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The method of differential diagnosis of the main forms of primary hyperaldosteronism by high performance liquid chromatography

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Abstract

Objective. To provide a method of differential diagnosis of the main forms of primary hyperaldosteronism (PHA) based on the high effective liquid chromatography (HELC). **Design and methods.** We evaluated 98 patients with PHA and with essential hypertension (EHTN). Aldosterone and plasma renin activity were measured by radioimmunoassay, renin levels by immunoassay. The blood levels of cortisol (F), cortisone (E), corticosterone (B), 11-deoxycorticosterone (DOC), 11-dehydrocortitocosterone (A), 11-deoxycortisol (S), 18-hydroxycorticosterone (18-OH-B), the urinary excretion of free cortisol (UFF), free cortisone (UFE), 18-hydroxycorticosterone (U18-OH-B) were measured by HELC. Dexamethasone test, saline infusion test, postural test, computed tomography of adrenal glands were performed. All PHA patients underwent adrenal vein sampling (AVS). **Results.** PHA patients had higher blood levels of B, DOC, 18-OH-B and urinary excretion of U18-OH-B than EHTN patients. Moreover, patients with aldosteroma had combined excess of blood B, DOC, 18-OH-B and urinary excretion of U18-OH-B, patients with idiopathic hyperaldosteronism (IHA) showed a reduction of F/E and B/A blood ratios and UFF/UFE urine ratio. The blood levels of B, DOC, 18-OH-B and urinary excretion of U18-OH-B showed the highest sensitivity and specificity for the diagnosis of PHA. Patients with aldosteroma showed higher levels of B and 18-OH-B, higher ratios B/A, B/F and 18-OH-B/F in the adrenal vein blood at the tumor side as compared with those in patients with IHA. **Conclusions.** B, DOC, 18-OH-B blood levels and U18-OH-B urinary excretion determined by HELC are informative and reliable indicators for early diagnosis of PHA. The use of HELC method in a complex examination of PHA patients is necessary in case of aldosterone-renin ratio between 30 and 50 ng/dl per ng/(ml per hour) and in case of borderline values of lateralization coefficient at AVS.

Key words: secondary hypertension, primary aldosteronism, differential diagnosis, high performance liquid chromatography

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Способ дифференциальной диагностики основных форм первичного гиперальдостеронизма с применением высокоэффективной жидкостной хроматографии

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Резюме

Цель исследования — разработка способа дифференциальной диагностики основных форм первичного гиперальдостеронизма (ПГА) на основании метода высокоэффективной жидкостной хроматографии (ВЭЖХ). **Материалы и методы.** В исследование включены 98 пациентов с ПГА и с эссенциальной артериальной гипертензией (ЭАГ). Уровни альдостерона и активности ренина плазмы измеряли радиоиммунологическим анализом, уровень ренина — иммуноферментным анализом, уровни кортизола (F), кортизона (E), кортикостерона (B), 11-дезоксикортикостерона (DOC), 11-дегидроксикортикостерона (A), 11-дезоксикортизола (S), 18-гидроксикортикостерона (18-OH-B) в крови, экскрецию с мочой свободного кортизола (UFF), свободного кортизона (UFE), 18-гидроксикортикостерона (U18-OH-B) методом ВЭЖХ. Проводились функциональные пробы с дексаметазоном, физиологическим раствором, «маршевой» нагрузкой; компьютерная томография надпочечников с контрастированием. Всем больным с подтвержденным ПГА выполняли сравнительный селективный забор крови из надпочечниковых вен (ССЗВК). **Результаты.** У пациентов с ПГА установлено повышение уровней B, DOC, 18-OH-B в крови и экскреции U18-OH-B с мочой по сравнению с обследованными с ЭАГ по данным метода ВЭЖХ. Кроме того, у пациентов с альдостеромой установлено сочетанное повышение уровней B, DOC, 18-OH-B в крови и экскреции U18-OH-B с мочой, у обследованных с идиопатическим гиперальдостеронизмом (ИГА) выявлено снижение соотношений F/E и B/A в крови, UFF/UFE в моче. Наиболее высокие чувствительность и специфичность для подтверждения диагноза ПГА были выявлены при определении B, DOC, 18-OH-B в крови и экскреции U18-OH-B с мочой. У больных с альдостеромой выявлено увеличение уровней B и 18-OH-B, соотношений B/A, B/F и 18-OH-B/F в крови из надпочечниковой вены на стороне образования по сравнению с аналогичными показателями у пациентов с ИГА. **Выводы.** Уровни B, DOC, 18-OH-B в крови и экскреции U18-OH-B с мочой, определяемые методом ВЭЖХ, являются информативными и надежными показателями для ранней диагностики ПГА. Использование метода ВЭЖХ в комплексе обследования пациентов с ПГА необходимо при значении альдостерон-ренинового соотношения в диапазоне от 30 до 50 нг/дл на нг/мл/час и при пограничных значениях коэффициента латерализации по данным ССЗВК.

