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Circulating Stromal Cells as a Potential Blood Based Biomarker for Screening Invasive Solid Tumors

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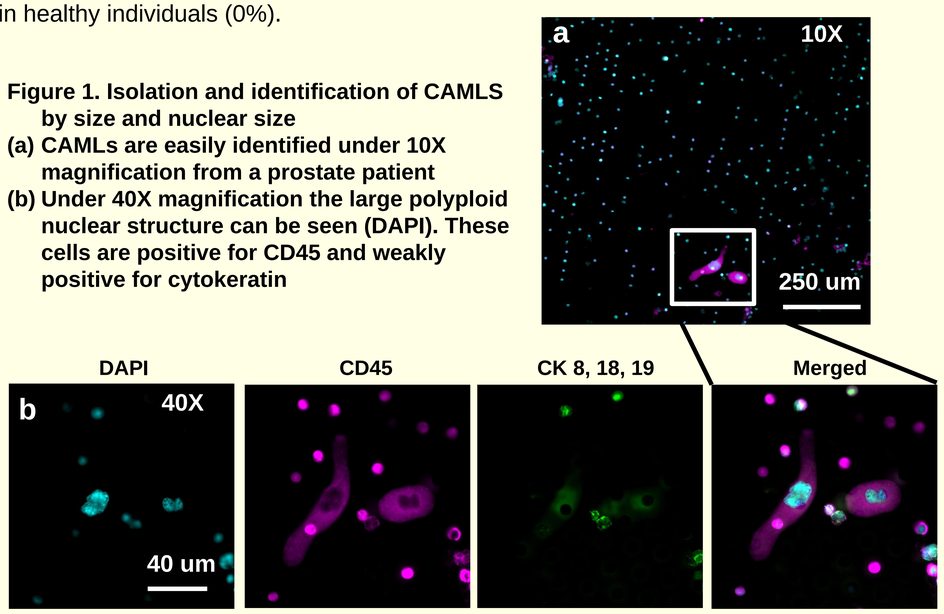
ABSTRACT

Peripheral blood allows for a simple non-invasive method for isolating various cancer associated circulating stromal cells (CStCs) which may predict for cancer presence. Cancer Associated Macrophage-Like cells (CAMLs), a specific CStC, are phagocytic myeloid cells that derive from an immunological response to cancer and emanate from tumors sites. Using a filtration platform we screened the peripheral blood of untreated newly diagnosed cancer patients (n=308) for CAMLs. In parallel, we screened patients with newly diagnosed non-malignant diseases, i.e. lupus, benign cysts, etc. (n=39), and healthy control samples (n=76). We found that CAMLs are highly prevalent (87%) in the blood of cancer patients, but uncommon in non-malignant conditions (20%) & absent in healthy individuals (0%).

Figure 1. Isolation and identification of CAMLS by size and nuclear size
(a) CAMLs are easily identified under 10X magnification from a prostate patient
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are positive for CD45 and weakly positive for cytokeratin



INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients with various types of solid malignancies and appearing in all stages of cancer[1-4]. However, while CAMLs are easy to identify by their large size and polyploid nucleus (**Fig. 1**), their expression of multiple heterogeneous markers have defied conventional characterization and have made study difficult using most isolation technologies. 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 which efficiently isolate CAMLs from whole blood, making it possible to study CAMLs in conjunction with and in relation to malignant disease.

References

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4. Cristofanilli M, "Liquid Biopsies in Solid Tumors" *Humana Press*. 2017

MATERIALS & METHODS

Anonymized peripheral blood were taken from 308 cancer patients after confirmation of invasive malignancy [stage I (n=76), stage II (n=73), stage III (n=72), stage IV (n=65) and unstaged non-metastatic (n=22)] with pathologically confirmed lung (n=65), pancreas (n=53), breast (n=52), prostate (n=40), esophageal (n=30), renal cell (n=18), hepatocellular (n=15), neuroblastoma (n=10), melanoma (n=8), and other (n=17). Further, anonymized blood was taken from patients with untreated non-malignant conditions including benign breast masses (n=19), lupus (n=11), liver cirrhosis (n=5), benign prostatic hyperplasia (n=3), and viral infection (n=1); or from healthy control volunteers (n=76). CAMLs were isolated from whole peripheral blood by the CellSieve™ microfiltration technique and defined as enlarged, multinuclear cells with cytokeratin and/or CD45/CD14 positive.

