An assessment of diagnostic performance of a filter-based antibody-independent peripheral blood circulating tumour cell capture paired with cytomorphologic criteria for the diagnosis of cancer

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ABSTRACT

Objectives: Circulating tumour cells (CTCs) are reported to be predictive for prognosis and response to treatment in advanced lung cancer. However, the clinical utility of the CTCs detection remains unknown for early stage lung cancer as the number of CTCs is reported as low, providing challenges in identification. We have evaluated diagnostic performance of filtration-based technology using cytomorphologic criteria in patients undergoing surgery for lung cancer.

Material and methods: We processed blood from 76 patients undergoing surgery for known or suspected lung cancer using ScreenCell® Cyto filter devices. Captured cells were stained using haematoxylin and eosin and independently assessed by two pathologists for the presence of atypical cells suspicious for cancer. Diagnostic performance was evaluated against pathologist reported diagnoses of cancer from surgically obtained specimens.

Results: Cancer was diagnosed in 57 patients (77.0%), including 32 with primary lung cancer (56.1%). The proportion of patients with early stage primary lung cancer in which CTCs were identified was 18 and 21 (56.3% and 65.6%, respectively) as reported by two pathologists. The agreement between the pathologists was 77.0% corresponding to a kappa-statistic of 53.7% indicating moderate agreement. No significant differences were found for the percentage of CTCs for primary and metastatic cancer as well as for cancer stages. On sensitivity weighted analysis, a sensitivity and specificity were 71.9% (95% CI 60.5–83.0) and 52.9% (95% CI 31.1–77.0), respectively. On specificity weighted analysis, a sensitivity and specificity were 50.9% (95% CI 39.3–64.4) and 82.4% (60.4–96.2), respectively.

Conclusion: The performance of the tested filter-based antibody-independent technology to capture CTCs using standard cytomorphologic criteria provides the potential of a diagnostic blood test for lung cancer.

1. Introduction

Lung cancer is the leading cause of cancer death worldwide with the lowest survival rate compared to other cancer types [1]. By the time symptoms develop, patients are usually in the advanced stages of disease. Currently, efforts are directed at early diagnosis where more treatment options are available, reinforced by results of the National Lung Cancer Screening Trials that reported a 20% improvement in cancer related survival in participants randomised to low dose CT, a more sensitive method (compared to chest X-ray) for early cancer detection [2].

At our institution, we have an established translational science research programme [3,4] and questions posed include how early do cancers start metastasising and how soon can this be observed in the blood of patients? The most straightforward way would be to use current established methods using circulating epithelial cell counts [5] but cells need to be identifiable in suing a methodology that allows robust diagnosis using accepted

cytological criteria for malignancy. Moreover CTC load is expected to be very low in early cancer and current technology is limited by ability to accurately identify and extract suspicious cells for conventional clinical processing. It has been demonstrated that filtration-based antibody-independent methods for CTC detection outperform EpCAM-antibody-based approach in terms of the number of the cells detected [6]. This allows considering filtration-based approaches as method of choice in routine clinical practice. However, their performance in standard clinical settings needs to be identified.

Recently designed size-based capture of CTCs by ScreenCell® microporous filters may represent a clinically relevant approach due to its simplicity and such the advantages as its ability to preserve cell morphology and cell characterisation [7]. Similar investigations have been undertaken by other groups [8], but there is currently a lack of consensus of the diagnostic criteria used, and some of the criteria applied are not widely accepted (e.g. E-cadherin or vimentin expression) [9].

We sought to evaluate the presence of circulating lung cancer cells using conventional cytomorphologic criteria assessed through haematoxylin and eosin staining and light microscopy in thoracic surgical pathology and define the test performance against an underlying diagnosis of cancer in patients with known or suspected lung cancer.

2. Material and methods

The study was performed in Royal Brompton and Harefield NHS Foundation Trust. The project was approved under the auspices of National Institute of Health Biomedical Research Unit Advanced Disease Biobank (NRES 10/H0504/9).

Eighty one patients who underwent surgery for suspected lung cancer were enrolled in the study and donated blood. Due to artefacts of blood processing, seven specimens were excluded, so the final cohort comprised 74 patients. The baseline characteristics of the patients are summarised in Table 1.

