



Cancer associated macrophage-like (CAMLs) cells in circulation predict progression and survival in all stages of solid tumors

Daniel L. Adams¹, Steven H. Lin², R. Katherine Alpaugh³, Thai Ho⁴, Jeffrey R. Marks⁵, Stuart S. Martin⁶, Susan Tsai⁷, Saranya Chumsri⁸, Cha-Mei Tang⁹, Massimo Cristofanilli¹⁰, Raymond C. Bergan¹¹

¹ Creatv MicroTech, Monmouth Junction, NJ 08852, ² MD Anderson Cancer Center, Houston, TX 77030, ³ Fox Chase Cancer Center, Philadelphia, PA 19111, ⁴ Mayo Clinic, Phoenix, AZ 85054, ⁵ Duke University, Durham, NC 27710, ⁶ University of Maryland, Greenebaum Cancer Center, Baltimore, MD 21201, ⁷ Medical College of Wisconsin, Milwaukee, WI 53226, ⁸ Mayo Clinic Cancer Center, Jacksonville, FL 32224, ⁹ Creatv MicroTech, Rockville, MD 20850, ¹⁰ Northwestern University, Robert H Lurie Cancer Center, Chicago IL 60611, ¹¹ OHSU, Portland, OR 97239

ABSTRACT

We have previously demonstrated that cancer associated macrophage-like cells (CAMLs) are a cancer specific giant polyploid cell that circulates in the blood of patients with solid tumors. Building on our initial discovery, others have shown that these hyperploidy cells are an innate immune response that is associated with decreased survival. However to date, no study has been done to elucidate their clinical significance as in relation to the various stages of malignant disease. We established a 2 year prospective study of 315 patients from a variety of solid tumors (breast, prostate, lung, renal cell, pancreas, and esophageal) comparing the morphological properties of CAMLs in both early and late stage disease as they relate to progression free (PFS) and overall survival (OS). These data suggest that large CAML size has a significant negative correlation with PFS and OS in a variety of solid tumor regardless of stage, which indicates their broad use as a non-invasive blood based biomarker in solid tumors.

