

# Identifying, Subtyping and Classifying Tumor Associated Circulating Endothelial Cells in Solid Tumors

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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), Circulating Cancer Associated Macrophage-like cells (CAMLs), and Tumor endothelial cells (ECs) from the blood of cancer patients. In blood, circulating ECs (CECs) are normal constituents of healthy individuals, although a specific Cancer Associated Vascular Endothelial cell (CAVE) subtype has been observed as being isolated from cancer patients using microfiltration. However, there have been limited efforts to differentiate and subtype this CAVE population. This is not surprising, as in-depth phenotyping of ECs requires an array of biomarkers that until recently has not been feasible. To better evaluate CAVEs, a multi-phenotypic screening of various EC markers was tested of cells isolated from 116 blood samples in 3 different types of solid tumors. This data suggests that CAVEs exist as a common and diverse subtype of tumor derived CECs that may express cytokeratin (CK) and various subtypes of EC biomarkers.

## MATERIALS & METHODS

Peripheral blood samples from 116 cancer patients (stage I-IV) were drawn from 2012-2014 including breast (n=42), lung (n=39) and prostate (n=35), as well as blood from 34 healthy controls. Blood was processed by an established filtration approach, i.e. the CellSieve™ microfiltration technique (Creatv MicroTech), filtering blood by size exclusion and staining cells for CK 8, 18 & 19, EpCAM and CD45 (Fig. 1A). After identification and imaging, the QUAS-R (Quench, Underivatize, Amine-Strip and Restain) technique was used to remove fluorescence signal and restain all cells with CD146, CD14, vimentin, & DAPI (Fig. 1B). After reimaging, QUAS-R was again used to remove fluorescence and restain the cells for CD144, CD34 (or CD105), CD31, & DAPI (Fig. 1C). Multinucleated clusters of CAVEs were differentiated from cancer associated macrophage-like cells using CD14+ and the polyploid nucleus structure observed with CAMLs.

## RESULTS

- CAVEs were identified in 63 of 116 patients (54%) based on positivity of CD31, CD144 or CD146, but none were found in healthy controls.
- CAVEs were found in 43% of stage I, 66% of stage II, 74% of Stage III, and 82% of Stage IV patients (Fig. 2).
- CAVEs were found in 69% of breast, 60% lung, and 77% prostate samples.
- CAVEs were all negative for CD14 and CD45.
- CD31 was the most present marker, found on 96% of CAVEs, followed by CD144 (85%), Cytokeratin (68%), CD34 (64%), CD146 (45%), EpCAM (23%) & CD105 (4%) (Fig. 3).

