https://doi.org/10.23934/2223-9022-2020-9-4-593-597

Characteristic Curves and Criterion of Critical Difference in Assessing the Informativity of Markers of Renal Damage in Lithotripsy

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AIM OF STUDY Determination of the diagnostic significance of laboratory biomarkers of renal tissue damage in remote nephrolithotripsy in patients with urolithiasis.

MATERIAL AND METHODS On the basis of the urology department of the Republican Clinical Hospital (Ufa), 35 patients with urolithiasis were examined, who underwent remote shock wave lithotripsy sessions. The laboratory parameters were determined in patients: the number of erythrocytes, leukocytes in the blood, the level of lipocalin and microalbumin in the urine, as well as alpha2-microglobulin and cystatin C in the blood serum. The control group included 14 healthy donors. To determine the diagnostic efficacy of biomarkers of renal injury, characteristic curves were plotted, and lipocalin level shifts were interpreted taking into account the data on the critical difference criterion value.

RESULTS When studying the urinary level of lipocalin in patients with urolithiasis, it was found that the difference in the concentration of the biomarker in them and in healthy individuals is statistically insignificant (0.68 pg/ml versus 0.4 pg/ml). After the first session of extracorporeal lithotripsy, an increase in urinary excretion of lipocalin by 5 times is noted, after the second - by an additional 1.6 times, and after the third - by another 1.7 times (the differences are statistically significant). To analyse the prognostic efficiency of markers of renal injury, characteristic curves were plotted. The area under the ROC curve for lipocalin varied from 0.77 to 0.80 depending on the number of sessions, which indicates a high diagnostic efficiency of this biomarker. The determination of the criterion of critical difference (RCV) showed that an increase in the level of lipocalin in the urine after the first session of lithotripsy more than 2.1 times is statistically significant. The concentration of the specified biomarker in urine exceeding 4.5 pg/ml, 6 pg/ml and 10 pg/ml after the first, second and third sessions of lithotripsy, respectively, can be considered as a basis for changing treatment tactics (delaying the second procedure) or performing lithotripsy in another way.

CONCLUSION Urinary lipocalin, associated with neutrophil gelatinase, is an informative biomarker of renal injury in the assessment of complications associated with the lithotripsy procedure.

Keywords: kidney damage, biomarkers, extracorporeal lithotripsy, NGAL, ROC, RCV

For citation Iskhakova RS, Gilmanov AG. Characteristic Curves and Criterion of Critical Difference in Assessing the Informativeness of Markers of Renal Damage in Lithotripsy. *Russian Sklifosovsky Journal of Emergency Medical Care.* 2020;9(4):593–597. https://doi.org/10.23934/2223-9022-2020-9-4-593-597 (in Russ.)

Conflict of interest Authors declare lack of the conflicts of interests

Acknowledgments, sponsorship The study had no sponsorship

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AUC ROC — Area under the curve
CT — computed tomography
ECLT — extracorporeal lithotripsy
NGAL — Neutrophil gelatinase-associated lipocalin
RCV — Reference change values
ROC — Receiver operating characteristic

INTRODUCTION

Extracorporeal lithotripsy (ECLT) is the most effective and well established method of minimally invasive removal of kidney stones. ECLT has revolutionized the treatment of patients with urolithiasis and in many cases it is the treatment of choice [1, 2]. At the same time, mechanical forces that cause stone fragmentation in ECLT contribute to damage to the renal parenchyma [3]. Despite the proven micro- and macroscopic damage to the kidney tissue, functional parameters (glomerular filtration rate, etc.) after ECLT usually do not change, particularly, due to the large functional reserve of organs [5, 6]. In this regard, it is relevant to search for sensitive and specific biomarkers of renal tissue damage after exposure to acoustic waves [7], as well as the action of other damaging factors, since kidney pathology is one of the most common complications of various diseases and pathological conditions.

There are no clear clinical criteria for renal damage after lithotripsy in national and international guidelines for the use of ECLT in urolithiasis [4]. Determination of urinary excretion of albumin, lipocalin (NGAL), serum β_2-microglobulin,

cystatin C and other substances associated with trauma can be useful for predicting and diagnosing acute kidney damage in patients with nephrolithiasis undergoing sessions of shock wave nephrolithotripsy and possible dysfunction of renal tissue, but a comparative study of the effectiveness of these biomarkers using modern evidence-based statistical methods is required.

