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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

Maxim B. Freidin[a,b], Andee Tay[a,b], Dasha V. Freydina[a,b], Dimple Chudasama[a], Andrew G. Nicholson[a,b], Alexandra Rice[a], Vladimir Anikin[a], Eric Lim[a,b,*]

[a] Royal Brompton and Harefield NHS Foundation Trust, London, UK
[b] National Heart and Lung Institute, Imperial College London, London, UK

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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

* Corresponding author at: Imperial College London and The Academic Division of Thoracic Surgery, Royal Brompton Hospital, Sydney Street, London SW3 6NP, United Kingdom. Tel.: +44 0207 352 8591; fax: +44 0207 352 8560.
E-mail address: e.lim@rbht.nhs.uk (E. Lim).

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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 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].

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.

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)

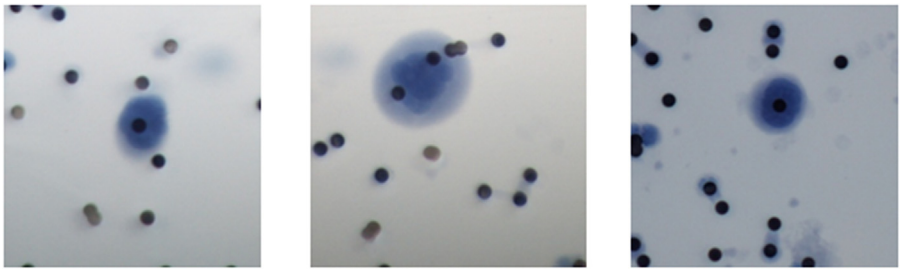

Fig. 1. Atypical cells suspicious for cancer trapped on ScreenCell® Cyto device filters (400×, H&E staining).

There were no statistically significant differences between patients with primary and metastatic cancer regarding the presence of atypical cells in peripheral blood (Table 2). There was an increased amount of the cells in advanced stage patients as compared to early stage patients (Table 2). However, these differences were not statistically significant (Fisher's exact $p = 0.569–0.999$).

The percentage of atypical cells was higher in cancer patients as compared to patients with benign lung diseases (Table 2). These differences were statistically significant for the assessments undertaken by the second pathologist and by two pathologists together ($p = 0.013$ and 0.024, respectively), but not for the assessments undertaken by the first pathologist and by at least one of the pathologists ($p = 0.165$ and 0.080, respectively).

Sensitivity, specificity, positive and negative predictive values were estimated for each pathologists independently and in sensitivity and specificity weighted manners (Table 3). Highest sensitivity was achieved when at least one pathologist reported positive findings (71.9%, 95% CI 60.5–83.0), and the highest specificity was achieved when both pathologists reported positive findings (82.4%, 95% CI 60.4–96.2). High positive predictive values (83.7–90.6%) and low negative predictive values (31.4–37.5%) were achieved in all cases.

4. Discussion

This study was designed to assess the ability to detect CTCs in peripheral blood of a prospective cohort of surgical patients suspected of having lung cancer, using ScreenCell® Cyto filter devices to isolate cells and standard cytomorphologic criteria and analysed in a clinical setting.

We were able to detect atypical cells suggestive of cancer in more than 50% of cancer patients. High specificity was achieved using weighted analysis (82.4%). Recently, we used another antibody-independent approach to detect CTCs in peripheral blood of lung cancer patients, which is based on a microfluidic biochip [4]. A high specificity (up to 100%) was achieved using this method, while sensitivity was low (up to 46%). This study as well as our current findings justifies that antibody-independent size-based filtration techniques with cytomorphologic assessment for the detection of CTCs can be used as a diagnostic tool for lung cancer.

The results of our study identified atypical cell suggestive of cancer in a high proportion (55.6–66.7%) of our patients with early stage primary lung cancer. This observation would be consistent in the seed and soil hypothesis where the cancer needs to invade the basement membrane and elude host cell immunity to be able to continue in the metastatic process, and the CTCs that we have captured represent cells from the underlying cancer "en route" to a secondary site [14]. If so, then our findings add to the increasing body of literature that supports the concept of cell based cancer detection despite no clinical evidence of secondary tumour.

We also report a number of atypical cells with cytomorphologic features suggestive of cancer identified in the cohort with "benign" disease, and we are unable to confidently determine if these represent true cancer cells in patients who may subsequently develop clinical features of cancer or simply false positive results in patients with genuinely benign disease. We plan to follow this sub-cohort carefully with time.

The results from our work suggest that we are not yet ready for clinical use. Test sensitivity is poor, and can be due to either analytic sensitivity of the method used or the genuine absence of CTCs in the blood. A diagnostic method can have 100% sensitivity but if only 2 out of 100 patient shed circulating tumour cells in the system will have a test performance sensitivity of 2%, both variables are beyond the investigators control. Specificity however can be improved. Current lack of confirmatory diagnostic ability limits the use of this method in clinical practice at individual pathologist's specificity ranging from 64.7 to 70.6%, regarded as moderate at best (but remains short of the performance required for widespread clinical adoption). With the addition of immunohistochemistry, false positive results are expected to reduce. Certainly, cell characterisation remains a challenging area in this research space, other similar studies report a between-observer agreement for cancer diagnosis as kappa of 0.64 [15], which is not far from what we observed at 0.537.

Getting this process correct has a number of important clinical implications, pre-operative identified increasing CTC load was associated with increasingly poor prognosis and shorter disease free interval [16]; a monitoring of CTC number before and after chemotherapy was shown to be of essential prognostic significance for small cell lung cancer [17]. However, our current test performance remains insufficient for clinical application and further work needs to be undertaken with cell characterisation before this can become effective tool for clinical diagnosis.

Table 3
Test performance statistics (95% CI) for the diagnosis of cancer using filter-based capture of rare atypical cells in peripheral bloodstream.

Statistic	Pathologist 1	Pathologist 2	Both pathologists[a]	At least one pathologist[b]
Sensitivity	57.9 (46.1–70.9)	64.9 (53.2–77.1)	50.9 (39.3–64.4)	71.9 (60.5–83.0)
Specificity	64.7 (42.0–85.8)	70.6 (47.8–89.7)	82.4 (60.4–96.2)	52.9 (31.1–77.0)
Negative predictive value	31.4 (23.7–42.2)	37.5 (28.9–49.0)	33.4 (27.3–41.4)	36.0 (25.2–50.9)
Positive predictive value	84.6 (75.6–91.6)	88.1 (79.6–94.1)	90.6 (79.9–96.5)	83.7 (76.6–89.7)

[a] Specificity weighted analysis.
[b] Sensitivity weighted analysis.

5. Conclusion

The results of our study suggest that an antibody-independent filter-based technology to capture CTCs coupled with standard H&E staining and light microscopy assessment has potential as a diagnostic blood test for lung cancer.

Conflict of interest statement

None declared.

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