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Brief Communication

Circulating tumour cells in patients with lung cancer undergoing endobronchial cryotherapy

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ABSTRACT

Early diagnosis of lung cancer still poses a major issue, with a large proportion of patients diagnosed at late stages. Therapeutic options and treatment remain limited in these patients. In most cases only palliative therapies are available to alleviate any severe symptoms. Endobronchial cryotherapy (EC) is one form of palliative treatment offered to patients with obstructive airway tumours. Although successful, the impact on circulating tumour cell (CTCs) spread has not been investigated in detail. This study recruited 20 patients awaiting EC treatment. Baseline and post EC blood samples were analysed for presence of CTCs. Results showed an increase in CTCs following EC in 75% of patients. Significant increases were noticeable in some cases. Although EC is a well-accepted modality of treatment to alleviate symptoms, it may lead to an increase in CTCs, which in turn may have implications for tumour dissemination and metastatic spread.

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CTCs are a well-established phenomenon which may influence the outcome of cancer treatments [9]. CTCs are tumour cells that shed directly from the primary tumour. It has been well established that metastasis is propagated by CTCs, these are so called 'seeds' that detach from a primary tumour to form a subpopulation of tumour cells which intravasate to allow lymphohematogenous dissemination to other areas of the body [4,5]. A large variety of techniques are available for CTC detection and isolation. The main categories are based on physical properties and surface antigen expression of the tumour cells [9]. There is a growing body of evidence that the number of CTCs in patients with lung cancer depends significantly on the stage of the tumour and can be influenced by therapeutic surgical interventions [7].

Endobronchial cryotherapy (EC) is a technique to treat and restore the bronchial patency and to control symptoms of obstructive endobronchial tumours, with minimal complications [7,9]. In the majority of cases, EC is offered to patients with tracheal and bronchial tumours obstructing airways in advanced stages (T3 and T4 metastatic cancers), where surgical intervention is not an option.

EC is a local treatment modality and is administered by introducing a cryoprobe into the trachea close to the area of the tumour, freezing the tumour and following a repeated freeze–thaw cycle.

There are no significant reported side effects or complications, other than cough and general results and findings are positive. However, the influence of EC on CTCs in the peripheral blood of patients with lung cancer is unknown and has never been reported. The main aim of this investigation was to establish the incidence of CTCs in the peripheral blood of patients with lung cancer undergoing EC before and after the procedure.

We carried out a prospective, observational, pilot study (Ethical approval number 10/H0504/9). Patients with symptomatic tracheo-bronchial obstruction and morphologically confirmed diagnosis, undergoing EC, were recruited (June to November 2014). Ten patients who were EC naïve and 10 who had previous EC were included. Specific informed consent was obtained from all the patients. EC (via rigid and fibre-optic bronchoscopy) was performed under general anaesthesia (GA). Cryoapplication (tip temperature -70°C) was performed with a flexible cryoprobe (ERBE Elektromedizin GmbH, Tübingen, Germany) inserted via the operating channel of a 2.8 mm fiberoptic bronchoscope. Standard exposure time was 4–5 min and the number of applications ranged from 2 to 4. Two 3 ml samples of peripheral blood were obtained into EDTA anticoagulation tubes from each patient (baseline sample = after GA, before procedure; second sample = end of cryotherapy).

Samples were incubated for 8 min in buffer (ScreenCell[®], Paris, France), then vacuum filtered through a microporous filter (8 μm pores) within 1 h of collection. Filters were rinsed with

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Table 1
Pathology, location and staging of patients with lung cancer and respective CTC counts detected in peripheral blood, before and after undergoing endobronchial cryotherapy.