Ключевые слова: вторичная артериальная гипертензия, первичный гиперальдостеронизм, дифференциальная диагностика, высокоэффективная жидкостная хроматография

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Background

Primary hyperaldosteronism (PHA) was first described by J. Conn in 1955 [1]. He supposed the 20% prevalence of this syndrome in patients with arterial hypertension (AH). Therefore he recommended all patients with AH to be screened on PHA [2]. However, his views found no support among his contemporaries. Since then, for over 30 years PHA was considered to be a rare disease, occurring only in 1% of patients with AH [3]. Nowadays different authors describe the incidence of the disease ranges from 7 to 15% among individuals with AH [4–8] and 20 to 30% among persons with secondary (symptomatic) forms of AH [9, 10].

The Endocrine Society guidelines recommend to screen for PHA groups with a relatively high prevalence [11]: AH II and III stage; AH resistant to medical therapy; in case of hypokalemia in a patient with AH (including those caused by diuretics); with AH in a patient with adrenal incidentalomas; in the case of a positive family history of early development of AH, acute cerebrovascular disorders under the age of 40 years; the I-degree relatives of patients with PHA suffering from AH. Japanese Association of Endocrinologists suggests screening for PHA in all patients with AH [12]. Researches showed that patients with PHA had a higher risk of developing cardiovascular disease compared to the patients with essential AH (EAH). This fact is the main argument in the justification for the widespread screening of PHA [10, 13].

Currently the most reliable screening test is considered to be aldosterone-renin ratio (ARR) [4, 8, 14]. The Endocrine Society guidelines recommend confirming the diagnosis in patients with an increased ARR using one of four load tests: with fludrocortisone, with captopril, saline infusion test and oral sodium loading [11]. The oral sodium loading test is difficult to perform in the absence of preformed drugs because there is the necessity of consumption of not less than 12.8 grams of sodium chloride per day for three days [15]. Frequent cases of QT interval prolongation according to the electrocardiography, and left ventricular dysfunction were described during a test with fludrocortisone, therefore most of the researchers currently refused to use it [15, 16]. Several studies have demonstrated the presence of a large number of false negative and doubtful results obtained by using the confirmatory test with captopril [11]. The saline infusion test ("water loading") is the cheapest and the easiest [5]. However, there is the risk of acute volume overload, which limits the use of this test in patients with cardiac or renal failure, unstable AH and severe hypokalemia [16].

Two main forms of the disease account for up to 95% of all cases of PHA. They are solitary aldosterone-producing adenoma (APA), or Conn syndrome, and idiopathic hyperaldosteronism (IHA) due to bilateral diffuse or diffuse-nodular hyperplasia of the adrenal cortex [10, 17]. Differential diagnosis of major forms of PHA is the determining criterion in the choice of further tactics: conservative treatment or surgical intervention. To visualize adrenal gland computed tomography (CT) or magnetic resonance imaging (MRI) are used. At the moment, these methods considered to be insufficiently informative in detection of small masses in the adrenal gland [18] and differential diagnosis of bilateral lesions in patients older than 40 years due to a higher incidence of hormonally inactive adrenal adenomas [19].

The "gold standard" differential diagnosis of APA and IHA is considered to be adrenal vein sampling (AVS) which allows to reliably determine the side of the pathological process [20–22]. Despite the diagnostic value of this procedure it has many drawbacks [13, 19, 21]. First, the method is technically difficult and invasive [13, 20]. The age of the patient, the presence and severity of comorbidity should be taken into account [19]. According to the literature, the complication rate of this diagnostic manipulation ranges from 0.2 to 13%, whereas the success of the AVS varies from 30.5 to 78% [17, 22]. Difficulties in catheterization can be associated with the anatomical features of the location of the adrenal veins, as well as the lack of an experienced specialist [19]. Secondly, so far there is no single standardized protocol for performing and interpreting

the obtained results, which leads to high variability in the evaluation of the success of obtaining blood samples [13, 17, 19].

