

Polyploid Circulating Stromal Cells in Blood Identifies Invasive Solid Tumors and Prognosticates for Highly Aggressive Cancer Subtypes

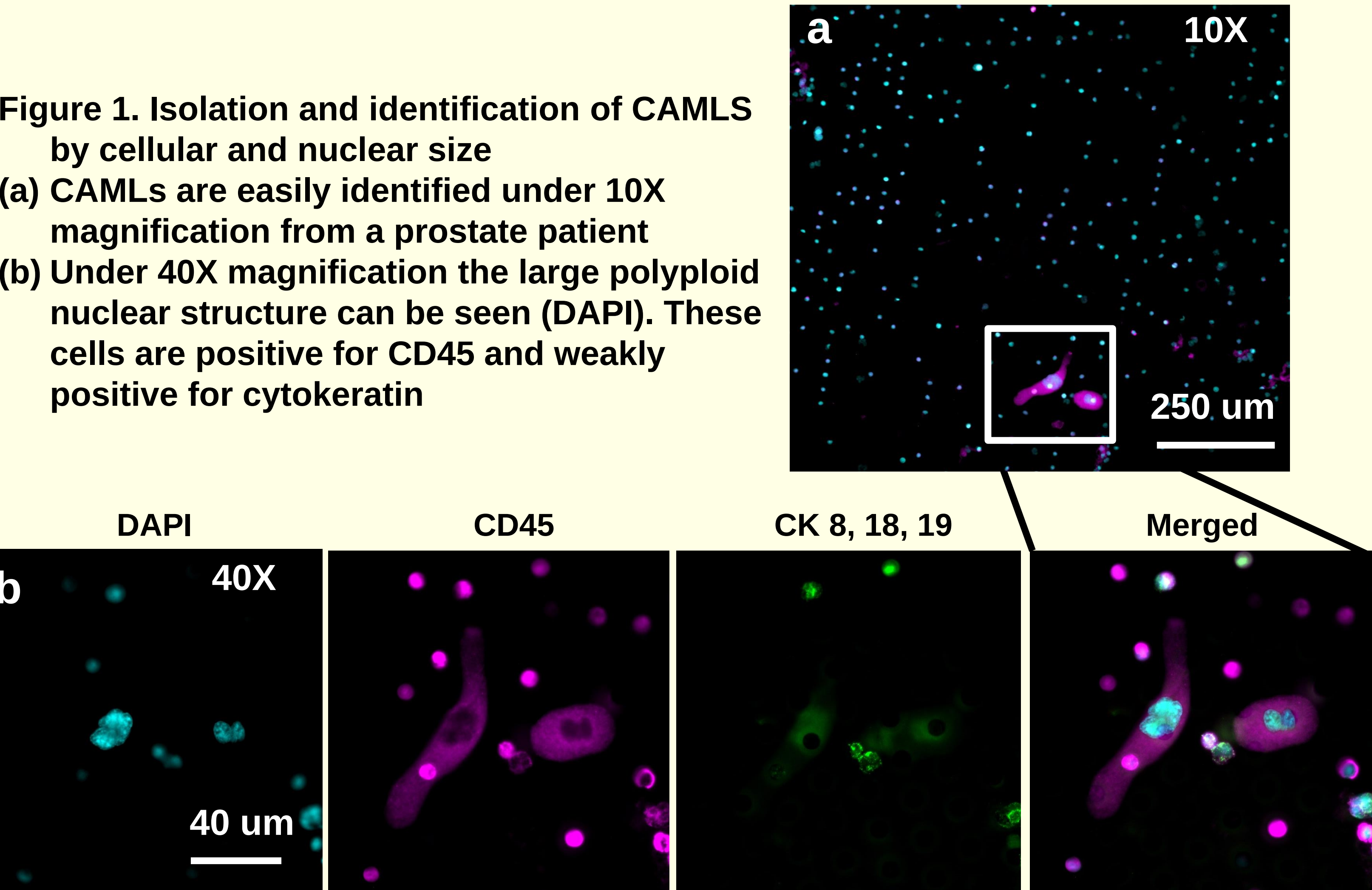
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ABSTRACT

Recent studies have identified circulating stromal cells (CStCs), i.e. Cancer Associated Macrophage-Like cells (CAMLs), in patients (pts) in newly diagnosed invasive cancers, which are polyploid phagocytic myeloid immune cells emanating from primary tumors. However, studies have not yet evaluated CAMLs as a pan solid tumor screening tool or if CAML presence has clinical impact on pt outcomes. We analyzed the blood of a) untreated newly diagnosed cancer pts (n=441), b) patients in non-malignant conditions (i.e. pancreatic cysts, benign breast masses, viral infections, etc. (n=77), and c) healthy controls (n=100). We found that CAMLs are prevalent in blood (82%) in cancer pts, absent in healthy controls (0%), are less common in non-malignant conditions (36%), and that CAMLs are highly correlated with worse clinical outcomes.