RESULTS

- CAMLs were found in 87% of all cancer patients (~5.4 CAMLs/7.5mL blood)
  - CAMLs were found in 79% of Stage I, 89% of Stage II, 88% of Stage III, and 95% of Stage IV patients (**Fig 2**).
- No CAMLs were found in any healthy controls (**Fig 2**)
- CAMLs were found in 21% of benign conditions
  - Specifically, 26% of benign breast masses, 18% of lupus patients, but 0% in benign prostatic hyperplasia and 0% in cirrhosis.
- CAML sensitivity in cancer vs healthy was 87% (CI95 82-90%), specificity=100% (CI95 95-100%), PPV=100% (CI95 100%), NPV=67% (CI95 58-71%). (**Fig 3**)
- CAML sensitivity in cancer vs benign was 87% (CI95 82-90%), specificity=80% (CI95 64-91%), PPV=97% (CI95 95-98%), NPV=43% (CI95% 33-51) (**Fig 3**)

Figure 2. Percentage of patients with CAMLs by Stage or Cancer

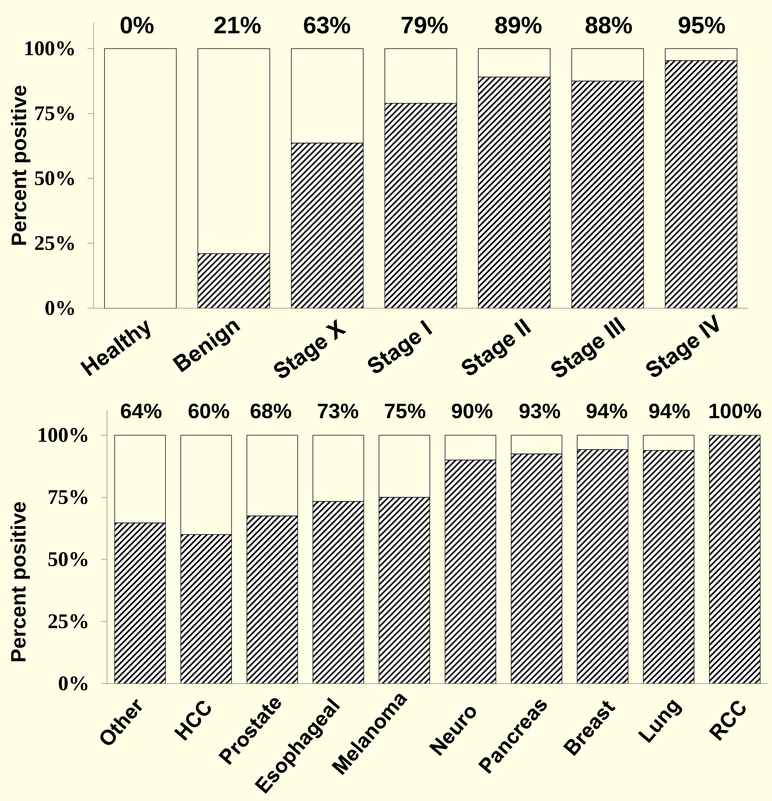
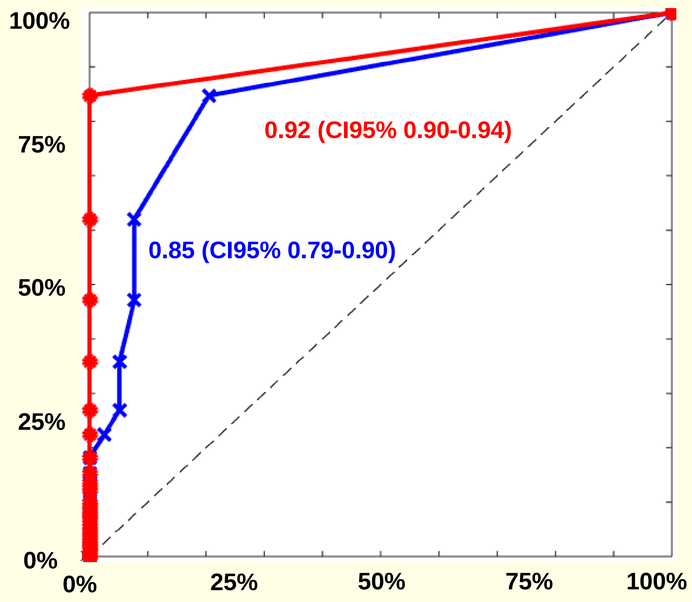


Figure 3. AUC chart-patients with carcinoma vs healthy control or carcinoma vs benign



CONCLUSIONS

- Using combination of clinical studies, we found CAMLs (a Circulating Stromal Cell subtype) are a sensitive blood biomarker specific to patients with confirmed malignancy.
- CAMLs were uncommon in non-malignant conditions and absent in healthy individuals
- CAMLs appear to be a sensitive and specific blood based biomarker for persons with solid cancers

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