Detection of Circulating Tumour Cells and Survival of Patients with Non-small Cell Lung Cancer

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Mortality from cancer remains high and accounts for 29% of all deaths (1). The development of metastatic disease is responsible for 90% of these deaths (2).

Circulating tumour cells (CTCs) are cancer cells of epithelial origin that are present in the peripheral blood samples of cancer patients to form a subpopulation of tumour cells which intravasate to allow lymph haematogenous dissemination in other areas of the body (3).

CTC counts in peripheral blood prior treatment can independently predict the early recurrence in patients with cancer (3, 4). Overall survival in patients with stage III and IV non-small cell lung cancer (NSCLC) was significantly longer (8.1 months vs. 4.3 months) in patients with fewer than 5 CTCs compared to those with five or more CTCs before chemotherapy (5). Clusters of CTCs, also known as circulating tumour microemboli (CTM), involve a process of collective cell migration which is important in tumour cell invasion (6, 7). CTMs have fewer apoptotic markers than CTCs, and their ability to survive is enhanced. As a result detection of CTMs is associated with a poorer prognosis (8, 9). More advanced and/or metastatic cancers express higher levels of CTCs and CTMs (10).

Multiple platforms for CTC isolation exist with varying functionalities. In this study, we used ScreenCell® (Paris, France), which is an antibody-independent device to avoid bias introduced by antibody-dependent techniques such as the CellSearch (Paris, France). The aim of our study was to examine the relationship between the number of CTCs detected by ScreenCell® and survival percentage rate in a NSCLC cohort of patients with surgically resectable lung cancer. Based on our knowledge there are no previous studies that have examined such relationship.

Patients and Methods

Patients. Twenty-three patients undergoing surgical treatment for suspected or confirmed NSCLC were recruited from February to May 2014. Patients had a confirmed diagnosis of NSCLC either pre-operatively or on an intra-operative frozen section. Ethical approval was sought prior (10/H0504/9) and consent was obtained pre-
operatively. Surgery was performed under the care of four thoracic surgeons at a tertiary thoracic centre. All operations were performed via a thoracotomy beside one, which was done via a video-assisted thoracotomy approach.

The cohort consisted of nine males and fourteen females, with a median age of 66±11 years. All patients had primary lung cancer, with a subtype of adenocarcinoma in thirteen (56.5%) and squamous cell carcinoma in ten (43.5%) patients. Early stage (I-II) was diagnosed in eighteen and late stage (III-IV) in five patients (Table I). Patients were followed up for a median time period of 31.8 months post-operatively. Survival information and cause of death was obtained by contacting the patient’s General Practitioner.

**Results**

There was no perioperative mortality and all patients were operated on. Surgery was performed under the care of four thoracic surgeons at a tertiary thoracic centre. All operations were performed via a thoracotomy beside one, which was done via a video-assisted thoracotomy approach.

The cohort consisted of nine males and fourteen females, with a median age of 66±11 years. All patients had primary lung cancer, with a subtype of adenocarcinoma in thirteen (56.5%) and squamous cell carcinoma in ten (43.5%) patients. Early stage (I-II) was diagnosed in eighteen and late stage (III-IV) in five patients (Table I). Patients were followed up for a median time period of 31.8 months post-operatively. Survival information and cause of death was obtained by contacting the patient’s General Practitioner.

CTC detection using the ScreenCell device. The detailed technique of CTC detection using the ScreenCell device is described elsewhere (11). In brief, three millilitres of blood were collected from the peripheral vein of patients immediately prior to surgery in EDTA-containing tubes. Samples were incubated with 7 ml of a lysis and formaldehyde fixation buffer for 5 min, provided by ScreenCell. Samples were then filtered through the ScreenCell device per the manufacturer protocol.

Post-filtration filters were removed and stained with Haematoxylin and Eosin (H&E, Sigma Aldrich, St Louis, MO, USA) staining. Stained filters were then mounted on to slides and viewed by two independent consultant pathologists to assess the presence or not of CTCs. Filters were scored as either positive or negative for atypical cells suspicious for cancer, based on cell morphology and size, typically much larger cells, with nucleus to cytoplasmic ratio larger than normally were deemed as positive (Figure 1).

Statistical analysis was performed using GraphPad Prism v5 (GraphPad, San Diego, CA, USA), survival rate was analysed by Kaplan-Meier curves, statistical significance was calculated using a Chi-square test, and a Hazards Ratio (HR) calculated with a 95% confidence interval (CI).

**Table I. Details patient demographics and diagnosis.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Mean age (±SD) years</td>
<td>66±11</td>
<td>-</td>
</tr>
<tr>
<td>Males/Females</td>
<td>9/14</td>
<td>39.1%/60.9%</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary lung cancer</td>
<td>23</td>
<td>100.0%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>13</td>
<td>56.5%</td>
</tr>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>10</td>
<td>43.5%</td>
</tr>
<tr>
<td>TNM (Tumour, node, metastasis) staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early stage (IA-IIB)</td>
<td>18</td>
<td>78.3%</td>
</tr>
<tr>
<td>Late stage (III-IV)</td>
<td>5</td>
<td>21.7%</td>
</tr>
</tbody>
</table>

The Kaplan-Meier survival curves, showed a poorer survival proportion in the CTC negative group compared to the CTC positive group, with a chi-square value of 11.08, shown as being statistically significant (p<0.0009) and a HR of 0.04453 (95% CI; range, 0.007131 to 0.2781) (Figure 2).

