Accuracy of extrapolation of circulating tumor cell count from small blood volumes: statistical estimation using the AccuCyte®– CyteFinder® system

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Abstract 1732

Background
Baseline and post-treatment counts of circulating tumor cells (CTCs) are prognostic of patient outcome in cancer. The FDA-approved CellSearch® system has defined CTC count as the number of cells per 7.5 mL of blood, with poor prognosis being 5 or more for breast and prostate cancers, and 3 or more for colon cancer. There is little reported data on whether it is reasonable to extrapolate CTC counts from smaller blood volumes, particularly when CTC count is low, given sampling variability. The AccuCyte®–CyteFinder® system (RareCyte) collects 7.5 mL of blood for analysis, isolates theuffy coat and smears it onto 8 slides, which are then stained with markers for CTCs that are identified by fluorescence microscopy. We investigated the statistical accuracy of extrapolation of total CTC count from fewer than 8 slides.

Methods
172 blood samples from various cancers were processed to microscopic slides, stained, and analyzed by CyteFinder®. Counts were made on a per slide basis, with CTC count “CTC count being the sum of all 8 slides. Samples with no CTCs were excluded. To extrapolate the total CTC count from k (k < 8) slides, all possible combinations of k slides were used to estimate the total count of 8 slides. This was done for k = 1-7 for all 172 samples and extrapolated counts were rounded to the nearest integer. The proportion of time that extrapolated counts fell within a specified percentage of the true CTC count was calculated across the entire sample set, as well as for 6 sub-categories defined by true CTC count range.

Results
The 8 CTC count categories (number of samples) were: 1-4 (35), 5-10 (21), 11-25 (21), 26-50 (14), 51-100 (24), > 100 (53). Cumulative proportions within k, 10, 25 and 50% of the true total CTC count were determined for the entire sample set as well as by category. In the entire sample set, the proportion of extrapolated counts that fell within 25% of the true count ranged from 0.687 (4 slides) to 0.999 (7 slides). Generally, the fewer the true total count, the lower the fraction of extrapolated counts that fell within the specified percentage of the true count. For example, for true count 9-10, the fraction that fell within 25% of the true count ranged from 0.961 (7 slides) to 0.997 (2 slides). In contrast, for true count >100, the fraction that fell within 25% of the true count ranged from 0.751 (1 slide) to 0.986 (4 slides) to 1.000 (7 slides).

Sample breakdown by patient cancer type and CTC count

- In this sample set, nearly three quarters of cases could be estimated within 25% of the true CTC count from extrapolating from 4 slides.
- Estimating each slide to represent ~1 mL of processed whole blood, our analysis suggests that ~4 mL sample will have this same level of accuracy.
- Counts were compared to the true CTC counts. The percent of these estimates that are within ±10%, ±25% and ±50% are displayed.

Conclusions
- Whole slide "CyteMap®" imaging
- Candidate cells are identified by staining for nuclear and epithelial markers – cytokeratin and/or epithelial cell adhesion molecule (EpCAM) – and absence of the leukocyte marker (CD45). User interface presents in rank order of "CTC scores" that predicts likelihood that a cell is a "real" CTC.
- Automated slide staining
- For the entire sample set and the estimated CTC counts were compared to the true CTC counts. The percent of these estimates that are within 5%, 10% and 25% and 50% are displayed.

Biomarker detection from the CTCs
- Cytokine and cell surface markers
- CTCs were identified by staining for nuclear and epithelial markers – cytokeratin and/or epithelial cell adhesion molecule (EpCAM) – and absence of the leukocyte marker (CD45). User interface presents in rank order of "CTC scores" that predicts likelihood that a cell is a "real" CTC.