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Laboratory Diagnostics in Arterial Hypertension

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Arterial hypertension – a practical approach

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Arterial hypertension is a global healthcare burden. With a prevalence of 30% to 45% among the adult population, it represents the most common cause of doctor visits. Incidence increases by age, and >60% of people over the age of 60 suffer from hypertension. It is well known that arterial hypertension is a major independent risk factor for cardiovascular morbidity and mortality.

Hypertension is defined as a systolic pressure ≥140 mmHg and/or dyastolic pressure ≥90 mmHg. The optimal arterial pressure value within the classification is defined as <120/80 mmHg, a normal range is between 120 to 129 mmHg for systolic and/or 80 to 84 mmHg for dyastolic pressure, while a high normal is between 130 and 139 and/or 85 to 89 mmHg. The grade I of arterial hypertension is a systolic pressure of 140-159 mmHg and/or a dyastolic pressure of 90-99 mmHg; the grade II includes a systolic pressure of 160-179 mmHg and/or 100-109 mmHg; the grade III refers to a value >180 mmHg for systolic and/or >100 mmHg for dyastolic pressure. Isolated systolic hypertension applies to people with systolic pressure >140 mmHg and dyastolic pressure <90 mmHg.

The use of mercury-based sphygmomanometers is abandoned today within the European Union, and it is recommended that office arterial pressure measurement should be performed using automated oscillometric devices, which are validated and calibrated at least once a year. Increasingly, continuous measurement of arterial pressure has become the diagnostic standard, which is also indicated in the follow-up of patients with arterial hypertension, reevaluation of poorly controlled arterial hypertension and assessment of treatment efficacy.

According to etiology, hypertension can be primary (essential or idiopathic), which account for 85% of cases; and secondary, which is present in 15% of patients. The most common causes of secondary hypertension are chronic kidney disease, obesity and endocrine disorders. Namely, arterial hypertension can be a presentation of 15 different endocrine disorders, of which primary aldosteronism is the most common.

The diagnostic algorithm includes a thorough patient history and physical examination, focusing mostly on information regarding potential damage to target organs and an increased cardiovascular and renal risk, and basic blood analysis with ECG examination. It is recommended to assess the overall cardiovascular risk as well. It is considered that patients with hypertension and documented cardiovascular disease, diabetes type 1 or 2, or chronic kidney disease have a very high 10-year cardiovascular risk (>10% mortality from cardiovascular causes) or high risk (5%-10% mortality from cardiovascular causes).

Treatment of arterial hypertension includes non-pharmacological and pharmacological measures. Non-pharmacological approaches refer to weight loss, reduction of salt, alcohol, and fat intake, and regular physical activity. Pharmacological therapy can be initiated using any available type of antihypertensive drug.

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Measurement of arterial pressure

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Measurement of arterial pressure is a fundamental test in diagnosing arterial hypertension, determining therapy and monitoring patients. Impractical mercury sphygmomanometers are being abandoned, while the use of hybrid semi-automatic and automatic oscillometric devices that are digital and easy to use is on the rise. Aneroid devices often break down and should be calibrated more often and devices for measuring blood pressure on the finger or wrist are unreliable so both of them are not recommended for use. Survey about the general knowledge on measurement of arterial pressure was conducted among participants during the lecture. Out of 103 participants who completed the survey, 71 (69%) of them knew that hybrid semi-automatic and automatic oscillometric devices were recommended due to its simplicity and precision.

Before measuring arterial pressure, the patient should sit relaxed without talking for 3-5 minutes. 85 (83%) participants knew this recommendation. The measurement is most often performed in a sitting position, with back leaning on the back of a chair. The legs should be placed calmly touching the floor with full feet. The patient should not sit cross-legged while measuring blood pressure and 97 (94%) participants answered the question related to that fact correctly. The arm on which the arterial pressure will be measured, should be at heart level and placed on a table. The sleeve must be taken off and the upper arm must be free of clothing. Hand above the level of the right atrium leads to a false decrease in arterial pressure and 74 (72%) participants answered that question correctly. The patient should avoid food consumption, exposure to cold, physical exertion, abstain from cigarettes, caffeine and alcoholic beverages 30 minutes before the measurement. 68 (66%) participants knew that it was necessary to abstain from coffee and cigarettes for at least 30 minutes before measuring blood pressure.

