

# Preliminary Finding: Detection of Circulating Cancer Cells in Blood from a Patient with Peritoneal Carcinomatosis Treated with Cytoreductive Surgery and Intraperitoneal Chemotherapy

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## Abstract

**Background:** Patients with Peritoneal Carcinomatosis (PC) from colorectal cancer have a poor prognosis. Aggressive treatments by Cytoreductive Surgery (CRS) and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) offer a cure in selected patients with PC. However, in the great majority of patients the disease will recur in liver or lung. The underlying cause for recurrence could be the existence of Circulating Cancer Cells (CTCs) in PC patients prior to or at the time of CRS and HIPEC. There is a need for new cell-surface marker independent techniques to detect and isolate CTCs. We decided to try one such new technique, developed by Liquid Biopsy, and made available to us as a pre-production model. This method isolates both EpCAM positive and EpCAM negative CTCs potentially detecting a wider, more representative, sample of cells than samples restricted to certain known cell surface markers (Lab Chip, 2011, 11, 375).

**Methods:** This report focuses on a PC patient treated by CRS and HIPEC. The patient presented with PC from caecal cancer. Prior to CRS and HIPEC, the patient was treated with neoadjuvant chemotherapy. CTCs were isolated from peripheral blood preoperatively, at one week and one month after CRS and HIPEC using the novel marker independent method (Liquid Biopsy, patent pending). Conventional soluble serum tumour markers were also taken and analysed at the same time as the CTCs.

**Findings:** The preoperative level of CTCs was 25 cells/5 ml bloods. One-week post CRS and HIPEC, CTCs level was 21 cells/5 ml blood and one-month after CRS and HIPEC no CTC cells could be detected in 5 ml blood. Serum tumour marker analysis of preoperative CEA showed 5.8 (ref <3.8 ug/L), and CA72-4 was >600 (ref <6.9 KE/L). One week post CRS and HIPEC, CEA was normalised (1.6) and CA72-4 was significantly reduced to 31.1.

**Interpretation:** It appears that CTCs can be detected successfully in peritoneal carcinomatosis opening up the possibility to study the molecular characteristics of these CTCs. CTC numbers are also seen to drop to undetectable levels following radical surgery. Thus the occurrence and characteristics of CTCs in blood have the potential to assist decision making when considering treating patients with postoperative adjuvant systemic chemotherapy.

## Introduction

Peritoneal carcinomatosis (PC) from colorectal cancer is usually regarded as an incurable component of intra-abdominal malignancy [1-3]. However, over the past two decades, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have emerged as a treatment option, with an encouraging 5-year survival rate of 30-40%. Previous reports have revealed that the long-term survivors are those patients who undergo macroscopic radical surgery (R1) [4,5]. Despite the fact that preoperative investigation has shown no appearance of haematogenic disease (no lung or liver metastases) and that R1 surgery is performed, the majority of the patients die from systemic or loco-regional disease recurrence [6]. Furthermore, because evidence is lacking on postoperative adjuvant chemotherapy treatment after CRS and HIPEC, most of the patients do not receive an adjuvant treatment.

One of the reasons for haematogenic disease recurrence could be circulating tumour cells (CTCs) that are released into the blood prior to or at the time of CRS and HIPEC. Since the critical molecular nature of the pro-metastatic CTCs is not known a priori, the ability to isolate and study a wide spectrum of CTCs, both EpCAM positive and negative, seems to be a prerequisite to maximize the utility of the samples. Furthermore, finding a method to detect CTCs could further optimise, revolutionise and individualise not only the treatment of PC from colorectal cancer but also other cancer diagnoses and therapies. One commonly used method of capturing CTCs is by means of ferrofluidic nanoparticles conjugated to a monoclonal antibody against epithelial cell adhesion molecule (EpCAM) [7]. However, the downside of this

method is that it selects only EpCAM-positive CTCs, i.e. EpCAM-negative CTCs cannot be captured. EpCAM negative cells may include some of the most important cells to diagnose, since they may include cells with elevated stem cell or invasive properties.