Patient number	Sex	Pathology	Staging	Total number of cryotherapy cycles	Baseline CTC count	CTC count after cryotherapy	Status
1	M	Squamous	T3 N2 M0	7	0	0	Alive
2	M	Squamous	Tracheal tumour*	1	0	0	Alive
3	M	Adeno	Tracheal tumour*	6	0	0	Alive
4	M	Squamous	T4 N0 M0	3	0	0	Died
5	F	Adeno	T3 N3 M1b	1	0	0	Died
6	M	Squamous	T2 N0 M1A	3	50	2	Alive
7	M	Adeno	T4 N2 M1b	5	0	2	Died
8	M	Squamous	T3 N0 M1b	0	0	3	Alive
9	F	Adeno	T3 N2 M0	0	0	3	Alive
10	M	Squamous	Tracheal tumour*	0	0	3	Alive
11	M	Adeno	Tracheal tumour*	0	2	5	Alive
12	F	Squamous	T2 N2 M0	0	0	10	Alive
13	M	Adeno	Tracheal tumour*	2	0	10	Alive
14	M	Squamous	Tracheal tumour*	0	0	>50	Died
15	F	Squamous	Tracheal tumour*	0	3	>50	Died
16	M	Squamous	T4 N2 M1a	0	0	>100	Alive
17	F	Squamous	T2 N2 M1a	0	2	>100	Died
18	M	Inv Adeno	T4 N2 MX	4	0	>100	Died
19	M	Squamous	T4 N2 M0	0	2	>100	Died
20	F	Squamous	T4 N1 M0	5	0	>100	Died

* No staging available on tracheal tumours.

phosphate-buffered saline and captured cells stained (Haematoxylin & Eosin) directly on the filter. All filters were viewed and reported by a Consultant Pathologist (AR). Data are expressed as mean \pm standard deviation for continuous and percentages for categorical data. Differences between the baseline and post cryotherapy cell counts were compared with paired *t*-test. Statistical analysis was performed with GraphPad Prism[®] 6.0 and a *p*-value <0.05 was considered to be significant.

Twenty lung cancer patients (mean age = 67 \pm 12 years) were recruited. There were no deaths or serious complications and all patients were discharged on the day of procedure. The median number of cryotherapy sessions was 3, in patients who had previous cryotherapy. Cryotherapy sessions were planned at 4–6 weekly intervals and dependent on patient need and compliance.

Five patients showed baseline presence of CTCs pre-procedure. Fifteen of the 20 patients (75%) had an increase in atypical suspicious cancer cells (see Fig. 2 – image of cells). An increase in atypical, suspicious cancer cells was seen in all patients who had no previous EC treatment (Table 1). Of the 15 patients that had detectable CTCs, 10 had a diagnosis of squamous cell carcinoma (SCC), and 5 of adenocarcinoma (AC). A significant increase (>50 cells) in post cryotherapy cell counts was seen in 7 patients, 6 of whom died during follow-up.

The study confirmed increases in cell count following cryotherapy in 15 patients (*p* = 0.0086) (see Fig. 1). A significant increase in

CTCs (>100 fold) compared to the baseline cell counts was seen in 7 patients. This data would correlate with general findings that increased CTC counts in the blood are associated with poorer prognosis. Other studies have shown that cell counts of >5 are implicated with much shorter survival times [4,6,8]. Current evidence suggests that presence of CTCs at baseline is also indicative of poor prognosis and survival [3]. Our data shows a similar trend, whereby 3 of the 5 patients with positive baseline samples are deceased.

The majority of the patients recruited were diagnosed in advanced stages of their cancers, stage T3 or T4 and some with metastatic disease. Typical median survival for these patients is quoted at 13 months and 8 months, for T3 and T4 metastatic patients [2]. In most cases these patients are inoperable and cryotherapy is offered as a means of palliative care. The median time to death calculated in all patients was 369.5 days (13.6 months), and 393.3 days (13.1 months) for patients with a diagnosis of T3 or T4 metastatic disease, which is in line with National statistics [1]. Considering a larger proportion of the patients were in stage T3 to T4 (40% and 60%, respectively) it may be arguable that cryotherapy may slightly extend survival.

