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Cancer associated macrophage-like cells in the early detection of solid tumors from numerous malignancies

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ABSTRACT

Blood-based biopsies can be used as a non-invasive method to recover a variety of cancer associated circulating cells, including Circulating Tumor Cells (CTCs) and Circulating Cancer Associated Macrophage-like cells (CAMLs) from the blood of cancer patients. CAMLs are a newly-defined circulating immune cell type, described as a subtype of circulating stromal cells, known specifically to circulate in patients with malignant disease. We studied the peripheral blood of 100 cancer patients to ascertain the prevalence, specificity and sensitivity of CAMLs in relation to their disease status at presentation. We compared a variety of benign and malignant diseases, along with matched healthy control blood samples. We supply evidence that this previously unidentified circulating cell can be used as a screening tool to detect solid tumors in numerous malignancy subtypes in all disease stages.

MATERIALS & METHODS

Peripheral blood samples from cancer patients were provided by University of Maryland, Greenebaum Cancer Center, Northwestern University, The Medical College of Wisconsin, Fox Chase Cancer Center, and Duke University. We ran a prospective blinded study to isolate CAMLs from patients with known invasive carcinomas (n=117), healthy control samples (n=40) and patients with benign non-malignant conditions (n=21). The patient distribution included Stage I (n=39), Stage II (32), Stage III (16), Stage IV (30); breast (n=31), pancreatic (n=22), lung (n=38), and prostate (n=26) cancers. CellSieve™ microfilters were used to isolate CTCs and CAMLs from 7.5 mL of whole peripheral blood. The 7 µm pore size of the membrane allows isolation of both CTCs and CAMLs based on size. Collected cells were fixed, permeabilized, and stained with DAPI and antibodies against cytokeratin 8, 18 and 19, EpCAM, and CD45. CAMLs were defined as enlarged, multinuclear cells with *diffuse* cytoplasmic cytokeratin staining; they can be either CD45+ or CD45-. CTCs were defined as *filamentous* cytokeratin cells that are CD45-.

