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Monitoring kidney-transplant patients using metabolomics and dynamic modeling

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ABSTRACT

A kidney transplant provides the only hope for a normal life for patients with end-stage renal disease, i.e., kidney failure. Unfortunately, the lack of available organs leaves some patients on the waiting list for years. In addition, the post-transplant treatment is extremely important for the final outcome of the surgery, since immune responses, drug toxicity and other complications pose a real and present threat to the patient. In this article, we describe a novel strategy for monitoring kidney transplanted patients for immune responses and adverse drug effects in their early recovery. Nineteen patients were followed for two weeks after renal transplantation, two of them experienced problems related to kidney function, both of whom were correctly identified by means of nuclear magnetic resonance spectroscopic analysis of urine samples and multivariate data analysis.

1. Introduction

The kidney is a crucial organ that plays key homeostatic roles, through the removal of waste products from the blood stream and maintenance of a constant volume of body fluids and constant concentrations of various electrolytes, despite variations in dietary intake [1]. A number of diseases and conditions can cause kidneys to fail to perform their function suddenly (known as acute kidney failure) [2], and other conditions cause slow failure, most commonly diabetes or high blood pressure, which eventually leaves the patients unable to survive on their own [3].

Once kidney failure has progressed to the (chronic) end stage, the only opportunities for survival are to receive frequent dialysis [4], or a kidney transplant. However, dialysis is expensive and has a major impact on the patient's daily life. Thus, if possible, transplantation is the treatment of choice, as it is the patient's only hope for a normal life, but even today, more than 50 years after the first successful kidney transplantation, many problems are associated with the procedure [5], an obvious one being the scarcity of available organs. Often a candidate has to wait a long time for an organ, and it is therefore of utmost importance that the procedure and follow-up treatment are optimal. Once the transplantation has occurred, conditions such as immunological rejection, ischemia/reperfusion injury and immunosuppressant-mediated nephrotoxicity are common reasons for loss of the kidney graft [6,7].

Treatment with appropriate amounts of immunosuppressant medications is necessary for the rest of the life of the kidney graft in order to avoid immunological rejection. Too little suppression will lead to rejection, whereas too much can lead to problems with toxicity. Therefore it is of great importance to develop methods for continually monitoring the condition of the transplant patient, and thus allow doctors to optimize the treatment.

A technique that may be useful for monitoring transplant patients is metabolomics, which has proven utility for diagnosing and monitoring diseases. For example, measurements of the concentrations of metabolites, obtained with NMR or mass spectrometry, have been used to separate groups of patients according to disease state or severity [8]. The possibility of surveying kidney-transplanted patients using NMR spectroscopy was first demonstrated in 1993 [9,10], when two groups showed that it could be used to measure predictive markers for graft function. Since then metabolomics has been frequently used to study patients with kidney transplants, especially in attempts to identify new biomarkers [11]. For instance, Lenz et al. used NMR, mass spectrometry and chemometric methods to identify cases of nephrotoxicity caused by the commonly used drug cyclosporine A, and in 2005 Serkova et al. showed oxidative stress due to ischemia/reperfusion in rats could be detected by NMR-based methods. NMR spectroscopy is very well suited

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Table 1
List of samples analyzed for each patient, identified by the patient ID and the day after kidney transplantation.

ID	Day l	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1308			1					2	3	4	5	6			
1309						1	2.3		4		5		6		
1310	1	2.3				4		5		6			7		8
1311	1			2	3		4	5			6	7			8
1312								1	2.3				4		5
1313	1		2			3	4.5			6					
1314		1.2	3	4		5	6		7						
1315	1	2		3	4	5	6					7.8			9
1316			1	2	3				4	5				6	
1317	1				2			3	4	5		6			
1318		1	2.3			4	5	6	7.8	9.10					
1319		1			2	3		4	5			6			7
1320		1	2		3				4				5		
1322	1	2				3	4	5				6			
1323	1	2.3			4	5	6	7.8		9.10					
1324						1	2	3	4	5					6
1325	1				2			3	4	5	6	7			
1326				1	2					3	4	5			6
1327	1	2						3	4		5				6

It can be seen that samples were taken from patients at irregular intervals, since urine production had not yet been fully established.

for studies in clinical settings because of its high degree of reproducibility (and for this reason it was also used in the study reported here). Since, previous studies have demonstrated the feasibility of identifying both toxicity and reperfusion damage using metabolomics, opening up the possibility for following a patient's recovery using a single technique.