In connection with the restrictions in the use of AVS the search for new methods of differential diagnostics of the main forms of PHA continues. Recently the role of aldosterone precursors in the differential diagnosis of major forms of PHA has been discussed actively. In the study by Y. Nakamura et al. it was shown that the ratio of 18-oxocortisol and 18-oxocortisol to cortisol in the blood taken from the adrenal veins is significantly higher in patients with APA compared to patients with IHA [23]. R. J. Auchus et al. evaluated the level of 18-hydroxycorticosterone (18-OH-B) in AVS with stimulation by synthetic analogue of ACTH. It was suggested to use the odds ratios 18-OH-B to cortisol of more than 2 and 18-OH-B to aldosterone of less than 0.5 in addition to the aldosterone/cortisol ratio to establish the side of mineralocorticoids hypersecretion [24].

Objective: to provide a method of differential diagnosis of the main forms of primary hyperaldosteronism (PHA) based on high performance liquid chromatography (HPLC).

Design and methods

We evaluated 387 patients with elevated blood pressure (135 men and 252 women) aged 18 to 65 years who were referred to the department of endocrinology of the clinic named after E. E. Eichwald of North-Western State Medical University named after I. I. Mechnikov and Leningrad Regional Clinical Hospital. The control group consisted of 30 healthy people.

For evaluation of hormonal secretion before blood sampling in all patients were cancelled diuretics for 4 weeks, for 2–3 weeks the previous antihypertensive therapy was replaced by doxazosin and/or verapamil. All patients had a normal salt diet.

Aldosterone and plasma renin activity (PRA) were measured by radioimmunoassay, renin levels by immunoassay. The blood levels of cortisol (F), cortisone (E), corticosterone (B), 11-deoxycorticosterone (DOC), 11-dehydrocortitocosterone (A), 11-deoxycortisol (S), 18-hydroxycorticosterone (18-OH-B), the urinary excretion of free cortisol (UFF), free cortisone (UFE), 18-hydroxycorticosterone (U 18-OH-B) were measured by HPLC. Dexamethasone test and saline infusion test were performed. All patients underwent contrasted computed tomography of adrenal gland by X-ray computer tomograph "SOMATOMAR" (Siemens).

All PHA patients underwent AVS. An access to adrenal veins was performed through the right femoral vein using the Seldinger method. Catheterization of both adrenal veins was performed using diagnostic explorer STORQ, introducer 5F, catheters SIMMONS, COBRA, HOOK. The position of the tip of the catheter was verified with a minimum number of contrast agent. In accordance with the Endocrine Society guidelines used a selectivity of 3:1. The gradient of lateralization over 2 testified unilateral overproduction of aldosterone, less than 2 — bilateral lesions of the adrenal gland [11]. In blood samples from the right and left adrenal veins obtained at AVS we measured the levels of F, E, B, DOC, A, S and 18-OH-B by HPLC.

The received clinical results were processed using the software system STATISTICA for Windows (version 10). To create data matrix we used the program Microsoft Excel (version 7.0). Comparison of frequency characteristics of the quality indicators was conducted using non-parametric methods Chi-square (χ^2), χ^2 with Yates correction (for small groups), odds ratios (OR). Comparison of quantitative parameters between groups was performed using Mann-Whitney test. Quantitative performance results are presented as M±m, where M is the arithmetic mean value, m — standard error of mean; Me (LQ; UQ), where Me — median, LQ — lower quartile, UQ — upper quartile. In the processing of data the correlation analysis was performed using Spearman's rank correlation. The criterion for statistical reliability of the findings was considered to be the value of the confidence probability (p) less than 0.05.

Results and discussion

We evaluated 387 patients with hypertension. The patients with impaired renal function, renal artery stenosis, syndrome Cushing's, pheochromocytoma, impaired function of the thyroid gland were excluded from the study. In 197 patients increased blood pressure was regarded as EAH. 122 of these people had hormonally inactive adrenal lesion. In our study the statistical processing and analysis of the data of 98 patients with PHA and with EAH without adrenal disease was performed.

Among the studied hypertensive patients the prevalence of PHA was equal to 11.8%, which corresponds to the literature [4–8]. A 219 of 387 pa-

tients (56.6%) had a secondary AH associated with disorders of the endocrine or urinary systems. The share of PHA among the symptomatic forms of hypertension was 21.0%, which corresponds to the data of other authors [9, 10].

In 27 of 46 (58.7%) of patients with aldosterone hypersecretion APA was detected, the remaining 19 (41.3%) were diagnosed IHA. The obtained results confirm the data of world literature on the number of patients with different forms of PHA [10]. The group of patients with EAH included 52 patients which were divided into two subgroups: 27 with low-renin EAH and 25 with the form of EAH with normal renin level.

