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A Pilot Study of Circulating Tumor Cells in Stage IV Non-Small Cell Lung Carcinoma

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Purpose	Measurement of the number of circulating tumor cells (CTCs) in the bloodstream has been shown to have prognostic significance in treating breast carcinoma. This pilot study was formulated to determine if stage IV non-small cell lung carcinomas similarly shed malignant cells into the circulation and if their presence has prognostic significance.
Methods	Patients with stage IV non-small cell lung carcinomas were tested once for CTCs in 7.5 ml of their blood prior to receiving any treatments. A proprietary blood collection kit produced by Veridex LLC (Raritan, NJ), which manufactures the instrument that performs the immunomagnetic CELLSEARCH [®] CTC assay, was used. Tumor measurements were determined in three dimensions by the same radiologist using computerized axial tomography. The three-dimensional sum was used to represent tumor size. Survival from the date of the pretreatment CTC assay was monitored and recorded. Data were analyzed statistically using NCSS8 statistical software (NCSS LLC, Kaysville, UT).
Results	Of 19 evaluable patients, 10 had no detectable CTCs. There was no relation between intrapulmonary primary tumor size and the number of CTCs, nor between tumor size and survival. Survival was not affected by gender or age at entry into the trial. The mean survival of those with no detectable CTCs was 536 ± 91.1 days versus 239 ± 96.0 days for those with 1 or more detectable CTCs, a statistically significant advantage (P=0.034) favoring those without CTCs.
Conclusions	Patients with a CTC score of 0 survived significantly longer than those with a CTC score of ≥ 1. Survival was not correlated with gender, age or primary tumor size. Recovery of CTCs potentially provides a noninvasive source of tumor cells for genomic profiling, which may enable development of a custom treatment plan for the individual patient. Further investigations are warranted and needed. (<i>J Patient Cent Res Rev.</i> 2016;3:136-141.)
Keywords	lung; carcinoma; non-small cell; survival; circulating tumor cells; CELLSEARCH assay

In the United States, lung cancer is the most common noncutaneous malignancy in both men and women. Our ability to treat this disease currently depends on imaging technologies such as plain film radiography, computerized axial tomography, magnetic resonance imaging, radionuclide bone scans and positron emission tomography (PET).¹ These tests tell us about the location and extent of the disease. While PET

scanning gives limited information regarding disease activity, the other imaging techniques do not. None of them determine whether tumor cells are actively being shed into the bloodstream, which may disseminate the neoplasm to previously uninvolved areas. In clinical practice, we monitor changes in the size and number of sites of disease as indicators that imply control, response or resistance to a given treatment regimen.

There are good data supporting the concept that quantitation of circulating tumor cells (CTCs) can provide meaningful information regarding the prognosis and potentially the response of malignancy to therapy. Cristofanilli et al.^{2,3} conducted a study of

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177 patients with metastatic breast cancer. They found that detection of CTCs before the initiation of first-line therapy was highly predictive of progression-free survival (PFS) and overall survival. There appeared to be a clear advantage in progression-free and overall survival favoring those patients with less than 5 CTCs (per 7.5 ml blood sample as collected) in comparison to those with 5 or more CTCs. (Unless otherwise specified, all references herein to the number of CTCs will be understood to indicate per 7.5 ml blood sample.) The median progression-free survival of patients with less than 5 CTCs was 9.5 months versus 4.9 months for those with 5 or more CTCs. Similarly, the median overall survival was greater than 18 months for those with less than 5 CTCs versus 14.2 months for those with 5 or more CTCs. These results were highly statistically significant.

Cristofanilli's group also observed that a decrease in CTCs was associated with response to therapy as determined by subsequent conventional imaging follow-up study. The report does not, however, indicate the temporal relationship between CTC reduction and the conventional imaging tool. We do not know if the change in CTC level precedes tumor reduction on imaging.³

Reports by others have corroborated Cristofanilli's findings as well as offered new insights into tumor biology. The CTC method offers the prospect of monitoring nonradiographically measurable breast cancer.⁴ Breast cancer patients completing adjuvant chemotherapy were found to have levels of CTCs that were statistically the same as pretreatment values.⁵ While CTC levels predict survival, serum tumor markers do not.⁶ Analysis of CTCs was readily available in the United States⁷ when this study was first proposed in 2007.