**Discussion**

Detection of CTCs can be difficult as only one CTC may be found in a background of 1×10^5 to 1×10^6 peripheral blood mononuclear cells (12). Another challenge lies in the biological characteristics of CTCs that can differ from those of the primary tumour. CTCs acquire genetic mutations equipping them to respond to local growth factors and stimulate neovascularisation. Several studies in breast cancer patients have found that HER2 expression differed between CTCs and the primary tumour. It has thus been postulated that CTCs expressing different biomarkers from the primary tumour may respond differently to treatments (13-16).

Several studies have compared the performance of different technologies to find varying results, in particular between antibody-dependent and antibody-independent devices. It is becoming increasingly evident that some epithelial properties are lost from cancer cells, in some cancers more than others (17, 18). Hence, epithelial expression-based methods such as the CellSearch device, may have a disadvantage (despite being the only FDA approved CTC isolation device) over expression-independent, filtration-type platforms such as ISET (RareCells, Paris, France) or ScreenCell which rely on cell size and other physical cellular properties. There are a multitude devices available on the market, with varying functionality (19-25).
The CTC detection rate by ScreenCell in our study, was higher compared to similar studies looking at patients with early-stage NSCLC. Freidin et al. (2014) reported CTC detection rates of 56% and 65% by two separate pathologists using ScreenCell in patients undergoing surgery for early-stage lung cancer in comparison to 78.3% (N=15) and 73.9% (N=14) detection rates in our study (Table II) (26). This could partly be explained by the subjective nature of the method of identification, particularly in the absence of any special or specific antibody stains. However, our
detection rates were similar in comparison to studies looking at patients with more advanced NSCLC with 60% for both Pathologists, although it must be noted the advanced cancer sample numbers comprised a very small proportion of the cohort, Mascalchi et al. (2015) reported detection rates of CTCs using ScreenCell of 65% in a cohort of 26 patients with stage III and IV NSCLC. The authors found no correlation between tumour stage and the number of CTCs detected (27). Our detection rates, in comparison to other studies, support the idea that the release of CTCs is not related to tumour stage. A study by Wendel et al. (2012) found no significant differences in the median number of CTCs in patients with different stages of NSCLC detected by using a fluid phase biopsy approach (28). However, there is contradictory evidence since Krebs et al. (2011) found that the number of CTCs correlated with NSCLC stage. Patients at a higher NSCLC stage, had a greater number of CTCs. CTCs were detected using a semi-automated, epithelial cell adhesion molecule-based immunomagnetic technique (5). The conflicting evidence may be due to the different methods of CTC detection and that CTCs detected by different methods may have different biological characteristics.

In our study, patients negative for CTCs had a higher mortality rate during the study period, with 40% (N=2) in patients negative or CTCs vs. 22.2% (N=4) % in patients positive for CTCs (Table III). Additionally, in patients who died the median survival was shorter in those who were positive for CTCs. These results differ from other studies where patients who tested positive for CTCs had a worse prognosis. Krebs et al. (2011) found that prognosis was significantly worse in patients with stage III and IV NSCLC who had more circulating tumour cells (5).

These differences in survival may reflect the way that CTCs are analyzed. ScreenCell detects CTCs based on size exclusion, not allowing larger epithelial cells to pass through the micropores of the filtration device. On the other hand, Krebs et al. (2011) used an antigen-based technique. It may be that although ScreenCell is detecting CTCs it may not detecting clinically relevant CTCs that have the biological properties to influence the survival of NSCLC by establishing metastatic disease. Additionally there may be differences in the characteristics of CTCs in early-stage lung cancer and more advanced NSCLC (5). An alternative hypothesis that could explain the lower mortality rate would be that CTC detection in early-stage NSCLC could prime the immune system to respond more effectively against CTCs.

Our results indicate that detection of CTCs using ScreenCell is not necessarily prognostic and the detection of CTCs should not preclude the patient from undergoing surgical resection. The limitations of our study is the small cohort size, as well as analysing retrospective data. Additionally, our analysis was performed based on whether the patient had CTCs or not rather than looking at the effect of the total number of CTCs on prognosis.

In conclusion, our results do not support the idea that detection of CTCs using ScreenCell equates to a worse prognosis in comparison to patients who tested positive for CTCs using ScreenCell. Detection of CTCs by ScreenCell should not prevent patients from undergoing surgery with curative intent. More research is needed into this field to investigate the application of ScreenCell in other forms of cancers and in particularly to check whether in more advanced cancers this technology does show a correlation between CTC detection and poor prognosis. Additionally, it would be useful to look in the future at the different biological characteristics of CTCs detected by different methods.

### Table III. Patient follow-up and survival data based on CTC presence or absence.

<table>
<thead>
<tr>
<th></th>
<th>CTC positive</th>
<th>CTC negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Alive at follow up</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Median survival (days)</td>
<td>1011</td>
<td>711</td>
</tr>
</tbody>
</table>

References


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