Contribution to the definition of diagnostic criteria for Balkan endemic nephropathy

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Abstract

Background. Diagnostic criteria for Balkan endemic nephropathy (BEN) have not been precisely established. In the present study the predictive value of variables previously proposed as diagnostic criteria for BEN was examined.

Methods. The study involved 182 patients: 98 patients with BEN, 57 patients with other kidney diseases (20 with glomerulonephritis, 17 with tubulointerstitial diseases and 20 with hypertensive nephrosclerosis) and 27 healthy subjects. The BEN group comprised patients who fulfilled criteria for BEN and suspected BEN, together with patients with proteinuria and at least two tubular abnormalities or one tubular abnormality and a history of urothelial tumour. Demographic, clinical, laboratory and ultrasound variables of examined groups were combined in univariate/multivariate logistic regression analysis.

Results. Out of 28 analysed variables only urine alpha1-microglobulin (MG) and kidney length were selected as significant predictors in differentiating BEN from other kidney diseases and healthy controls. Using ROC curves the cutoff values of these variables and proteinuria and kidney volume, variables collinear with them, were found. Moderate sensitivity and specificity characterized all these cutoff values except for proteinuria, which provided high sensitivity and specificity in combination of BEN and healthy persons. The predictive value of different combinations of selected variables was not significantly different from the predictive value of each variable individually.

Conclusions. Proteinuria, urine alpha1-MG, kidney length and volume were selected as significant predictors of BEN. Variables related to kidney failure as well as several tubular disorders (urine specific gravity, FENa and TRP) had an insignificant predictive value and could not be used for differential diagnosis of BEN.

Keywords: Balkan endemic nephropathy; diagnostic criteria

Introduction

The definition of criteria for the diagnosis of Balkan endemic nephropathy (BEN) has remained an unsolved problem although significant attention was paid to it from the very beginning of investigations into the disease [1]. Danilović was the first who described BEN in Serbia and proposed criteria for its diagnosis. These involved epidemiological criteria (residence in an endemic settlement and a positive family history), proteinuria, low urine specific gravity, anaemia, azotaemia and shrunken kidneys [2]. Similarly, Radonic and co-workers used proteinuria, anaemia, azotaemia and a positive family history for diagnosis of BEN [3]. Both of these teams applied these criteria to classify patients into BEN patients with manifested disease, suspected BEN patients and patients with proteinuria and positive epidemiological criteria [2,4]. Although the insufficiency of these criteria has been discussed frequently, they have been used in many studies. Stefanović extended these criteria and included tubular abnormalities, proteinuria of the tubular type, scarce urinary deposits, absence of persistent urinary infection and symmetric reduction of the kidneys with apparently normal pelvic and calyceal systems among diagnostic criteria for BEN [5].

Recently, we have demonstrated that Danilović’s criteria only enable detection of BEN in the advanced phase of the disease and differential diagnosis between BEN and healthy persons. None of Danilović’s criteria provided high sensitivity and specificity for differential diagnosis between BEN and other kidney diseases [6]. Also, the examination of different BEN groups showed that the disease was manifested not only in BEN patients but also in BEN-suspected patients. Moreover, tubular disorders and hypertension were found frequently in patients with proteinuria, indicating that they might be considered as patients in the early phase of BEN, especially if some tubular disorders accompany the proteinuria [7].

An International Panel of BEN Investigators has agreed recently that the establishment of diagnostic criteria is of utmost importance and will improve clinical work and further
scientific investigations of BEN [8]. In order to contribute to these efforts the present study was undertaken with the aim of examining the predictive value of variables previously proposed as diagnostic criteria for BEN.

