



Applied methodology

Diagnostic potential of major and trace elements in the serum of bladder cancer patients



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ABSTRACT

Major and trace elements may play a role in the diagnosis of diseases. In this study, we investigated the concentration of 26 major and trace elements in the serum by inductively coupled plasma (ICP) – optical emission spectrometry (OES) and ICP-sector field-mass spectrometry (sf-MS). We analyzed the serum from a discovery cohort of 6 bladder cancer (BCa) patients and 12 healthy controls as well as from a validation cohort of 21 BCa patients, 29 non-tumor bladder patients (with acute and chronic inflammation) and 18 healthy controls. Patients were recruited after written consent was obtained at one medical center. Serum was prepared from peripheral blood prior to surgical treatment.

Differences in the levels of major and trace elements were determined by a nonparametric Mann-Whitney test and Kruskal-Wallis statistics. In the discovery cohort, we measured significantly increased levels of calcium, mercury, potassium, lithium, nickel, phosphorus and strontium and a significantly decreased level of sodium in BCa patients compared with healthy controls.

These findings were reassessed in our validation cohort. We measured significantly increased levels of boron, calcium, cadmium, copper, chromium, lead, lithium, potassium, magnesium, nickel, sulfur, strontium, titan, vanadium and zinc and significantly decreased levels of iron and molybdenum.

When we studied the concordance for the discovery and validation cohorts, concentrations of five elements were detected as significantly increased in BCa patients compared with healthy controls: calcium, lithium, potassium, nickel, and strontium. Interestingly, the levels of three elements (calcium, potassium and strontium) were also significantly increased in non-tumor bladder patients compared with healthy controls. But no element was significantly altered between non-tumor bladder patients and BCa patients.

In summary, we suggest that determination of the elements calcium, lithium, nickel and strontium in the serum could be a new and promising tool for the early diagnosis of BCa.

1. Introduction

Urothelial carcinoma of the bladder (bladder cancer; BCa) is the ninth most frequently diagnosed cancer worldwide, with 439,000 new cases and 165,000 deaths estimated for 2012 [1]. Conventional diagnosis for BCa is still based on morphologic and pathologic criteria such

as histology, tumor stage and tumor grade [2]. An improvement in diagnosis was achieved by the application of fluorescence endoscopy [3]. Molecular studies have focused on pathohistological classification and outcome prediction of BCa patients by gene expression analysis of coding genes or noncoding genes and characterization of chromosomal aberrations or mutational alterations [2,4,5–8]. A noninvasive

Abbreviations: BCa, bladder cancer; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma – optical emission spectrometry

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approach in studying urologic cancers is the molecular analysis of a liquid biopsy, e.g., blood and its components, such as serum [9]. However, there are only a few reports using serum from BCa patients for biomarker studies. The utility of the serum CRP value for assessing the prognosis and therapeutic response of urological malignancies, including BCa, has been reported [10]. Serum DNA analysis for hypermethylation of a set of genes shows promise as an indicator of cancer progression and mortality [11]. In addition, the analyses of cell-free circulating DNA (cfDNA) and the molecular characterization of circulating tumor cells (CTCs) (RNA, DNA and protein levels) are promising for biomarker identification in BCa [12,13]. Furthermore, the analysis of bodily fluids for inorganic substances, i.e., major or trace elements, is another promising process to identify potential biomarkers for diagnosis, prognosis and prediction of cancers including BCa [reviewed in 14]. In this study, we analyzed the serum concentration of 26 major and trace elements in BCa patients by mass/optical emission spectrometry and compared them to those of healthy controls and non-tumor bladder patients.

2. Material and methods

2.1. Patients

The use of patient serum samples for the molecular analyses was approved by the institutional ethical review board, and the study was conducted according to the standards set by the Declaration of Helsinki. The first patient set of 6 BCa patients and 12 healthy control probands was designated the discovery set. A second set of 21 BCa patients, 29 non-tumor bladder patients (with acute and chronic inflammation) and 18 healthy control probands was designated the validation cohort (Table 1).

