Antibody-independent microfiltration biochip for the diagnosis of lung cancer

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Background

Circulating tumour cells (CTCs) originate from primary or secondary tumours, which when present in peripheral blood has the potential to be used as a non-invasive liquid biopsy for cancer diagnosis.

CTC detection typically involves a two-stage process of enrichment and immunological detection. Based on the high expression levels of the cell surface marker Epithelial Cell Adhesion Molecule (EpCAM) on many epithelial cancers, including lung cancer, it is a common target for the immunological enrichment of CTCs from EpCAM negative cells normally present in the blood. Platforms such as the Herringbone chip and Cell Search rely on EpCAM for CTC isolation.[1,2]

Following isolation of EpCAM-positive cells from blood, immunostaining is carried out and CTCs are defined as cells that are positive for cytokeratin (CK) and negative for CD45. EpCAM+/CK+/CD45- cells only represent a subpopulation of CTCs and there has been evidence of CTCs that are EpCAM and CK negative.[3] In order to avoid reliance on these markers, we used an antibody independent microfluidic system that exploits differences in size and deformability between CTCs and normal blood cells for enrichment. Here we describe initial results utilizing the Clearbridge Biomedics System and identifying CTCs using conventional clinical criteria with haematoxylin and eosin staining.

The microfluidic System utilizes an antibody-independent microfluidic biochip to filter peripheral blood and trap CTCs. The version of the biochip used in this study comprises 9000 crescent-shaped traps spread across 6 chambers. Individual traps have a diameter of approximately 20µm and are positioned 20µm apart. Each trap consists of 3 microposts spaced 5µm apart, to allow red blood cells to pass through. The majority of white blood cells can also squeeze through the gaps due to their deformability, while CTCs get retained due to their relatively larger size and rigidity.

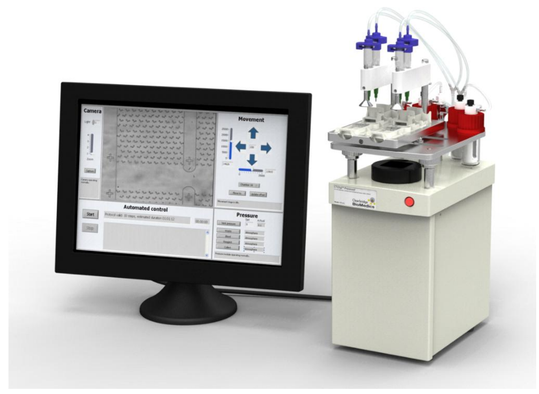

Figure 1. The Clearbridge Biomedics ClearCell System. Image used with permission from Clearbridge BioMedics.

Methods

Samples of blood were collected and processed from patients who had consented to donate blood for research (NRES 10/H0504/9). Following cell trapping using the ClearCell System, haematoxylin and eosin solutions are passed through the biochip to stain the cells. Each biochip was reviewed by two pathologists, using conventional histopathological criteria, e.g. high nucleus to cytoplasm ratio, large or irregular nuclei, presence of nucleoli etc. to identify trapped CTCs. Pathologists classified each biochip as negative, positive or suspicious for presence of trapped CTCs (examples in figure 2). Between-pathologist agreement was estimated using kappa statistic. To help position the technology in clinical practice, sensitivity-weighted (either pathologist classifies "suspicious" or "positive") and specificity-weighted (both pathologists classify "positive") analyses were undertaken.

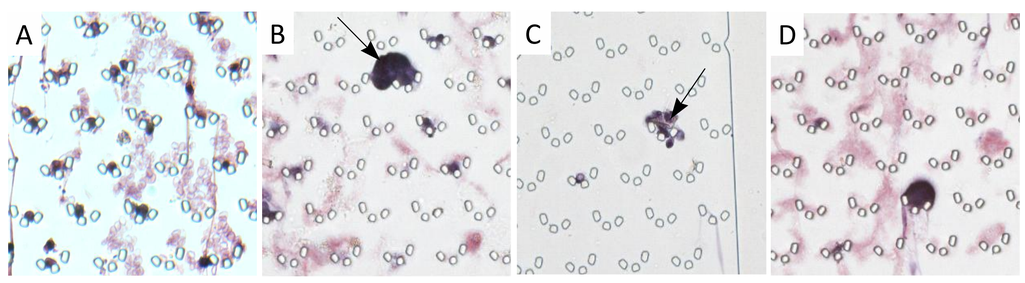

Figure 2. A: Example of CTC "negative" chip. Small trapped cells are lymphocytes. B: Example of CTC "positive" chip containing a cell with large nucleus to cytoplasm ratio. Solid arrow points to darkly staining nuclear region. C: Example of CTC "positive" chip with cells containing distinct nucleoli. Dashed arrow points to a nucleolus. D: Example of a chip "suspicious" for CTCs. Abnormally large cell observed but nuclear characteristics cannot be visualized.

Results

From March to October 2011, 52 samples of blood were collected and processed from patients who had consented to donate blood for research (NRES 10/H0504/9). Of 52 samples, 4 were from patients that did not have cancer, 35 from patients with primary lung cancer and 11 from patients with secondary lung cancer. Agreement between the two pathologists was 73%, with a kappa value of 0.58 (95% CI: 0.26, 0.91) indicating moderate agreement. Sensitivity and specificity values for sensitivity-weighted test performance were 33% (20 to 48) and 50% (7 to 93) respectively, while specificity-weighted test performance values were 13% (5 to 25) and 100% (49 to 100) respectively.

Discussion

Initial results suggest this as a promising marker for the diagnosis of lung cancer with high specificity (positive test rules in disease). Confidence intervals remain wide and a corroborative clinical study will be required to evaluate the test performance in a clinical (cancer network) setting.

References

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Acknowledgements

Project was funded by

- Point Hope Investment Company (Singapore)
- Peter Goldstraw

Supported by

- National Institute Health Research Respiratory Disease Biomedical Research Unit
- Royal Brompton and Harefield NHS Foundation Trust
- Imperial College London

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