The average age of patients with PHA and EAH was comparable (48.4 ± 1.7 vs 47.0 ± 2.2 years respectively). There were no significant differences between study groups on sex composition.

In the group of patients with a PHA the potassium blood level was lower (3.35±0.13 and 3.95±0.07 mmol/l, respectively; p<0.001), and the concentration of aldosterone in plasma (CAP) was significantly higher (392.1 ± of 75.4 and 76.2 ± 4.7 ng/ml, respectively, p<0.001) compared with the group of patients with EAH. In a subgroup with IHA the potassium blood level was higher compared with the patients with APA (3.81 ± 0.21 vs of 3.03 ± 0.14 mmol/l respectively, p < 0.05), CAP in both subgroups was comparable. This data matches with the data of other authors [5, 6, 16].

ARR was increased in all patients (100.0%) with PHA and in 18 EAH patients (34.6%). Thus, the sensitivity of the measurement of ARR (threshold differential diagnostic value of the index was chosen equal to 30 ng/dl to ng/ml/h as the test for establishing PHA) was 100.0%, whereas specificity was only of 65.4%. When choosing a threshold of ARR 50 ng/dl to ng/ml/h, the sensitivity was of 60.9% and specificity was of 88.5%. It can be difficult to compare these data with those of other authors, as in the works of different authors, there are significant differences in the study design. Overall, our results do not contradict the data of other authors [6, 14]. At the same time, several studies have indicated the worst performance of sensitivity and specificity when using ARR for the diagnosis of PHA [26]. According to the literature the ARR is higher in APA than in IHA [14]. In our study, differences in the values of ARR in these subgroups have been identified. The average value of the ARR in low-renin EAH patients was significantly higher than that in the group of EAH with normal renin level (42.1 ± 4.9 vs. 10.5 ± 1.2 ng/dl to ng/ml/h, respectively; p < 0.001).

All patients underwent a night test with 1 mg of dexamethasone to exclude glucocorticoid-suppressed hyperaldosteronism. The disease was not detected in any of the patients.

After the saline infusion test in the PHA group the CAP was 314.8 ± 31.3 pg/ml, but 6 patients with hypersecretion of aldosterone had the CAP of 91.5 ± 2.3 pg/ml (less than 100 pg/ml) that required an additional confirmatory test. After the saline infusion test in the EAH group the CAP was 40.5 ± 5.2 pg/ml, but in 9 patients the decrease of the hormone level less than 50 pg/ml was not reached, that fact does not allow to exclude the presence of PHA in these patients. Thus, the election threshold CAP equal to 100 pg/ml in saline infusion test for the diagnose of PHA makes the sensitivity of the test to be 87.0%, whereas the specificity of 100.0%. When the threshold is equal to 50 pg/ml the sensitivity of the test was 100.0%, while specificity was 82.7%. It can be stated that the method was highly specific at a threshold level of aldosterone in the blood equal to 100 pg/ml and highly sensitive at a threshold of 50 pg/ml. In clinical practice that means that the CAP is less than 50 pg/ml after the saline infusion the diagnosis of PHA is extremely unlikely; if the level of aldosterone is higher than 100 pg/ml the diagnosis of PHA can be considered confirmed. The disadvantage of the test is that up to 1/3 of cases are accompanied by a reduction in the CAP to intermediate values (from 50 to 100 pg/ml), in this case it is impossible to establish any diagnosis. This lack of the test was observed by other authors. Thus, our findings in respect of the saline infusion test consistent with the literature [11, 16]. However, it is worth mentioning that 100% sensitivity or specificity of this test were not described by any of these researchers. Summary data on sensitivity and specificity of the tests for the diagnosis of PHA are presented in the table 1.

HPLC method allows to determine the content of corticosteroids in biological fluids. It is important for the diagnosis of diseases of the pituitary–adrenal axis and the renin–angiotensin–aldosterone system when traditional research methods perform the questionable data.

We found an increase of B level in blood in patients with APA and with IHA in comparison with the level of this indicator in patients with EAH (6.5 ± 0.8 vs 3.0 ± 0.6 ng/ml, respectively; p<0.001). A positive

correlation between the B blood level and the CAP was noted (r = 0.75; p = 0.031). We evaluated the diagnostic value of the B level (as the threshold was set the level of 5 ng/ml) for the diagnosis of PHA. The sensitivity was equal to 71.7%, the specificity was 67.3%. These rates are quite low for the use of B in order to validate PHA, but they can be used as an additional diagnostic criterion in the examination of the patient with hypersecretion of aldosterone. In the literature we have not found information about the level of B in the blood of patients with PHA, however, N. M. Kaplan et al. found a significant increase of this hormone in aldosteromas' tissue [27].

