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Prediction of clinical outcomes using the pyrolysis, gas chromatography, and differential mobility spectrometry (Py-GC-DMS) system

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ABSTRACT

Biological and molecular heterogeneity of human diseases especially cancers contributes to variations in treatment response, clinical outcome, and survival. The addition of new disease- and condition-specific biomarkers to existing clinical markers to track cancer heterogeneity provides possibilities for further assisting clinicians in predicting clinical outcomes and making choices of treatment options. Ionization patterns derived from biological specimens can be adapted for use with existing clinical markers for early detection, patient risk stratification, treatment decision making, and monitoring disease progression. In order to demonstrate the application of pyrolysis, gas chromatography, and differential mobility spectrometry (Py-GC-DMS) for human diseases to predict the outcome of diseases, we analyzed the ionized spectral signals generated by instrument ACB2000 (ACBirox universal detector 2000, ACBirox LLC, NJ, USA) from the serum samples of Mantle Cell Lymphoma (MCL) patients. Here, we have used mantle cell lymphoma as a disease model for a conceptual study only and based on the ionization patterns of the analyzed serum samples, we developed a multivariate algorithm comprised of variable selection and reduction steps followed by receiver operating characteristic curve (ROC) analysis to predict the probability of a good or poor clinical outcome as a means of estimating the likely success of a particular treatment option. Our preliminary study performed with small cohort provides a proof of concept demonstrating the ability of this system to predict the clinical outcome for human diseases with high accuracy suggesting the promising application of pyrolysis, gas chromatography, and differential mobility spectrometry (Py-GC-DMS) in the field of medicine.

1. Introduction

Despite advances in the treatment of hematological malignancies, a subset of patients continues to develop progressive disease, to be refractory to treatment, or to relapse shortly after treatment. Hematological malignancies often pose a challenge in terms of early detection, diagnosis, and prediction of clinical outcomes [1]. With robust biomarker-based detection methods, extremely sick patients can be stratified prior to treatment and recognized as a high-risk group with poor prognosis. However, conventional methods to detect clinical biomarkers usually involve invasive procedures for procuring blood or tissue biopsy, and require lengthy laboratory diagnostic confirmation [2]. Recently, corroborative research efforts in the field of oncology have led to use of molecular biomarkers for early diagnosis and accurate prediction of

Abbreviations: bcl-1, b-cell lymphoma-isoform 1; CDK4, Cyclin dependent kinase 4; CDKN2A, cyclin dependent kinase inhibitor 2A; MIB-1, mindbomb 1; SOX-11, sry-related HMG box; IL-2Rα, interleukin-2 receptor alpha; IL-8, interleukin-8; MIP-1β, macrophage inflammatory protein-1 beta.

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prognosis for various cancers [3]. However, no simple, rapid, and non-invasive test exists that is capable of performing initial patient selection, accurately predicting treatment response, and assigning the probability of good or poor outcome. We used a technology that combines pyrolysis, gas chromatography, and differential mobility spectrometry (Py-GC-DMS) for detecting ionized particles from human bio-specimens derived from Mantle cell lymphoma (MCL) patients as an example to assess the diagnostic ability of this technology to directly predict or supplement other biological biomarkers to predict clinical outcomes.

MCL is cytogenetically characterized by a t(11;14) translocation and a bcl-1 rearrangement resulting in the overexpression of cyclin D1 [4–6]. Presence of complex karyotypes, deregulation of key cell cycle regulators associated with p53 mutations, CDK4 activation, p16/CDKN2A inactivation, and inactivation of DNA damage response pathways are associated with aggressive clinical behavior and poor prognosis [7–9]. A high proliferation index as assessed with Ki67/MIB-1 immunostaining, SOX11 overexpression, p53 alterations, and blastoid morphology have been reported to predict a poor outcome in MCL patients [10–12]. Other serum biomarkers such as higher levels of β-2 microglobulin, free immunoglobulin light chain in serum (either monoclonal or polyclonal), IL-2Rα, IL-8, and MIP-1β correlate with poor prognosis and inferior outcomes [13–15]. Although morphological features along with genetic and serum biomarkers appear to be useful for predicting outcomes, their prognostic significance has not been confirmed in all studies [16,17]. Furthermore, assessment of overall prognosis using the MCL International Prognostic Index (MIPI) lacks accuracy and is dependent on the treatment regimen [18]. In view of these deficits, it is evident that there is a lack of diagnostic methodology that can predict the clinical outcome before initiation of treatment for MCL.