Figure 1. Isolation and identification of CAMLs by cellular 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 cancer¹⁻⁴. 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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MATERIALS & METHODS

Anonymized peripheral blood was taken from n=441 pts before or after confirmation of invasive malignancy [stage I (n=105), stage II (n=125), stage III (n=114), stage IV (n=97) with pathologically confirmed lung (n=84), pancreas (n=61), breast (n=73), prostate (n=65), esophageal (n=34), Renal Cell (n=29), Sarcoma (n=27), or Other (n=68). In addition, anonymized blood was taken from pts with untreated non-malignant conditions including benign breast masses (n=19), Lupus (n=11), liver Cirrhosis (n=6), rising PSA (n=24), or other benign masses (n=17); and from healthy control volunteers (n=100). CAMLs were isolated by CellSieve™ microfiltration, defined as enlarged polyploid cells with cytokeratin+ and/or CD45/CD14+. Pts were monitored for 0-8 years (median=2.6) for progression free survival (PFS) and overall survival (OS).

RESULTS

- CAMLs were found in 82% of cancer pts (~5.4 CAMLs/7.5mL blood).
- CAMLs were found in 66% of Stage I, 84% of Stage II, 92% of Stage III, and 92% of Stage IV patients (Fig 2 & 3).
- No CAMLs were found in any healthy controls.
- CAMLs were found in 36% of non-malignant pts.
- CAML specificity vs healthy controls was 100% (CI95% 96-100%).
- CAML specificity vs benign pts was 64% (CI95% 52-74%).
- CAML presence and phagocytic engorgement of CAMLs (>50um) were both significantly associated with worse & OS (Fig 4).
- In the benign pts positive for CAMLs, 2 pts were in situ, 16 had histology's with increased cancer risk (i.e. IPMN, atypical hyperplasia), 4 pts progressed with cancer and 5 pts died within 3 years.