**Patients and methods**

The study involved 182 patients: 98 patients with BEN, 57 patients with other kidney diseases [20 with glomerulonephritis (GN), 17 with chronic tubulointerstitial diseases (TID) and 20 with hypertensive nephrosclerosis (HNS)] and 27 healthy subjects. The patients with kidney diseases other than BEN and healthy controls were from non-endemic settlements. The BEN group comprised patients who fulfilled criteria for BEN and suspected BEN according to Danilović [2], together with the patients from BEN families who had proteinuria and at least two tubular abnormalities or proteinuria, one tubular abnormality and a history of urothelial tumour but after exclusion of other kidney diseases. For exclusion of adult polycystic kidney disease, obstructive uropathy, nephrolithiasis ultrasound and if necessary other imaging methods were used, in addition to a medical and family history and laboratory examination. In patients with proteinuria >1 g/24 h, primary and secondary glomerulonephritis were excluded using routine methods including kidney biopsy. All patients from BEN families with diabetes mellitus as well as those with the criteria for HNS quoted below were excluded from the study. Also, all patients in whom overlapping of BEN and other nephropathies could not be excluded at the time of the study were not involved.

GN was diagnosed using routine methods including kidney biopsy. The histopathological diagnoses of patients with GN were membranous nephropathy in nine, IgA nephritis in four, non-IgA mesangioproliferative GN in four and mesangiocapillary glomerulonephritis in three patients. A diagnosis of HNS was made using the previously defined sets of clinical criteria that best characterize the hypertensive HNS phenotype: a history of hypertension for >10 years, a positive family history for hypertension, the presence of renal abnormalities and other organ evidence of hypertensive damage (hypertensive retinopathy and left ventricle hypertrophy) [9,10]. Only patients with hypertensive retinopathy grade 2 or more and patients with left ventricle hypertrophy diagnosed by two-dimensional echocardiography were involved. In all cases, the clinical history of hypertension preceded the known onset of renal abnormalities, such as proteinuria or renal insufficiency. Exclusion criteria for this group were renovascular hypertension, other definite causes of hypertension including primary and secondary glomerular diseases. The TID group consisted of 13 patients with obstructive uropathy and 4 patients with reflux nephropathy. The diagnosis of TID was based on the history, laboratory tests and imaging procedures, including ultrasound and if necessary X-ray and radionuclide cystography and excretory urogram that revealed parenchymal scars and deformation of the calyces and papillae. Patients with bacteriuria and kidney stones were excluded. The healthy controls were members of the medical staff and kidney donors who were preparing for donation in the Department of Nephrology. All healthy persons had a negative medical history for kidney disease and hypertension and no pathological finding detected by objective, laboratory and ultrasound examination.

Patients were considered eligible for inclusion if they had no acute illness and they were recruited consecutively in the Outpatient Department of the Institute for Endemic Nephropathy, Lazarevac, and the Institute of Urology and Nephrology, Clinical Center of Serbia, Belgrade.

Arterial hypertension was diagnosed when systolic blood pressure was ≥140 mmHg and/or diastolic pressure was ≥90 mmHg or if patients were on antihypertensive treatment previously prescribed by their physicians. Out of 62 BEN patients with hypertension only 26 used previously prescribed antihypertensive drugs, most frequently angiotensin-converting enzyme inhibitors (ACEI) occasionally together with beta-blockers. Fifteen GN patients with hypertension and all patients with HNS used ACEI often in combination with beta-blockers and/or calcium channel blockers. Five patients with TID and hypertension used calcium channel blockers.

Body mass index (BMI) was calculated according to the formula: weight (kg)/height^2 (m^2). Body surface area (BSA) was estimated according to weight and height by using a nomogram based on the formula of DuBois [11].

The Ethics Committee of the Clinical Center of Serbia evaluated and approved this study, and both patients and healthy controls gave their informed consent.

