What's in a vein?

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Feature Editor Note—*In this Invited Expert Opinion article, Wakeam and colleagues review the basic science of circulating tumor cells in non–small cell lung cancer in light of a recent randomized study from Wei and colleagues on the impact of a vein-first versus artery-first surgical lobectomy technique on circulating tumor cells and survival recently published in the* Journal of the American Medical Association Surgery. *The group at University of Michigan has expertise in the isolation of circulating tumor cells using intraoperative aspiration from the pulmonary vein. The potential effect of circulating tumor cells on survival is crucial, since 90% of all cancer-related deaths are thought to be due to metastatic disease. Circulating tumor cells may also serve as a prognostic and therapeutic biomarker. Wei and colleagues hypothesized that ligating the pulmonary veins first would help prevent shedding of circulating tumor cells. They report a significant difference in circulating tumor cells in a randomized trial of patients undergoing artery-first versus vein-first lobectomy. They also performed a separate retrospective review correlating the surgical approach with overall and progression-free survival using the Western China Lung Cancer Database and found significantly worse overall and lung cancer–specific survival in the artery-first patients. Wakeam and colleagues review the advantages and disadvantages of the unique study design combining a randomized trial evaluated circulating tumor cells with a separate retrospective, propensity-matched study evaluating overall and disease-specific survival based on a cancer registry and how the study design could impact interpretation of the results. They conclude that the possibility of affecting long-term survival by altering one's surgical technique is interesting but needs to be validated in a larger study with clear documentation of the surgical approach and that the measurement of circulating tumor cells should be validated using other methods.*

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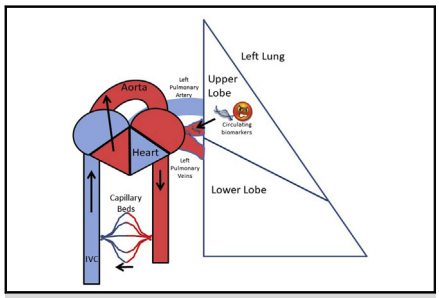

Modifying surgical technique without further validation of CTC changes is not warranted.

CENTRAL MESSAGE

This review examines the basic science of circulating tumor cells in NSCLC. We review a study from Wei et al on the impact of surgical technique on CTCs.

See Commentaries on pages 354 and 356.

Metastasis is theorized to account for up to 90% of all cancer-related deaths.[1] The rate of tumor recurrence for surgically resected cancers ranges from 30% to 77%, leaving patients with a long-term survival rate of only 50%.[2] The exact mode of recurrence for these cancers is still fairly undefined and remains a critically unmet challenge in the realm of lung cancer treatment. A current theory on hematogenous cancer metastasis links the development of metastatic lesions to the seeding by circulating tumor cells (CTCs). CTCs are shed by the primary tumor into circulation and are able to extravasate into distant tissue to seed secondary tumor sites. The vast majority of CTCs released from the primary tumor are likely destroyed in circulation,[3] with only a rare subset of the total CTC population that are able to develop metastatic lesions.

Some surgeons hypothesize that surgical technique during pulmonary lobectomy could influence tumor dissemination. Specifically, surgeons debate whether ligating the pulmonary vein or artery first could influence the levels of CTCs, and hence metastasis. The vein-first (VF) technique has emerged as the technique of choice for video-assisted/minimally invasive resections, because it enables lobectomy without direct fissural dissection, which decreases air leaks.[4] However, ligating the vein first has a theoretical

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risk of increasing venous congestion in the lobe during the rest of the operation.

CIRCULATING TUMOR CELLS IN NON–SMALL CELL LUNG CANCER (NSCLC)

The clinical utility of CTCs in NSCLC may be associated with their ability to predict patient prognosis from their presence in patients with advanced-stage NSCLC,[5,6] but this paradigm has not been as well studied in patients with early-stage lung cancer. CTCs have also been identified as a useful biomarker for disease progression and therapeutic effectiveness following treatment,[7] but the sensitivity of current CTC isolation technologies limits the applicability of CTCs as a mode of reliable early detection and prognosis, along with a lack of clear prognostic thresholds.