The aim of this study is to determine the diagnostic significance of a number of biomarkers of renal tissue damage in ECLT in patients with urolithiasis.

MATERIALS AND METHODS

We examined 35 patients with urolithiasis, where laboratory parameters were determined before ECLT and after one, two and three sessions: in urine – RBC and WBC (Nechiporenko method), the levels of uroalbumin and lipocalin associated with neutrophil gelatinase (NGAL), and in blood serum - the content of β_2-microglobulin and cystatin C by enzyme immunoassay (test systems Aquatest, BioPorto, Orgentec, Vector-Best). The control group included 14 healthy donors. The results were statistically processed using the Statistica 6.0 computer program. For indicators not having a normal distribution, the median was calculated. When assessing the statistical significance of intergroup differences, pairwise comparisons were made using the Mann – Whitney test and the Bonferroni correction for multiple comparisons; the level of significance of differences $p < 0.05$ was taken as the threshold [9]. To determine the diagnostic and prognostic efficiency of markers of renal injury, the characteristic curves (ROC curves) were plotted and the critical difference criterion (RCV) was calculated.

Currently, the scientific literature contains information about an increase in urinary concentration of the level of lipocalin associated with neutrophil gelatinase in the urine (NGAL), in various conditions directly or indirectly associated with damage to kidney tissue, including such interventions as ECLT. At the same time, it is necessary to interpret the shifts in the NGAL level taking into account the values of the critical difference criterion (RCV) [8], which was calculated in this study.

RESULTS AND DISCUSSION

According to clinical protocols and recommendations, all patients before and after lithotripsy are examined for clinical symptoms, blood and urine tests, ultrasound and X-ray examinations are performed in order to assess the quality of stone fragmentation and identify complications. To determine the body's response to the ECLT procedure, the patients' well-being and general manifestations of inflammation in response to trauma were assessed. There were no signs of deterioration in the general condition of patients with nephrolithiasis after ECLT procedures; statistically significant shifts in markers of the systemic inflammatory response (leukocytosis, increased ESR (erythrocyte sedimentation rate) and C-reactive protein level in the blood) were also absent.

In ultrasound scanning, signs of kidney injury are:

1. Detection of areas of increased echogenicity in the parenchyma. This type of ultrasound changes in patients with nephrolithiasis after ECLT may be associated with the formation of areas of fibrosis or parenchymal calcifications and should not be considered as a reliable criterion for diagnosing renal damage, which is confirmed by the low frequency or absence of differences in the occurrence of this pattern in patients before and after ECLT (3.4% versus 2.7%).

2. Hypoechoic linear structures extending from the capsule deep into the kidney. This type of injury, detected in 7.4% of the examined patients, may be associated with the foci of intraparenchymal hemorrhages and heart attacks.

3. Subcapsular accumulation of hypo- or anechoic fluid, in case of damage to the capsule, spreading into the perirenal tissue. This type of ultrasound changes in our study was observed in 1.7% of cases in the form of a subcapsular strip of fluid not thicker than 5 mm. In general, the ultrasound picture of kidney damage as a result of ECLT is not always specific and difficult to standardize.

In our study, computed tomography (CT) showed renal trauma after ECLT in 21.3% of patients. It was manifested by the perirenal tissue streaking on the side of lithotripsy, enlarged kidney according to measurements using CT segmentation, the appearance of areas of parenchyma of increased density (using the Hounsfield scale), uneven contrasting of the parenchyma in the arterial and nephrographic phases, as well as the appearance of a subcapsular strip of fluid. There were no signs of perirenal hematomas, since severe renal injuries (grade II – V according to Moore) were not observed in our patients.

For complete fragmentation of kidney stones, in some cases, several sessions of ECLT are required, which undoubtedly leads to an increase in the amount of damage to the renal structures. At the same time, clear and reliable criteria defining the number of permissible crushing procedures have not yet been developed. As part of solving this problem, we analyzed the diagnostic and prognostic effectiveness of markers of renal damage by constructing characteristic curves (ROC analysis) with calculating the area under the curve (AUC) for each laboratory indicator characterizing possible disruption of tissue integrity or impairment of function (the number of RBC and WBC, the level of microalbumin and NGAL in the urine, the concentration of β_2-microglobulin, cystatin C in the blood serum) after the first, second and third sessions of ECLT.