2.2. Serum samples

Serum was prepared from fresh blood samples collected in S-monovettes Z with clotting activator (Sarstedt, Nümbrecht, Germany). The serum was prepared by centrifugation (2000g for 10 min) and the samples were stored at -80°C . Serum vials were checked in the Central Laboratory, University Hospital Erlangen, to exclude hemolysis and contamination with peripheral blood cells. This was performed at measuring LDH (lactate dehydrogenase) with a normal range of 75–195 U/L and HBDH (alpha-hydroxybutyrate dehydrogenase) with a normal range of < 250 U/L. Only serum samples that complied with these

Table 1
Clinicopathological data of the BCa patients.

N	Discovery cohort 6	Validation cohort 21
gender		
female	2	5
male	4	16
tumor stage		
pTa	2	1
pT1	1	4
pT2	2	9
pT3	1	2
pT4	0	3
n.d.	0	2
tumor grade		
G1	2	0
G2	1	1
G3	3	18
n.d.	0	2
low grade	2	1
high grade	4	18
n.d.	0	2

n.d.-not determined.

quality criteria were included. Serum samples were stored at -80°C until used, and samples were sent on dry ice to Helmholtz Zentrum München (Research Unit, Analytical BioGeoChemistry/Prof. B. Michalke). The samples were thawed slowly at 4°C in a refrigerator before being diluted 1:10 with Milli-Q water; the diluted samples were used for element determination.

2.3. Sample analysis by ICP-OES

An ICP-AES “Spectro Ciros Vision” system (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) was used for element determination in 1:10 diluted (Milli-Q water) samples. Sample introduction was carried out using a peristaltic pump connected to a Meinhard nebulizer with a cyclonic spray chamber. The spectral element lines measured were (nm) Al: 167.078, B: 249.773, Ba: 455.404, Ca: 183.801, Cu: 324.754, Fe: 259.941, K: 766.491, Li: 670.770, Mg: 279.079, Mn: 257.611, Na: 589.592, P: 177.495, S: 180.731, Sr: 407.771, Ti: 334.941, and Zn: 213.856 (Table 2). The RF power was set to 1400 W, and the plasma gas was set to 13 L Ar/min; the nebulizer gas was approximately 0.6 L Ar/min after daily optimization. In the majority of samples, barium concentrations were found to be below the internal quantification limit of $11.2\ \mu\text{g/L}$. Therefore, barium analysis was not considered further.

2.4. Sample analysis by ICP-sf-MS

An ELEMENT 2, Thermo-Electron (Bremen, Germany) ICP-sf-MS instrument was employed for determination of elements that were below the level of detection from ICP-OES. The high-resolution capability of the ICP-sector-field-mass spectrometer provided element determinations unaffected from resolved spectral interferences. ^{103}Rh was administered to each sample at a concentration of $1\ \mu\text{g/L}$ as an internal standard. Sample introduction was carried out using a peristaltic pump connected to a Seaspray nebulizer with a cyclonic spray chamber. The RF power was set to 1300 W, the plasma gas was 15 L Ar/min, whereas the nebulizer gas was approximately 0.9 L Ar/min after daily optimization. Measured element isotopes were ^{75}As , ^{114}Cd , ^{59}Co , ^{52}Cr , ^{202}Hg , ^{55}Mn , ^{98}Mo , ^{60}Ni , ^{208}Pb , ^{120}Sn , ^{77}Se , ^{47}Ti , and ^{51}V (Table 2).

2.5. Quality control for element determinations

The determination method had been validated previously by regular laboratory intercomparison studies and by regular analysis of adequate certified reference materials.

Routinely, after every ten measurements, three blank determinations and a control determination of a certified standard for all mentioned elements were performed. Calculation of results was carried out on a computerized lab-data management system, relating the sample measurements to calibration curves, blank determinations, and control standards.

2.6. Statistical analysis

The differences between the major and trace element levels in the serum of BCa and non-tumor bladder patients and healthy volunteers were estimated by a Mann-Whitney *U* test or a Kruskal-Wallis test and determined in ROC analyses. For establishing classification trees, we used recursive partitioning methods as implemented in the RPART package. Classification trees were pruned in order to minimize the relative misclassification error. All calculations were performed with the R statistical framework Ver. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>).

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