**Laboratory analyses** included peripheral blood cell count, serum and urine levels of urea, sodium and phosphate measured with a commercially available kit (Beckman, Germany) using Synchron CX Beckman Brea, CA, USA. Serum creatinine was determined on a Beckman Creatinin Analyzer II (Brea, CA, USA) with the modified Jaffe rate method. Urine glucose was determined as a ‘spot test’ with a dipstick containing a colour-sensitive pad. Urine alpha1-microglobulin (alpha1-MG) was measured by immunonephelometric assay (BN II nephelometer, Dade Behring) (normal value <0.37 U/mmol creatinine) and alkaline phosphatase (AP) by a colorimetric method (normal value <0.37 U/mmol creatinine). Fresh morning urine specimens were used for all the above-mentioned analyses.

The fresh urine specimens were centrifuged and the sediment was examined under a microscope. Less than five red or white blood cells per high-power field were considered normal. Urine-specific gravity was measured in a morning specimen after 8-h water deprivation using an Assistent Urineproby hydrometer (model 242; Sondheim/Rhon, Germany).

In the sample of urine collected over 24 h, protein was measured by the colorimetric method with pyrogallol red and creatinine by the Jaffe method and 24-h urinary creatinine clearance (Ccr) was calculated. Proteinuria >200 mg/day was considered pathologic. Fractional sodium excretion (FENa) and tubular phosphate reabsorption (TRP) were calculated using the standard formula.

Anaemia was defined as haemoglobin <130 g/l for males and post-menopausal women and <120 g/l for pre-menopausal women, as proposed by the World Health Organization [12]. Azotaemia was defined as serum urea >7.5 mmol/l or creatinine level >124 µmol/l for males and 106 µmol/l for females.
Kidney ultrasound was performed by one dedicated doctor, using a Vivid 3-General Electric ultrasound machine with a sector probe of 3.5 MHz. Cranio-caudal measurements of length and cross-sectional measurements of width and depth were made on each kidney in the sections visually estimated to represent the largest diameter. The thickness of the kidney parenchyma was determined as the shortest distance from the renal sinus fat to the renal capsule. The measurements were expressed in centimetres. Kidneys of length <10 cm were considered shrunken. Kidney volume was calculated using the ellipsoid formula: length × depth × mean width × 0.523. Relative kidney length was calculated using absolute kidney length (cm) and body height (m) ratio and relative kidney volume using absolute kidney volume (cm³) and body size ratio (m²). The mean absolute and relative kidney length, volume and parenchymal thickness were calculated using the sum of the right and left kidney values divided by 2.

Statistical analysis

Descriptive statistics are reported as frequency for categorical data and as mean and standard deviation for continuous data. A comparison of the variables among the four groups was made with one-way analysis of variance (ANOVA) accompanied by Bonferroni multiple comparison tests. The statistical significance of the differences between the group frequencies was determined using the chi-square test. The correlation between variables was tested using Pearson’s or Sperman’s correlation coefficients as appropriate. A value of $P < 0.05$ was considered significant.

All demographic (sex and age), clinical (BMI, BSA, systolic and diastolic blood pressure and presence of hypertension), laboratory (haemoglobin, serum urea, creatinine levels, urine protein, alpha1-MG, AP level, glucosuria, urine specific gravity, FENa, TRP, Ccr, presence of anaemia and azotaemia) and ultrasound (absolute kidney length and volume for right and left kidney, mean absolute and relative kidney length and volume, parenchymal thickness and presence of shrunken kidney) variables determined in the study were combined as independent variables in a univariate/multivariate logistic regression analysis. The dependent variable was ‘belonging to the group’: belonging to the BEN group was coded as 1 and belonging to the group of healthy persons or the group of other kidney diseases was coded as 0. As a positive family history and proteinuria overlapped with belonging to the group, these variables could not be included in the logistic regression. Also, collinearity was found between serum urea, creatinine and Ccr, and between all kidney dimension variables. Therefore, only Ccr and mean absolute kidney length were involved in the analysis. The majority of variables were used as continuous variables. The presence of hypertension, pathological urine sediment, glucosuria, anaemia, azotaemia and shrunken kidney was recorded as 1 and their absence as 0. A receiver operating characteristic (ROC) curve was generated to identify the optimum cutoff value for variables found as significant diagnostic predictors of BEN. Sensitivity, specificity, area under the curve (AUC), standard error and $p$ were calculated not only for the cutoff values obtained but also for their combination. The AUC of two ROC curves is statistically compared using a formula provided by Hanley and McNeil [13].