The area under the ROC-curve for the leukocyturia index after the first session was 0.41, after the second session - 0.13, after the third session - 0.24; and for erythrocyturia - 0.36, 0.45 and 0.54, respectively. This indicates a low diagnostic efficiency of determining the level of WBC and RBC in urine for diagnosing complications of nephrolithotripsy.

A different situation was observed with biochemical markers of renal damage. The area under the curve (AUC) after the first session of ECLT in patients with urolithiasis was: for NGAL - 0.80, for β_2-microglobulin - 0.32, for microalbumin - 0.24, for cystatin C - 0.23. After the second session, the AUC value for NGAL was 0.77, for β_2-microglobulin - 0.57, for microalbumin - 0.37, cystatin C - 0.20. After three crushing sessions, the area under the ROC curve for NGAL was also 0.77, for β_2-microglobulin - 0.56, for microalbumin - 0.30, cystatin C - 0.37. The data obtained clearly demonstrate that only NGAL has sufficient effectiveness as a marker of kidney damage after ECLT: the area under the ROC-curve (hence, the probability of making a correct conclusion) for which, depending on the number of sessions, varies from 0.77 to 0.80 (Fig. 1). For β_2-microglobulin and microalbumin, AUC was below 0.60, which indicates a low diagnostic efficiency of studying the level of these substances for diagnosing complications of nephrolithotripsy.

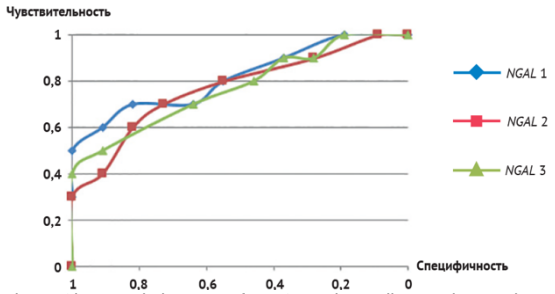

Fig. 1. Characteristic curves for NGAL, depending on the number of sessions of extracorporeal lithotripsy

The threshold value of the NGAL level, which has diagnostic significance for detecting damage to the renal parenchyma during ECLT and calculated from the results of the analysis of ROC curves, was: after one session - 4.5 pg/ml, after two sessions - 6 pg/ml, and after three sessions - 10 pg/ml. Thus, the concentration of NGAL in urine, equal to or higher than the indicated levels after the ECLT sessions, can be regarded as the basis for postponing the repeated procedure or changing the treatment tactics and performing another method of lithotripsy (depending on the effect of the previous ECLT sessions).

The distribution of urine NGAL level indicators in patients with nephrolithiasis, depending on the number of sessions by quartile, is presented in Table. 1. Based on these data, the critical difference coefficient for urinary NGAL was calculated after one, two and three sessions of ECLT in patients with nephrolithiasis.

Table 1

Quartile distribution of NGAL concentration in urine in patients with nephrolithiasis depending on the number of sessions of extracorporeal lithotripsy, pg/ml

After session	Median	Q1	Q3	σ	2 σ
1	3.48	1.93	4.84	1.30	2.60
2	5.47	4.80	6.77	1.07	2.14
3	9.54	8.10	10.42	1.16	2.32

As follows from the Table 1, the median urine NGAL level increased nonlinearly, but very significantly in accordance with the number of ECLT sessions performed. At the same time, the dispersion of the indicator remained practically unchanged, which may be an indirect confirmation of the biological causality and regularity of the observed shifts.

Currently, according to scientific publications [1, 5], researchers pay serious attention to changes in NGAL concentration in various conditions accompanied by damage to kidney tissue, including such interventions as surgical treatment, contact and extracorporeal lithotripsy. At the same time, the shifts in the NGAL level can be correctly interpreted only in combination with data on the criterion of the critical difference (reference change value, RCV). The RCV calculation includes biological and analytical changes in biomarkers in a specific clinical setting.