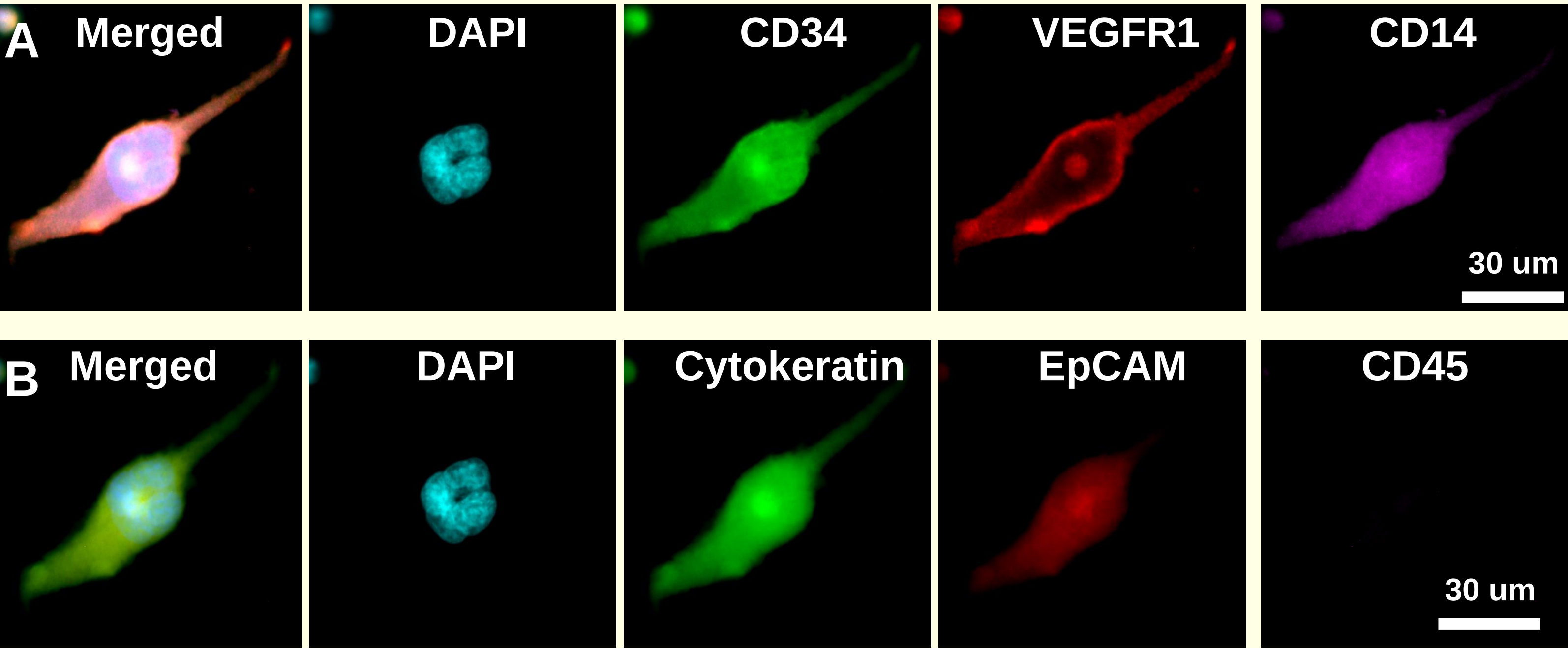
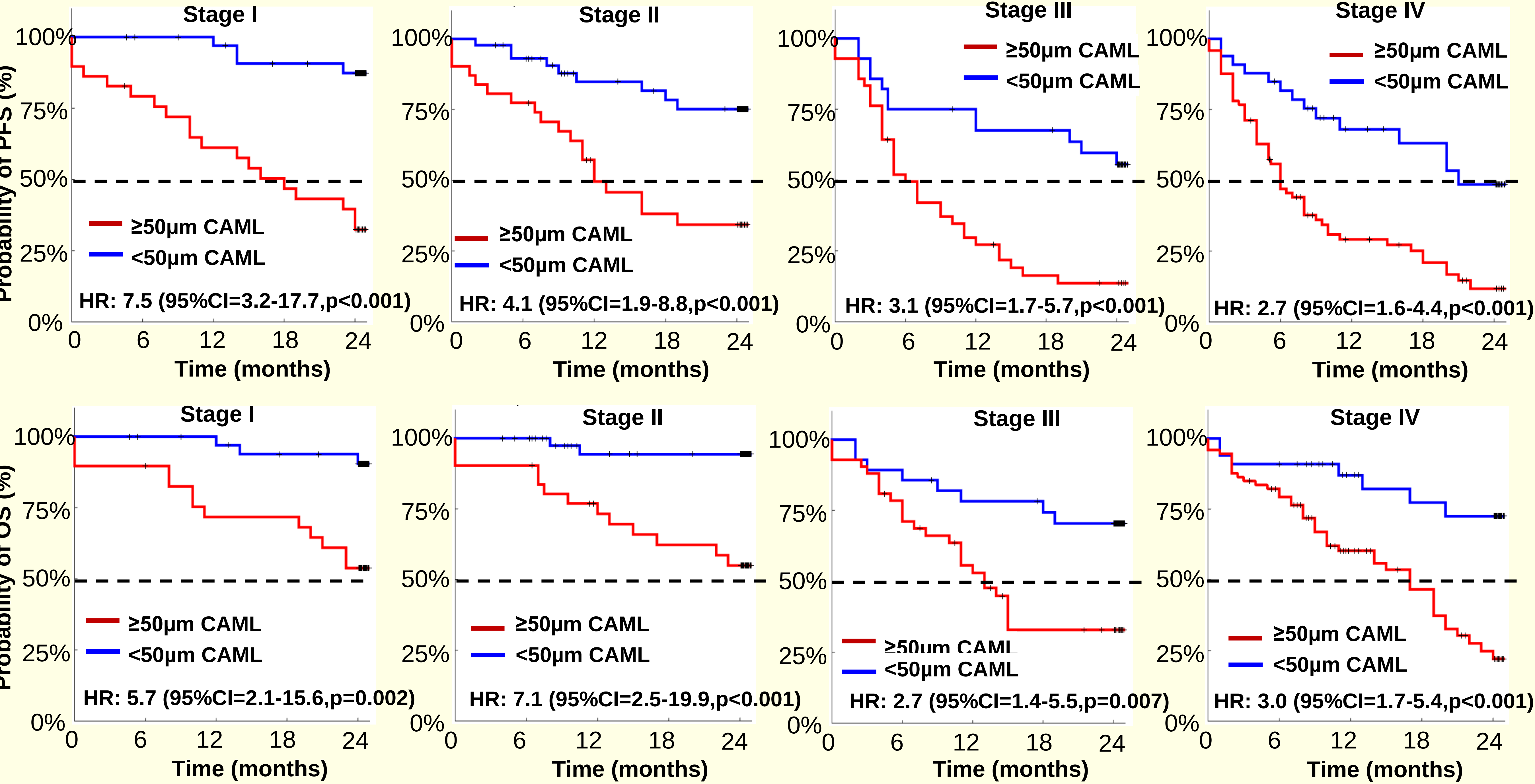


