Circulating Tumour Cells in Patients with Malignant Lung Tumors Undergoing Radio-frequency Ablation

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Abstract. *Background/Aim: Radiofrequency ablation (RFA) is an increasingly utilised technique in patients with surgically-untreatable lesions. The effect of this therapy on circulating tumor cells (CTCs) is unknown. As far as we are aware of, this is the first study to evaluate the effects of RFA on CTCs in patients with malignant lung tumors immediately post-treatment. Patients and Methods: Nine patients with primary or metastatic lung tumors underwent RFA therapy from June to November 2013. Blood samples were taken before and after RFA, and filtered through the ScreenCell CTC capture device. Results: A general increase in CTCs in 7 out of the 9 cases was found, the largest increases were seen in the metastatic group. Conclusion: This study demonstrates that the manipulation and ablative procedure of lung tumors leads to immediate dissemination of tumor cells, the effects of which are unknown and require further investigation.*

Cancer treatment options available to patients have increased over time, with surgery, chemotherapy and radiotherapy no longer being the only modalities offered. Ablation techniques are becoming increasingly popular, due to their technical success, low risk and few complications. There are various forms of ablative techniques available. Radiofrequency ablation (RFA) and Microwave ablation (MWA) deposit energy into the tumour causing frictional heating and then coagulative necrosis and cell death. Percutaneous cryotherapy creates temperatures as low as –40˚ centigrade to freeze and destroy cells (1). Ethanol (alcohol) ablation whereby concentrated alcohol is injected directly into the tumour and irreversible electroporesis have also been utilised with varying degrees of success(2). In many cases, ablative procedures are offered to patients who are not functionally suitable for surgical intervention.

RFA is a very commonly used technique and has been utilised in tumours all over the body. In the lung it has been used in both primary and secondary tumours. The technique involves introducing a probe into the tumour and connecting the free end to an RF generator. Short- and long-term follow-up reports have shown excellent results, with minimal side-effects or complications (3, 4).

Circulating tumor cells are a well-established phenomenon which may influence the outcome of cancer treatments (5). CTCs are a potential cancer biomarker present in the peripheral blood of patients with cancer. These are cells that shed from a primary tumor and have the potential to enter the vasculature, spread to distant sites and form distant metastases (6). Understanding CTCs has become a fundamental part of cancer research, as diagnostic blood assays for cancer are a growing area of interest. The role of CTCs in the formation of metastases has long been hypothesised, and the invention of reliable CTC capture devices has led to a renewed interest in the area.

A large variety of techniques are available for CTC detection and isolation. The main categories are based on the physical properties and surface antigen expression of the tumour cells (7). There has been continuing debate about the superiority of any one of these platforms, and to date only one has received FDA approval, for metastatic breast, prostate and colon cancers, the Veridex CellSearch device (Janssen Diagnostics company, USA) (8). There is a growing attraction towards antibody-independent devices, in an attempt to eliminate antibody bias (particularly in the cases of lung cancer where epithelial markers are thought to be lost, owing possibly to the infamous epithelial-mesenchymal transition paradigm) and a move towards filtration devices to isolate CTCs with minimal processing to preserve cell integrity (9, 10, 12).

Tumor cell clusters, known as circulating tumor microemboli (CTM; contiguous groups of tumour cells), involve a process of collective cell migration, an important mechanism in tumour cell invasion (13-15). Individual studies

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Table I. *Patients with primary and metastatic lung tumors and circulating tumour cell counts per sample in the peripheral blood, before and after Radiofrequency ablation.*

Patient number	Sex	Age, years	Tumour	Pathology	Baseline CTC count	CTC count after RFA	Status[a]
1	F	60	Primary	AC	0	0	Alive
2	F	76	Primary	AC	0	1	Alive
3	F	74	Primary	SCC	2	5	Alive
4	M	60	Primary	AC	0	3	Alive
5	M	79	Primary	AC	50	50	Alive
6	F	78	Metastatic	AC	0	10	Alive
7	M	72	Metastatic	SCC	10	20	Died
8	F	52	Metastatic	AC	0	10	Alive
9	M	72	Metastatic	AC	0	3	Alive