In this study, we demonstrate the feasibility of identifying a profile reflecting biochemical changes that occur in the first two weeks following a kidney transplant. The profile is based on the many metabolites detectable in urine and is highly sensitive. The profile is typical for the normal progress of recovery, hence, if a patient has a different progression it may be indicative of a problem in the recovery phase. By including profiles from patients that suffer from common complications, such as toxicity or rejection, it should also be possible to classify the types of problems patients have, but in this first study we have chosen to focus on normal recovery.

Since each patient is used as their own reference, the focus of our method is on variation around an individual starting point. This strategy makes the method more sensitive than normal multivariate modeling, in which data from a large group of patients with individual metabolic profiles are pooled. Here we focus solely on dynamic changes to detect whether the patient is moving towards recovery or some kind of complication.

We envision that following the dynamics in the patient's urine metabolite patterns will be useful in a wide range of medical areas, besides kidney transplantation as described in this paper. As new, highly specific and often expensive medications are developed it is becoming increasingly important to be able to assess if a treatment has the desired effect for the patient at an early stage. The development of metabolomic methods for continually determining a patient's health state could have an important impact on the emerging field of personalized medicine [12].

2. Experimental

Urine samples were collected from 19 patients during day 1 to day 15 following kidney transplantation, and analyzed by NMR using a Bruker DRX 600 MHz Avance spectrometer with a triple resonance inverse (TBI) ^{1}H {Broad Band, ^{13}C} probe head equipped with *X,Y,Z* gradient coils. Spectra were acquired at a constant temperature of 298.0 ± 0.1 K using a 90° pulse. A 10 s delay was included in the pulse sequence to allow T1 relaxation. The T1 values (in the range 1.5–2.8 s) of the considered metabolites are such that a 10 s delay allows full recovery of longitudinal magnetization after a 90° pulse, as verified by constant integral values for D1_5s. A 0.3 Hz line broadening function was applied before Fourier transformation. Suppression of the water signal was achieved by applying a saturation pulse of 2 s duration at the water resonance. 32 K data points per scan were used and 128 transients were accumulated. Each sample was measured after

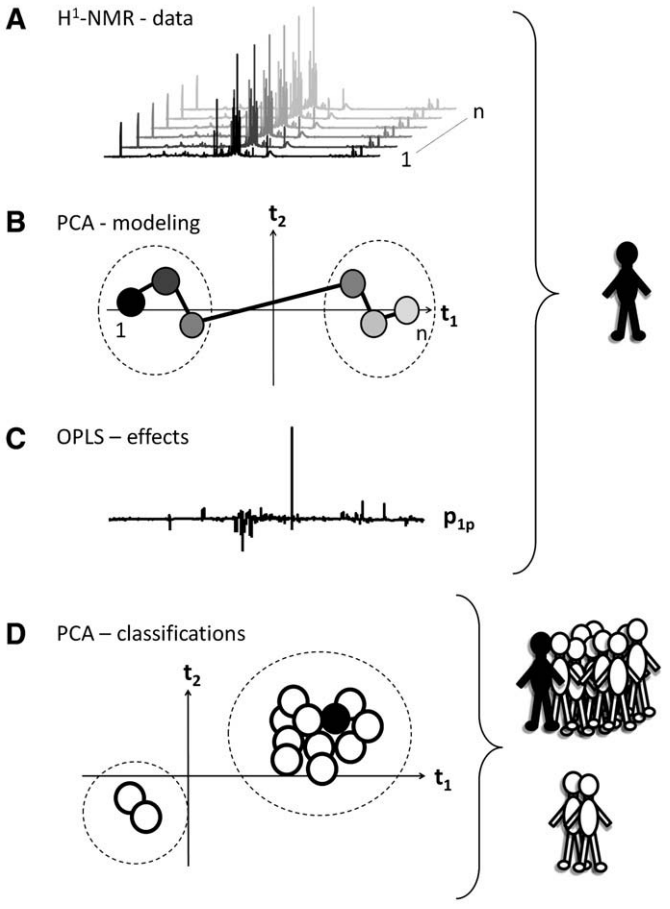

Fig. 1. Work-flow for the presented approach. First, urine is collected (in this study, in the schedule presented in Table 1). A) The urine is analyzed by NMR spectroscopy. B) Each spectrum is digitalized and the resulting data are analyzed by PCA modeling. C) The samples are grouped into before and after kidney function sets, by visual inspection. The differences between before and after function samples are identified by OPLS modeling. D) The recovery process is evaluated by modeling the recovery profiles with PCA and comparing the OPLS effect profiles.