HPLC analysis recorded an increase of DOC levels in the blood of patients with PHA compared with the rest of the patients (7.8 ± 1.4 vs. 2.0±0.8 ng/ml, respectively; p<0.01), mainly due to the patients with APA. This fact is consistent with the data of other authors revealed the hypersecretion of this hormone in PHA [28] and didn't find that in EAH [29]. The sensitivity of the measurement of the DOC blood level for diagnosis of APA (if the diagnostic threshold is 4 ng/ml) in our study amounted to only 51.9% and specificity of 74.6%. These indicators characterize the parameter as insufficiently accurate.

A number of researchers studied the blood levels of 18-OH-B and found its elevation in patients with PHA [30]. Given the multiple (6-fold) increase in the value of this indicator in APA in comparison with IHA, it was proposed to use it for differential diagnosis of different forms of PHA [30]. The results of our study obtained the data on the elevated level of 18-OH-B in patients with PHA compared with patients with EAH (2.1 ± 0.5 vs 4.8 ± 0.5 ng/ml, respectively; p < 0.001). This indicator was higher in APA than in IHA (5.7±0.6 versus 3.4 ± 0.4 ng/ml, respectively; p = 0.02).

Similar patterns were found in relation to excretion U 18-OH-B with urine. We observed an increase excretion of U 18-OH-B with urine in patients with PHA compared with patients with EAH (44.3 ± 3.0 vs 17.4 ± 2.0 mg/24h, respectively; p < 0.001). The average value of the daily excretion of U 18-OH-B in the subgroup of patients with the established aldosteroma exceeded the average rate of this indicator in the patients with IHA (47.1 ± 2.0 vs 40.5 ± 3.9 mg/24h, respectively; p = 0.02).

The correlation analysis revealed a positive linear relationship between 18-OH-B and U 18-OH-B (r = 0.62; p<0.03).

The sensitivity of the 18-OH-B level in blood samples to the exclusion of PHA (diagnostic threshold of 3 ng/ml) was 82.7% and specificity of 80.9%. The use of this indicator for the confirmation of PHA (diagnostic threshold is 5 ng/ml) showed a sensitivity of 70.4% and a specificity of 94.4%. Thus, assessment of blood levels of 18-OH-B can be used as a fairly reliable method of confirmation of PHA and as an additional way of exclusion of the disease.

The use of U 18-OH-B (a diagnostic threshold of 30 mg/24h) to confirm PHA is characterized by similar sensitivity and specificity (71.7 and 96.2%, respectively). Thus, the results of our work show the informativeness of determination of the content of 18-OH-B in blood and excretion U 18-OH-B with urine for diagnosis of PHA.

We evaluated the diagnostic significance of blood levels of 18-OH-B in the range of 3–5 ng/ml for the detection of IHA: the sensitivity amounted to 63.2% and the specificity was 84.8%. Low sensitivity index

Table 1

SENSITIVITY AND SPECIFICITY OF THE SCREENING IMMUNOASSAY AND RADIOIMMUNE TESTS FOR PRIMARY ALDOSTERONISM

Parameter	Threshold level	Sensitivity	Specificity
Aldosterone-renin ratio	30 ng/dl : ng/(ml/hour)	100.0%	65.4%
	50 ng/dl : ng/(ml/hour)	60.9%	88.5%
Intravenous saline test	Plasma aldosterone 100 pg/ml	87.0%	100.0%
	Plasma aldosterone 50 pg/ml	100.0%	82.7%

does not recommend evaluating the blood content of the investigated hormone for the differential diagnosis of different forms of PHA.

Patients with PHA had higher levels of A in the blood compared with patients with hypertension (6.5±0.8 vs 2.5±0.3 ng/ml, respectively; p<0.001). In group with APA this hormone level was lower than in patients with IHA (5.0±0.5 vs 8.6±0.9 ng/ml, respectively; p<0.05). In the literature we have not found sources, evaluating the content of A in patients with PHA.