Statistical analysis was performed using the SPSS software version 15 for Windows, Rel. 15, 2007 (SPSS Inc., Chicago, IL, USA).

Results

The data for the five examined groups are presented in Table 1. There were no differences in sex and BMI among the groups but they differed significantly in age. BEN patients and patients with HNS were significantly older than patients in the two remaining patient groups and the healthy controls.

Means (±SD) for all variables determined in the study are presented in Table 2. The presence of glucosuria and scarce urine sediment are the only variables given as frequency. A comparison of the mean values of each of the variables between the five groups by ANOVA revealed significant differences for all variables except FENa and TRP. This significance of the differences was not only due to the significant differences in mean values and frequencies between the healthy subjects and the patient groups but also due to significant differences between individual patient groups. In Table 2 the mean values or frequencies of each of the variables that differed significantly from the values for healthy subjects and BEN patients are designated, but not all other significant differences. All variables presented in Table 2 (except those excluded due to collinearity), in addition to sex, age, BMI, BSA, presence of hypertension, anaemia, azotaemia and shrunken kidney, were combined in a univariate logistic regression model in order to find those that significantly differentiate patients with BEN from those with other kidney diseases and BEN patients from healthy persons. Age, BSA, systolic blood pressure, urine-specific gravity, urine glucose, AP, alpha1-MG, Ccr and kidney length appeared as significant predictive variables in the analysis of BEN and other kidney diseases, as well as for BEN patients and healthy persons. Haemoglobin, anaemia, azotaemia, shrunken kidney, FENa and TRP appeared as significant predictors of BEN in the combination with healthy persons but not for other kidney diseases. BMI was a significant predictor of BEN in the combination with other kidney diseases.

<table>
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<th>Table 1. Data on examined groups</th>
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<td><strong>Group</strong></td>
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GN: primary glomerulonephritis; TID: chronic tubulointerstitial diseases; HNS: hypertensive nephrosclerosis.

$^*$ $P < 0.05$ as compared with the BEN and HNS groups.

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The variables found to have a significant predictive value in univariate logistic regression analysis were used in the multivariate analysis. This showed that age, urine alpha1-MG and kidney length were significant independent predictors for differentiation of BEN both from other kidney diseases and from healthy persons. When the variables were adjusted by sex and age, alpha1-MG and kidney length appeared again as significant predictors of BEN in combination with both healthy persons and other kidney diseases, but BMI was a significant predictor of BEN in the combination with other kidney diseases (Table 3).

Univariate/multivariate logistic regression was also used to find out predictors that discriminate BEN from a particular kidney disease after adjusting all the variables by sex and age. The analysis revealed the following: proteinuria [B: −0.05; odds ratio (OR): 0.95; P: 0.0144] as significant independent predictors for differentiation of BEN from GN; systolic blood pressure (B: 0.06; OR: 1.05; P: 0.0167) and kidney length (B: −1.74; OR: 0.17; P: 0.0263) for differentiation of BEN from TID; alpha1-MG (B: 0.35; OR: 0.96; P: 0.0201) for differentiation of BEN from HNS.

Using receiver-operating characteristic (ROC) curves, the sensitivity and specificity were calculated for the variables selected by multivariate analysis combining BEN and other kidney diseases, as well as BEN and healthy persons. Proteinuria, which was found to overlap with the group and kidney volume that was collinear with kidney length, was also included in this analysis. The cutoff values corresponding to the best combination of high sensitivity and high specificity of these variables were found and are presented in Table 4. The results showed moderate sensitivity and specificity in both combination BEN and healthy persons and BEN and other kidney diseases. The only exception was proteinuria, which had high sensitivity and specificity in combination BEN and healthy persons.

