Urine metabolomics as a predictor of patient tolerance and response to adjuvant chemotherapy in colorectal cancer

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Abstract. Colorectal cancer is the third leading cause of cancer-associated mortality in the western world. The ability to predict a patient's response to chemotherapy may be of great value for clinicians and patients when planning cancer treatment. The aim of the current study was to develop a urine metabolomics-based biomarker panel to predict adverse events and response to chemotherapy in patients with colorectal cancer. A retrospective chart review of patients diagnosed with stage III or IV colorectal cancer between 2008 and 2012 was performed. The exclusion criteria included chemotherapy for palliation and patients living outside of Alberta. Data was collected concerning the chemotherapy regimen, adverse events associated with chemotherapy, disease progression and recurrence and 5-year survival. Adverse events were subdivided as follows: Delays in treatment, dose reductions, hospitalizations and chemotherapy regime changes. Patients provided urine samples for analysis prior to any intervention. Nuclear magnetic resonance (NMR) spectra of urine samples were acquired. The 1H NMR spectrum of each urine sample was analyzed using Chenomx NMRSuite v7.0. Using machine learning, predictors were generated and evaluated using 10-fold cross-validation. Urine spectra were obtained for 62 patients. The best predictors resulted in area under the receiver operating characteristic curve values of: 0.542 for chemotherapy dose reduction, 0.612 for 5-year survival, 0.650 for cancer recurrence and 0.750 for treatment delay. Therefore, predictors were developed for response to and adverse events from chemotherapy for patients with colorectal cancer patients. The predictor for treatment delay has the most promise, and further studies will aid its refinement and improvement of its accuracy.

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Introduction

Colorectal cancer (CRC) is the third leading cause of cancer death in Canada (1). Optimal treatment for CRC is dependent on the stage of disease at the time of diagnosis. For stages III and IV CRC, the addition of chemotherapy to surgical resection has been demonstrated to improve survival (2). Unfortunately, not all patients who receive chemotherapy respond and certain patients develop severe and debilitating side effects while on chemotherapy. In a number of patients the side effects are so severe that they are not able to continue treatment.

Being able to predict if an individual patient will respond to chemotherapy as well as being able to predict if a patient will have severe chemotherapy side effects would be of great value to clinicians when counseling patients with CRC about their treatment options.

Metabolomics is the science of analyzing small molecule metabolites present in the human body. These metabolites may be measured from various sources, including serum, saliva, stool and urine. Metabolomics is able to provide an up-to-the-minute profile of a patient's health as the presence and concentration of metabolites changes based on current disease states. This has led to a number of studies examining the use of urine metabolomics as potential screening tools for various cancer types (3-5).

Metabolic alterations are evident in cancer cells throughout tumorigenisis (6). Metabolomic studies are a natural fit for examining cancer cells not only for the purpose of diagnosis, but also as a way of predicting response to therapy (7).

The purpose of the present study is to examine urine metabolomes of patients diagnosed with stage III or IV CRC and use those metabolomes to create predictors that will indicate whether a patient will respond to chemotherapy and/or have significant adverse reactions to chemotherapy prior to the initiation of treatment.

Patients and methods

Patients. A retrospective review of the charts of 91 patients was performed, with prospectively collected urine samples

from an existing colorectal cancer tissue bank. Adult patients with clinical stage III or IV colorectal cancer (based on preoperative imaging, colonoscopies and biopsy) who presented to Edmonton hospitals (Grey Nuns Hospital, Misericordia Hospital, University of Alberta Hospital and Royal Alexandra Hospital, Alberta, Canada) between January 2008 and December 2012 were included. Patients who did not reside in Alberta were excluded, as their charts were not available, as well as those who were deemed palliative at the time of their initial operation. Patients with end stage renal disease had also been excluded from the initial tissue bank (8). All of the patients provided a urine sample following the diagnosis of colorectal cancer, but prior to any operation, chemotherapy or radiation. Ethical approval was obtained from the Health Research Ethics Board at the University of Alberta, and patients provided informed consent.

Charts were reviewed for chemotherapy regimens, delays or discontinuation of chemotherapy, chemotherapy dose reductions, hospitalizations during chemotherapy treatment, cancer progression, cancer recurrence and five-year survival. Patients who had cancer detected while still on chemotherapy were deemed to have progression, whereas patients who had cancer detected on post-treatment surveillance were deemed to have recurrence.

Metabolite analysis. Urine samples were thawed at room temperature. A 1:10 ratio of internal standard (2,2-dimethyl-2-silapentane-5-sulfonate) was added, following which the urine sample was pH adjusted to 6.7-6.8 using 1.0 M HCl or 1.0M sodium hydroxide buffers as required. The urine was pipetted into a Wilmad 528-pp 4-inch nuclear magnetic resonance (NMR) tube (Wilmad, Buena, NJ, USA).