Interesting data was obtained when analyzing the ratios of various steroids. Despite the fact that blood levels of F and E had no statistically significant differences in PHA and EAH groups, in the subgroup of patients with IHA the increase in the content of E (25.8 ± 2.8 vs 15.6 ± 1.7 respectively; p < 0.05) and the decrease of F/E (3.6 ± 0.2 vs 5.8 ± 0.4 respectively; p < 0.05) was detected when compared with all other subgroups. These data were confirmed by the results of urine analysis. In the subgroup with IHA a decrease in the ratio UFF/UFE was detected (when compared to the same ratio in the subgroups with APA, normo-renin and low-renin forms of EAH: 0.41 ± 0.12 vs 0.86 ± 0.09; 0.70 ± 0.24 and 0.64 ± 0.19, respectively; p < 0.05). The revealed differences in patients with IHA show a decrease in the activity of 11β-HSDH type 1, carrying out the conversion of E to F. In the proof of this fact we found a statistically significant decrease in B/A ratio in the subgroup of patients IHA (1.3 ± 0.2 vs 4.6 ± 0.6, respectively; p < 0.05), as it is known on the participation of 11β-HSDH type 1 in the conversion of A to B. In the literature we have not found the data with analysis of these indexes or with study of the activity of 11β-HSDH type 1 in patients with PHA.

The results of our study revealed the informative value of determination of corticosteroids in blood and excretion of their metabolites in urine by HPLC method for early diagnosis of PHA (tab. 2). This method is useful when the data of the classical diagnostic tests is questionable, such as ARR in the range of 30 and 50 ng/dl per ng/(ml per hour), the intermediate values of the samples with saline (CAP from 50 to 100 pg/ml after test), and in the presence of contraindications to the stress tests. Summary data on sensitivity and specificity of studies of the various steroids to confirm the diagnosis of PHA is presented in table 3.

All patients with laboratory-confirmed PHA underwent AVS. On the basis of AVS the subgroups of the research were established: APA when revealed unilateral overproduction of aldosterone and IHA in a bilateral form (tab. 4). The coefficient of selectivity of AVS in our study amounted to 3.7 ± 0.3.

The results of AVS allowed to establish the diagnosis of APA in 27 patients. The blood samples from the dominant side naturally revealed increased levels of aldosterone (3541.9 ± 76.9 against 1139.3 ± 35.3 pg/ml, respectively; p < 0.05) and aldosterone corrected by cortisol (3.3 ± 0.5 from the overproduction vs 0.8 ± 0.1 at the side of the normal secretion; p < 0.05). The coefficient of lateralization was 2.3 ± 0.2 and the ratio CAP/cortisol in different adrenal veins exceed 4. ARP, renin level and cortisol level were not significantly different on both sides. Of the 27 patients with APA the left hyperproduction of aldosterone was detected in 15 people (55.6%), and right-sided in 12 (44,4%).

19 patients with the lateralization ratio of less than 2 were classified in the subgroup with IHA. The level of aldosterone in serum from the adrenal veins in these patients was 1344.8 ± 76.1 pg/ml, whereas CAP corrected by the level of cortisol was 1.5 ± 0.3.

A linear relationship between aldosterone and its level corrected by the level of cortisol was established in both groups of patients with PHA (r = 0.76; p < 0.05). Thus, the results obtained in AVS meet the commonly used criteria of differential diagnostics of different forms of PHA [11, 21].

The main criterion of differential diagnosis of different forms of PHA in AVS is the coefficient of lateralization. If the value of this indicator is significantly more than 2, the diagnosis of APA is not in doubt. Unilateral form of PHA is an indication for surgical treatment. When the coefficient of lateralization is much less than 2 the existence of a bilateral form is considered to be proven, which requires the prolonged conservative therapy. When questionable data of AVS with a gradient of lateralization of around 2 the use of additional methods of establishing the side of the hyperproduction of aldosterone is necessary. In our study we evaluated the additional coefficients of lateralization using data from the HPLC (tab. 5).

The analysis of blood samples obtained at AVS by HPLC revealed higher levels of B (64.7 ± 9.8 vs 38.4 ± 4.9 ng/ml, respectively; p < 0.05), DOC (20.6 ± 3.7 vs 7.9 ± 2.8 ng/ml, respectively; p < 0.05) and 18-OH-B (62.1 ± 7.1 vs 31.9 ± 5.3 ng/ml, respectively; p < 0.05) in the blood from adrenal vein from the side of hypersecretion of aldosterone.