At home arterial pressure measurement in the form of a seven-day diary is used to diagnose arterial hypertension, determine therapy, and detect white-coat hypertension and masked hypertension. Pressure is measured 2 times in 2 minutes in the morning and 2 times in the evening, before taking the drug if already prescribed, over 7 days. 54 (52%) participants knew the protocol for home arterial pressure measurement. The values measured the first time are discarded and the mean value of all measurements is calculated. Hypertension is defined if values ≥135/85 mmHg are obtained. For stable and well-regulated patients with arterial hypertension, measuring blood pressure at home 1 to 2 times a week is sufficient. When measuring arterial pressure at home, the device used by the patient must be calibrated and the patient educated about self-measurement. The rules and techniques of measurement at home are the same as when measuring the pressure at the clinic. 70 (68%) participants knew those facts about measuring blood pressure at home. If performed correctly, measuring blood pressure at home can provide more information about the therapy effect, it improves patient compliance, hypertension control and can detect white-coat hypertension and masked hypertension. 83 (81%) participants answered correctly that measuring blood pressure at home has advantages in comparison to measurement at the clinic.

The values of arterial pressure measured by the patient at home are lower than those measured in the presence of a doctor. Therefore, the cut-off value for diagnosis of arterial hypertension is different for at home measurement (≥135/85) and in the clinic (>140/90). The reason for this is that the measurement of blood pressure in the patient's daily environment increases the reliability of the measured value. Also, the possibility of multiple measurements at home and obtaining a mean pressure value over time allows for better risk assessment. Only 19 (18%) participants knew the exact limit value for diagnosing hypertension based on values measured at home, while the rest of participants thought that this limit was higher.

Results of this survey showed that the general knowledge about correct measurement of arterial

blood pressure is satisfactory among the population of medical biochemists. Small number of participants knew the limit value for normal arterial pressure so it is important to further educate people about this global growing problem.

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Laboratory diagnostics of arterial hypertension

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More than half of the patients with arterial hypertension (AH) go undiagnosed, so it is important to investigate the presence of other risk factors, comorbidities or organ damage at the time of diagnosis. In only 5-15% of patients with AH it is possible to determine the cause of the hypertension (secondary hypertension) where laboratory diagnostics can significantly contribute. In the remaining 85-95% of patients, the cause of AH cannot be determined. This condition is called primary hypertension, where the laboratory plays an important role in risk stratification for adverse cardiovascular, cerebrovascular and peripheral vascular events, in assessing asymptomatic hypertension-mediated organ damage (HMOD) and in determining the therapeutic approach for a patient.

According to the 2018 guidelines for the management of arterial hypertension issued by the European Society of Hypertension and the European Society of Cardiology, the following routine laboratory tests must be performed in every patient when diagnosed with AH: haemoglobin, haematocrit, glucose, HbA1c, creatinine, eGFR (calculated using the formula CKD-EPI (Chronic Kidney Disease-Epidemiology Collaboration)), triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, potassium, sodium, uric acid, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT and GGT. It is also recommended to perform a qualitative urine dip-stick test, microscopic examination and albumin-creatinine ratio (ACR) in the spot urine sample. Based on laboratory test results, blood pressure, other risk factors, asymptomatic HMOD and comorbidities, patients should be assessed for 10-year risk of a fatal cardiovascular (CV) event using the Systematic Coronary Risk Evaluation 2 System (SCORE2). Risk assessment is not required in patients already diagnosed with cardiovascular disease (CVD), chronic kidney disease (CKD), dyslipidaemia and/or glucose intolerance. The latter two, together with hypertension, form a metabolic triad that multiplies the risk of a fatal CV event. SCORE2 risk assessment is useful for patients without proven comorbidities or strikingly high levels of the risk factors in order to identify asymptomatic HMOD that significantly affect the calculated risk. HMOD includes damage to the heart, brain, retina, kidney and blood vessels. Laboratory tests in the asymptomatic phase are important to determine the stage of CKD, as it is an independent risk factor for adverse CV events. eGFR values <60 mL/min/1.73 m^2 or ACR >3.4 mg/mmol indicate asymptomatic renal dysfunction.