The ability to isolate and effectively analyze CTCs from patients has been challenging but has dramatically improved over the past 2 decades. Currently, CellSearch, which uses anti-EpCAM (antibodies to Epithelial Cell Adhesion Molecule, a common CTC surface antigen)-coated magnetic beads to isolate EpCAM + CTC populations from patient blood, is the technology approved by the Food and Drug Administration in the United States and is considered the gold standard in CTC isolation. EpCAM + selection, however, has proven inefficient in regards to capturing CTC populations from patients with NSCLC, with some studies reporting as low as 23% to 39% positivity rates.[8,9] One proposed method to circumvent low EpCAM expression in CTCs is through the detection of folate receptors (FRs) on the surface of CTCs. The FR has been shown to be upregulated on CTCs and, outside of activated monocytes,[10] is not expressed on other cells found within circulation.[11] Previous studies have reported a sensitivity and specificity of 74.4% and 86.6%, respectively, for FR + CTC detection in NSCLC.[12]

STUDY SUMMARY

The current study by Wei and colleagues is presented in 2 parts.[13] The first is a direct comparison of CTC concentration in the blood of patients following both a pulmonary vein dissection and ligation-first technique, or VF, and a pulmonary artery-first, or "artery-first" (AF), technique. The second is a retrospective study correlating surgical approach (VF or AF) with overall survival (OS), progression-free survival, and lung cancer–specific survival of patients registered in the Western China Lung Cancer Database following a propensity matching. In the first part of their study, the authors quantified the change in CTC concentration between a cohort of 86 patients using Genosaber's Cytoplorare FR + CTC detection kit (Genosaber Biotech Co, Ltd, Shanghai, China). To summarize, erythrocytes are lysed from whole blood, followed by the immunomagnetic depletion of leukocytes using CD45-coated magnetic beads. The enriched sample is then quantified for CTCs using ligand-targeted polymerase chain reaction (PCR). The primer used for amplification in the PCR was specific for a synthesized oligonucleotide bound to folic acid. The tumor-specific folic acid–oligonucleotide conjugates are added to the enriched sample and washed to remove excess. The oligonucleotide is then cleaved from the surface of the cells and quantified using ligand-targeted PCR. The results of the PCR can then be normalized to negative controls and samples with known concentrations of cancerous cells spiked in.[11]

CTC ANALYSIS IN WEI AND COLLEAGUES

The authors claim that they found a significant difference in the change in FR + CTC levels between patients undergoing AF and VF (0.73 vs –0.5 CTC units per 3 mL of blood; $P = .006$). It is important to note that these "CTC units" are not actually cells and that the small range of units is a concern in interpreting these results. Some of our previous work has shown a range of 0 (50% of samples) to 5422 EpCAM + CTCs per 7.5 mL in samples from the pulmonary vein.[14] These data are consistent with the ranges provided in a number of other publications, but we are unable to compare this to the range of data presented by the authors, which uses CTC units and the samples were taken from peripheral arterial, which can have a 200-fold lower yield of tumor cells than the pulmonary veins. Furthermore, the use of a "CTC unit" in their study leaves the results vulnerable to interpatient variability in FR expression. Previous reports have shown variable FR expression among adenocarcinoma samples.[15] Due to the narrow ranges in changing FR + CTC levels between patients, it is difficult to discern what proportion of these variations are associated with cell number differences and/or FR expression variability. Also of concern is that there is little discussion on where and why the authors set their "threshold" for a positive capture unit rate. In the published literature, there is a baseline "noise" level of antibody-positive cells that range from 2 to 5 "CTCs" per 7.5 mL of blood.[11] Varying this baseline level can dramatically affect what the reported rates of patients with positive or negative circulating tumor cells. For example, at a baseline threshold of 5 CTCs per 7.5 mL of blood, the result of 3 CTCs would be "negative," whereas a threshold set of 2 CTCs would result in a "positive." Without understanding their thresholds and why they set them, it is very hard to interpret the data set. In their retrospective study, they analyzed a total 1691 patients—210 patients undergoing AF and 1481 patients undergoing VF. Following a propensity-matching process, they found patients undergoing AF to have a significantly worse OS (57.6% vs 73.6; $P = .002$), progression-free survival (48.4% vs 64.6%; $P = .001$), and lung cancer–specific survival (59.9% vs 76.4%; $P = .002$) than patients undergoing VF,

respectively. They also determined AF to be an independent prognostic factor for poorer OS in a multivariate analysis, alongside stage II and stage III disease.

STATISTICAL METHODOLOGY

The authors present an unusual combination study—the first part, a randomized trial, and the second, a propensity matched study—and they should be commended on a creative approach to the difficult issues around sample size in the trial. However, each separate study method has advantages and disadvantages that impact the interpretation of the results.