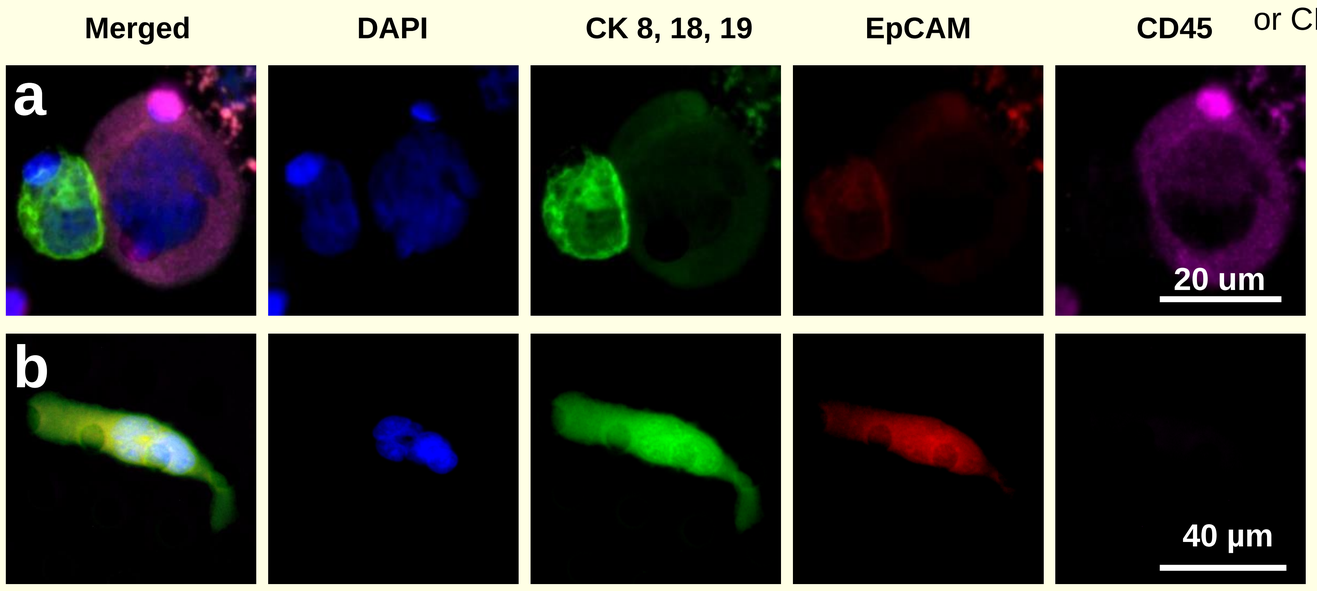

Figure 1. Representative examples of CTCs and CAMLs. (a) CTC attached to a CAML. CTC, left cell, has a single nucleus, filamentous cytokeratin and no CD45. CAML, right cell, has enlarged multinuclear structure, diffuse cytokeratin, and is CD45 positive. (b) CAML with enlarged multinuclear structure, EpCAM expression and no CD45.

INTRODUCTION

CAMLs are specialized myeloid cells transiting the circulation of patients in all stages of cancer. They are responsive to cancer treatment and are found in multiple cancer types[1,2]. However, though seen by numerous groups, these cells have remained largely unstudied, and their clinical and biological value in malignancies remains uninvestigated.

Size exclusion is a technique for isolating large cells from peripheral patient blood irrespective of their surface marker expression. CellSieve™ microfilters are size exclusion membranes capable of rapidly and efficiently isolating both CTCs and CAMLs from whole blood, making it possible to study both cell types in conjunction with and in relation to malignant disease[1-4].

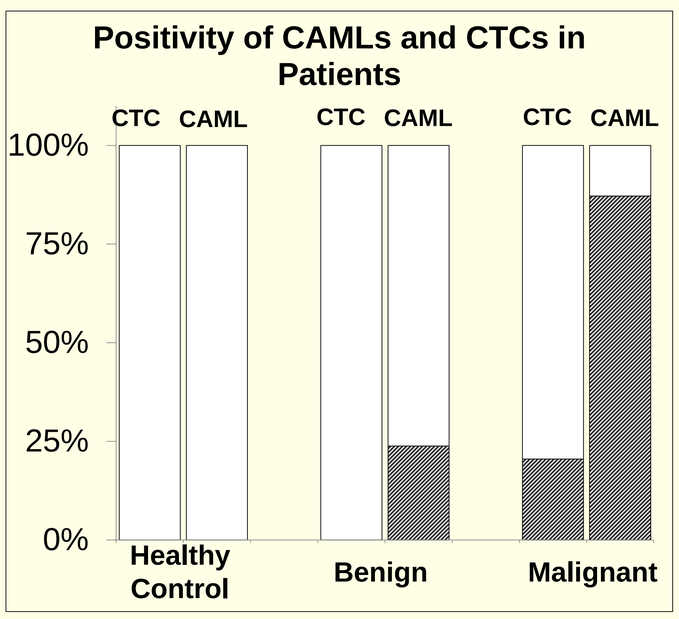

Figure 2. Presence of CTCs and CAMLs in healthy controls, benign and malignant diseases.

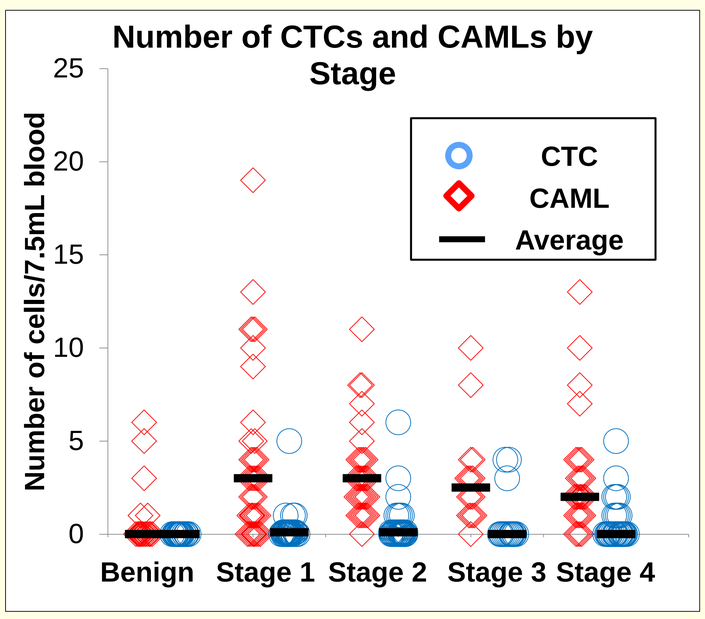

Figure 3. Number of CTCs and CAML in each patient (n=138).