Metabolite spectra were collected using a 600 MHz NMR spectrometer (Oxford Instruments, Abingdon, Oxfordshire, UK) and a VNMRS two-channel console (Varian Inc.; Agilent Technologies Inc., Santa Clara, CA, USA) that was running VNMRJ software version 2.2C (Agilent Technologies, Inc.) on a RHEL 4 (Red Hat) host computer. The spectrometer was equipped with an HX probe and Z-axis gradients. The first increment of a 2D-^{1}H, ^{1}H-NOESY pulse sequence acquired the 1H-NMR data and suppressed the solvent signal. Each run used a 100 ms mixing time along with a 990 ms pre-saturation (~80 Hz γB_1). Spectra were collected at 25˚C for a total of 32 scans over a period of 3.5 min.

Quantification of 69 validated and previously confirmed metabolites from the spectra was completed using targeted profiling with Chenomx NMR Suite v7.0 software (Chenomx Inc, Edmonton, Alberta, Canada). Quantification was completed by one individual and verified by a second; both individuals were blinded to the provenance of the samples.

Predictor determination. The open source software R was used for all analyses (9). Several prediction tasks were examined separately, using 10-fold cross-validation. For each of these analyses, 12 metabolites were removed from the entire dataset as these were previously found to be inconsistently quantified (10). Metabolites with nil values were replaced with half the value of the lowest concentration for that metabolite. The prediction was done using four different machine learning algorithms: least absolute shrinkage and selection operator (LASSO), Support Vector Machine (SVM), Decision Trees and Random Forest. The metabolite concentrations were also log transformed and urea-normalized. Predictive performance results for each prediction task were compared using area under the receiver operating characteristic (ROC) curve (AUC).

Creation of a predictor for each task was performed as described previously (11). For each prediction task (e.g., treatment delay, hospitalization, cancer recurrence), the performance of each combination of machine learning algorithms and data processing was examined (log-transformation/urea-normalization). In 10 fold cross-validation, nine-tenths of the urine samples from each group (with and without the clinical indication being predicted) were randomly assigned to the un-blinded training set. This un-blinded training set was used to generate the metabolite profile that was diagnostic for this prediction task. The remaining one-tenth of the urine samples from each group (with and without the clinical indication) formed the blinded testing set. This blinded testing set was used to validate the metabolite profile diagnostic for the feature of interest. This was repeated 10 times, so that each one-tenth split of the data set acts as the testing set once.

Results

Patients. After applying the exclusion criteria, 62 patients remained. As presented in Table I, 34 were male (54.8%) and the age range was 44-87 years with a median age of 66. The primary site of cancer was the colon in 41 patients, and rectum in 22 patients (1 patient had synchronous colonic and rectal lesions).

Chemotherapy. Of the 62 patients whose charts were reviewed, 45 received chemotherapy. Of the 17 who did not receive chemotherapy, 5 declined for personal reasons, 5 were not offered chemotherapy due to medical co-morbidities, 3 were not offered chemotherapy due to post-operative complications and 4 had no reason given for not receiving chemotherapy.

Treatment delays. Of the 45 patients who received chemotherapy, 13 completed their course with no delays, 30 had a delay in treatment and 2 patients had no data. Reasons for treatment delay included reactions to chemotherapy, other medical conditions and patient choice. Reactions to chemotherapy included neutropenia, diarrhea, hand and foot syndrome, mucositis and peripheral neuropathy. Other medical conditions included cellulitis, hip fracture, rib fracture, central line infection and eye surgery. One patient chose to delay treatment to go on vacation.

Hospitalizations. Of the 45 patients who received chemotherapy, in our predictor for hospitalization, 7 patients required hospitalization, 37 did not and 1 did not have data recorded. Reasons for hospitalization included cellulitis, dehydration, diarrhea, mucositis, SVC obstruction and a hip fracture.

Dose reductions. Twenty-three of the 45 patients receiving chemotherapy had dose reductions, 19 did not and 3 had no data recorded. Reasons for dose reduction included hand and foot syndrome, diarrhea, nausea and vomiting, neuropathy, facial rash and reduced creatinine clearance.

Regime change. Three patients changed chemotherapy type due to drug intolerances, and 5 changed chemotherapy type due to disease progression. No regime change was required in 35 patients, and for 2 patients there was no data recorded.

Cancer recurrence and progression. Five patients had cancer progression while on chemotherapy, and 14 had a recurrence of cancer identified on post-treatment surveillance. Eight patients had no data collected. Recurrences or progressions were detected with colonoscopy, sigmoidoscopy, computed tomography scan, magnetic resonance imaging or physical exam.

