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Original Articles

Circulating tumour cells and lung microvascular tumour cell retention in patients with metastatic breast and cervical cancer

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ABSTRACT

We have shown that in up to half of the patients with metastatic breast cancer (MBC), higher numbers of circulating tumour cells (CTCs) are present in the central venous blood (CVB) compared to the peripheral venous blood (PVB), suggesting that the lungs might retain a substantial number of CTCs. Here we report the presence of tumour cell emboli (TCE) in the microvasculature of the lungs in three out of eight patients with MBC and one patient with metastatic cervical carcinoma who had markedly elevated numbers of CTCs in the blood. All these patients suffered from symptomatic dyspnoea not easily attributable to other causes. No TCE were observed in five patients with MBC and elevated CTC counts and three patients with MBC who had low CTC counts (<5/7.5 ml). To investigate whether CTCs derived from CVB or PVB exhibit different transcriptional characteristics that might explain selective CTC retention, paired CTC samples from CVB and PVB of 12 patients with advanced breast cancer were subjected to gene expression analysis of 105 genes. No significant differences in CTC gene expression were observed. Together, these data suggest that potentially clinically relevant CTC retention in the microvasculature of the lung can occur in a subset of patients with advanced metastatic breast and cervical cancer, which seems to be transcriptionally non-selectively.

Introduction

Circulating tumour cells (CTCs) are increasingly being recognised for their prognostic and predictive potential in the management of patients with cancer. In addition to their clinical utility, CTCs provide a unique opportunity for researchers to study biological processes and molecular mechanisms involved in metastasis.

We have shown that in up to 50% of the patients with metastatic breast cancer (MBC), significantly higher numbers of CTCs can be detected in blood samples taken from the central venous blood (CVB) compared to peripheral venous blood (PVB) samples [1]. In this study, we also showed that CTCs measured in CVB were on average larger in size than CTCs measured in the PVB. Given the anatomical flow pattern of CTCs shedding from systemic metastases (Fig. 1), we hypothesised that the microvasculature of the lung might retain a substantial number of CTCs from the circulation. Particularly in patients with highly elevated numbers of CTCs, the cumulative entrapment of CTCs in the microcirculation of the lungs might in turn provide one possible explanation for some otherwise unexplained respiratory distress syndromes sometimes observed in patients with metastatic cancer. In addition, several genes and gene signatures have been described to selectively mediate metastasis of breast cancer cells to the lung or to predict breast cancer recurrence in the lungs [2–4].

The primary aim of this study was to evaluate to what extent retention of CTCs in the lung microvasculature could be documented in patients with metastatic (breast) cancer. Therefore, we performed autopsies to evaluate the presence of intravascular tumour cell emboli (TCE) in the lungs in a series of 11 patients with MBC and 1 patient with metastatic cervical cancer in whom CTCs were

Abbreviations: CTC, circulating tumour cell; MBC, metastatic breast cancer; CVB, central venous blood; PVB, peripheral venous blood; TCE, tumour cell emboli; ABC, advanced breast cancer; RT-qPCR, reverse transcription quantitative real-time polymerase chain reaction.

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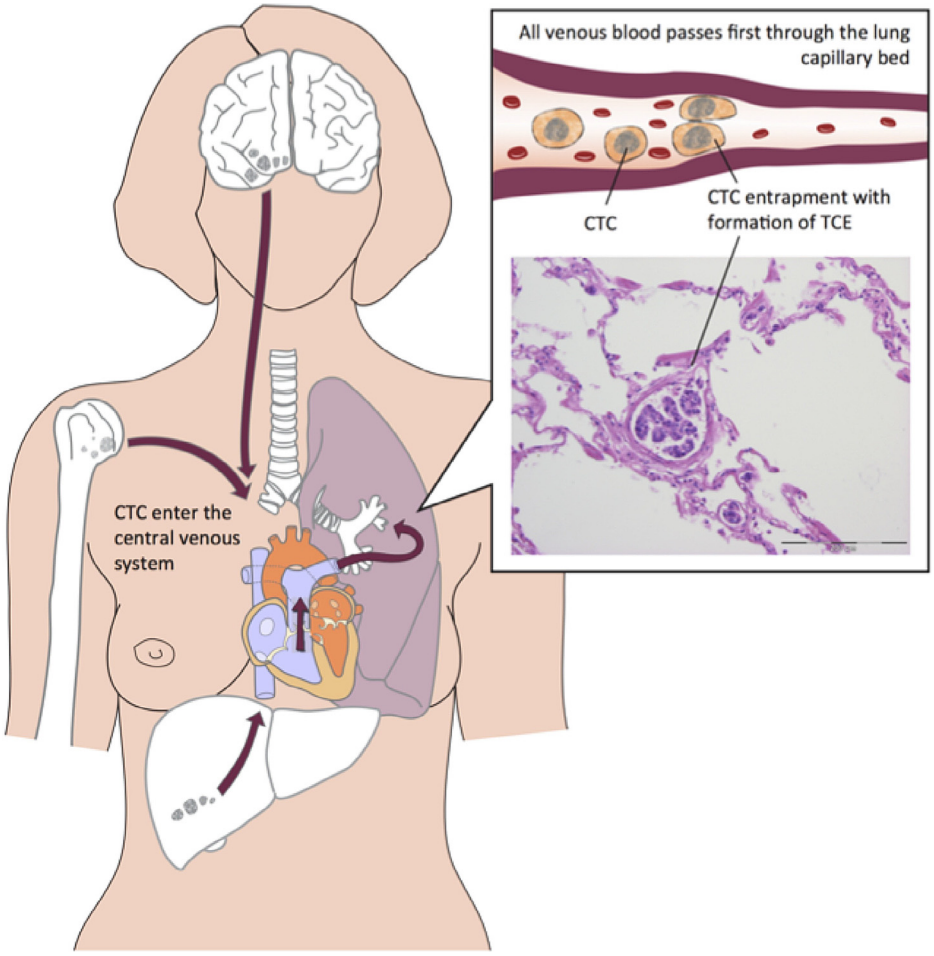