The most significant increase in CTC counts were seen in patients who had not previously been treated with cryotherapy. Interestingly, patients who had previous cryotherapy appeared to show a smaller increase in CTCs post-cryotherapy. The implications of this CTC spread and increased presence in the blood is presently unclear. Although not investigated, the elevated CTCs may contribute or promote distant metastasis. The viability of the cells and ability to give rise to metastasis remains questionable.

CTCs have been the focus of much research over the recent years for their potential clinical utility as a biomarker for diagnostic and prognostic benefits. Detecting CTCs is difficult, due to the very small numbers found in the blood. There is evidence to suggest tumour manipulation during surgery contributes to tumour cell dissemination.

However there is a paucity of evidence on the effects of various lung cancer diagnostic or therapeutic interventions, and their impact on the release of CTCs into the circulation. EC involves direct contact with the tumour, which could potentially lead to unknown tumour cell disruption and shedding.

The limitations of this pilot study include relatively small sample size and short follow-up. Further research in this area with a

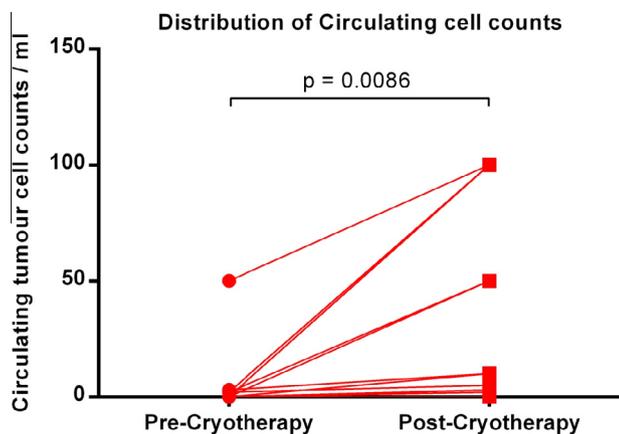


Fig. 1. Pre and post cryotherapy cell counts.

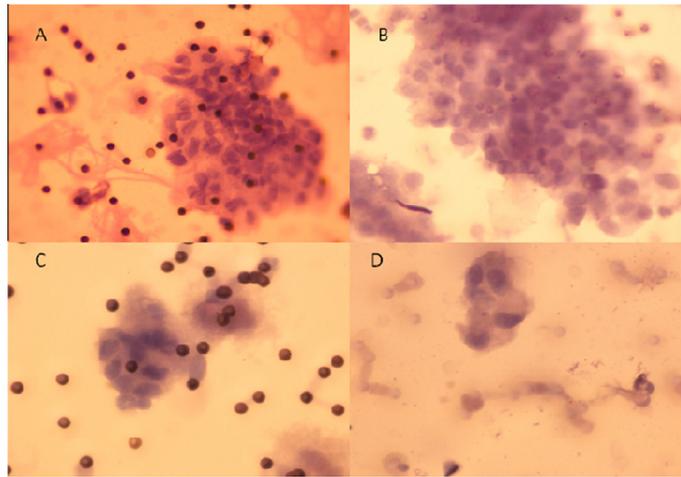


Fig. 2. Results of captured suspicious cancer cells on ScreenCell® filters (haematoxylin and eosin staining). Individual cell features can be seen such as large irregular nucleus compared to small cytoplasmic layer, general size of cells is much larger ($>8 \mu\text{m}$, comparable to dark circular pores on the filter), to normal cells. Panel A (Pt 3) – a large cluster of atypical cancer cells captured on the ($20\times$ magnification). Panel B (Pt 4) – also with a large cluster of many atypical suspicious cancer cells ($40\times$ magnification). Panel C – shows a much smaller cluster of cells ($40\times$ magnification). Panel D – shows a few scattered atypical suspicious cancer cells ($40\times$ magnification).

larger patient cohort would be desirable to understand the implications of elevated CTCs following endo-bronchial interventions.

Disclosures

The authors have no disclosures to make.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency Document

The [Transparency document](#) associated with this article can be found in the online version.

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