Other methods of analyzing CTCs have been reported from diverse investigators around the world. Mutant allele-specific amplification analysis by polymerase chain reaction (PCR) has detected CTCs in blood and lung samples in rats.⁸ However, in that study there was no correlation between the finding of circulating cell-free DNA and the number of CTCs. The reverse transcriptase PCR Southern blot assay has been used to detect CTCs.⁹ Using

this technique, 8 of 17 lung carcinoma patients had evidence of CTCs at the time of diagnosis. After three cycles of adjuvant chemotherapy, however, the proportion showing CTCs increased to 12 of 17 patients. This observation is disturbing. Still, it does not necessarily imply that those CTCs are clonogenic or even viable. We must consider the possibility that the chemotherapeutic agents may have mobilized them into the circulation in a fashion analogous to mobilization of stem cells for bone marrow harvesting, which is accomplished by administering chemotherapy.¹⁰ The Rare Event Imaging System,¹¹ the GEN-S hematology analyzer (Beckman Coulter Inc., Brea, CA)¹² and a laser scanning cytometer (CompuCyte Corp., Westwood, MA)¹³ are some other methods that have been studied. The GEN-S study found a correlation between the number of CTCs and the weight of the primary tumor as well as with the number of lung metastases.¹²

More recently, others have reported the use of circulating micro-RNA as a marker for lung cancer.^{14,15} Cell-free nucleic acids also have been investigated.¹⁴ We initiated our pilot research when it was not known if stage IV non-small lung carcinoma would shed quantifiable numbers of tumor cells into the circulation and whether those results would have prognostic value.

METHODS

Between January 29, 2010, and October 15, 2012, one 7.5 ml sample of blood was drawn from 20 consecutive patients diagnosed with stage IV non-small cell lung carcinoma after obtaining informed consent and before initiation of any treatment. The consent and the study itself were approved by the local institutional review board. The sample was drawn using a proprietary collection kit and sent to Quest Diagnostics/SJC (San Juan Capistrano, CA). The device used for the CTC count determination was produced by Veridex LLC (Raritan, NJ).

A malfunction of the CTC counting equipment could not be remedied before the time window for sample testing expired on the blood from one patient; as a result, no assay could be run and the patient was excluded from analysis. The majority of the patients in this study had their histologic confirmation of non-

small cell lung carcinoma obtained via fine needle aspiration. As such, further histologic typing was not available and therefore is not reported here. This would render meaningless attempts at statistical correlations based upon histology. Likewise, we have not reported biomarker testing for which adequate tissue was not available for most of the study population. Thus, 19 patients were available for data analysis.

The CELLSEARCH® CTC Test (CS-CTC, Veridex) was conducted by our reference laboratory using a system approved for clinical use by the U.S. Food and Drug Administration for the identification, isolation and counting of CTCs. The sensitivity of the test detects the rare CTC, estimated at 1 per billion normal blood cells.¹⁶ The CS-CTC detects CTCs of epithelial origin (CD45-, EpCAM+ and cytokeratins 8, 18+ and/or 19+) from a 7.5 mL blood sample with a high level of sensitivity and specificity. It uses unique immunomagnetic and fluorescence imaging technology to provide rapid, precise and reproducible analysis of CTCs.

The sum of the three largest perpendicular diameters of the intrathoracic tumor (measured by a radiologist [E.C.] on computerized axial tomography) was used in our analysis of tumor size as a RECIST score. Age (in years), survival (in days from the date of obtaining the blood sample for this study), CTC value and tumor size were analyzed using NCSS8, a Windows-based statistical software program (NCSS LLC, Kaysville, UT).

RESULTS

The patients' data, demographics, CTC count, tumor size and length of survival are shown in Table 1. A plus sign after the number of days of survival indicates that the patient was still alive as of September 20, 2013, when the data analysis was performed.

The mean age of all the patients was 64.1 ± 2.2 years (range: 51–82 years). The mean survival of the entire group \pm standard error of the entire group was 395.9 ± 73.1 days. Their RECIST scores of tumor size ranged from 5.4 to 30.6 cm, with a mean of 15.1 ± 1.5 cm. Ten patients had a CTC score of 0, and 9 had scores of 1 or greater. Mean CTC score was 11.7 ± 7.0 .