Figure 1. Example of subtyping markers on CAMLs, including myeloid (CD14), epithelial (Cytokeratin/EpCAM), white blood cell (CD45), & stem (CD34), and angiogenic (VEGFR1).

MATERIALS & METHODS

A prospective multi-institutional study used anonymized peripheral blood (7.5 mL) from 315 cancer patients [stage I (n=62), stage II (n=73), stage III (n=67) & stage IV (n=103), unknown stage (n=10)] from subjects with breast (n=59), esophageal (n=27), lung (n=59), renal cell carcinoma (n=37), prostate (n=74), pancreas cancers (n=59). CAMLs were isolated by the CellSieveTM microfiltration technique at 5 institutions and stained for cytokeratin 8, 18, & 19, CD14 and CD45. After imaging, a size based threshold ≥50μm was used to separate the patient cohorts.

Figure 3. Analysis of OS and PFS based on size of CAMLs.



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INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients in various types of solid malignancies and appearing in all stages of cancer¹⁻⁴. While CAMLs are easy to identify by their large size and polyploid nucleus, they appear to present as stem cell like phenotype with multiple heterogeneous epithelial, myeloid, and endothelial markers.

Size exclusion is the only known technique for isolating large cells from peripheral patient blood irrespective of their surface markers. CellSieveTM microfilters are size exclusion membranes which efficiently isolate CAMLs and circulating tumor cells (CTCs) from whole blood, making it possible to study both cell types in relation to malignant disease¹⁻⁴.