This report describes the detection and isolation of CTCs from a patient with PC from caecal cancer, treated by CRS and HIPEC. The new method used to capture CTCs irrespective of surface markers, used in this report, is described in detail in patent application PCT/SE2010/051473.

## Clinical History and Treatment

A 60-year old male was diagnosed with PC and further investigation showed a primary tumour originating from caecal cancer. The diagnosis of the primary tumour as well as the PC was

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verified histopathologically. A CT scan of the thorax and abdomen ruled out detectable liver and extra-abdominal metastases. The patient was treated preoperatively for three months with a combination of systemic chemotherapy of folinic acid + 5-fluorouracil (5-FU) + oxaliplatin (FOLFOX). A PET scan was performed and showed no signs of metastases in liver or extra-abdominal organs. Six weeks after neo-adjuvant chemotherapy, laparotomy was performed and the extent of the abdomino-pelvic disease was quantified by using the peritoneal cancer index (PCI) as described by Sugarbaker [8]. The PCI was 36. An extensive CRS was performed according to Sugarbakers [9] methodology and the procedures included the following: greater omentectomy; splenectomy; cholecystectomy; lesser omentectomy and stripping of duodenal-hepatic ligament; parietal peritonectomy; right and left upper quadrant peritonectomy; total colectomy; pelvic peritonectomy with rectosigmoid resection. The surgery was macroscopic radical i.e. R1.

Intra-operative HIPEC treatment was given for 30 minutes using a combination of oxaliplatin 360 mg/m<sup>2</sup> body surface area (BSA) + irinotecan 360 mg/m<sup>2</sup> BSA. Concomitant intravenous 5-FU (400 mg/m<sup>2</sup>) and isovorin (30 mg/m<sup>2</sup>) were given. The carrier solution for the HIPEC drugs was 50 mg/ml glucose. The intra-abdominal temperature during perfusion ranged from 42°C to 44°C.

#### CTC analysis

Peripheral blood samples were taken in EDTA tubes. The first sample was discarded. The blood was processed through a prototype

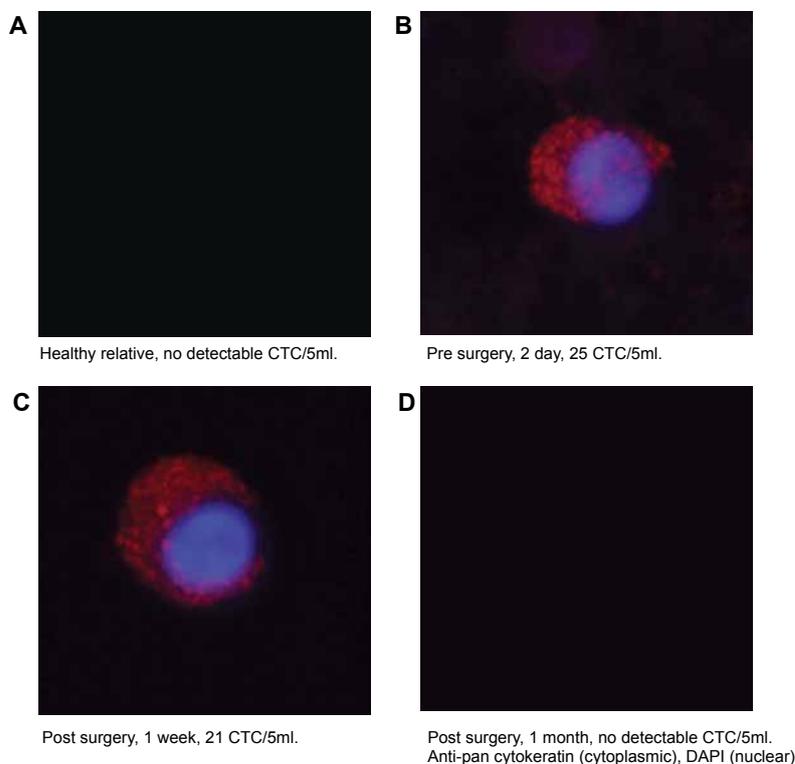
Liquid Biopsy sample preparation instrument. The resulting concentrated CTCs were suspended in proprietary buffer, were sedimented onto slides, washed and stained using anti-cytokeratin antibody, anti CD45 antibody and using DAPI, visualized in a confocal microscope and counted (Figure 1a). The isolated cells from the patient samples were cytokeratin positive and CD45 negative, compatible with being PC CTCs (Figure 1b). No cytokeratin negative/CD45 negative, nucleated, morphologically abnormal, cells were found in this study. The preoperative level of CTCs was 25-cells/5 ml blood (Figure 1b). The level of CTCs one week post CRS and HIPEC was 21-cells/5 ml blood (Figure 1c) and one month after CRS and HIPEC, no detectable cells/5 ml blood (Figure 1d). No CTCs were found in a healthy first order relative (Figure 1a).

