High-sensitivity C-reactive protein and total antioxidant status in patients with essential arterial hypertension and dyslipidemia

Kuklinska AM\textsuperscript{1*}, Mroczko B\textsuperscript{2}, Musial WJ\textsuperscript{1}, Sawicki R\textsuperscript{1}, Kozieradzka A\textsuperscript{1}, Waszkiewicz E\textsuperscript{1}, Szmitkowski M\textsuperscript{2}

\textbf{ABSTRACT}

\textbf{Purpose:} To assess low-grade, systemic inflammation and antioxidant status as additional factors contributing to pathophysiology of essential arterial hypertension (HTN) and compare them with traditional risk factors, like abnormal lipids profile, considering their potential diagnostic usefulness.

\textbf{Material and Methods:} Serum high-sensitivity C-reactive protein (hs-CRP) concentrations and total antioxidant status (TAS) were measured in 143 subjects – 71 patients with diagnosed HTN and in 72 healthy controls.

\textbf{Results:} In hypertensive patients, as compared to healthy control group, the median hs-CRP concentration was higher (2.0 mg/L, 25%; 75% quartile range: 0.1; 27.1 vs 0.4 mg/L, 25%; 75% quartile range: 0.0; 4.6, respectively, \textit{p}<0.001) and TAS concentration lower (1.4 mmol/L, 25%; 75% quartile range: 1.0; 2.1 vs 1.5 mmol/L, 25%; 75% quartile range: 0.5; 1.8, respectively, \textit{p}=0.048). Hypertensives had higher low-density lipoprotein cholesterol concentration (LDL-C) as well as triglycerides concentration (TG) and lower high-density lipoprotein cholesterol concentration (HDL-C). Higher diagnostic sensitivity was found for hs-CRP (87\%) and for TAS (89\%). According to the global linear regression analysis, age, gender, hs-CRP, TAS and HDL-C were the only parameters influencing the occurrence of HTN. ROC analysis identified hs-CRP, HDL-C and TG as statistically significant to diagnose HTN (0.839; 0.816 and 0.855, respectively). Moreover, in ROC analysis there were no differences in hs-CRP and TAS in females and males.

\textbf{Conclusions:} These results indicate that low-grade, systemic inflammation measured by hs-CRP as well as antioxidant status assessed by TAS, in the presence of traditional risk factors, are significant factors contributing to pathophysiology and diagnosis of essential arterial hypertension.

\textbf{Key words:} arterial hypertension; dyslipidemia; hs-C-reactive protein; total antioxidant status

\textbf{INTRODUCTION}

Essential arterial hypertension (HTN) is associated with an increased risk of atherosclerosis and thus directly included in the pathophysiology of various cardiovascular diseases. The etiology of hypertension is multifactorial, complex, and poorly understood. Recently it has been suggested that inflammation of the arterial wall is implicated in the development of endothelial dysfunction, which leads to hypertension [1]. Besides, the presence of impaired endothelium-dependent vasodilation in essential hypertension is often a causal mechanism responsible for the high blood pressure values [2]. On the other hand, antioxidants and free radicals play an important role in cardiovascular system. Determination of the balance between the formation and release of these substances is important for understanding their role in patients with HTN. The antioxidants are being extensively studied because of their potential importance and pathogenetic role in several non-communicable diseases, like cardiovascular diseases and cancer, but the data on hypertension is scanty.
Hypertension frequently coexists with obesity, diabetes, hyperlipidemia, or the metabolic syndrome. In spite of hypertension and dyslipidemia are interrelated and share common pathophysiologic mechanisms, the role of elevated lipid levels in hypertensive patients is unclear.

Therefore the present study aimed to assess the inflammation and antioxidant status as well as changes in lipids profile among patients with essential arterial hypertension. We assessed new markers possibly contributing to the pathophysiology of arterial hypertension, i.e. high-sensitivity C-reactive protein (hs-CRP) and total antioxidant status (TAS), in context of the traditional, like lipids concentrations.