M: Male, F: female, SCC: squamous cell carcinoma, AC: adenocarcinoma. Life status as of 1st Feb 2015[a]

isolating cancer cells from patient samples by Brandt *et. al.* (1996), Molnar *et. al.* (2001), and Kats-Urgurla *et. al.* (2009), have reported the presence of CTMs in prostate, colorectal and renal cancers respectively (16-18). CTMs could potentially reflect the collective migration of tumour cells in to the lymphatic system, *via* channels such as the tumor vessels, a feature of highly angiogenic tumors (7, 19). Earlier pre-clinical studies on animal models, suggest that intravenously injected CTMs have a greater tendency to form metastasis, than the equivalent number of single tumour cells (20).

There is a growing body of evidence that the number of CTCs in patients with lung cancer depends significantly on the stage of the tumor (21-23) and may be influenced by therapeutic surgical interventions (5).

However, whether treatment with RFA increases CTCs in the peripheral blood of patients with lung cancer is unknown. This is a proof-of-concept study with the main aims of establishing the incidence of CTCs in the peripheral blood of patients with lung tumours undergoing RFA before and after the procedure.

Patients and Methods

This was a prospective, observational, pilot study (ethical approval number 10/H0504/9). Nine patients with a pathologically confirmed diagnosis of primary or metastatic tumour of the lung (Table I) were treated with RFA from June to November 2013. Five patients had primary lung cancer and four patients had a solitary metastasis from colorectal adenocarcinoma. The mean age of the patients was 71±14 years, with a 4:6 male to female ratio. RFA was performed as these patients were not functionally fit to undergo resection. Specific informed consent was obtained from all patients.

RFA was performed under general anaesthesia. One to three Cool-Tip needles (Covidien, Boulder, CO, USA) were used in sequence. Complete tumour ablation based on physiological and radiological criteria was achieved for all treated lesions.

Three millilitre blood samples were taken 30 min before and immediately after treatment from a peripheral vein. All samples were filtered through the ScreenCell filtration device (ScreenCell®; Paris, Sarcelles, France). The blood samples were incubated for 7 minutes in buffer provided by manufacturers (ScreenCell®; Paris, Sarcelles, France), then vacuum-filtered through a microporous filter (8-μm pores) within 1 hour of collection. Filters were rinsed with phosphate-buffered saline and captured cells stained with haemotoxylin and eosin directly on the filter. All filters were viewed and assessed by a Consultant Pathologist (AR), by means of microscopy. Cells were counted manually, using cytopathological criteria, cell size, cell and nuclear morphology and nuclear size. Clusters of cells were counted by visible cell nucleus.

Data are expressed as the mean±standard deviation for continuous data and as percentages for categorical data. Differences between cell counts in the baseline samples and samples obtained post RFA were compared with a paired t-test. All statistical analysis were performed with GraphPad Prism® 6.0 (GraphPad Software®, San Diego, CA, USA), and a p-value less than 0.05 was considered to be significant.

Results

There were no deaths recorded or serious complications and all patients were discharged, with a mean hospital length of stay of three days. Characterisation of CTCs were made based on physical properties *e.g.* cell size, and nucleus to cytoplasmic ratio (Figure 1).

Out of the five patients with primary lung cancer, two (40.0%) showed the presence of CTCs before the procedure, with an increase seen in three (60.0%) after RFA. In the metastatic group, surprisingly only one (25.0%) patient had CTCs present before the procedure, but all four did after RFA (Table I). A two tailed t-test calculated a *p*-value of 0.0152, suggesting strong statistical significance of the difference in CTCs before and after RFA.

Discussion

This study evaluated the effects of RFA in nine patients and confirmed an increase in CTCs freely-circulating in the blood, after RFA. To our knowledge this is the first study investigating the presence of CTCs in patients with lung tumours undergoing RFA. A study in patients with colorectal liver metastases reported a decrease in CTC levels following surgical resection, but an increase in those having RFA (24). There was a variation in the level of increase of CTCs after RFA, however, there appeared to be a larger increase in the patients with metastatic tumours.

