Clinical utility of circulating tumor cell measurement in the diagnosis of indeterminate lesions of the pancreas

Satvinder Mudant[†], Alexandros Giakoustidis, Andrew V Thillainayagam, Jimmy Jacob & Justin Stebbing*

[†]Author for correspondence: Department of Surgery, Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK ■ Tel.: +44 20 7811 8015 ■ Fax: +44 20 7808 2805 ■ satvinder.mudan@rmh.nhs.uk

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Case Report Future Oncology

The quantification of circulating tumor cells has been historically problematic due to the different methods applied to their measurement. Following the development of standardized technology, they are now becoming well-established prognostic and predictive markers in patients with breast, colon and prostate cancer. While they represent a real-time noninvasive test, their use in diagnostics has seldom been reported. We report their use to help diagnose an indeterminate pancreatic mass. The use of an automated circulating tumor cell platform as described is likely to have utility as an aid to differential diagnosis, although larger studies will be required to ascertain its positive or negative predictive value.

The prognostic role of circulating tumor cells (CTCs) is now established [1,2], and there are data supporting their role as more reproducible indications of disease status than current imaging methods, raising the possibility that changes in CTCs could be used to predict clinical benefit [3].

The ability to isolate, quantify and molecularly categorize CTCs is a tremendous challenge [4]. A number of methodologies have been proposed for their measurement, including quantitative real-time PCR-based assays [5–7], immunomagnetic separation and laser scanning cytometry [8–11]. Following these data, the US FDA has now approved the routine use of the bead-based fluorescence CellSearch® system (Veridex, NJ, USA) in a number of clinical settings, including breast and prostate cancer – diseases that are considered incurable when metastatic – in order to determine prognosis. Their role in prediction has become established following the discovery that therapies that decrease their number are associated with improved outcomes [4,12–14].

The use of CTCs measured by this system in patients with pancreatic cancer has seldom been reported [15]. As pancreatic lesions are often difficult to biopsy, we report on the use of CTCs in an individual with a pancreatic mass to facilitate diagnosis.

Patient & methods

A 72-year-old man with an unremarkable past medical history, apart from chronic alcohol use, presented with intermittent abdominal pain

for several months, leading to an unrevealing gastroscopy, colonoscopy and abdominal ultrasound. He had few systemic symptoms and routine laboratory tests gave no further information. The pain then radiated to his back, and computed tomography revealed a 7 × 8 × 5 cm mass at the tail of his pancreas with features compatible with either a large cyst, similar to those often seen in alcoholic pancreatitis, or a carcinoma (FIGURE 1). At that time, his carcino-embryonic antigen (CEA) was 27.2 ng/ml and his carbohydrate antigen (CA)19–9 measured 1413 IU/ml.

The CellSearch system was then used to enrich and enumerate his CTCs, as we have described previously [16]. A 7.5 ml peripheral blood sample was taken in a CellSave® (Veridex LLC, NJ, USA) preservative tube, kept at room temperature and processed within 72 h. The system enriched for epithelial cell adhesion molecule (EpCAM)-positive epithelial cells by incubating the sample with ferrofluid conjugated to anti-EpCAM antibodies. Cells were stained with the following fluorescent-labeled monoclonal proprietary antibodies: CD45-APC, to distinguish the CTCs from leukocytes, and pan-cytokeratin 8, 18 and 19 (CK-PE), to stain epithelial cells. Nucleic acids were stained using 4,6-diamidino-2-phenylindole (to exclude red blood cells). The sample was then scanned on the CellTracks analyzer II® (Veridex) fluorescent microscope for analysis, as described previously [14,17].

Keywords

- circulating tumor cells
- diagnosis ■ pancreatic cancer

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Figure 1. A computed tomography image of the indeterminate mass arising from the tail of the pancreas (arrow).

Results

Based on the computed tomography appearances (FIGURE 1), the diagnosis was unclear. A peripheral blood sample was obtained for CTC analysis. A total of four CTCs in 7.5 ml of blood were observed. As CTCs have not previously been used in diagnostics, a multidisciplinary team decision was made to continue with 'standard routine care'. A subsequent laparoscopic biopsy revealed adenocarcinoma, and the patient commenced on gemcitabine/capecitabine chemotherapy and, at the time of writing, is continuing with this.

Discussion

We report the use of CTCs as an aid to the differential diagnosis in a patient with a pancreatic lesion. The fact that four CTCs were obtained specifically indicates that the lesion was an epithelial carcinoma and not a cyst, and the patient was informed of this result and advised accordingly. However, we did obtain a biopsy to confirm the diagnosis – the recognized gold standard for any pancreatic lesion.

Pancreatic lesions are well known for their lack of accessibility, and often the diagnosis of carcinoma is only made following surgical resection. Fine-needle aspirates are often inconclusive, and radiologic appearances of benign and metastatic lesions are often similar. Major surgical procedures with associated morbidity, such as pylorus-preserving pancreaticoduodenectomies or Whipple's procedures, have been performed for benign but suspicious-appearing lesions. However, technologies such as this are limited by false-positives and -negatives, and the possibility that nonepithelial malignant cancers, such as neuroendocrine tumors, may arise from the pancreas. In addition, the CellSearch system would not distinguish primary and metastatic lesions, and does not allow light microscopy of the cells detected. While a cut-off of five CTCs is typically used for both prognostic and predictive purposes in breast cancer [1,2], such delineation requires further study in this setting.

Allard *et al.* described the existence of CTCs in the peripheral blood of all patients with major carcinomas, but not in healthy subjects with nonmalignant diseases [17]; CTCs, as assessed by the CellSearch System, are not found in nonmalignant pancreatic diseases, such as chronic pancreatitis [15].

Despite years of research and hundreds of reports on tumor markers in oncology, the number of biomarkers that have emerged as clinically useful is very small, with initial promise replaced by inconsistent data [18,19]. While the use of CTCs as a prognostic and predictive marker is now accepted, we report here its use in defining the likely underlying pathology of an indeterminate abdominal lesion. The use of a standardized and automated CTC platform as described is likely to have utility in facilitating diagnosis, although prospective studies will be required to ascertain its positive or negative predictive value and determine the true clinical benefit of CTC measurement.

While it is difficult to draw conclusions from cohort studies, if CTC assays could lead to the earlier diagnosis of pancreatic cancer, this would be a remarkable accomplishment. The presence of CTCs is unlikely to be a false-positive and, as such, larger studies will be required to determine their predictive value.

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Affiliations

- Satvinder Mudan
Department of Surgery, Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK
Tel.: +44 20 7811 8015
Fax: +44 20 7808 2805
satvinder.mudan@rmh.nhs.uk
- Alexandros Giakoustidis
The London Clinic, London, UK
- Andrew V Thillainayagam
Gastroenterology and Hepatology Unit, Hammersmith Hospital, Imperial College Healthcare Trust, London, UK
- Jimmy Jacob
Department of Oncology, Hammersmith Hospital, Imperial College Healthcare Trust, London, UK
- Justin Stebbing, MA MRCP FRCPath PhD
Imperial College Healthcare NHS Trust, Charing Cross Hospital, 1st Floor, E Wing, Fulham Palace Road, London, W6 8RF UK
Tel.: +44 208 746 8295
Fax: +44 208 846 1433
j.stebbing@imperial.ac.uk