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Clinical decision making based on circulating endothelial (progenitor) cells: a help or a struggle?

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Background: There is an increasing need for biomarkers that can aid clinicians in the management of patients treated with anti-angiogenic agents. Circulating endothelial (progenitor) cells [CE(P)Cs] are believed to be a biomarker that not only reflects angiogenic activity of a tumor but also is a target of treatment. However, this biomarker is not properly validated yet. This is largely due to differences in technical procedures and also to the remarkable inter- and intrapatient variability. To obtain more insight in these variabilities, we critically re-evaluated all analyzed patient-samples in our center.

Methods: We have measured CE(P)Cs as part of clinical studies in 450 patients and 90 volunteers, comprising more than 1000 samples. The mononuclear cell fraction was isolated from whole blood, frozen in RPMI/DMSO and CE(P)C numbers were determined by flowcytometry as reported previously (Rademaker-Lakhai et al. Clin Cancer Res, 2007). Most samples were analyzed in duplo. To determine the variability of the assay we analyzed 214 patient samples taken on two different time points before start of therapy. The intraprocedure, intrapatient and interpatient variability was assessed by comparing the duplo results from each patient.

Results: There is limited intraprocedure and intrapatient variability in CE(P)C numbers in both duplo and two baseline measurements (Spearman's correlation coefficient for intraprocedure variability 0.937 (CEC) and 0.68 (EPC) and for intrapatient variability 1.0 (CEC) and 0.93 (EPC) ($p = 0.01$). Bland–Altman analysis showed a good repeatability. No significant difference was observed between the duplo measurement groups (Wilcoxon rank test $p = 0.16$ for CEC and $p = 0.078$ for EPC). However, interpatient variability was substantial [mean 1.8E+07 (CEC), 1.4E+05 (EPC); SD 2.6E+07 (CEC), 1.2E+05 (EPC); variance 7.0E+14 (CEC), 1.6E+10 (EPC); range 8.0E+07 (CEC), 4.0E+05 (EPC)].

Conclusions: Our findings may explain the controversy around CE(P)C as a biomarker. Although flow cytometry of frozen samples seems to be an accurate and reproducible assay, the interpatient spread is considerable. This prohibits the use of reference values which in turn leads investigators to use "percentage change" as an outcome measure. However, this may confound findings because the absolute CE(P)C numbers most likely reflect their biological activity.