzed. There were three cases included in the study with tumor tissue but missing clinical data.

1.2. Tissue processing

Researchers in Stockholm collected tumor tissue blocks and tissue sections were placed on Silane-prep slides treated with DEPC in thicknesses of 5, 20, or 50 microns. Slides were baked at 60 °C for 2 h. The slides were then sent to GU by express shipping. Hematoxylin and eosin (H&E) slides were prepared either at KI before shipping or by the histopathology shared resource facility at GU.

1.3. Immunohistochemistry

IHC was done to detect ER protein expression in breast and lung tumors and TTF-1 in lung tumors. Five micron slides were deparaffinized with xylene and ethanol. Antigen retrieval was achieved by a Citra-Plus Buffer microwave protocol and slides were treated with peroxide and protein block (Biogenex; San Ramon, CA). The ER antibody (Clone F-10, Santa Cruz Biotechnology; Santa Cruz, CA) was used for 60 min at room temperature. The slides were then treated with a biotinylated second antibody link, enzyme-conjugated label, and Chromagen DAB (Biogenex). Slides were analyzed according to the Allred system, where intensity of staining is recorded as a score of 0–3 (0 is no staining, 1 is weak, 2 is moderate, and 3 is strong) and proportion of cells stained is recorded as a score of 0–5 (0 is no staining, 1 is 20%, 2 is 40%, 3 is 60%, 4 is 80%, and 5 is 100%) [33]. These scores are added together for a total of 0 or 2–8; scores of 2 or higher were considered positive for ER expression. Immunohistochemistry with the TTF-1 antibody (Clone 8G7G3/1, Invitrogen; Carlsbad, CA) was conducted using a similar protocol. Tissues were positive for TTF-1 if 10% or more of the cells were stained.

Table 1
Description of the five score categories used to classify cases with breast and lung cancer in the joint pathology review.

Criteria for pathology review scores	
1	Definitely metastatic: significantly similar morphology, TTF-1 negative.
2	Probably metastatic: similar morphology, TTF-1 negative.
3	Undecided: unclear morphology comparison, poorly preserved tissue.
4	Probably primary: different morphology or a weak TTF-1 stain with similar morphology.
5	Definitely primary: significantly different morphology and/or positiveTTF-1 stain.

1.4. Pathology review

Two pathologists collaborated to confirm histology, one with expertise in breast cancer (BS) and one in lung cancer (AH). First, the initial diagnosis of breast cancer was confirmed morphologically and by ER staining. An algorithm was established to classify lung tumors based on morphology, ER and TTF-1 lung tumor staining. A scoring system was used that ranged from 1 to 5 for the lung tumors, indicating a definite diagnosis of breast cancer metastasis (score of 1) to a definite diagnosis of a primary lung cancer (score of 5) (Table 1). Positive TTF-1 staining was considered indicative of primary lung tumor status. A case with an ER positive, TTF-1 negative lung tumor, an ER positive matched breast tumor, and a finding of no significant morphologic differences was considered a probable metastasis. Different ER staining for the breast and lung tumors from an individual was considered indicative of a probable lung primary. All tumors were initially reviewed independently. When the initial diagnoses differed, the pathologists reviewed these cases together, following repeat immunohistochemical staining using newly sectioned tumor blocks.

2. Results

2.1. Clinical characteristics

Cases ($n = 110$) were women who had breast cancer and developed lung cancer at least 1 year after breast cancer diagnosis and whose original breast tumor was diagnosed between 1958 and 2000. Their clinical characteristics are shown in Table 2. Mean age at breast cancer diagnosis for all cases was 57 (+/−13) and 68 (+/−11) for lung cancer. Availability of information on menopausal status in medical records was inconsistent, so age greater or less than 50 was used as a surrogate for menopausal status; 35% of cases were premenopausal at the time of breast cancer diagnosis. Mean time to diagnosis of lung cancer after breast cancer for all cases was 13 years (range 1–35). About 48% of lung cancers were ipsilateral (occurring on the same side in the lung as the breast cancer), 38% were contralateral (occurring on the opposite side from the breast cancer), and 14% were of unrecorded location.

2.2. Pathology verification of cancer diagnosis

Upon the initial morphology review, 61 cases were given concordant diagnoses by the two pathologists. For cases where the two pathologists initially disagreed on the diagnosis, an algorithm was applied for establishing a final diagnosis. The first step in the algorithm was to examine morphology and TTF-1 staining in the 49 lung tumors that had discordant diagnoses. There were 7 squamous cell cancers in the review, which were subsequently considered primary lung cancers based solely on the squamous histology (score = 5). For the non-squamous cell cancers, there were 25 adenocarcinomas and 17 tumors with mixed histology or other non-small cell lung cancer. For these, 50% of the adenocarcinomas were positive for TTF-1 and so were considered to be primary lung