RESULTS

- CAMLs were found in 87% patients with confirmed malignant disease.
  - CAMLs were found in 77% of stage I, 97% of stage II, 94% of Stage III, and 87% of Stage IV patients, regardless of cancer type.
  - CAMLs were found in 77% of prostate, 100% of pancreatic, 82% of lung, and 90% of breast patient samples.
- Neither CAMLs nor CTC were found in healthy individuals (n=40).
- CTCs were found in 21% of the same patient cohort, averaging 0.9 cells per sample.
- CAMLs have vacuoles containing biomarkers from the primary tumor sites.

Invasive Carcinomas (IC) vs Healthy Controls					
	Sensitivity (CI95%)	Specificity (CI95%)	AUC	PPV (CI95%)	NPV (CI95%)
CAMLs	87% (80-93%)	100% (91-100%)	0.93 (0.89-0.96)	100% (97-100%)	73% (59-84%)
CTCs	21% (14-29%)	100% (91-100%)	0.60 (0.57-0.64)	100% (86-100%)	30% (22-39%)
Invasive Carcinomas vs Benign conditions					
CAMLs	87% (80-90%)	76% (53-92%)	0.82 (0.67-0.89)	95% (89-99%)	52% (33-70%)
CTCs	21% (14-29%)	100% (84-100%)	0.60 (0.57-0.65	100% (86-100%)	18% (12-27%)

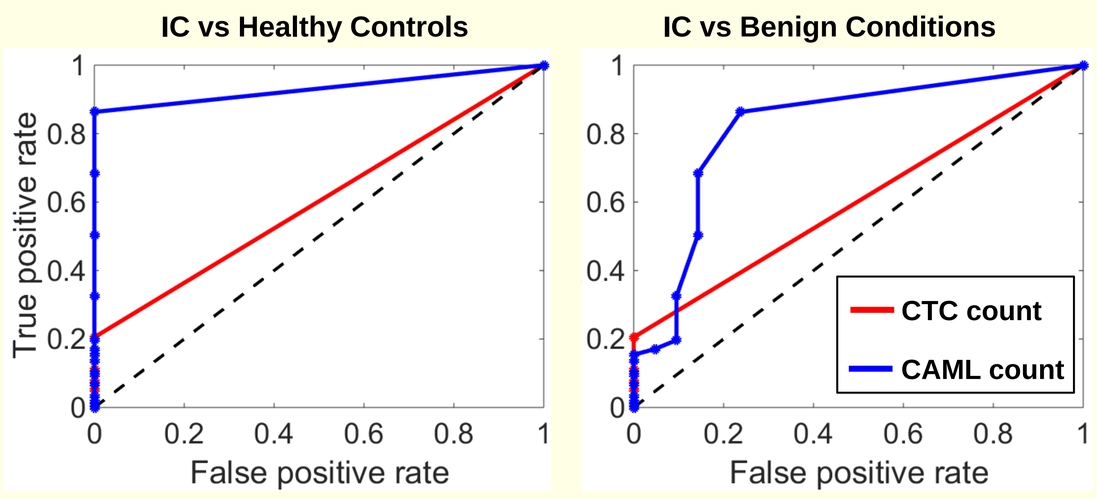

Figure 4. ROC curves using CAMLs (blue) or CTCs (red) for Invasive carcinomas vs healthy controls (left) or Invasive carcinomas vs benign conditions (right)

CONCLUSIONS

- Highly differentiated myeloid cells transit the blood of cancer patients
- CAMLs can be used as a non-invasive blood based biopsy, to detect the anatomical presence of solid malignancies.
- CAMLs sensitivity and specificity suggests a use as a blood biomarker for early stage cancer screening in a broad population.
- This data suggests that larger validation studies should be done to determine the use of CAMLs as a screening tool for cancer.

References

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