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Original article

Inflammation and Apoptosis in Hypertension. Relevance of the Extent of Target Organ Damage

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ABSTRACT

Introduction and objectives: To investigate the relationship between inflammatory and apoptotic parameters and the severity and extent of target organ damage in patients with essential hypertension.
Methods: We studied 159 consecutive patients with treated essential hypertension. An exhaustive evaluation of damage to heart, kidney, and blood vessels was performed and plasma levels of inflammatory (interleukin 6 and soluble receptor of tumor necrosis factor-alpha type 2) and apoptotic markers (soluble receptor of tumor necrosis factor-alpha type 1 and soluble Fas receptor) were determined. Patients were categorized into four groups: *a)* no organ damage (33 patients); *b)* 1 organ damaged (52 patients); *c)* 2 organs damaged (44 patients), and *d)* 3 organs damaged (30 patients).
Results: Serum levels of interleukin 6, soluble receptor of tumor necrosis factor-alpha type 1 and soluble receptor of tumor necrosis factor-alpha type 2 were higher in patients with target organ damage than in hypertensive patients without organ damage. Increasing levels of these molecules were progressively associated with an increase in the number of organs damaged, and the highest levels were observed in the group with damage to 3 organs (heart, kidney, and blood vessels). There were no differences in soluble Fas receptor levels between groups. Logistic regression analysis showed that age, smoking, diabetes mellitus, abdominal circumference, interleukin 6, and soluble receptor of tumor necrosis factor-alpha type 1 were independently related to the number of target organs damaged.
Conclusions: Extensive hypertensive disease with involvement of more target organs was associated with greater inflammatory and apoptotic activation in these hypertensive patients.
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Inflamación y apoptosis en la hipertensión arterial. Importancia de la extensión de la lesión de órgano diana

Palabras clave:
Hipertensión arterial sistémica
Inflamación
Apoptosis

RESUMEN

Introducción y objetivos: Analizar la relación entre parámetros inflamatorios y marcadores de apoptosis con la gravedad y la extensión de la lesión de órgano diana en pacientes con hipertensión arterial esencial.
Métodos: Se ha reclutado, de manera consecutiva, a 159 pacientes hipertensos tratados, a los que se realizó un estudio exhaustivo de daño orgánico cardiaco, renal y vascular, y se determinaron las concentraciones plasmáticas de diferentes moléculas relacionadas con la inflamación (interleucina 6 y receptor soluble tipo 2 del factor de necrosis tumoral alfa) y apoptosis (receptor soluble tipo 1 del factor de necrosis tumoral alfa y receptor soluble Fas). Se dividió a los pacientes en cuatro grupos: *a)* sin lesión de órgano diana (33 pacientes); *b)* lesión a un nivel (52 pacientes); *c)* lesión a dos niveles (44 pacientes) y *d)* lesión a tres niveles (30 pacientes).
Resultados: Los pacientes con lesión de órgano diana presentaban valores plasmáticos significativamente más elevados de interleucina 6, receptor soluble tipo 1 del factor de necrosis tumoral alfa y receptor soluble tipo 2 del factor de necrosis tumoral alfa que los pacientes hipertensos sin lesión orgánica. Además se objetivó un incremento progresivo de estos marcadores a medida que aumentaba el número de lesiones con las cifras plasmáticas más elevadas en los pacientes con lesión de órgano diana a tres niveles (cardiaco, renal y vascular). No hubo diferencias en el receptor soluble Fas entre las diferentes poblaciones. El análisis de regresión logística mostró que los valores plasmáticos de interleucina 6 y receptor soluble tipo 1 del factor de necrosis tumoral alfa, junto con edad, diabetes,

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tabaquismo y perímetro abdominal, se asociaban de manera independiente con el número de lesiones de órgano diana.

Conclusiones: Una enfermedad hipertensiva más generalizada y con mayor número de órganos diana afectados se asocia a mayor activación inflamatoria y apoptótica en pacientes hipertensos.

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Abbreviations

ABI: ankle-brachial index
HT: hypertension
IMT: intima-media thickness
LVMsa: left ventricular mass adjusted for body surface area
TOD: target organ damage

INTRODUCTION

Inflammation and apoptosis both play an important role in the pathophysiology of cardiovascular diseases such as atherosclerosis, heart failure, and hypertension (HT).[1–4] These processes can induce inflammatory and apoptotic changes in heart function, in peripheral vascular resistance, and in renal control mechanisms of electrolytes and plasma volume. Moreover, certain concentrations of inflammatory molecules and certain cytokines are predictive of future cardiovascular events.[5] Several studies suggest that immunological and inflammatory mechanisms are also crucial in the development and progression of organ damage in hypertensive patients.[6] There is currently substantial evidence of increased levels of inflammation and apoptosis markers in hypertensive patients, and correlations between these parameters and different target organ damage (TOD) have been found, indicating that these molecules may participate in the development of hypertensive lesions.[7–10] However, most of these studies focused on a single clinical aspect (ventricular hypertrophy or microalbuminuria), without taking into account the fact that plasma concentrations of these molecules depend on the sum of molecules released from both cardiac and noncardiac sources (blood vessels, kidneys, etc.). Therefore, their findings do not reveal the total burden of hypertensive remodeling.