Five-year survival. At the time of data collection, 5 patients had not reached 5 years since the date of their surgery. Of the 57 patients for which 5 years had elapsed since surgery, 36 were still alive and there were 21 mortalities.

Predictors. Individual predictors were created for each variable as outlined in the methods section. Predictors were created for 5-year survival, cancer recurrence, chemotherapy dose reduction and treatment delay. The sample size was too small for predictors to be created for hospitalization, regime change and cancer progression.

Each predictor used 4 different machine-learning algorithms (LASSO, SVM, decision trees and random forest) for log-transformed and urea-normalized concentrations of the measured metabolites, for a total of 8 AUC values for each predictor. The AUC values are presented in Tables II and III.

For 5-year survival, the most effective predictor had an AUC of 0.612. The AUC values for other variables included: Cancer recurrence, 0.650; chemotherapy dose reduction, 0.542; and treatment delay, 0.750.

Table I. Patient demographics (n=62).

Characteristic	No. of patients (%)
Male	34 (54.8)
Median age (range)	66 (44-87)
Primary tumor site[a]	
Colon	41(66.1)
Rectum	22(35.5)

[a]One patient had synchronous tumors in the colon and rectum.

Table II. Log-transformed concentrations for the 4 machine learning algorithms.

Predictor	AUC (LASSO)	AUC (SVM)	AUC (DT)	AUC (RF)
Five year survival	0.450	0.592	0.490	0.456
Cancer recurrence	0.450	0.325	0.519	0.412
Chemotherapy dose reduction	0.483	0.508	0.304	0.367
Treatment delay	0.750	0.467	0.642	0.700

AUC, area under the curve; DT, decision tree; RF, random forest; LASSO, least absolute shrinkage and selection operator; SVM, Support Vector Machine.

Table III. Urea-normalized concentrations for the 4 machine learning algorithms.

Predictor	AUC (LASSO)	AUC (SVM)	AUC (DT)	AUC (RF)
Five year survival	0.504	0.612	0.506	0.421
Cancer recurrence	0.412	0.650	0.431	0.262
Chemotherapy dose reduction	0.458	0.358	0.392	0.542
Treatment delay	0.483	0.317	0.517	0.717

AUC, area under the curve; DT, decision tree; RF, random forest; LASSO, least absolute shrinkage and selection operator; SVM, Support Vector Machine.

Discussion

The rise of 'omics' research (genomics, transcriptomics, proteomics, metabolomics and epigenomics) has led to increased emphasis on tailoring treatments to individual patients (12). Not only are clinicians increasingly recognizing that each patient's disease is varied, but it is clear that patient's personal values, expectations and goals of therapy differ as well. Where one patient's treatment goal may be length of survival, another patient may value quality of life over quantity.

The current study examined urine metabolomics as a method of predicting a patient's tolerance and response to adjuvant chemotherapy in colorectal cancer. The aim was to determine whether urine metabolomics has the potential to provide an additional tool for clinicians to use in counseling patients about which treatment strategy will best aid them in meeting their treatment goals.

Numerous decision aids have been developed to provide patients with individualized information to help them make informed treatment decisions (13). Several tools have been developed for various types of cancer (14), including colorectal cancer (15). These tools combine information about patient demographics and patient specific tumor characteristics. The present study considers that there is a definite role for predictors such as those presented to be included in these decision aids.

The AUC from the ROC that the current predictors generated provides a measure of the predictor's accuracy. A guideline for the accuracy of tests based on AUC values is as follows: Perfect (AUC=1), highly accurate (AUC, 0.9-1), moderately accurate (AUC, 0.7-0.9) less accurate (AUC, 0.5-0.7), and non-informative (AUC=0.5) (16). According to this guideline, the predictors the present study generated for chemotherapy dose reductions, cancer recurrence, and five-year survival were less accurate and the predictor for treatment delay was moderately accurate.

As the current study revealed that less than a third of patients (13/45) who are eligible for adjuvant chemotherapy were able to complete the course without any delays, the

predictor for treatment delay is a very important piece of information to guide the patient and clinician in deciding whether to pursue chemotherapy and aid planning for future procedures (including reversal of ileostomy) or life events.

The present study was limited by a small sample size. Unfortunately, there were not enough patients in the cancer progression, hospitalization, or chemotherapy regime change categories to create predictors. However, it is promising that even with the small sample size in this pilot project, the predictor for treatment delay was moderately accurate. Future studies with an increased number of patients may allow the determination of predictors with greater accuracy, and have the potential to elucidate individual reasons for treatment delay.

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