MICROSAMPLING FOR RNA BIOMARKER PROFILING IN BLOOD

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Background Multi-omic analysis of microsamples of lancet-induced blood drops allows frequent capture and quantitation of numerous metabolites, lipids, cytokines, and proteins. Microsample-based transcriptome profiling would facilitate use of RNA biomarkers in diagnosis and treatment of immunotherapy patients, but a suitable method has not been available. We tested a targeted sequencing protocol for this purpose in human blood microsamples.

Methods We tested normal blood collected either by phlebotomy into standard vacutainer tubes or by absorption of 30 μL of blood onto a Mitra device pre-treated with a RNA-stabilization reagent. We tested detection of gene expression changes by incubating anti-coagulated blood for 18 hours with endotoxin (LPS, 100 ng/ml) followed by isolation of identical volumes of blood using a standard method (Qiagen kit) or from Mitra devices air dried for 24 h to mimic a home-use scenario. RNA was extracted from the microsamplers by immersion of the tips into a high NaCl buffer containing proteinase K, NP-40 and vigorous shaking at 60°C for 30 minutes. After RNA purification, a panel of 274 immune/inflammatory genes was quantified by NGS after PCR using targeted primers following the Cellecta DriverMap protocol, which avoids counting of abundant rRNA and mitochondrial sequences. The targeted sequencing results were normalized to counts per million and used to compare results in standard vs microsamples tested in triplicate.

Results We found robust detection of gene expression and correlation (figure 1) using the two methods in both unstimulated ($r^2 = 0.94$) and LPS-stimulated blood ($r^2 = 0.97$, figure 1). Comparison of the top up- or down-regulated genes showed comparable changes in microsamples vs standard method analysis (e.g., log2 ratio of LPS-stimulated to control for CXCL10, IL1B, HMOX1, FCGR3B, respectively: 8.77 vs 8.72; 7.99 vs 7.83; -3.84 vs -3.32; -3.47 vs -3.18). The correlation was strongest for genes with high counts (>1K) but lower for the subset of low-count genes ($r^2 = 0.7$ for <1K). The differentially expressed genes identified in both standard and microsample methods showed high overlap with DEGs reported in public datasets in similar experiments.

Conclusions RNA transcriptome profiling using targeted sequencing allows sensitive detection of gene expression levels in normal and activated blood samples. Additional testing of a whole transcriptome panel (~19K protein-coding genes) is underway and will be reported. Because microsamples can be air-dried and mailed in for analysis, this approach has great promise for easy and repeated monitoring of RNA biomarkers in immunotherapy.