The predictive value of different combinations of variables, for which cutoff values were determined, was then analysed by logistic regression. The predictive value of these combinations was not significantly different from the predictive value of each variable individually. A comparison of areas under ROC curves (AUC) of different combinations of variables presented in Table 4 revealed that the
AUCs differed insignificantly (z statistics: 0.483–1.854, P > 0.05).

Discussion

The main goal of the present study was to discover the variables with the significant predictive value in the differential diagnosis of BEN. Twenty-eight demographic, clinical, laboratory and ultrasound variables of patients with BEN, GN, TID or HNS and healthy persons were combined in univariate/multivariate logistic regressions. Although these variables were previously involved among diagnostic criteria for BEN, only alpha1-MG and kidney length were selected as significant BEN predictors. These results confirm our recently published data that showed insufficient sensitivity and specificity of Danilović’s criteria for differentiating between BEN and other kidney diseases and indicated alpha1-MG as a variable that significantly discriminated BEN from other kidney diseases [6].

In the selection of variables for the present study, we used those proposed by different authors as diagnostic criteria. The first proposed WHO criteria were established on the basis of a comprehensive analysis of the initial investigations of BEN [1]. The subsequent definitions of criteria proposed by Danilović and Radonić involved most of them [2,3]. These three definitions involved epidemiological criteria (positive epidemiological and family history), proteinuria, azotaemia and anaemia. The failure of concentrating ability was included in the WHO and Danilović criteria, but the absence of oedema was included only in the WHO criteria. In addition, Danilović involved symmetrically shrunken kidneys among the criteria. In order to improve the diagnostic criteria for BEN, Stefanović proposed numerous epidemiological, clinical, laboratory and morphological characteristics of BEN that could be used in diagnosis of the disease [5]. In subsequent studies, this author’s team diagnosed BEN in patients with positive epidemiological criteria and unremarkable urinary sediment, low-molecular weight (LMW) proteinuria, tubular abnormalities, sterile urine, anaemia, azotaemia and symmetric reduction of the kidneys with apparently normal pelvic and calyceal systems [14]. Almost all diagnostic criteria used by the above-mentioned authors were involved in the present analysis. In addition, several variables that more precisely determine some of these criteria (Ccr, kidney dimensions and parenchymal thickness), some demographic variables (age, sex, BSA and BMI) and systolic and diastolic blood pressure were involved. Although normal blood pressure has been considered to be a characteristic of BEN patients [2,15], recent studies showed a higher prevalence of hypertension not only in BEN patients [7,16] but also in BEN offspring [17]. In the BEN population presented here, 63% of the patients had hypertension and therefore blood pressure was involved among the independent variables in logistic regression analysis.

Another problem was the selection of patients with BEN. According to Danilović, patients with BEN are those who fulfilled at least five of his criteria. BEN-suspected patients were those with positive epidemiological criteria, proteinuria and at least one of the remaining criteria [2]. Radonić’s group diagnosed BEN if proteinuria, anaemia and azotaemia were found and suspected BEN if proteinuria and either anaemia or azotaemia were present [3,4]. These definitions and our previous analysis indicated that BEN-suspected patients had numerous disorders of kidney function and among them some even had chronic renal failure and reduced kidney size [6]. Therefore, these patients could not be considered BEN suspected but BEN diseased. Moreover, different tubular disorders and frequently hypertension were found among the patients that Danilović designated as persons with proteinuria, indicating that these patients might be considered as patients in the early phase of BEN [7,18]. On the basis of these results, the present study comprised patients that fulfilled criteria for BEN and suspected BEN, as well as patients from BEN families who had proteinuria and at least two tubular abnormalities or proteinuria, one tubular abnormality and a history of urothelial tumour and in all of them other kidney diseases were excluded.