MATERIAL AND METHODS

Selection of the study population
Between October 2006 and September 2008 – seventy-one patients with already treated essential arterial hypertension were included in the study. Patients' baseline characteristics is shown in Tab. 1. Overall, 143 subjects were studied – 71 patients (median age 53 years- 25%; 75% quartile range: 42; 64) with ambulatory diagnosed HTN and 72 young healthy control group (median age 23 years – 25%; 75% quartile range: 22; 32). In a group of patients there were 37 (52%) males and 34 (48%) females and in healthy control group – 15 (21%) and 57 (79%), respectively. Patients had a good controlled disease – median SBP (systolic blood pressure) was 129.1 mmHg (25%; 75% quartile range: 120.5; 135.2) and median DBP (diastolic blood pressure) was 74.2 mmHg (25%; 75% quartile range: 68.3; 81.3). All hypertensive females were postmenopausal. The exclusion criteria were: smoking, secondary reason of HTN, any history of symptoms of coronary artery disease, diagnosed diabetes mellitus, renal dysfunction estimated glomerular filtration rate (eGFR) < 60mL/min. or symptoms of heart failure (HF), any acute or chronic inflammatory diseases or a history of chronic anti-inflammatory treatment. These patients were selected from among those referred to our hypertension outpatient clinic. The mean time of a history of arterial hypertension was 5.0 ±3.2 years. All patients were treated according to the current guidelines [4]. Patients were not taking statins and fibrates before enrollment. The proportion of used anti-hypertensive agents were as follows: 65.9% angiotensin converting enzyme inhibitors (ACEI), 6.8% angiotensin receptor blockers (ARBs), 29.6% calcium antagonists (CA), 35% beta-blockers (BB) and 50% diuretics. None of the patients received alpha-blockers. In young healthy control group – median SBP was 115 mmHg (25%; 75% quartile range: 107.5; 118) and median DBP was 68.5 mmHg (25%; 75% quartile range: 65.5; 74). Moreover, to assess subclinical organ damage in hypertensives we performed ECG (electrocardiography) and echocardiography (the detection of left ventricular hypertrophy, LVH) and serum creatinine of glomelural filtration rate (diagnosis of hypertension-related renal damage). The controls were non-smoking, young, healthy people.

Blood pressure was measured in ambulatory conditions using 24-hour ambulatory blood pressure measurement device (ABPM, Tracker Reynolds NIBP2, Reynolds Medical, Hertford, UK). Cuffs of appropriate size were used on the non-dominant arm with the automatic readings provided during 10-minute intervals during the day (from 6.00 a.m. to 10.00 p.m.) and 20-minute intervals during the night (from 10.20 p.m. to 5.40 a.m.). Automatic deflation of the equipment was no more than 2 mmHg per second. All patients were instructed to engage in normal activities, refrain from strenuous exercise and keep the arm extended at the time of cuff inflations. Only recordings with more than 85% of valid values were analyzed.

Based on the recent recommendations HTN was diagnosed when mean 24-hour value of systolic blood pressure (SBP) was 125-130 mmHg and/ or mean value of diastolic blood pressure (DBP) exceeded 80 mmHg [4]. Patients with known secondary reason of HTN, any history of symptoms of coronary artery disease, diagnosed diabetes mellitus, renal dysfunction or symptoms of heart failure (HF) were excluded. The study design was compliant with the Helsinki Declaration of 1975 as revised in 1996 and it was approved by the local institutional committee on human research (Institutional Review Board – Local Bioethics Committee of Białystok Medical University). Informed consent of all participants studied for the report was obtained.

Blood sampling and biochemical measurements
Venous blood samples were obtained between 8.00 a.m. and 10.00 a.m. from fasting patients and healthy volunteers. Peripheral venous blood samples were obtained in the following conditions: after 15 minutes of resting in horizontal position a Viggo needle was introduced percutaneously into an elbow vein. After 20 minutes venous blood samples for hs-CRP, TAS, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) assays were collected into tubes with clotting activation system. All samples were centrifuged within 2h after drawing and stored at -80°C until assayed.