STUDY PART I: RANDOMIZED TRIAL

In the randomized trial portion, patients were randomized in a 1:1 fashion between the VF and AF groups. The power calculation was done using standard parameters and based on differences observed in a preliminary study that suggested that there might be at least a 10% difference in the change in "folate units" (FU) between the groups, a difference whose meaning is unclear. How they determined what the expected distribution of CTC changes would be is unclear, and while in a large sample one might be safe to assume a normal distribution of results for the purposes of calculating power, in a sample of 40 this may not be the case and would impact the power required to show a true difference. As stated earlier, other CTC studies have shown a wide variation in the detection of CTCs that don't follow a normal distribution curve.[12] Also, the false-positive "rate" or "level" is not clearly defined, which is a common concern in CTC identification. Due to the small size of the trial and short follow-up, surrogate end points were chosen rather than recurrence or survival. The authors only analyze recurrence and survival in the retrospective portion of the study, which is misleading. The trial itself measured FUs and only followed patients for the short term, so survival outcomes cannot be studied. There are no previously published data that support changes in "FU" impacting survival. One must be careful in how these results are interpreted. Previous work from our group[14] found a statistically significant greater number of CTCs in patients at the time of surgery who had a previous bronchoscopy-guided biopsy, versus those with a computed tomography–guided biopsy. While these were interesting results, given the possibility that they are the result of a multiple testing phenomenon, they must be validated by other studies before being used to change practice. The current study similarly needs to be validated before surgeons make changes to their practice.

In addition, the included Figure 2—which is the trial's version of what an oncology trial would include as a "waterfall plot"—displays the individual responses to surgery in terms of CTCs. Based on this figure, it is hard to appreciate a marked difference between the groups—while the averages may favor VF, this result may be driven by a select few patients in whom CTCs were markedly greater, whereas most patients seem to get little to no elevation with either technique, and the margin of error for measurement is also not displayed on this figure. Lastly, it should be noted that several patients with stage IV disease were included in the study, after finding pleural nodules at the time of surgery. These patients underwent resection anyway, which would not be standard treatment. Patients with stage IV disease may have greater or fluctuating levels of CTCs independent of surgical technique. A subgroup analysis of more advanced-stage patients (stages III and IV) would have clarified this issue.

STUDY PART II: RETROSPECTIVE REVIEW

The authors then conducted a propensity-matched examination of a registry "to limit the effect of potential confounding factors." Propensity matching by definition cannot do this; it can only balance the known covariates on which the matching is done. It is important to note that the variables for matching appear to be selected based on their relationship to the outcome (survival) rather than the exposure variable (AF or VF technique). This is an incorrect approach, and there is no description of how the authors attempted to determine which patient or tumor characteristics were important in determining whether a surgeon chose the AF or VF technique. This is an important source of bias, which has gone unaddressed—and would be very difficult or impossible to tease out of retrospective operative reports.

Using the framework proposed by McMurry and colleagues,[16] other deficiencies in the propensity matching in this study are evident. There is no explanation as to how these variables were selected, how the models were decided upon, how they decided to use a 1:1 match (vs a 1:2 or 1:3 match), as there were more than 9000 patients in the original sample and many more potential controls than the 210 used in the 1:1 match. There was also no information on the adequacy of the matching, as there seems to have been minimal to no description of whether the match produced 2 balanced cohorts of patients, usually done by assessing standardized differences in the pre- and post-matched samples. Finally, the authors neglect to address 2 major issues in thoracoscopic lobectomy: training and lymph node dissection. Many thoracoscopic surgeons use a VF approach to minimize the fissure dissection, and training paradigms could result in VF surgeons being technically more adept at video-assisted thoracoscopic surgery lobectomy, which could bias the results. Second, there is no mention of the extent of lymph node dissection or pathologic staging of those nodes, which could impact OS results.

CONCLUSIONS

The authors of this study should be congratulated for conducting a randomized trial on a difficult but important topic.

Despite this, the fact remains that many questions are still unanswered. The idea that a surgeon could alter their technique with long-lasting oncologic impact is intriguing and could easily be validated by a larger trial where surgeons document their surgical approach. Ultimately, a randomized trial for VF versus AF approach focusing on survival, recurrence, and lung cancer-specific survival could have a significant impact but would require a multihospital consortium to realistically complete. The "FU" differences seen here with different surgical approach must be validated by other CTC measurement (antibody or physical property-based collection). This study's findings of increased CTC "units" is intriguing, but unclear if it is clinically meaningful.

Conflict of Interest Statement

Dr Reddy reported Intuitive Surgical, Auris Health, and Medtronic, nonrelevant to this manuscript. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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