Laboratory diagnostics can identify additional risk factors that are not direct markers of organ damage but have an important influence on the development of CVD. The current guideline recommends re-evaluation of SCORE2 risk in the presence of elevated uric acid or elevated C-reactive protein. There is also evidence that the lipid profile should be supplemented by measuring lipoprotein (a) and apolipoprotein B.

The guidelines define the recommended laboratory tests for detection of risk factors, but also therapeutic targets. This shows the importance of laboratory diagnostics in the assessment and monitoring of patients with AH.

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Arterial hypertension and chronic renal disease

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Chronic kidney disease (CKD) is a common clinical condition in developed countries and a significant public health problem because it affects almost 11% of the adult population, and due to the many complications occurring during disease development. The definition of CKD includes decreased glomerular filtration rate (<60 ml/min/1.73 m^2) and/or renal impairment (histological abnormalities of the kidneys, structural abnormalities of the kidneys, history of kidney transplantation) accompanied by albuminuria (>30 mg/day) for more than three months.

CKD is not an independent disease, but it shows significant cause-and-effect relationships with a number of diseases, of which diabetes mellitus should be particularly emphasized (causing diabetic nephropathy), but also hypertension and atherosclerosis. The development of CKD is facilitated by general risk factors including age, obesity, hyperuricaemia, dyslipidaemia, tobacco use, family history and male gender, but also risk factors for kidney damage such as albuminuria, anaemia, bone mineralization disorders, malnutrition, toxic metabolites, inflammation and oxidative stress, and arterial hypertension. In patients with CKD, arterial hypertension leads to faster progression of renal disease with cardiovascular complications such as cardiomyopathy, atherosclerosis, arterial stiffness, calcification and subsequent ischemic heart disease, heart failure, cerebrovascular and cardiovascular death.

The most common causes of arterial hypertension are disorders of sodium homeostasis, increased activity of the renin-angiotensin-aldosterone system, increased activity of the sympathetic system, mediators of inflammation and fibrosis, and the action of various drugs, especially those that stimulate erythropoietin production.

Arterial hypertension is diagnosed if systolic pressure values ≥140 mmHg and diastolic pressure value ≥90 mmHg. Arterial pressure typically increases with declining glomerular filtration rate whereas persistently elevated arterial pressure accelerates the development and progression of CKD. Generally speaking, arterial hypertension leads to remodelling of the myocardium and blood vessels and nephrosclerosis. Almost 90% of patients with glomerular filtration rate <30 ml/min have confirmed arterial hypertension. Even normal and high-normal blood pressure (120-139/80-89 mmHg) increases the risk of developing CKD and cardiovascular changes, and the range of blood pressure values that can be considered ideal for patients with CKD is very narrow. Existing KDIGO guidelines („KDIGO 2021 clinical practice guideline for the management of blood pressure in CKD") recommend a target blood pressure <120 mmHg in patients with stable renal function, <130/80 mmHg for renal transplant patients and a reduction of 24-hour mean arterial pressure in children according to the 50th percentile for the age, sex and height.

Achieving optimal blood pressure in patients with CKD is often very demanding and includes non-pharmacological measures (reduced salt intake, weight loss in obese people, regular physical activity and reduced alcohol intake) and pharmacological measures (ACE inhibitors, diuretics, calcium channel blockers) but also the constant education and support by health professionals.

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Diagnostics of hypertensive disorders in pregnancy

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Hypertensive disorders of pregnancy are among the most common medical disorders in pregnancy

with serious maternal and perinatal morbidity. There are some differences among national and international clinical practice guidelines, but the most common classification includes four categories: gestational hypertension, chronic hypertension, chronic hypertension with superimposed preeclampsia and preeclampsia.

Hypertension is defined as a persistent systolic blood pressure of 140 mmHg or higher and a diastolic blood pressure of 90 mmHg or higher. Gestational hypertension is a new-onset hypertension after 20 weeks of pregnancy in the absence of proteinuria or other signs of preeclampsia. It should be followed carefully as it can develop into preeclampsia or future chronic hypertension. Chronic hypertension in pregnancy is a high blood pressure that predates pregnancy or occurs before 20 weeks of pregnancy and persists after delivery. It can develop into superimposed preeclampsia which is diagnosed based on the findings of symptoms suggestive of preeclampsia.

