CTC identification enhanced specificity by using a double exclusion biomarker assay

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Background & objectives

Cancer is a leading cause of death worldwide, with metastasis playing a significant role. Circulating Tumour Cells (CTCs) can provide important real-time insights into tumour heterogeneity and clonal evolution, making them an important tool for early diagnosis and patient monitoring. However, the low concentration of CTCs in blood and the lack of universal cancer-specific markers make their isolation and detection challenging. After isolation, CTCs are typically identified by immunocytochemistry using positive biomarkers such as Cytokeratin (CK) and exclusion biomarkers like CD45. However, some white blood cell (WBC) populations can express low levels of CD45 and stain non-specifically for CK, increasing their risk of misclassification as CTCs.
The purpose of this study was to improve CTC detection and enumeration by introducing a second exclusion marker, CD15, to eliminate interfering WBCs populations.

Methods

CTCs were isolated using the RUBYchip™, and exclusion rate was evaluated by immunocytochemistry. Different CK and CD45 antibodies were tested to optimise the specificity of the assay.

Results

This study showed that, indeed, some granulocyte subpopulations expressed low levels of CD45 and stained non-specifically for CK, misidentifying them as CTCs. These same cells, however, strongly expressed CD15, allowing them to be identified as WBCs and excluded from CTC classification. False positive rates were reduced to 0.2% by combining a highly specific CD45 antibody with a CD15 antibody. The addition of a second CK antibody improved specificity even more, resulting in no false positives. Flow cytometry experiments confirmed the specificity of the CD15 antibody for the granulocyte subpopulation and enabled comparison of CK antibody specificity. The study emphasises the importance of a robust exclusion criteria and high antibody specificity in CTC immuno-assays for accurate identification of CTC candidates and thorough exclusion of interfering white blood cell subpopulations.

Conclusion

In conclusion, this study demonstrated how misidentifying a granulocyte subpopulation can lead to inaccurate CTC evaluation. However, using highly specific and high-performance antibodies, as well as including a second exclusion biomarker, can improve CTC detection and enumeration, allowing for more comprehensive clinical applications of CTCs.