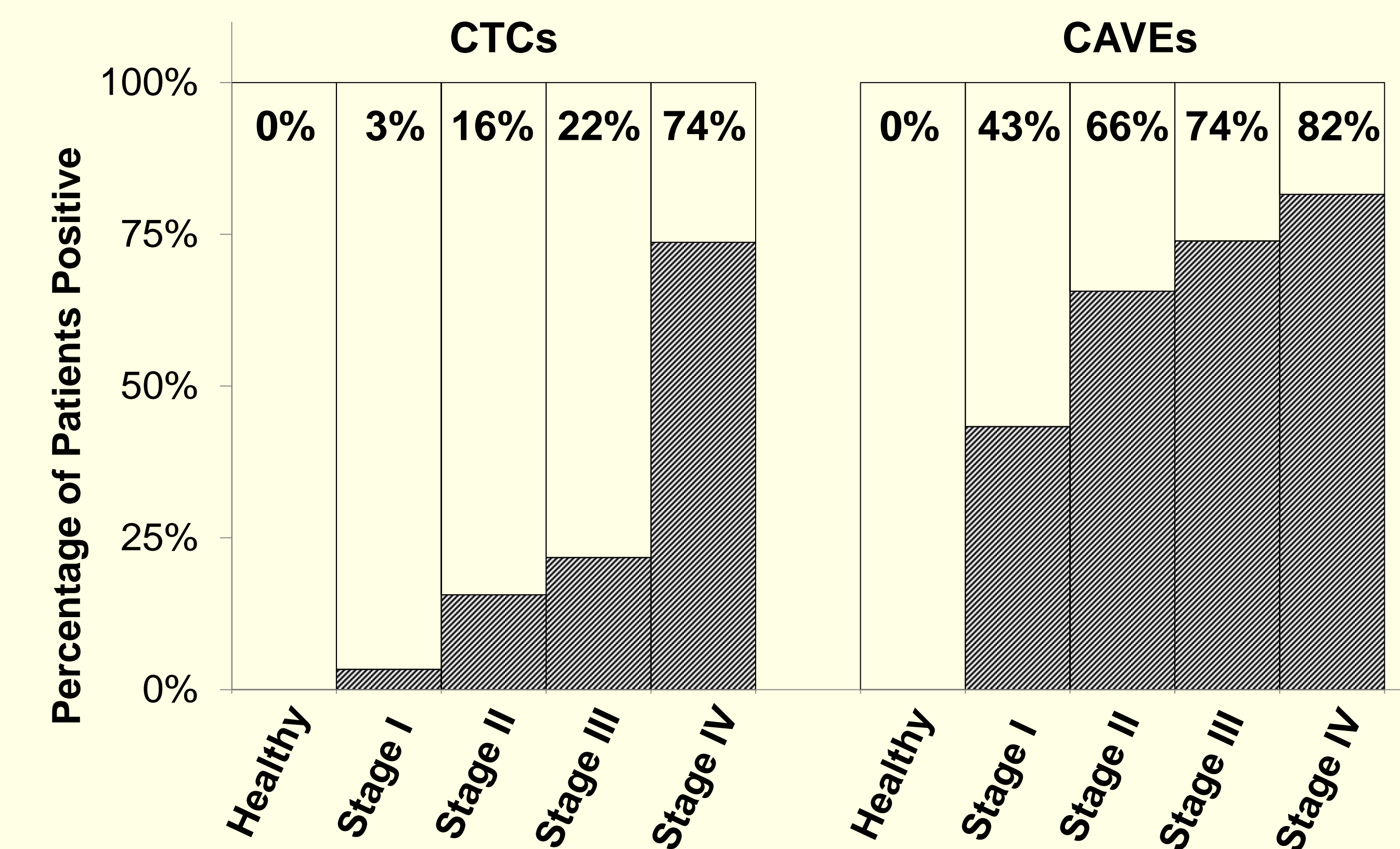


Figure 2. Percentages of CTC and CAVEs in 116 patient sample by stage.