The serum concentration of hs-CRP was determined using immunoturbidimetric method using CRP High-Sensitivity assay kits (Thermo Electron Corporation, Tstartie, Finland) according to manufacturer’s instructions. The intra-assay coefficient of variation (CV) % is reported by the manufacturer of assay kits to be 2.3 % at hs-CRP mean concentration of 2.41 mg/L.

The serum concentration of TAS was assayed using enzymatic method with peroxidase by commercially available Randox TAS kits (Randox, Ardmere, United Kingdom) according to manufacturer’s instructions. In this method ABTS® (2,2 Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase metmyoglobin and H₂O₂ to produce the radical cation ABTS®⁺. This has a relatively
stable blue-green colour, which is measured at 600 nm. Antioxidants in the added serum sample cause suppression of this colour production to a degree which is proportional to their concentration.

Concentration of the serum total cholesterol was measured using enzymatic method with cholesterol esterase whereas serum LDL-C levels using indirect LDL-C method by Beckman Synchron CX® Systems assay kits (Beckman Coulter, Inc., Fullerton, CA, USA). The intra-assay CV% is claimed as 3.0%, 1 SD=5.0 mg/dL for determinations of total cholesterol.

The serum concentration of HDL-C was measured using timed-endpoint method. The method depends on a unique detergent which solubilizes only the HDL lipoprotein particles and releases HDL-C to react with cholesterol esterase and cholesterol oxidase in the presence of chromogens, to produce a color product. The same detergent also inhibits the reaction of the cholesterol enzymes with LDL, VLDL, and chylomicrons lipoproteins by adsorbing to their surfaces. A polyanion contained in the reagent enhances the selectivity for HDL-C assay by complexing LDL, VLDL, and chylomicrons lipoproteins. HDL-direct reagent is used to measure the cholesterol concentration. The intra-assay CV% is referred to as 3.0%, 1 SD=3.0 mg/dL for determinations of HDL-C.

The serum concentration of triglycerides was determined using a timed endpoint method by Beckman Synchron CX® Systems assay kits. Triglycerides in the sample are hydrolyzed to glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase, glycerophosphate oxidase and horseradish peroxidase causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid with 4-aminobenzoic acid to form a red quinoneimine dye. The intra-assay CV% is referred to as 3.0%, 1 SD (standard deviation) = 5.0 mg/dL for determinations of TG.

In the receiver operating characteristic (ROC) report the cut-off values for measured parameters (hs-CRP, TAS, TC, LDL-C, HDL-C and TG) corresponding to the highest accuracy (minimal false-negative and false-positive results) were indicated by a sign.

**Statistical analysis**
Because of an asymmetrical distribution of variables in a group of hypertensive patients and young healthy control (assessed in Shapiro – Wilk test) results were expressed as medians with 25% to 75% interquartile ranges (continuous variables) or as proportions (categorical variables). Associations between continuous variables were examined using the U Mann-Whitney test and associations between categorical variables using $\chi^2$ test. Diagnostic sensitivity, specificity, as well as positive and negative predictive values (PPV, NPV) of cut-off points characterizing patients with or without hypertension were computed. The receiver operating characteristic (ROC) curves were constructed. The area under curve (AUC), a measure of the diagnostic efficiency, was also computed. Global linear regression analysis was performed to indicate factors associated with the presence of hypertension. All analyses were carried out using Statistica 8.0 (StatSoft, Tulsa, OK, USA) and MedCalc 8.0 (MedCalc Software, Mariakerke, Belgium). $P \leq 0.05$ was considered statistically significant.