Preeclampsia is a hypertension developed in previously normotensive women after 20 weeks of pregnancy. Traditionally findings of proteinuria were considered mandatory for the diagnosis, but recent guidelines have broadened the definition of preeclampsia, questioning necessity of proteinuria for its diagnosis. Also, multiple studies reported that accepted standard cutoffs for proteinuria in pregnancy are based on limited data in small studies rather than on evidence for prognostic significance of maternal or perinatal complications.

Preeclampsia is redefined as multisystem disease with proteinuria or end-organ damage - renal insufficiency, impaired liver function, thrombocytopenia and neurologic complications. Evaluation of end-organ damage includes findings of abnormal low platelet count (lower than $100x10^9$/L), raised serum creatinine and elevated liver transaminases (twice to normal concentrations).

In clinical practice pregnant women are still tested for proteinuria to assess preeclampsia. Historically, the gold standard for proteinuria is considered measurement of protein excretion in 24-hour urine collection with cutoff at 300 mg/24h or higher. Most guidelines recommend urine dipstick testing for initial screening of proteinuria with 1+ as threshold. Dipstick testing is inexpensive, easy to use, giving fast results, but with low sensitivity and specificity. Furthermore, these qualitative results should be confirmed with quantitative testing. 24hour urine collection is challenging, time consuming, inconvenient and misleading if collected inaccurately. The spot protein-to-creatinine ratio with cutoff of 30 mg/mmol or higher is convenient alternative and reasonable rule-out test.

To date, considerable efforts are put into improvement of maternal and perinatal outcome considering hypertensive disorders. It is important to understand both strengths and limitations of current diagnostics, as to offer adequate diagnosis, monitoring and treatment at present and to set future research goals.

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Hyponatremia in arterial hypertension

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Hyponatremia is defined as a serum sodium concentration <135 mmol/L. It is characterized by weakness, nausea, confusion and, in severe cases, seizures and coma. It is the most common disorder of water and electrolyte balance. Sodium is the most abundant cation of extracellular fluid and plays a role in maintaining water distribution and osmotic pressure of extracellular fluid. Sodium concentration is regulated by the kidneys and aldosterone. Water homeostasis is regulated by osmoreceptors and baroreceptors which, depending on the osmolality of the extracellular fluid and arterial pressure, stimulate the thirst and the secretion of antidiuretic hormone (ADH).

Hyponatremia may be hypoosmotic, hyperosmotic, or isoosmotic. Hypoosmotic hyponatremia is the most common type and is characterized by increased sodium loss relative to water loss with sodium being lost through the skin, digestive tract or kidneys, or increased extracellular volume due to water retention. When diagnosing hyponatremia, glucose, total proteins and lipids should be measured in serum to rule out hyperglicemia and pseudohyponatremia. Also, serum osmolality should be measured. If it is low, it is recommended to measure osmolality and sodium in a spot urine sample in order to differentiate between possible causes of hypoosmotic hyponatremia. Measurement of ADH is not recommended.

First-line antihypertensives include thiazide diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists (ARB) and calcium channel blockers. It is recommended to use two antihypertensive drugs combined in a single tablet.

Hyponatremia is most commonly caused by thiazide diuretics. Thiazide diuretics inhibit the NaCl cotransporter in the distal tubule and prevent the reabsorption of sodium and chloride, while also reducing the reabsorption of water in the distal tubule. The reduced volume of extracellular fluid leads to the secretion of ADH and water retention, so the water is reabsorbed in the collecting ducts. Hyponatremia occurs within a few days to two weeks after starting thiazide therapy, so it is important to monitor such patients regularly. Hyponatremia caused by thiazides is more common in women, the elderly, patients with low body weight and with impaired renal function. However, other groups of antihypertensives may sometimes contribute to hyponatremia because they indirectly reduce sodium concentration and are often used in combination with thiazides.

Hyponatremia is a common finding in laboratory, so when thinking about a possible cause, one should not forget the use of drugs, especially diuretics.