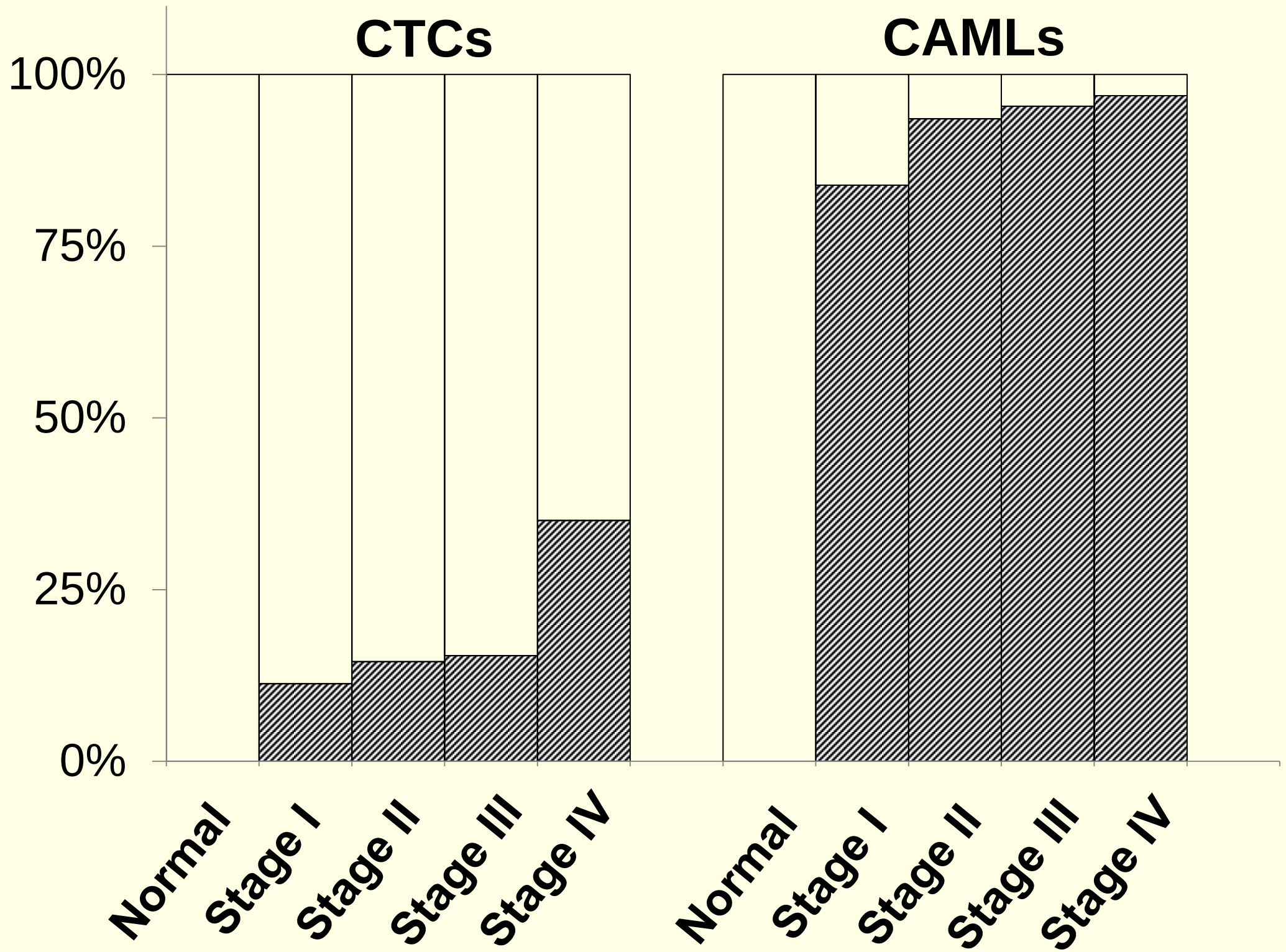


Figure 2. Patients positive for CTCs and CAMLs

RESULTS

- CAMLs were found in 92% (n=289/315) of all cancer patients (n=266/293), but in none of the healthy control samples (Fig. 2)
- CAML sensitivity: Stage I (86%), Stage II (90%), Stage III (97%) & Stage IV (95%), Figure 2.
- Overall, CAMLs of <50μm in size had superior PFS (HR: 3.8; 95% CI 2.8-5.3; (p<0.001) and OS (HR=3.8; 95% CI 2.6-5.7;p<0.001)
- Using a <50μm cut off was prognostic for PFS and OS in each stage.

CONCLUSIONS

- In a large scale prospective study on the clinical utility of CAMLs, CAMLs were common in all stages of invasive solid cancers.
- Smaller CAML size appeared associated with improved survival outcome and longer PFS.
- In a multivariate analysis, CAML size was the most predictive variable for PFS & OS and independent of other clinical variables.
- CAMLs assessment may constitute a new real time predictor of progression. and survival in both early and late stage disease.
- Larger scale clinical validation studies are underway to determine predictive value of CAMLs in specific cohorts

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