#### Tumour markers analysis

Two serum tumour marker analysis (CEA and CA-72-4) were performed at the same time periods as CTCs and the preoperative CEA showed 5.8 (ref <3.8 ug/L), and CA72-4 was >600 (ref <6.9 KE/L). One week post CRS and HIPEC, CEA was normalised (1.6) and CA72-4 was significantly reduced to 31.1.

#### Discussion and Conclusion

Determination of the level of CTCs in this case report was successful. Furthermore, the sampling of CTCs at different time periods correlated with changes in tumour markers after the CRS and HIPEC. The level of CTC is found to drop to undetectable following macroscopically radical surgery. It is now possible to study in more detail the CTCs isolated



**Figure 1:** Fluorescent detection of circulating tumour cells (CTCs) in 5 ml of peripheral blood from a patient with peritoneal carcinomatosis from ceacal cancer. Anti-keratin epithelial marker (red) and nuclear marker (blue).

- No CTCs are detected in healthy relative control.
- 25 CTCs detected two days preoperatively by anti-keratin, anti-CD45 and DAPI staining.
- 21 CTCs detected seven days postoperatively with the same staining as B.
- No CTCs detected 30 days postoperatively

with this technology. The observed reduction in CTC count following apparently radical surgery suggests that monitoring peripheral blood samples may be a new independent readout of how effective in eradicating the tumours the surgery was. Monitoring the peripheral blood CTC count may constitute an early readout of treatment success or failure. If the method can detect micro metastases, undetectable by imaging techniques, remains to be determined. It is interesting that the CTC count remained elevated for a limited period of time following surgery, but then declined. This suggests that some CTCs may have an extended half-life. This can now be studied further. Future studies should address the molecular constitution of the isolated CTCs, including for potential disease mechanism, prognosis prediction, and treatment prediction and to follow treatment. It would seem important to be able to assay an as wide a sample of CTCs as possible, irrespective of surface markers. A cell surface marker independent method, like the present in principle would therefore seem preferable over methods that rely on specific cell surface markers, and therefore are blind to those cells that may not express that particular marker. With the method used in this report, it seems that CTCs are identified easily. However, this result should be confirmed in a larger series before being used as a routine method to monitor the radical removal of tumor. Molecular studies of the isolated CTCs are required to determine the impact on prognosis, treatment prediction and follow-up.

Although in about two-thirds of colorectal cancer patients the diagnosis is made at a stage when all apparently diseased tissue can be surgically removed, metastases are present in about 25% of the patients at the time of diagnosis and the disease will recur in about 40% at some point in time [10]. Therefore, there is a need to find a way to detect the disease at an early stage, before signs of metastasis appear in radiological imaging, in laboratory findings or in clinical symptoms. Detecting CTCs properly may also be used in the future as a method for the early diagnosis of colorectal cancer.

The proper selection of PC patients for CRS and HIPEC is critical in order to offer the patients improved survival. In addition to poor performance status, radiological imaging is currently used for the selection of patients for CRS and HIPEC [11]. Moreover, data is lacking both on the value of preoperative systemic chemotherapy as well as on systemic chemotherapy after CRS and HIPEC. However, detecting

CTCs properly could play a significant role in selecting patients who may benefit from preoperative treatment, and in the early diagnosis of recurrence. This might provide a rational for additional postoperative treatment and may this further improve the outcomes of PC patients from CRC, since most of these patients develop haematogenic disease despite macroscopic radical surgery.

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