Table 2

BLOOD CORTICOSTEROIDS IN PATIENTS WITH PRIMARY HYPERALDOSTERONISM AND HYPERTENSION ASSESSED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Group	Parameter, M ± m (ng/ml)						
	Cortisol (F)	Cortisone (E)	Corticosterone (B)	11-deoxycorticosterone (DOC)	11-deoxycortisol (S)	18-hydroxicorticosterone (18-OH-B)	11-dehydrocorticosterone (A)
Patients with primary aldosteronism (n=46)	95.5 ± 6.5	17.1 ± 1.3	6.5 ± 0.8* $p_{1-2} < 0.001$	7.8 ± 1.4* $p_{1-2} < 0.01$	1.4 ± 0.2	4.8 ± 0.5* $p_{1-2} < 0.001$	6.5 ± 0.8* $p_{1-2} < 0.001$
Patients with essential hypertension (n=52)	83.0 ± 4.9	16.8 ± 0.8	3.0 ± 0.6	2.0 ± 0.8	1.0 ± 0.3	2.1 ± 0.5	2.5 ± 0.3
Healthy control(n=30)	82.7 ± 4.3	19.9 ± 0.9	2.5 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	1.7 ± 0.1	2.0 ± 0.2

Note: * — $p < 0.01$ (vs. healthy control).

In addition, a similar pattern continued when comparing the levels of these indicators, corrected for the level of F (also measured by HPLC). This phenomenon confirms once again discovered and previously documented patterns in relation to the blood levels of intermediate products of steroidogenesis in the patients with PHA.

The correlation analysis revealed a linear relationship of the level of aldosterone with the levels of B ($r = 0.93$; $p<0.05$), DOC ($r = 0.83$; $p < 0.05$), S ($r = 0.96$; $p < 0.05$) and 18-OH-B ($r = 0.81$; $p < 0.05$) in the blood from the suppressed side. Such correlations were not detected in the blood from the dominant side, which may be due to increased production of various mineralcorticoids depending on the presence and type of the defect of steroidogenesis in aldosterone.

Thus, our results show that the lateralization of the source of the overproduction of aldosterone in AVS can be confirmed by an estimation of blood levels of B, DOC and 18-OH-B. A method proposed R.J. Auchus et al. involving further assessment of the levels of 18-OH-B in the adrenal veins for a more accurate differential diagnosis of PHA [24], can be significantly extended by means of HPLC.

In patients with APA there was established the increase of B more than 4 times, DOC more than 2-fold and 18-OH-B more than 3 times in the blood of the adrenal vein with the hypersecretion of hormones compared to the levels of the same indicators in the blood of the opposite adrenal vein. Also in patients with aldosteroma there was an elevation in the ratio B/A in more than 3 times, B/F in more than 2-fold, 18-OH-B /F in more than 1.5 times in blood from adrenal vein with pathological hypersecretion of hormones compared to the data obtained from the adrenal vein on the other side. For patients with IHA there were established the ratios between the B levels in the adrenal veins of less than 2.5, DOC — less than 1.4, 18-OH-B — less than 0.4 (tab. 6). In addition, the analysis of the data revealed an increase of the ratios of the blood levels of B and 18-OH-B, ratios B/A, B/F and 18-OH-B /F in the adrenal veins in patients with aldosteroma compared to similar indicators in patients with IHA. Thus, the revealed regularities allow to perform differential diagnostics of different forms of PHA with the determination of corticosteroids in the blood of adrenal vein using AVS.

Table 3

SENSITIVITY AND SPECIFICITY OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY RESULTS FOR SCREENING OF PRIMARY ALDOSTERONISM

Parameter	Diagnostic threshold level	Sensitivity	Specificity
Corticosterone (B), ng/ml	5	71.7%	67.3%
11-deoxycorticosterone (DOC), ng/ml	4	51.9%	74.6%
18-hydroxycorticosterone (18-OH-B), ng/ml	5	70.4%	94.4%
Urine 18-hydroxycorticosterone (U18-OH-B), mcg/24 hours	30	71.7%	96.2%

Table 4

BLOOD SERUM HORMONES IN PATIENTS WITH ALDOSTERONE-SECRETING ADRENAL TUMOR (ASSESSED BY SELECTIVE ADRENAL VEIN BLOOD SAMPLING)

Site of blood sampling	Aldosterone, pg/ml	Plasma renin activity, ng/ml/h	Renin, pg/ml	Cortisol, nmol/l	Aldosterone corrected by cortisol level
Adrenal vein on the site of aldosterone hyper-production	3541.9 ± 76.9*	0.7 ± 0.2	9.6 ± 3.5	1423.9 ± 63.7	3.3 ± 0.5*
Adrenal vein on the site of normal aldosterone production	1139.3 ± 35.3*	0.7 ± 0.2	10.7 ± 4.2	1249.3 ± 42.2	0.8 ± 0.1*
Femoral vein	418.6 ± 25.1	0.4 ± 0.1	3.1 ± 0.4	379.2 ± 29.4	

Note: * — $p < 0.05$ for comparison of the samples from left and right adrenal veins.