**RESULTS**
In the group of hypertensive patients a significantly higher median serum hs-CRP, LDL-C and TG concentrations, as well as significantly lower median serum TAS and HDL-C concentrations were found, when compared to healthy control group (Tab. 1). No differences according to plasma TC concentrations were found (Tab. 1). Hs-CRP and TAS concentrations had the highest sensitivity (87% and 89%, respectively), while, according to the lipids profile, sensitivity of the measured parameters was relatively low: the highest one

* – ns, statistically not significant
hs-CRP - high-sensitivity C-reactive protein
TAS - total antioxidant status
TC - total cholesterol
LDL-C - low density lipoprotein cholesterol
HDL-C - high density lipoprotein cholesterol
TG - triglycerides
High-sensitivity C-reactive protein and total antioxidant status in patients with essential arterial hypertension and dyslipidemia

was found for TG (70%), while the lowest sensitivity among all measured parameters was found for LDL-C and TC (51 and 41%, respectively) (Fig. 1). However, these parameters had higher specificity: the highest was found for LDL-C and HDL-C (85 and 82%, respectively) (Fig. 1).

According to the global linear regression analysis, age, gender as well as hs-CRP, TAS and HDL-C were the only parameters influencing the occurrence of HTN (Fig. 2).

In the ROC analysis (Fig. 3; Fig. 4) the following cut-off values corresponding to the highest diagnostic accuracy (minimal false-negative and false-positive results) were indicated as follows: 0.71 mg/L for hs-CRP, 1.18 mmol/L – TAS, 199.0 mg/dL – TC, 120.50 mg/dL – LDL-C, 53.4 mg/dL – HDL-C, 80.50 mg/dL – TG. According to these values the areas under the ROC curves for hs-CRP, HDL-C and TG were larger (0.839, 0.816 and 0.794, respectively) than for TAS, TC and LDL-C concentrations (0.595, 0.568 and 0.665, respectively). Moreover, in ROC analysis there were no differences in hs-CRP and TAS in females and males, (Fig. 5).

**DISCUSSION**

Essential arterial hypertension (HTN) is prevalent in developed as well as developing countries. Hypertension is directly implicated in the pathophysiology of various cardiovascular diseases and it could contribute to an excess of both morbidity and mortality. The etiology of essential hypertension has been explored in depth, but the pathophysiology is multifactorial, complex and poorly understood.
Endothelial function is impaired in hypertensive patients. However, high blood pressure can arise from a primary defect in vascular function, which is related to endothelial dysfunction and also vascular smooth muscle cell contractile abnormal regulation [5]. There is large body of evidence indicating that inflammation plays a crucial role in all steps characterizing the atherosclerotic process. C-reactive protein is a circulating marker of inflammation which has been emerged as a powerful and independent determinant of cardiovascular events. Hypertension is suggested to be linked to inflammation. We have found higher hs-CRP levels in hypertensive patients. Hs-CRP levels are higher in patients with arterial hypertension [6].

Experimental data from cross-sectional studies in humans indicate a relationship between CRP levels and blood pressure [7]. In particular, CRP seems to be related with markers of arterial stiffness, thus suggesting a specific interaction between CRP and systolic blood pressure. In PRINCE Trial [8], pravastatin reduced CRP levels in long-term treatment in a largely LDL-C-independent manner. This effect was seen in primary and secondary prevention cohorts. Thus, because of their anti-inflammatory action, statins could have beneficial effect in treatment of arterial hypertension [9].

On the other hand, hypertensive patients are reported to have high circulating levels of other proinflammatory cytokines, such as interleukin-6, tumour necrosis factor-alpha, fibrinogen and other biomarkers, like brain natriuretic peptide (BNP) [10-12]. Moreover these inflammatory markers are elevated in patients with target organ damage, not in uncomplicated hypertension [11].