Multivariate analysis revealed only a few variables that significantly discriminated BEN from other kidney diseases and healthy persons. Those were alpha1-MG and kidney length. Thus, higher alpha1-MG and shorter kidney length were associated with BEN. Although previously proposed as diagnostic criteria, variables related to kidney failure (anaemia, haemoglobin, serum urea, creatinine level, Ccr, azotaemia and shrunken kidney), as well as unremarkable urinary sediment, low urine specific gravity, were not among the selected variables. Also, out of several indices of tubular function only alpha1-MG appeared as a significant independent predictor. Urine specific gravity, FENa, TRP had a insignificant predictive value, confirming already published data that disorders of these tubular functions appear in the advanced phase of BEN [19,20]. The present study selected kidney length as a significant predictor of BEN. Although there are
different opinions about kidney length in BEN, recent data indicate that reduced kidney length appears in the early stage of BEN and even in offspring of BEN families [7,17,21].

A ROC curve was generated to identify the optimum cutoff value for alpha1-MG and kidney length, i.e. variables found to have significant predictive values by multivariate analysis. Also, cutoff values were found for proteinuria, a variable that overlapped with ‘belonging to the group’ and for kidney volume that was collinear with kidney length. However, moderate sensitivity and specificity characterized all these cutoff values, except for proteinuria in the combination of BEN and healthy persons. The predictive value of different combinations of selected variables was not significantly different from the predictive value of each variable individually.

Despite the small number of patients in the groups with particular kidney diseases other than BEN, univariate/multivariate logistic regression was used to find predictors for differentiating BEN from these diseases. The analysis revealed that the same variables that appeared as significant predictors for differentiating BEN from the other kidney disease group differentiated BEN from the particular kidney disease groups but in different combinations. Kidney length was a significant predictor only for differentiation of BEN from TID, but proteinuria differentiated BEN from GN. Urine alpha1-MG was a significant predictor in differentiating BEN from HNS but not for differentiation of BEN from GN or TID. That could be expected because no significant difference was found for mean alpha1-MG between the BEN, GN and TID groups (Table 2). Increased urine alpha1-MG is a characteristic finding in TID but was also found in proteinuric glomerular diseases. This could be due to increased uptake of filtered proteins and saturation of the reabsorptive mechanism of the tubular cells or even their toxic damage [22]. The increased excretion of alpha1-MG in GN was also found to be significantly associated with the extent of tubulo-interstitial damage [22,23]. The remaining nine patients had mean proteinuria of 1.3 ± 0.8 g/24 h but also an increased urine alpha1-MG level (2.9 ± 3.7 mg/mmolCr). Many of our patients had hypertension treated with ACEI, agents that could lower proteinuria and consequently LMW proteinuria [24,25]. Therefore, the influence on proteinuria as well as alpha1-MG in these patients could be questionable. Nevertheless, the above-mentioned data on urinary protein and alpha1-MG excretion in patients with GN indicated that, regardless of ACEI therapy, patients with GN exhibited much higher proteinuria than BEN patients, so proteinuria appeared as a significant independent predictor of BEN.

Contribution to the definition of diagnostic criteria for BEN

The present study examined the predictive value of variables proposed by different authors as the criteria for the diagnosis of BEN. Multivariate logistic regression analysis selected alpha1-MG and kidney length as variables that significantly differentiate patients with BEN from those with other kidney diseases, as well as from healthy controls. The optimal cutoff values of selected variables had moderate sensitivity and specificity. Among several markers of tubular dysfunction (FENa, TRP, glucosuria, urine alkaline phosphatase and urine specific gravity) only alpha1-MG appeared as a significant independent predictor of BEN. Variables related to kidney failure, azotaemia, anaemia, shrunken kidney, which had been proposed as diagnostic criteria, had an insignificant predictive value and should not be used for differential diagnosis of BEN.

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Conflict of interest statement. None declared.

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