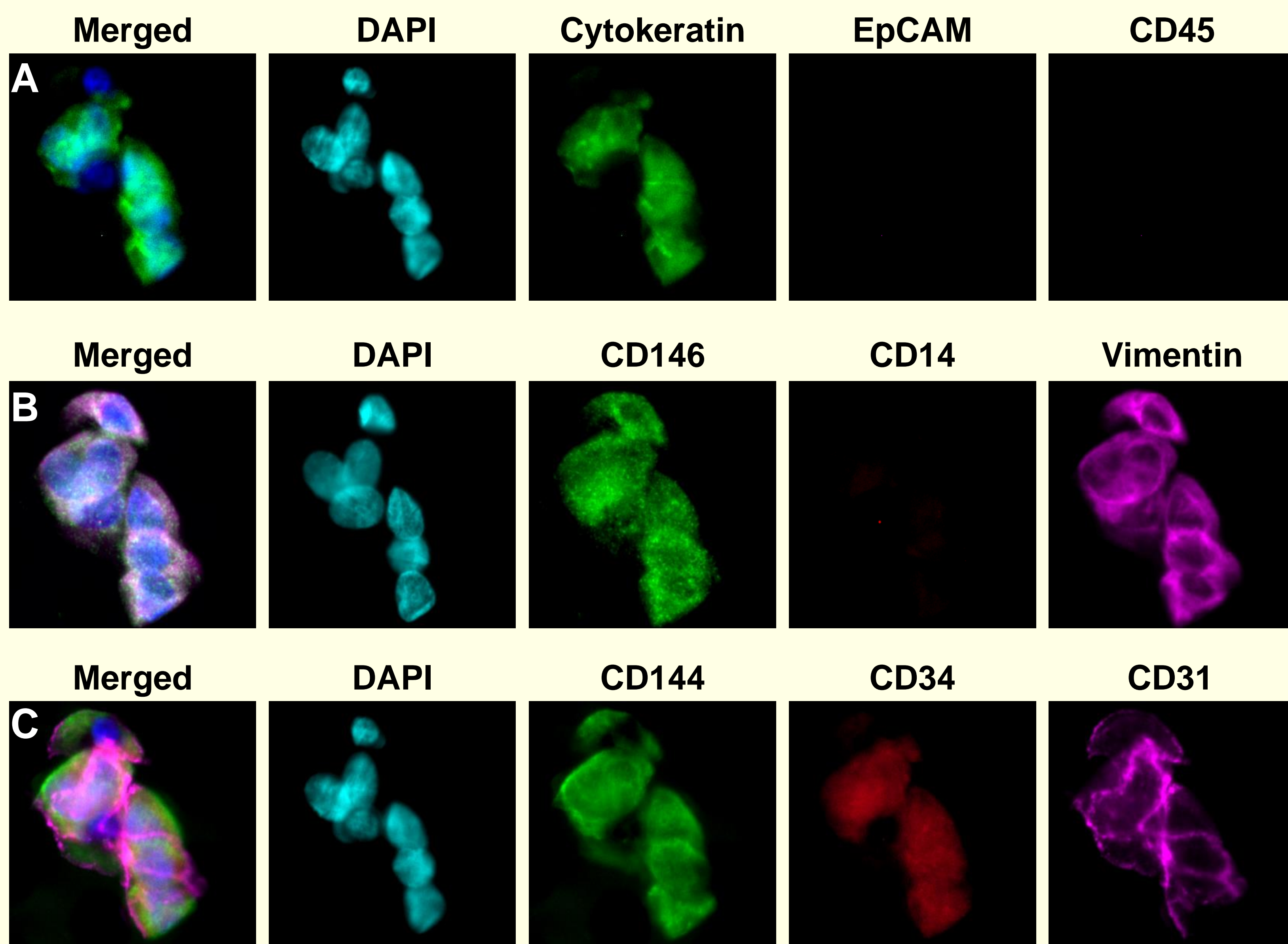


Figure 1. Representative examples of cytokeratin positive CAVEs that stain positive for CD31, CD146, Vimentin, and CD144, confirming their endothelial origin. All CAVEs are CD45 negative and CD14 negative. This CAVE appears EpCAM negative, although some CAVEs have been found to express EpCAM.