Recent studies conducted in Poland, indicate high distribution of hs-CRP among hypertensive patients [6]. Bautista et al. [13] for the first time showed that CRP level could be an independent risk factor for the development of hypertension. Moreover, in hypertensive patients being managed by drug therapy or lifestyle modification, CRP is an equivalent or superior independent predictor of the progression of carotid atherosclerosis [14]. These extensive data reveal hs-CRP as an important biomarker in hypertensive patients with regard to assessment of risk, the diagnosis and prediction of complications. In our study the statistical analyses confirmed the superiority of hs-CRP assessment in the biochemical evaluation of hypertensive patients. In fact, this biomarker has a very good diagnostic value in hypertensive patients. Low-grade inflammation seems to be independently related to increase the prevalence of arterial hypertension.

Free radical oxidative damage of the epithelium has been implicated in the atherogenic process. To gain information about the consequences of oxidative stress the investigation of plasma antioxidants seems to be plausible. Antioxidants and free radicals play an important role in cardiovascular system. The antioxidants are being extensively studied because of their potential importance in the pathogenetic mechanisms in several unrelated diseases, like cardiovascular diseases and cancer, but the data on hypertension is numerous. This supports the reason why we measured TAS in hypertensive patients. We have found significantly lower TAS concentrations in patients with hypertension. The imbalance in the pro-oxidant-antioxidant status shifts into the hypertensive patients. Lantos et al. [15] have found that, among other diseases, total antioxidant status values were below the normal range in hypertensive patients and increased gradually following antihypertensive treatment. Decreased TAS level was found in both patients with sustained arterial hypertension and white-coat hypertension [16]. Russo et al. [17] have shown that essential hypertension was associated with greater than normal lipoperoxidation and an imbalance in anti-oxidant status, suggesting that oxidative stress is important in the pathogenesis of disease or in arterial damage related to it. Lipid- and water-soluble antioxidants levels were lower in hypercholesterolemic and hypertensive patients as compared to normal subjects [3]. In our patients we found high sensitivity of TAS. Moreover, global linear analysis revealed TAS as one of the parameters influencing the occurrence of HTN.

It has been suggested that endothelial dysfunction is one of the main pathways in the pathogenesis of arterial hypertension [18]. Recent interest has been directed towards investigating the purported role of the endothelium, which acts as an important regulator of vascular homeostasis. Endothelial dysfunction is now recognized to occur in hypertension, regardless of whether the etiology is essential or secondary to endocrine or renal processes. On the other hand, increased production of contracting factors, like oxygen-derived free radicals and oxidized low density lipoprotein (ox-LDL), a marker of oxidative stress, could play an important role in the development and consolidation of HTN. This imbalance of counteracting mechanisms, normally designed to maintain vascular homeostasis, leads to vasoconstriction and impaired vascular function. It has become apparent that these changes may be effective in response to enhanced oxidative stress, possibly as a result of systemic and localized inflammatory responses.
Arterial hypertension is related to age. Messerli et al. [19] showed that 9 out of 10 patients aged over 55 years would become hypertensive later in life. That is why it is very difficult to find an age-matched control group for hypertensive patients. Even though, our aim was to assess the values of hs-CRP and TAS in selected hypertensive patients and compare them with young healthy control group. Our hypertensive patients, which were older, had higher hs-CRP levels. However, higher CRP concentrations can be also found in older, but healthy men. Cartier et al. [20] have found higher CRP levels in middle-aged than in younger healthy men. Moreover, CRP concentrations were found to be higher in premenopausal women than in men, which seems to be due to greater accumulation of subcutaneous fat than that observed in men [21]. We did not have any premenopausal women among hypertensives and in our young healthy control group there were more females than males. Nevertheless, median hs-CRP concentration was lower in young healthy control group than in hypertensive patients. Therefore the difference of hs-CRP concentration could be attributed not only to hypertension, but also to age and gender. Ageing is also related to increased oxidative stress [22], but it can’t be attributed to a decrease in the activities of antioxidant defense system [23]. The discrepancy data exists according to gender-related antioxidant power. Some antioxidant enzymes activities are higher in younger patients [24]. Our older hypertensive patients, almost fifty-fifty males and females, had lower TAS concentrations than healthy younger control group, in most cases females. It is worth to notice that in our study group gender did not influence hs-CRP and TAS concentrations.