In addition, to identify populations with high levels of cardiovascular risk, current HT guidelines recommend determining whether TOD is present during the initial assessment of hypertensive patients. However, these guidelines propose a dichotomous division between the presence and absence of TOD and include, within the same prognosis, patients with only one level of TOD and those with generalized (cardiac, renal, and vascular) damage.[11] The number of TOD in hypertensive patients who have suffered an acute coronary syndrome has recently been observed to be associated with a linear increase in mortality after 1 year, from 2% in those without TOD, to 7.6% in patients with level 1 TOD, 11.1% in those with level 2 TOD, and 20% in patients with 3 affected territories (left ventricular hypertrophy, glomerular filtration rate<60 mL/min, and ankle-brachial index [ABI]<0.9).[12] The aim of the present study was to analyze the association between distinct plasma markers of inflammation and apoptosis and the severity and extent of TOD (cardiac, renal, and vascular) in hypertensive patients.

METHODS

Patients

A total of 159 hypertensive patients over 18 years of age referred to our hypertension unit were prospectively recruited between January 2010 and September 2011. All patients underwent a complete physical examination, blood and 24-h urine microalbumin determination, ambulatory blood pressure monitoring, and a comprehensive assessment of the presence and extent of TOD. HT was defined as mean arterial pressure≥140/90 mmHg on at least 3 separate occasions, or receiving antihypertensive treatment. Data on the drug treatments received by the patients was also collected, together with the presence of other cardiovascular risk factors: diabetes mellitus (defined as glucose≥126 mg/dL, or taking antidiabetic drugs), dyslipidemia (defined as total cholesterol≥200 mg/dL, low density lipoprotein cholesterol≥150 mg/dL, or taking lipid-lowering drugs), and current or previous smoking. Blood pressure (BP) was measured with the patient seated using a validated automatic device. Three consecutive readings were recorded and the mean of the three recordings was used for the analysis. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease formula. Exclusion criteria were the presence of secondary HT, cardiovascular disease (heart disease, heart failure, stroke, or symptomatic peripheral arterial disease), acute or chronic inflammatory disease, recent infection, the presence of neoplasia, concomitant treatment with steroids or nonsteroidal anti-inflammatory drugs, alcoholism, or inability to cooperate. The study protocol was approved by the ethics committee of our hospital and informed consent was obtained from all patients.

Echocardiography

An echocardiographic study using a General Electric Vivid 7® apparatus (GE Healthcare, Waukesha, WI, United States) was performed in all patients. The study was conducted with the patient at rest in left lateral decubitus position and with the usual projections. A single observer blinded to the patients' clinical characteristics performed all the traces. Left ventricular mass adjusted for body surface area (LVMsa) was calculated using Devereux's formula. No patient had impaired contractility that would invalidate the theoretical assumptions underlying cardiac mass determination. We also determined the diameter of the left atrium in the parasternal long axis.

Carotid Ultrasound

With the patient supine and after 5 min of rest, we performed an ultrasound of both carotid arteries to determine carotid intima-media thickness (IMT). We also used a 5-10 MHz multifrequency linear probe to locate carotid plaques. IMT was measured in the posterior wall of the common carotid artery, 1 cm proximal to the start of the dilation of the carotid bulb. Using the cine-loop function and an optimal longitudinal image frozen at

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the end of diastole, IMT was measured using automated computer assisted edge detection software EchoPAC® (General Electric Healthcare, Waukesha, WI, United States). Mean IMT values were obtained. The investigation was repeated on the contralateral side. The highest mean IMT value was selected for both carotid arteries.

Ankle-brachial Index

The ABI was determined on both sides using a pocket Doppler BIDOP ES-100V3® and a BP cuff, following scientific recommendations.[13] In summary, systolic BP (SBP) was measured in both arms and both ankles (posterior tibial artery) with the patient supine. The ABI for each leg was calculated by dividing left and right ankle SBP by the highest SBP in both arms. We selected the lowest ABI value for each patient.

Definition of Target Organ Damage

TOD was defined as any of the following conditions: *a)* heart disease: LVMsa≥125 g/m^2 for men and ≥110 g/m^2 for women, or interventricular septal thickness>13 mm for men and >12 mm for women[11,14]; *b)* kidney disease: glomerular filtration rate<60 mL/h, microalbuminu-ria>30 mg/24 h[11], and *c)* vascular disease: ABI<0.9 and/or mean IMT>0.9 and/or carotid plaques.[11] On that basis, we divided patients into four groups: *a)* no TOD; *b)* level 1 TOD; *c)* level 2 TOD, and *d)* level 3 TOD.

Plasma Markers

The following plasma molecules were determined: *a)* inflammation: interleukin (IL) 6 and soluble receptor of tumor necrosis factor-alpha type 2 (sTNF-R2), and *b)* apoptosis: soluble receptor of tumor necrosis factor-alpha type 1 (sTNF-R1), and soluble Fas receptor (sFas).