Gas chromatography (GC)-related technologies have shown cost-effective utility in clinical diagnostic settings to resolve issues posed by the limitations of current clinical diagnostic procedures and laboratory associated costs. Several GC and other related "sniff and tell" technologies were previously developed for detection and biosensor related purposes [19–21]; these have included detection of infections and diagnosis of metabolic disorders [22,23]. A combination of gas chromatography and mass spectrometry (GC–MS) has been used for cancer detection via a "breath analyzer" that detects volatile organic compounds (VOC), which are elaborated from different types of cancer, including lung and breast cancers [24–26]. More recently, GC-related technologies have been more widely applied in clinical settings to identify serum metabolites and biomarkers for different cancer types, including gastric, colorectal, esophageal, and pancreatic cancers [27–30]. Similarly, GC–MS technologies have also been developed for diagnosis of hematological malignancies [31]. Ionization pattern recognition has been used in detection technologies, and has recently been under investigation for potential use in diagnosis of cancer and infectious diseases and for rapid analysis of potential bioterror agents [32–36]. For detection of pathogens or markers of interest, approaches to ionization pattern recognition have used a multivariate algorithm that can immediately differentiate the pathogens or markers only present in the experimental or case sets as opposed to control sets [37].

In this study, we analyzed twenty one serum samples of MCL patients using "ionized" biochemical signatures produced by the ACB2000 instrument. Signatures were first quantified, and the signal intensities were then differentiated using new multivariate data analysis algorithm approach and were finally associated with the clinical phenotype of interest to generate an ionization signature map. In combination with analysis of DNA, protein, or metabolite expression, the ionization signature map may provide a biomarker panel that could permit patient-stratification and prediction of clinical outcome with high sensitivity and specificity. The ionization signature map generated by this technology provides a "second opinion" that is independent of existing clinical biomarkers and established scoring systems, thus, enhancing clinical decision-making for human diseases. We thus illustrate the application of pyrolysis, gas chromatography, and differential mobility spectrometry system in the field of cancer medicine.

2. Materials and methods

2.1. Study population

This study was approved by the Hackensack University Medical Center's (HUMC) Institutional Review Board (IRB) (reference # CR00002851) and was performed under MCL study protocol number, PRO00001689 titled "Biomarker studies on Mantle Cell Lymphoma". Informed consents were obtained from all patients prior to enrollment. Aliquots of serum samples obtained under standard of care procedures were registered in the HUMC Tissue Repository and stored at −80 °C. Serum samples from untreated, newly diagnosed, chemo-naive (CN) (n = 15) and relapsed, chemo-exposed (CE) (n = 6) MCL patients (total n = 21) were studied. Clinical characteristics of the patients used in this study include leukemic phase status (increased WBC/lymphocyte count); blastoid (presence of blastoid cells) status; MIPI score (low, intermediate, and high); pre-treatment history (CN versus CE), type of treatment regimen administered, clinical outcome [good outcome (GO) versus poor outcome (PO)], progression-free survival (PFS, from date of first line treatment), and current status (alive or deceased) (Supplementary file 1, Table 1).

To evaluate the system, MCL patients were divided into GO (n = 12/21) and PO (n = 9/21) based on a median overall survival cut-off of 35 months irrespective of their prior treatment status. Among 15 Chemo-naïve MCL patients, 11 patients who subsequently received chemotherapeutic regimen, HCVAD (Hyper-fractionated–Cyclophosphamide, Vincristine, Adriamycin and Dexamethasone) with or without rituximab as first line treatment (n = 11) were further divided into good outcome (GO, n = 6/11) or poor outcome (PO = 5/11) according to the following clinical outcome criteria: GO = relapse-free/progression free survival > 35 months after initial treatment, or PO = more than 2 relapses or death < 35 months after initial treatment (Supplementary file 1, Table 1, gray portion). Since presence of leukemic phase, blastoid condition, and high MIPI score in MCL are associated with poor prognosis, we performed contingency analysis to determine the association of these clinical features with the prognosis for the MCL cohort used in our study. Our analysis suggested that there was no significant association between these clinical features and clinical outcome (as defined above) in this study (Fig. 1).

2.2. ACB2000 Py-GC-DMS detection system

A serum sample (2.5 μl) was applied to the sample probe, which was inserted into the pyro-tube (1) and heated to 300 °C to generate pyrolytic fragments in the vapor phase. Vaporized pyrolytic analytes were transported by a flow module (5) which injected into the GC module (3) where the analytes were separated. The separated analytes entered an ionization chamber inside the Differential Mobility Spectrum (DMS) module (4), and the ionized analytes were detected (Fig. 2). Typical DMS settings used for separation of analytes were based on ion differential mobility in alternating electric fields, in the compensation voltage (V) linearly increased from −40 V to +15 V in approximately one second. Positive and negative ion signal intensities were simultaneously recorded by DMS at each compensation voltage. The output spectrum generated by the DMS consisted of 60 signal intensity values each from the negative and