Hypertension frequently coexists with obesity, diabetes, hyperlipidemia, or the metabolic syndrome; their association with cardiovascular disease has been well established. Hypertension and hyperlipidemia are interrelated and share common pathophysiologic mechanisms, such as insulin resistance and endothelial dysfunction [25]. In spite of that, the data concerning lipids profile in patients with HTN is different. Thus the aim of the study was also to investigate lipids profile abnormalities, including total cholesterol concentration (TC), low-density lipoprotein cholesterol concentration (LDL-C), high-density lipoprotein cholesterol concentration (HDL-C) and triglycerides concentration (TG) in patients with essential hypertension. Some data show only significantly higher TG concentration in hypertensive patients, with no differences in total cholesterol, LDL-C and HDL-C, while some indicate higher levels of LDL-C and TG as well as lower levels of HDL-C in patients with essential arterial hypertension [26,27]. Lamourner-Zepter et al. [26] have shown that levels of LDL-C in the hypertensive obese women, among other measured parameters, like renin, aldosterone and insulin, were significantly increased in comparison to the obese normotensives. However no differences according to HDL-C or total cholesterol/HDL-C ratios between the two groups were found.

In our study group the levels of LDL-C and TG were significantly higher in hypertensives as compared to healthy, normotensive control group, while HDL-C concentrations were lower. We did not find any significant difference according to TC concentrations between both groups. In spite of low sensitivity of TC and LDL-C concentrations, specificity of all lipids fractions, especially LDL-C and HDL-C, was high. Moreover we showed the importance of HDL-C concentrations in the biochemical aspects of HTN. HDL-C cholesterol levels are inversely related to risk of coronary artery disease. HDL-C prevents atherosclerosis by reverting the stimulatory effect of ox-LDL on monocyte infiltration. The HDL-associated enzyme paraoxonase inhibits the oxidation of LDL. Platelet-activating factor acetyl hydrolase (PAF-acetyl hydrolase), which circulates in association with HDL and is produced in the arterial wall by macrophages, degrades bioactive oxidized phospholipids [29]. In our study various statistical analyses indicated HDL-C to be a valuable diagnostic parameter of HTN.

There is probably a link between dyslipidemia in hypertensive patients and impaired antioxidant efficacy. Hypertensive patients with abnormal lipids profile, have reduced protection from antioxidants, which may contribute to the predisposition for the development of various cardiovascular diseases [30].

We have shown that abnormal lipids profile, specified as high levels of LDL-C and TG and low levels of HDL-C together with low levels of TAS have important value in hypertensive patients. Although antioxidant status is important, it would be valuable to emphasize that long supplementation of antioxidants, such as vitamin C or E, were ineffective in primary prevention trials [31].

The main limitation of the study, that should be pointed out is that hypertensive group differs from young healthy control group concerning age and gender – the factors which could affect the variability of studied parameters. That’s why the interpretation of results and discussion should be performed with caution. However, the patients were included prospectively and were investigated thoroughly, so we consider the obtained results as being representative.

CONCLUSIONS

In this study we have pointed to the low grade systemic inflammation assessed by hs-CRP as well as antioxidant efficacy measured by TAS as meaningful evaluation of essential hypertension. It could be related to their important role in the pathophysiology of the disease. It is also suggested that hs-CRP as well as TAS concentrations could be a link between blood pressure and abnormal lipids profile.

The clinical implication of the study could be the statement that in hypertensive patients with borderline abnormal lipids profile, increased inflammation, and reduced protection from antioxidants, the treatment by statins may be beneficial beyond a reduction of abnormal lipid profile.
ACKNOWLEDGEMENTS

Work supported by The State Committee for Scientific Research (grant No N 402 075 31/2290).

REFERENCES


22. Mendoza-Núñez VM, Ruiz-Ramos M, Sánchez-Rodríguez MA, Retana-Ugalde R, Muñoz-Sánchez JL. Aging-