Table 5

BLOOD SERUM CORTICOSTEROIDS IN ADRENAL VEINS IN PATIENTS WITH ALDOSTERONE-SECRETING ADRENAL TUMOR (ASSESSED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)

Site of blood sampling	Parameter, M±m (ng/ml)				
	Corticos-terone(B)	11-deoxycor-ticosterone (DOC)	11-deoxy-cortisol (S)	18-hydroxycor-ticosterone (18-OH-B)	11-dehydrocorti-costerone (A)
Adrenal vein on the site of aldosterone hyper-production	64.7 ± 9.8*	20.6 ± 3.7*	33.8 ± 4.1	62.1 ± 7.1*	6.7 ± 2.7
Adrenal vein on the contralateral site	38.4 ± 4.9*	7.9 ± 2.8*	25.3 ± 7.2	31.9 ± 5.3*	12.8 ± 3.4

Note: * — $p < 0.05$ for comparison between left and right adrenal vein blood samples.

Table 6

CRITERIA OF LATERALIZATION IN SELECTIVE ADRENAL VEIN BLOOD SAMPLING (ASSESSED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)

Parameter	Me (LQ; UQ)	
	Patients with aldosterone-producing tumor (n = 16)	Patients with idiopathic aldosteronism (n = 9)
18-hydroxycorticosterone on the site of hyper-production ($18\text{-}OHB_1$)	15,2 (9,1; 22,2)**	2,1 (1,0; 2,5)
18- hydroxycorticosterone-1 / 18- hydroxycorticosterone-2 ($18\text{-}OHB_1$ / $18\text{-}OHB_2$)	3,8 (3,0; 4,8)** > 3,0	0,2 (0,1; 0,4) < 0,4
Corticosterone-1 / corticosterone-2 (B_1 / B_2)	7,8 (4,2; 18,8)* > 4,0	2,0 (1,6; 2,5) < 2,5
11-deoxycorticosterone-1 / 11- deoxycorticosterone-2 (DOC_1 / DOC_2)	4,5 (2,0; 8,5) > 2,0	1,2 (0,8; 1,4) < 1,4
Ratio of corticosterone / 11-dehydrocorticosterone-1 to corticosterone / 11-dehydrocorticosterone-2 (ratio B/A_1 : B/A_2)	8,4 (3,4; 12,5)* > 3,0	1,4 (0,9; 2,3) < 2,3
Corticosterone-1 corrected by the cortisol level / corticosterone-2 corrected by the cortisol level (ratio B/F_1 : B/F_2)	3,6 (2,0; 5,2)* > 2,0	1,2 (1,0; 1,4)
18- hydroxycorticosterone-1 corrected by the cortisol level / 18-hydroxycorticosterone-2 corrected by the cortisol level (ratio $18\text{-}OHB/F_1$: $18\text{-}OHB/F_2$)	1,8 (1,5; 2,7)* > 1,5	0,3 (0,1; 0,4) < 0,4

Note: * $p < 0,05$, ** $p < 0,01$ (vs. patients with idiopathic hyperaldosteronism).

1 — adrenal vein on the site of the higher aldosterone level corrected by the cortisol level, 2 — adrenal vein on the site of the lower aldosterone level corrected by the cortisol level.

Conclusion

1. PHA patients had higher blood levels of B, DOC, 18-OH-B and urinary excretion of U 18-OH-B that EAH patients.

2. Patients with aldosteroma had combined excess of blood B, DOC, 18-OH-B and urinary excretion of U 18-OH-B, patients with IHA showed a reduction of F/E and B/A blood ratios and UFF/UFE urine ratio measured by HPLC.

3. The blood levels of B, DOC, 18-OH-B and urinary excretion of U 18-OH-B showed the highest sensitivity and specificity for the diagnosis of PHA.

4. Patients with aldosteroma showed higher levels of B and 18-OH-B, higher ratios B/A, B/F and 18-OH-B/F in the adrenal vein blood at the tumor side as compared with those in patients with IHA.

5. B, DOC, 18-OH-B blood levels and U 18-OH-B urinary excretion determined by HPLC are informative and reliable indicators for early diagnosis of PHA. The use of HPLC method in a complex examination of PHA patients is useful in case of ARR in the range of 30 to 50 ng/dl per ng/(ml per hour) and in case of borderline values of lateralization coefficient in AVS.

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Conflict of interest
The authors declare no conflicts of interest

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