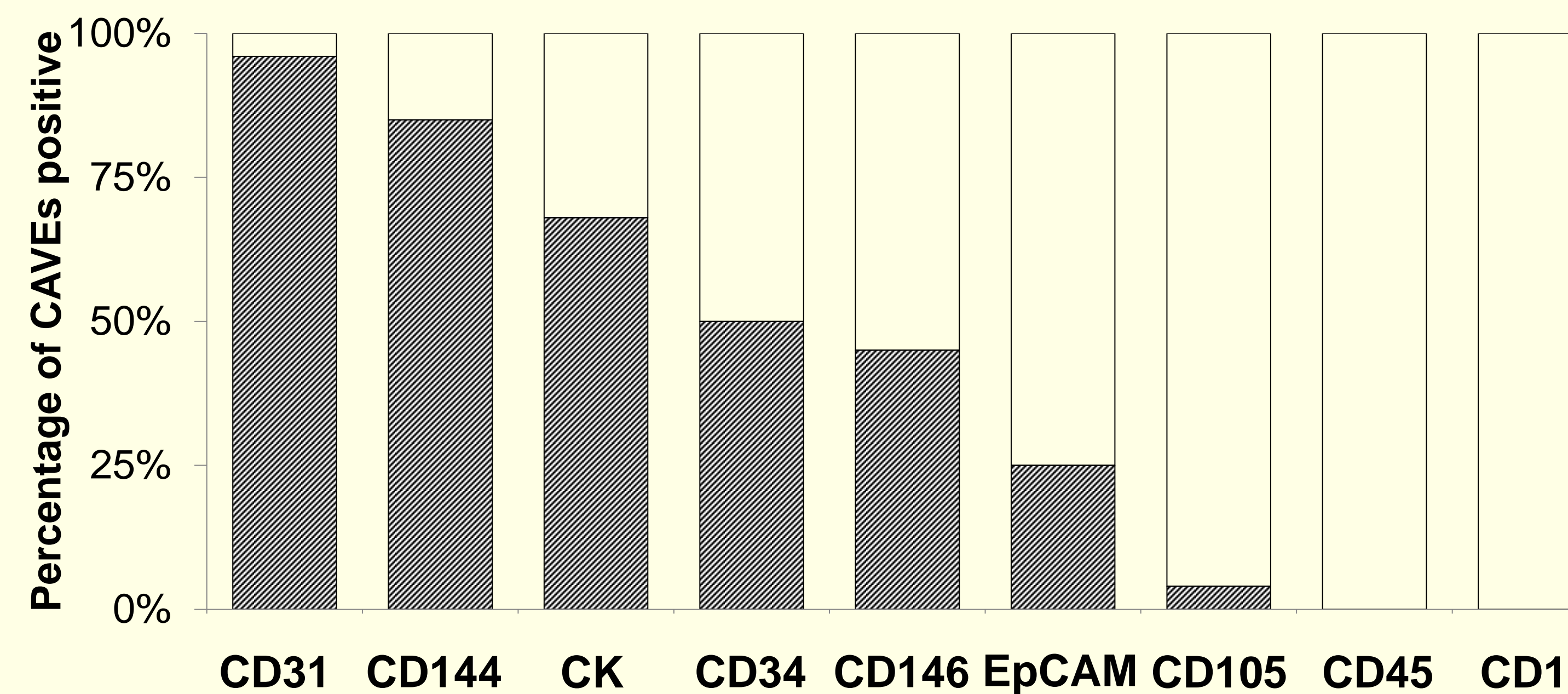


Figure 3. Percentage of each EC marker on the CAVE population (n=119 samples)

## CONCLUSIONS

- CECs isolated by microfiltration are positive for Cytokeratin and negative for CD45 which appear commonly in the circulation of patients with solid tumors but not in healthy controls.
- Multi-phenotypic subtyping can properly identify and subtype CECs in cancer patients with multiple solid tumor types.
- This data suggests that a subset of CECs, e.g. CAVEs, are found in circulation as CK+/CD45- and exist as a heterogeneous population of cancer specific circulating cells that require further study.

## INTRODUCTION

Tumor endothelial cells (TECs)<sup>1-3</sup> are a population of stromal cells required for tumor initiation, survival and growth by forming the vital structures for angiogenesis and neovascularization. TECs are mandatory constituents at all tumor sites, required for tumor vasculature, aid in priming metastatic niches, and contribute to the molecular instability of tumors. In the circulation, a common population of TECs has been identified and defined as CAVEs based on their large size, multicellular clustering, and the classical EC markers CD31 and Vimentin<sup>1</sup>.

Size exclusion is a technique for isolating large cells from peripheral patient blood irrespective of their surface marker expression, allowing for the capture of many subtypes of circulating tumor ECs. CellSieve™ microfilters are size exclusion membranes capable of rapidly and efficiently isolating CAVEs, CAMLs and CTCs from whole blood, making it possible to study all cell types in conjunction with and in relation to malignant disease<sup>1-4</sup>. Further, a multi-phenotyping technique has been developed using CellSieve™ microfilters allowing for a mass screening of subtyping biomarkers on isolated cells.

## References

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