Table 1. Age, Sex, CTC Count, Tumor Size and Survival Data for 19 Evaluable Patients

Patient #	Age	Sex	CTC count	Tumor size (cm)*	Survival (days)
1	65	F	1	30.6	434
2	60	F	7	20.1	37
3	63	M	4	13.2	264
4	80	M	1	14.7	67
5	56	M	0	12.5	1,236+
6	59	M	36	19.0	132
7	75	F	127	5.5	260
8	59	M	1	5.5	159
9	52	M	0	9.1	326
10	71	F	0	23.4	236
11	56	M	0	15.3	414
12	74	M	0	19.3	651
13	63	M	0	20.8	793+
14	76	F	1	14.1	759+
15	55	M	45	5.4	40
16	57	M	0	15.0	647+
17	64	M	0	15.9	624+
18	51	F	0	16.4	93
19	82	F	0	11.7	340

*Determined by the sum of the three largest perpendicular diameters.

A "+" symbol after the number of days of survival indicates the patient was still alive as of Sept. 20, 2013, when data analysis was performed.

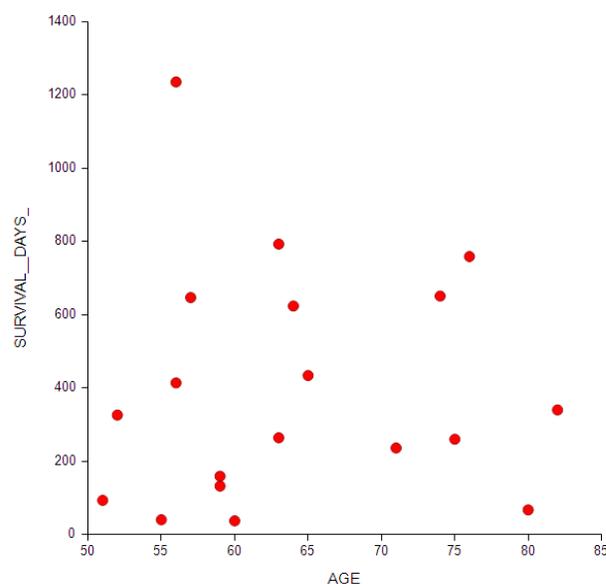


Figure 1. A scatterplot of age versus survival is shown. There was no correlation between age and survival.

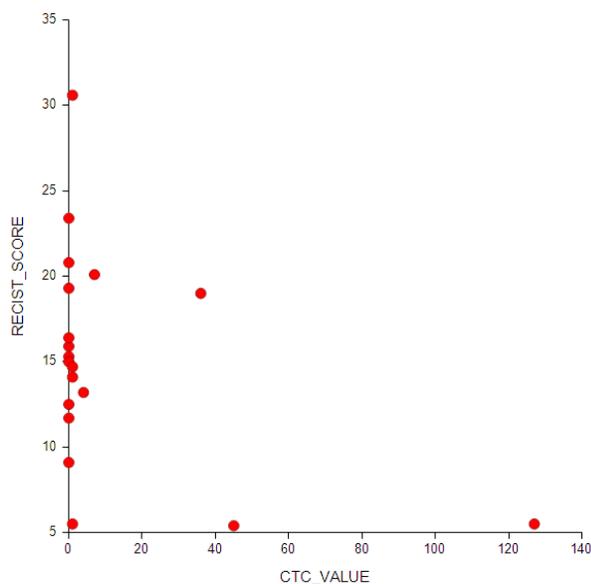


Figure 2. Tumor size (sum of the largest three perpendicular diameters in centimeters) versus CTC value is plotted. The size of the dominant mass had no relationship to the number of circulating tumor cells.

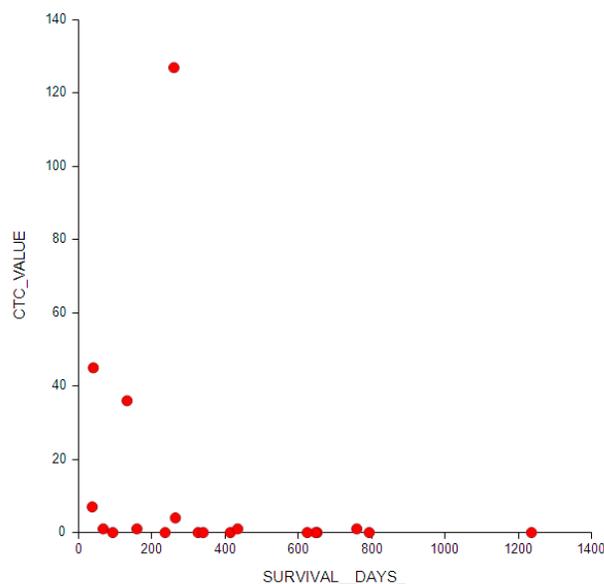


Figure 3. A scatter plot of circulating tumor cell (CTC) values versus survival (in days) first suggested that those patients with a CTC of 0 might have superior survival as compared to those whose CTC was ≥ 1 .