There appeared to be no correlation with baseline cell counts and cancer subtype or whether the patient's cancer was of primary or secondary origin. Nor was there any apparent correlation with CTC increase after RFA with tumour pathology or aetiology. Two patients showed no change in their cell count before and after treatment.

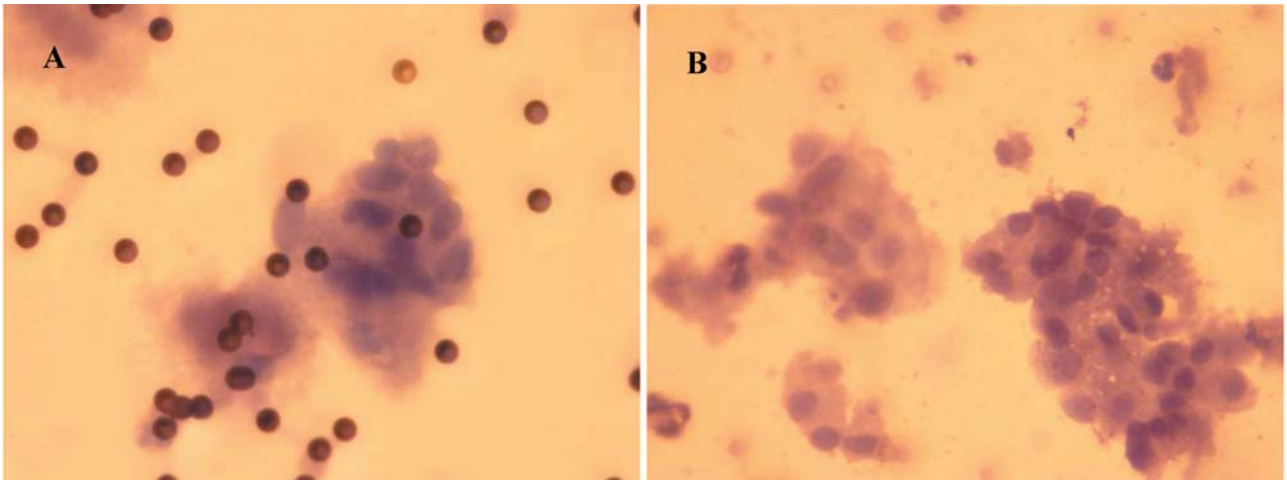

Figure 1. *Micrograph images of ScreenCell filters, stained with haemotoxylin and eosin, ×40 magnification. A: Patient with primary lung cancer, small cluster of (cell clusters were counted by visible nucleus) suspicious, atypical cells captured. B: Patient with metastatic colorectal cancer, with a large cluster of suspicious, atypical cells.*

RFA treatment can be used for treatment of a solitary nodule, or multiple nodules in both lungs in the same session. All of the patients with primary cancer were treated for one lung nodule, whereas three out of four of the patients with metastases were being treated for multiple nodules in single or bilateral lungs, the group in which the largest increase in CTCs were seen. These results raise suspicions as to whether RFA treatment of multiple nodules may have contributed to this or if this is due to the underlying nature of the advanced disease.

Patients were reviewed in follow-up clinics at 1, 3, 6, 9, 12 and 15 months. There was only one reported death at 13 months after RFA, with the remaining eight patients being alive, despite having metastatic cancer in some cases with advanced staging. A proportion of patients (4 of 9) have since returned for further RFA treatment of new lung nodules, mainly those already diagnosed with metastatic disease (3 of 4).

The implications of this CTC spread and increased presence in the blood is presently unclear. Although not investigated, the elevation in CTCs may contribute to the development of distant metastases. The viability of the CTCs and their ability to give rise to metastases remains questionable.

CTC detection could potentially be utilised to evaluate tumour evolution, involvement in drug development, investigating and monitoring acquired treatment resistance mechanisms and poor or no response to treatment (7). However, there is a paucity of evidence on the effects of various diagnostic or therapeutic intervention on patients with lung tumour and their impact on the release of CTC into the circulation.

The limitations of this pilot study include a small sample size and short follow-up. Further research in this area with a larger patient cohort would be desirable to understand the implications of the elevation of CTCs following RFA.

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