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Re: a Word of Caution on New and Revolutionary Diagnostic Tests

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In his letter "A word of caution on new and revolutionary diagnostic tests," Dr. Diamandis argues that discoveries and progress in the field of biomarker discovery for the early detection of cancer are hampered by multiple factors, including pre- and post-analytical and bioinformatics artifacts. He further indicates that careful patient selection is crucial.

Age can be an important confounding factor in the development of diagnostics tests, and we indeed observed a lower average age of the healthy donor cohort compared with the other cohorts. However, the healthy donor cohort included a wide range of ages (min-max 21–64 years), of which misclassified individuals were not correlated to older ages. Hence, we believe that, in this study, the biased age of the healthy individuals is not of significant influence to the presented results. Although recent work has shown that 139 age-related mRNAs can be identified in blood platelets (Simon et al., 2014), our pan-cancer classification algorithm included >1,000 RNAs for sample classification, far exceeding the number of differentially expressed age-related RNAs and possibly explaining why no age-related correlations were observed. Beyond doubt, validation studies will need to be performed using age-matched cohorts in well-defined prospective studies.

Clearly, it is of importance to determine the effect of inflammatory diseases, benign tumors or pre-malignant stages (such as colorectal polyps and pancreatic cysts or pancreatitis) on platelet RNA profiles, and to study whether this may interfere with tumor-educated platelet (TEP)-based cancer diagnostics. However, a single non-cancer disease will likely not be sufficient as a control for all different cancer classifiers described, hence we stated: "Systemic factors such as chronic or transient inflammatory diseases, or cardiovascular events and other non-cancerous diseases may also influence the platelet mRNA profile and require evaluation in follow-up studies, possibly also including individuals predisposed for cancer." Since we were also able to classify specific mutants and wild-type tumors (no healthy controls are part of this comparison), we are optimistic that the TEPs harness sufficient discriminatory power to distinguish cancerous from non-cancerous disease conditions. To provide insight into the differential levels of RNAs associated with non-cancerous diseases, we analyzed, visualized, and reported in the article the levels of RNA markers as collected from multiple publicly available studies: "Visualization of 22 genes previously identified at differential RNA levels in platelets of patients with various non-cancerous diseases (Gnatenko et al., 2010; Healy et al., 2006; Lood et al., 2010; Raghavachari et al., 2007) revealed mixed levels in our TEP dataset, suggesting that the platelet RNA repertoire in patients with non-cancerous disease is distinct from patients with cancer."

Positive and negative predictive values (PPV and NPV) can be more clinically relevant parameters than sensitivity and specificity, since they imply the rates of individuals correctly referred for follow-up diagnostics tests. We agree that those values can benefit the interpretation of the data if the study is set up as a clinical validation study. We also agree that diagnostic tests used for cancer screening have to reach a specificity of >99.5%, minimizing the number of false positive test results. The characteristics of our experimental first-generation algorithms are noted in the receiver operating characteristic (ROC) curves, allowing us to select any point on the ROC curve, including maximum specificity at the cost of sensitivity. For a dedicated screening set up, we will tune and validate our algorithms toward the most suited conditions, including minimizing false positives, using prospective age-matched screening cohorts. We may also consider developing diagnostic screening tests for high-risk individuals predisposed to cancer, e.g., mutant BRCA1/2 carriers, affecting NPV/PPV calculations. The data provided in our article are a first biosource evaluation of the power of TEPs for blood-based detection of cancer.

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