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Effect of antihypertensive therapy on laboratory results

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Arterial hypertension is one of the most common disorders in the world. Approximately 40% of the population is diagnosed with this disorder, making antihypertensives one of the most prescribed drugs worldwide, and in the Republic of Croatia.

There are several groups of antihypertensives available on the market which help regulate various organs and/or organ systems in the human body, including the renin-angiotensin-aldosterone system (angiotensin converting enzyme [ACE] inhibitors, angiotensin receptor or aldosterone antagonists), cardiovascular system (calcium channel blockers), kidneys (diuretics), and central nervous system (methyldopa, β-blockers).

Most of these drugs are not selective, and can cause undesirable side effects and affect laboratory findings. For example, ACE inhibitors decrease serum ACE levels, a test used in sarcoidosis diagnosis. Diuretics can cause hyponatremia, hypokalemia, as well as hyperuricemia and could possibly lead to gout development. Aldosterone antagonists besides hyperkalemia may affect steroid hormone levels and lead to fertility problems. Angiotensin receptor antagonists interact with TXA2/PGH2 thrombocyte receptors prolonging bleeding time and decreasing thrombocyte activity. Calcium channel blockers cause serum hypocalcaemia and hypoglycemia. Methyl-dopa, often recommended during pregnancy if needed, sometimes alters red blood cell membranes resulting in autoimmune haemolytic anaemia and a positive Coombs test via immune modulation, positive ANA and SMA, which can remain positive up to six months after last administration.

Current European guidelines for the management of newly diagnosed arterial hypertension from 2018 recommend dual drug therapy either with an ACE inhibitor or angiotensin receptor antagonist, and a thiazide diuretic or calcium channel blocker.

Croatian guidelines from 2017 also recommend multi-drug therapy based on medical history and different states of organ dysfunction.

With wide use of these drugs and since multi-drug therapy is common, it is necessary to be familiar with the pharmacokinetics, pharmacodynamics and side effects of antihypertensives to correctly interpret laboratory results.

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Screening for secondary hypertension

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Hypertension is one of the most prevalent chronic diseases, affecting nearly 1 billion people worldwide. Secondary hypertension (SH) makes up to 5%-10% of all hypertensive cases. It is a type of hypertension with known and potentially treatable causes.

SH should be screened in individuals with hypertension onset before puberty or 30 years of age, in stable hypertensive people with acute rise of blood pressure and in people with malignant hypertension. It is recommended that before screening for SH, an accurate measurement of blood pressure, basic diagnostic testing for hypertension and a review of patient diet and/or drug use should be done. The most common causes of SH are chronic kidney disease along with renovascular hypertension, obstructive sleep apnea and primary aldosteronism (PA). PA is a group of diseases with an autonomous production of aldosterone by adrenals, leading to renin suppression, hypertension, cardiovascular damage and occasionally hypokalemia. It contributes to up to 15% of all hypertensive patients. Screening for PA should be done in people with hypertension resistant to three or more hypertensive drugs, hypertension and hypokalemia or adrenal incidentaloma or obstructive sleep apnea, with family history of the early onset of hypertension or cerebrovascular disease and in hypertensive first-degree relatives of PA patients. In PA diagnostic workflow, the aldosterone-to-renin ratio (ARR) plays a role of triage test followed by a diagnostic test. The ARR preanalytical phase is complex due to several potentially confounding factors ranging from a patient's status, medications in use as well as blood collection, transport and storage. It seems that the antihypertensive drugs play the most critical role in a suboptimal approach to the screening. Furthermore, there are analytical matters that should be considered when measuring aldosterone and renin concentrations as well as plasma renin activity. They refer to the relatively low concentration of aldosterone compared to other steroids in blood and the role of aldosterone conjugates in an immunoassay performance. In the postanalytical phase, an appropriate cut-off level of ARR is essential for accurate screening for PA between hypertensive patients but it should be added that the ARR result of an individual may be highly influenced by confounding preanalytical factors and used methodology. However, the ARR introduction in the screening has made a contribution to a rise of PA incidence in recent decades.

In conclusion, despite the fact that SH takes a small proportion of hypertensive cases, the screening for particular underlying disorders may save many lives.

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