this, we will quantify tear cytokines in patients with oGVHD, with and without systemic JAK inhibition treatment. Patients with 'definite' oGVHD based on the international chronic oGVHD diagnostic criteria (ICOGVHD) whom we have collected tears will be grouped based on JAK inhibition treatment. Tear cytokines are analyzed using Iso spark Meteor bulk quantitative proteomic analysis. RESULTS/ANTICIPATED RESULTS: Seven patients were identified from our patient cohort who met inclusion criteria (oGVHD; tears collected while on Ruxolitinib), five patients were identified whom we have collected tears with oGVHD who have not taken ruxolitinib. The following 10 cytokines will be analyzed in the tears by the Iso spark Meteor bulk quantitative proteomic analysis: GM-CSF, IFN-g, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17A, TNF-a. The change in cytokine levels will be compared with the ICOGVHD score, corneal fluorescein staining, schirmers test (measurement of tear production), conjunctival injection score, ocular surface disease index score (validated symptomatic score of dry eye disease). DISCUSSION/SIGNIFICANCE: OGVHD is a major cause of morbidity for patients who undergo a hematopoietic stem cell transplant and is the result of a highly complex immune process including dysregulation of pro-inflammatory cytokines. It is critical to understand the effect of cytokine changes on the eyes to potentially identify a biomarker and possible treatment targets.

6

General Psychopathology Factor as a Mediator Between Polysubstance Use and Lower-Order Psychopathology Constructs†

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OBJECTIVES/GOALS: We aim to develop an understanding of how polysubstance use (PSU) relates to the general psychopathology factor (p-factor), as well as to individual components of the Hierarchical Taxonomy of Psychopathology (HiTOP) model (e.g., fear, distress). This insight can help identify treatment targets related to substance use and psychopathology. METHODS/STUDY POPULATION: Psychopathology and substance use data, collected at a Baltimore treatment center over several years, will be analyzed. The center aids about 6000 underserved clients per year, and the population is primarily African American clients of all genders. Structural equation modeling (using Mplus software) will be used to develop the latent models and identify relationships between psychopathology and PSU (i.e., direct and indirect pathways). The current latent HiTOP model was developed from symptom checklists completed upon entry at the treatment center. The PSU latent factor will be developed from a biopsychosocial assessment where clients list their drug of choice. Due to the varying organizations of the datasets, smaller-scale preliminary models will be developed to ensure an accurate large-scale final model. RESULTS/ANTICIPATED RESULTS: Current models being tested are derived from January to September 2023 data (i.e., completed months' data), with an N of 1,564. From symptom checklist data collected at the treatment center, a preliminary HiTOP model was derived with reasonable fit ($\chi^2 = 4532.35$ (df = 321, p<.001), CFI = .77, SRMR = .07, RMSEA = .09 (.089, .094)). Data analysis is being conducted to derive the PSU factor before relating PSU to the HiTOP model. Given previous work at a local treatment center (Pavuluri et al., 2022) and with the National Comorbidity Survey-Replication data, we expect all positive direct relationships, negative indirect relationships between internalizing factors (fear and distress) and PSU when accounting for p-factor, and a positive indirect relationship between antagonism and PSU when accounting for p-factor. DISCUSSION/SIGNIFICANCE: Given our previous work to develop such models, we want to establish proof of concept in alarger treatment center population. This confirmation will help provide a path towards conducting therapeutic trials to target psychopathology when treating substance use given the shared relations, some of which are less understood (e.g., fear and PSU).

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Flexible probabilistic methods to unlock the clinical potential of liquid biopsy sampling†

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OBJECTIVES/GOALS: Decoding the origins of cell-free DNA (cfDNA) released from dying cells in a liquid biopsy sample (e.g. blood) offers the potential to provide insight into the dynamic, organism-wide changes reflective of health and disease. Thus, making cfDNA an ideal target for serial, minimally invasive monitoring of disease-related changes. METHODS/STUDY POPULATION: We develop a probabilistic method that leverages the co-regulation of neighboring CpG sites on individual methylome-wide sequencing (WGBS) reads to more flexibly model cell-specific methylation compared to prior methods that focus on the methylation rate of a single CpG site. We then extend our cross-sectional model to account for sequential sampling within the same subject. The increased sampling frequency is critical to identifying the evolutionary dynamics of disease progression influencing treatment response and resistance, and disease recurrence. We utilize Bayesian inference techniques to model patient-specific longitudinal profiles of cell-type turnover in simulated serial samples. RESULTS/ANTICIPATED RESULTS: We found our model more effective at capturing a range of methylation patterns on cfDNA fragments with lower Root Mean Square Error across simulations compared to a single CpG model. We apply our model to detect significant ($p < 0.05$, Friedman's test) increases in cellular contributions from lung and cardiac tissue in breast cancer patients (n=15) undergoing radiation therapy compared to baseline. We also identify signals of radiation induced toxicity to the liver in right-sided breast cancer patients (n=8) receiving radiation treatment compared to left-sided breast cancer patients (n=7). Finally, we show our extended model results in more efficient estimates of simulated cell-type turnover profiles compared to analyzing serial samples cross-sectionally, ignoring the longitudinal nature of the data. DISCUSSION/SIGNIFICANCE: Here we address an unmet need in developing novel statistical methodologies to decode the origins of methylated cfDNA obtained from liquid biopsy samples. We demonstrate the far-ranging clinical utility of serial liquid biopsy sampling to complement and advance the standards of clinical care in oncology and other pathologies.

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