In accordance with the RCV concept, the results of repeated measurements of the patient's analyte content will be statistically significantly different from each other only if their relative (in percent) difference exceeds the RCV indicator in absolute value:

$$RCV = K \sqrt{(CV_i^2 + CV_a^2)},$$

where CV_i is the coefficient of intraindividual variation, CV_a is the coefficient of analytical variation, K is a constant equal to 2.77 with an assumed probability of 95%.

The coefficient of intraindividual variation CV_i shows the range of values of the indicator in a patient with repeated measurements within a certain time (most often one day) and characterizes the biological variability of the analyte concentration. In general, CV_i is an average value. For routine laboratory parameters, the CV_i value can be taken from the website www.westgard.com, for others it is calculated from the results of monitoring the parameter in the patient. The analytical coefficient of variation CV_a shows the fluctuations in the results of determining the analyte during its measurement, associated with random and systematic errors inevitable in analytical systems. Based on the characteristics of the analyzer and test systems, the CV_a value was taken to be 5%.

In the absence of information on RCV, it is incorrect to make clinical conclusions regarding the significance of changes in the biomarker level after ECLT. In the scientific press, we were unable to find information about RCV for

urinary NGAL, and one of the objectives of this study was to calculate it. Data on intraindividual variation and criterion of critical difference are presented in Table 2

Table 2
Intraindividual variation and criterion of critical difference for urinary NGAL after extracorporeal lithotripsy sessions

Sessions	CV_i, %	*RCV*, %
1	40	111.41
2	19	54.43
3	12	37.14

The high variability of the NGAL indicator after the first session of ECLT observed in the Table 2 is probably associated with the fact that the primary exposure causes the most significant shifts in the structure of the tubules, which differ depending on individual factors. The relatively lower CV_i values after the second and third procedures may associated with the fact that the zone of impact of the shock wave on the renal parenchyma is limited by the focal spot, which in subsequent sessions of ECLT is usually located in the same place as in the previous ones. Therefore, taking into account the time to recover between lithotripsy sessions, the total volume of damaged tubular tissue remains relatively stable. The coefficient of critical difference, calculated on the basis of intraindividual and analytical variation, allows you to establish whether a change in an indicator is diagnostically significant. The results obtained allow us to conclude that there is a clinically significant increase in the level of NGAL if the level of this biomarker in urine after the first session of ECLT increases by more than 111.41% (2.1-fold). After the second and third sessions of ECLT, the clinically significant change in the indicator was 54.43% and 37.14%.

It can be noted that in patients with chronic kidney disease, according to the literature, the levels of RCV and CV_i for urinary NGAL significantly exceed our values for patients with nephrolithiasis and are 69.3% and 86.3%, respectively [8]. Consequently, the shifts in the indicator for the ascertaining of renal tissue damage in CKD should be significantly greater, and its diagnostic efficiency should be less compared to ECLT procedures in nephrolithiasis.

CONCLUSION

The data obtained in the analysis of the parameters of the characteristic curves indicate the insufficient informativity of routine laboratory tests (the degree of leukocyturia and erythrocyturia) included in the standard protocols for lithotripsy, as well as such markers as β_2-microglobulin, microalbumin and cystatin C in urine. This allows us to recommend urinary NGAL as the most informative biomarker of renal injury in extracorporeal lithotripsy and procedure-related occlusive complications. It should also be noted that the measurement of NGAL in urine is one of the criteria for the quality of medical care in acute kidney injury in accordance with the order of the Ministry of Health of the Russian Federation dated May 10, 2017 No. 203n "On approval of criteria for assessing the quality of medical care."

FINDINGS

1. In accordance with the data obtained in this study, the threshold levels of NGAL, which have a diagnostic significance for detecting damage to the renal parenchyma after extracorporeal lithotripsy, were: after the first session - 4.5 pg/ml, after the second session - 6 pg/ml, after the third session - 10 pg/ml.

2. The coefficient of critical difference had the highest value after the first session of extracorporeal lithotripsy (accordingly, comparatively large shifts in NGAL concentration may be recognized as clinically significant), slightly less - after the second and third sessions of lithotripsy.

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Received on 10.06.2020

Review completed on 17.06.2020

Accepted on 30.06.2020