Fig. 1. Anatomical scheme of the flow pattern of CTCs from metastatic sites along the systemic circulation travelling towards the lung. CTCs shedding from systemic metastases in bone, liver or brain reach the heart through the central venous system. From the heart, the pooled venous blood is pumped into the pulmonary circulation. The lung capillary bed is the first capillary bed for the CTC rich blood to pass. The inlay shows CTCs blocking a small pulmonary blood vessel illustrated by an H&E stained microscopic image of a TCE in the lung microvasculature of one of the patients in this study. Abbreviations: CTC = circulating tumour cell; TCE = tumour cell embolus.

enumerated in the blood shortly before death. To explore whether CTCs isolated from CVB or PVB constitute of different subpopulations of tumour cells, one of which could be more likely entrapped in the lung microvasculature, we furthermore compared gene expression profiles of CTCs isolated from both vascular compartments in 12 patients with advanced breast cancer (ABC).

Materials and methods

Patient recruitment

Two patient cohorts were prospectively recruited for this study at GZA Hospitals Sint-Augustinus (Antwerp, Belgium). Appropriate local ethics committee approval was obtained and all patients provided written informed consent for CTC collection.

CTC enumeration

CTC enumerations were performed with the CellSearch system (Veridex LLC, Raritan, NJ, USA) as described previously [5]. Briefly, blood samples were drawn in CellSave Preservative tubes, (Veridex), stored at room temperature and processed within 72 h. CTCs were isolated and enumerated in 7.5 ml of blood using the CellSearch Circulating Tumour Cell kit (Veridex) according to the manufacturer's instructions. Criteria for an EpCAM positive object to be identified as a CTC include a round-to-oval morphology, a visible nucleus (DAPI), positive staining for cytokeratin (CK8/18/19), negative staining for CD45 and at least 50% overlap between nucleus and cytoplasm. All CTC image galleries were analysed by two readers (DP and GVdE). Questionable interpretations were evaluated again until consensus was reached.

Assessment of tumour cell retention in the lung microvasculature

In order to study to what extent CTC retention by the lung microvasculature could be objectified, we performed autopsy studies in 11 patients with MBC in whom CTCs had been enumerated shortly before death. Patients with elevated CTC counts were preferentially, but not exclusively, recruited for subsequent post-mortem examination of the lungs. In addition, as the mechanism of CTC retention in the lungs was considered presumably not to be exclusive for patients with MBC, we also included one patient with metastatic cervical carcinoma who presented in our hospital with symptoms of rapidly progressive, radiologically unexplained dyspnoea at the time this study was conducted.

All patients were terminally ill at the time of blood collection. Blood samples for CTC enumeration were drawn from a peripheral vein (PVB) and/or the central venous vascular access system (CVB), whichever was feasible at the time. Presence or absence of dyspnoea was recorded for all patients. After agreement of the patient's family, an autopsy limited to the lung was performed within 24 hours after death. The lungs were examined macroscopically for the presence of metastases and infarction areas and were subsequently fixed for 24 hours in formalin 4%. Tissue samples were taken from macroscopically suspect areas and/or at least two macroscopically normal areas per lobe for histological examination. Haematoxylin-eosin (H&E) stained slides were examined carefully for the presence of TCE by two experienced pathologists (GVdE and PV). If present, TCE were further characterised by immunohistochemistry for cytokeratins to confirm their epithelial (mammary) origin. In addition, immunohistochemical stainings for CD31 (vascular endothelium), CD34 (vascular endothelium) and D2-40 (lymphovascular endothelium) were performed according to routine pathology laboratory procedures, to confirm the