Figure 2. CAML Sensitivity and Accuracy vs Benign Pts by Stage

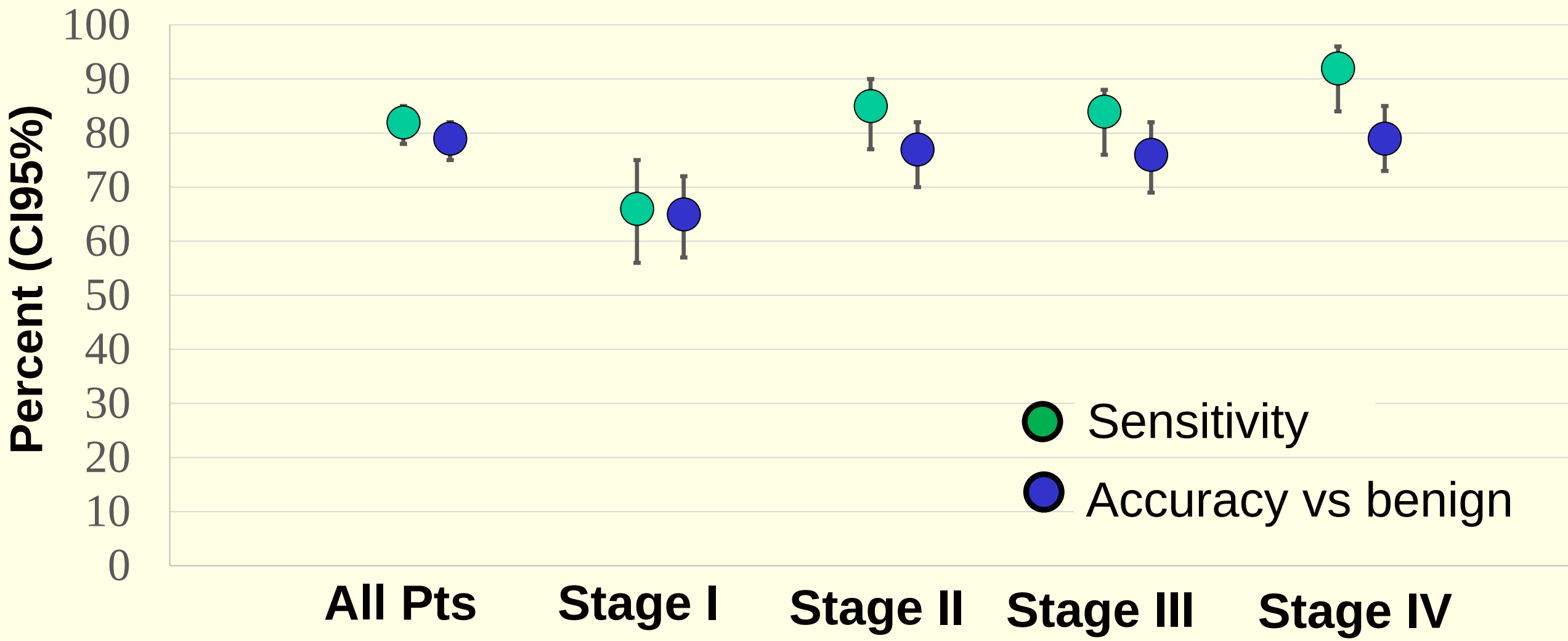


Figure 3. CAML Sensitivity and Accuracy vs Benign Pts by Cancer

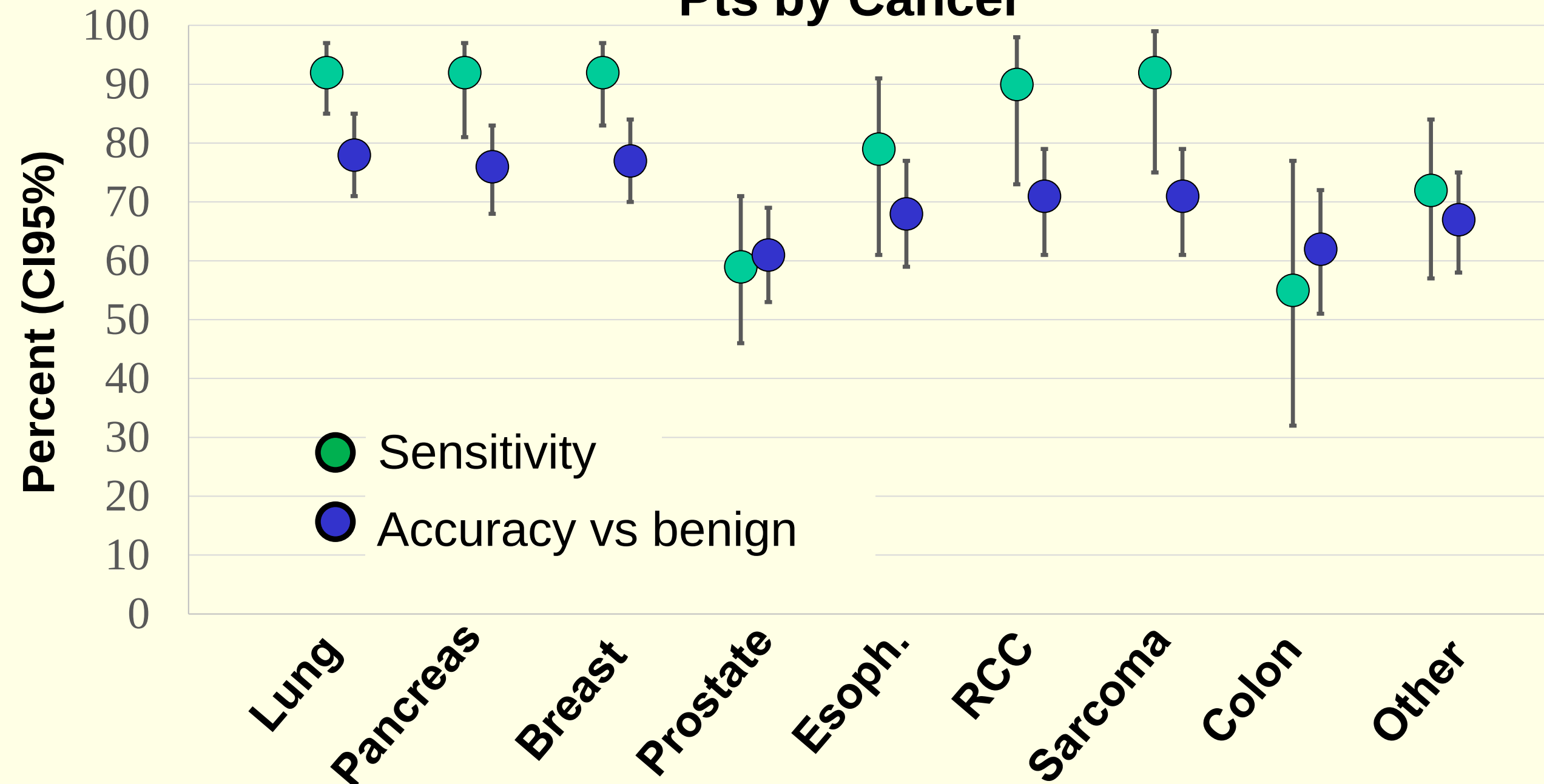
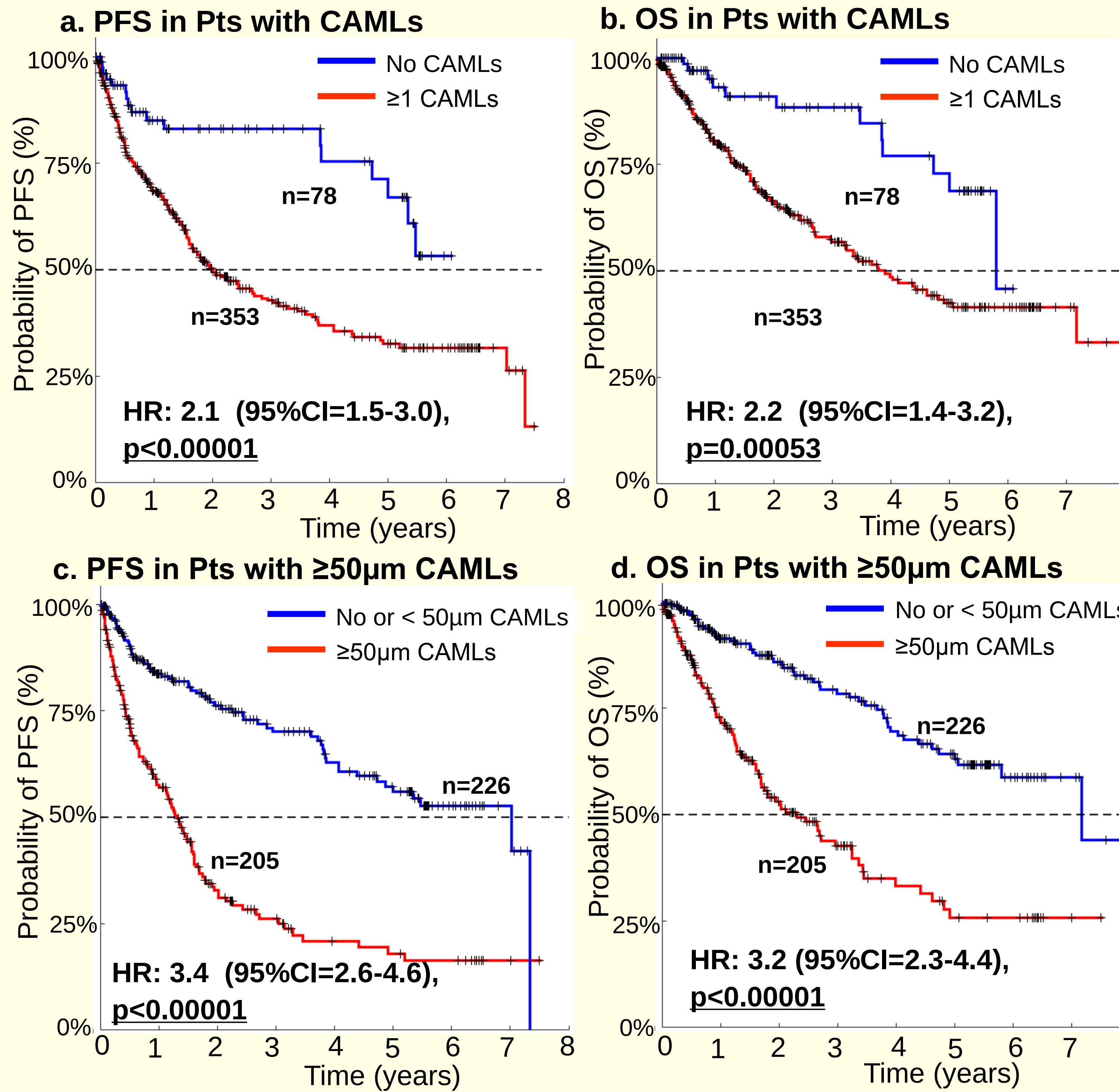


Figure 4. Cox Proportional Analysis by CAML Presence and Size



CONCLUSIONS

- CAMLs appear to be a highly sensitive blood based biomarker that can identify invasive carcinoma in all stages of cancer but absent in healthy individuals.
- CAMLs were rare in non-malignant conditions but appeared related to increased risk of cancer
- CAML presence and polynucleated enlargement of phagocytic CAMLs appeared to correlate with shorter PFS and OS prior to treatment induction.

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