There was no statistical difference in survival for men compared to women (325.3 ± 90.4 days vs. 515.4 ± 118.4 days, respectively, $P=0.219$). Age at entry into the study was evaluated and did not correlate with survival (Figure 1). Primary tumor size, once the patient had stage IV disease, did not influence survival (Figure 2). Our pilot study may have lacked adequate statistical power to detect differences in survival due to age or gender.

Inspection of a scatterplot of CTC versus survival suggested there might be a survival advantage favoring those with a CTC score of 0 versus those with a CTC score ≥ 1 (Figure 3). Statistical analysis showed that survival (measured in days \pm standard error of the mean) for those with a CTC score of 0 was 536 ± 91.1 versus 239 ± 96.0 for those whose CTC score was greater than or equal to 1. Tested by the Kruskal-Wallis one-way ANOVA on ranks-hypotheses, this difference was statistically significant ($P=0.034$).

DISCUSSION

Clearly, some non-small cell lung carcinomas shed tumor cells into the circulation. Mascalchi et al., using ScreenCell[®] methodology, found at least one CTC per 3 ml blood sample and one circulating tumor microembolus in 65% and 58%, respectively, in their study of 26 consecutive cases of stage III or IV non-small cell lung carcinoma.¹⁷ It is not surprising that different methods of analysis yield different rates of detection of CTCs. We found zero CTCs in 10 of our 19 subjects with advanced disease (52.6%). Okumura et al. found CTCs in the peripheral blood of 29.4% of patients with resectable lung carcinoma.¹⁸ Our study focused only on patients with stage IV carcinoma, i.e. unresectable patients. Size of the tumor did not correlate with the presence or absence of CTCs. Could the absence of measurable numbers of CTCs have been due to an artifact caused by false negatives? If the tumor cells lacked the specific surface antigens or contained other antigens to which the test's reagents were nonreactive, a zero result would have been obtained. Nonetheless, those of our subjects with a CTC score of 0 showed superior survival.

Other investigators before us have used 5 CTCs as the “break point” for determining low-risk versus high-risk prognosis. This has been rigorously determined in breast carcinoma.²⁻⁶ We tested our data comparing those with < 5 CTCs to those with \geq 5 CTCs. There was no apparent statistical difference in survival viewed according to those criteria. This may be a consequence of the small size of our pilot study or suggest a biologic difference based upon a different neoplasm in our study (lung) versus those in Cristofanilli’s group (breast). A larger, more robust study in lung cancer could potentially answer that question.

There are indications that the therapy applied may have an impact on the presence of CTCs in the peripheral blood. Using an adenoviral probe that detected the elevated telomerase activity present in almost all cancer cells but not in normal cells, the median number of CTCs was assayed in 30 patients with non-small cell lung carcinoma.¹⁹ Median CTC counts declined from 9.1 CTCs/ml to 0.6 CTCs/ml post-radiation therapy. Even the type of surgery appears to have an impact on the number of CTCs. Huang et al. studied 43 patients who underwent video-assisted thoracoscopic surgery (VATS) and 36 patients treated by open thoracotomy.²⁰ Blood samples obtained preoperatively, intraoperatively and 3 days postoperatively were studied for CTCs using an immunomagnetic method. The increase in the number of CTCs comparing pre- to postoperative assays was statistically significantly lower in the VATS group than in the open thoracotomy group (2.18 vs. 9.67, $P=0.015$). The application of radiofrequency was evaluated in a study of 9 patients by Chudasema et al., who found an increase in the number of CTCs in 7 of the 9.²¹ The long-term effects of manipulative and ablative therapeutic procedures in patients with primary pulmonary neoplasms are unknown.

CONCLUSIONS

There are circulating tumor cells in persons with stage IV non-small cell lung carcinoma, and these numbers can be influenced by the treatment employed. In our study, the absence of CTCs predicted superior survival in excess of one year. Further investigations are needed and indicated.

Patient-Friendly Recap

- Standard imaging techniques only tell us so much about the success of a patient’s cancer treatment.
- Circulating tumor cells (CTCs), cancer cells that may exist in a patient’s bloodstream, could be another indicator for prognosis.
- In this pilot study, the authors found that lung cancer patients without CTCs survive longer than patients with CTC.

Conflicts of Interest

None.

Funding Sources

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