Peripheral blood was collected in 9 ml EDTA vacutainers prior to surgery and processed within 4 h upon acquisition. ScreenCell® Cyto devices (ScreenCell, France) were used to capture circulating tumour cells according to the manufacturer's protocol. Briefly, 3 ml of blood was mixed with 4 ml of red blood cells lyses and fixation buffer provided with the kit and incubated for 8 min at room temperature. After incubation, blood was passed through the device filter to trap cells using vacuum force provided by a 9 ml vacutainer. Processed filters were subjected to standard haematoxylin and eosin staining and then reviewed by two histopathologists independently. Results were categorised into "negative" or "atypical cells suspicious for cancer" (nucleated cells with high nuclear/cytoplasmic ratios that are larger than resting lymphocytes; cells may also have irregular nuclear outline). An agreement between pathologists was measured by Cohen's kappa statistics [10].

The diagnostic performance of the blood test was also benchmarked against the reference of underlying cancer as a confirmatory diagnosis established by surgically biopsy and expressed

Table 1
Patient demographics.

Variable	Value	Percent (%)
Total	74	100.0
Mean age (±SD)	61.4 ± 14.5	–
Males/females	44/30	59.5/40.5
Pathology		
Primary lung cancer	32	43.2
Adenocarcinoma	17	53.1
Squamous cell carcinoma	6	18.8
Carcinoid tumour	6	18.8
Large cell undifferentiated carcinoma	1	3.1
Pleiomorphic carcinoma	1	3.1
Small cell carcinoma	1	3.1
Metastatic cancer[a]	21	28.4
Other cancer[b]	4	5.4
Benign	17	23.0
Staging[c]		
I–II	27	53.8
III–IV	23	46.2

[a] Non-lung origin; the majority was colorectal adenocarcinoma ($n = 7$, 31.8%); other types include carcinomas of bladder or larynx, large bowel, breast and prostate origin, melanoma, haemangiothelioma, myxofibrosarcoma, liposarcoma, teratoma, osteosarcoma, chondrosarcoma, leiomyosarcoma, post-radiation chest-wall cancer; for one case, organ of origin was not reported.

[b] Ganglioneuroma, two cases of mesothelioma, and chondrosarcoma of chest wall.

[c] For seven cancer patients staging was not reported.

as sensitivity, specificity, positive and negative predictive values. Secondary analyses were also performed by means of a sensitivity weighted analysis (at least one pathologist classifies the results as suspicious for cancer) and specificity weighted analysis (both pathologists classify the results as suspicious for cancer) were undertaken.

Prevalence of atypical cells suspicious for cancer between different groups was compared using two-sided Fisher's exact test. The significance level of $p < 0.05$ was applied. Statistical analysis was performed in R using *base*, *epicalc* and *bdpv* packages [11–13].

3. Results

From 06/03/2012 to 25/01/2013, blood specimens from 81 patients were processed. Seven filters were excluded due to artefacts of processing preventing pathology assessment and leaving 74 patients in the study (57 with a confirmatory diagnosis of cancer (77.0%) of which 32 (56.1%) were primary lung cancer). The artefacts were air bubbles under the cover slip (5), understaining (1), and a filter physical damage (1).

The results of the assessment for the presence of atypical cells suspicious for cancer are presented in Table 2.

Atypical cells suspicious for cancer (Fig. 1) were reported for 52.7% and 56.8% of patients by two pathologists, respectively. The between observer agreement was 77.0%, and corresponding Cohen's kappa-statistics was 0.537 ± 0.116 ($p < 0.001$) indicating moderate agreement according to Landis and Koch criteria [10].

Table 2
The number of cases (%) with atypical cells suspicious for cancer as established through the analysis of H&E stained filters catching atypical enlarged cells.

Group	Pathologist 1	Pathologist 2	Both pathologists	At least one pathologist
All samples, $n = 74$	39 (52.7)	42 (56.8)	32 (43.2)	49 (66.2)
Cancer samples, $n = 57$	33 (57.9)	37 (64.9)	29 (50.9)	41 (71.9)
Primary cancer, $n = 32$	18 (56.3)	21 (65.6)	16 (50.0)	23 (71.9)
Metastatic cancer, $n = 21$	12 (57.1)	13 (61.9)	10 (47.6)	15 (71.4)
Benign, $n = 17$	6 (35.3)	5 (29.4)	3 (17.6)	8 (47.1)
Early stage cancer (I–II), $n = 27$	15 (55.6)	18 (66.7)	14 (51.9)	19 (70.4)
Advanced stage cancer (III–IV), $n = 23$	15 (65.2)	16 (69.6)	13 (56.5)	18 (78.3)