For this study, blood samples were taken from seated subjects between 8.00 am and 11.00 am, using tubes with a gel matrix to facilitate removal of serum (Vacutainer®). The tubes were left for 15 min at room temperature for clot retraction. The samples were then centrifuged at 1500 g and the supernatants were stored immediately at −80° C until use. Serum levels of sTNF-R2, IL-6, sTNF-R1 and sFas were determined in the cell biology and pathology laboratory using a commercial ELISA test specific to each (Human sTNF-R1 ELISA kit, EuroResearch, HK302 Human sTNF-R2 ELISA kit; Hycult Biotechnology, The Netherlands; sDC95 (Apo/Fas) ELISA kit, Diaclone, France, Human IL-6 ELISA kit). Previously, samples were filtered in a Spin-X Centrifuge Tube Filter (Costar, New York, United States). The tests were quantified at 450 nm in a dual wavelength microplate reader (Sunrise, TECAN, Austria) using Magellan software (version 2.5; TECAN, Austria). The detection limits for sTNF-R1, sTNF-R2, sFas and IL-6 were estimated at around 40 pg/mL. Intra- and inter-assay coefficients of variation for sTNF-R1, sTNF-R2, sFas, and IL-6 were 5.8% and 8%, respectively.

Statistical Analysis

Continuous variables are expressed as mean (standard deviation) or median [interquartile range], while qualitative variables are expressed as percentage and absolute number. Continuous variables were compared using the Student *t* test or analysis of variance with Bonferroni correction. For variables with a non-normal distribution, we used the Mann-Whitney and Kruskall–Wallis tests. The χ^2 test was used to analyze qualitative variables. Spearman's correlation coefficient was used to investigate the linear relationship between plasma levels of inflammation and apoptosis and the distinct TOD. A multinomial logistic regression analysis with backward stepwise selection was used to assess factors independently associated with the number of TOD, using the group with no TOD as the reference group. The variables selected for inclusion in the model were those showing statistical significance in univariate analyses or those considered clinically relevant. Data were processed using SPSS 15.0 (SPSS Inc.; Chicago, Illinois, United States) and the significance level was set at $P<.05$.

RESULTS

Hypertensive patients included in the study had a mean age of 56 (13) years and 67.3% were men. A total of 87.4% of patients were on antihypertensive treatment at the time of the study: 60.4% were being treated with angiotensin receptor blockers, 47.2% with diuretics, 40.9% with calcium channel blockers, 36.5% with beta blockers, 18.2% with angiotensin converting enzyme inhibitors, and 5% with doxazosin. Slightly more than one quarter (26.4%) of the patients were receiving statins and 17.6% antiplatelet drugs.

After the study, 126 patients (79.2%) showed TOD: 39% had heart disease, 42.8% had renal impairment, and 62.9% had vascular disease (60.4% carotid disease and 14.5% ABI<0.9). Table 1 shows the patients' clinical and laboratory characteristics according to the number of affected territories. Age, SBP, waist circumference, duration of HT, prevalence of diabetes mellitus, and smoking increased with the number of affected territories, as did the severity of each TOD; patients with 3 affected territories had more LVMsa, microalbuminuria, and carotid IMT, and poorer ABI and glomerular filtration rates.

In the comparative study of the molecules analyzed, specific markers of inflammation were significantly higher in patients with TOD than in patients without TOD, i.e. IL-6 (2.34 pg/mL vs 1.6 pg/mL; P=.001) and sTNF-R2 (455.8 pg/mL vs 330.3 pg/mL; P=.001). There was also a significant increase in sTNF-R1 (marker of inflammation and apoptosis) in these patients (1598.3 pg/mL vs 1258.4 pg/mL; $P<.005$). There were no differences between the two populations in terms of sFas. Moreover, an increase in the number of affected organs was accompanied by a gradual increase in IL-6, sTNF-R1, and sTNF-R2, with higher plasma levels in the patient population with 3 levels of TOD (cardiac, renal and vascular); no differences were observed in sFas values (Table 2).

In the study of correlations between plasma levels of the inflammation and apoptosis molecules tested and the different TOD of hypertensive origin, we observed a positive correlation between plasma IL-6, sTNF-R1, and sTNF-R2, and LVMsa, microalbuminuria, and IMT (Table 3), and a negative correlation with the glomerular filtration rate and ABI (close to significant for sTNF-R2). By contrast, no correlations were found with sFas values, with the exception of the glomerular filtration rate.

Logistic regression analysis showed that the factors significantly associated with the number of TOD were age, current smoking, diabetes mellitus, waist circumference, and plasma concentration of sTNF-R1 and IL-6. Table 4 shows the evaluation of such associations by number of TOD.

DISCUSSION

The present study shows increased activation of apoptosis and inflammation in hypertensive patients suffering from more severe and widespread cardiac, renal, and vascular organ disease, and establishes an association between plasma levels of IL-6 and sTNF-R1 and the number of TOD. This is one of the first studies to show the possible relationship between the extent of TOD and activation

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Table 1
Comparison of Populations by Number of Target Organs Damaged

	No TOD (n=33)	1 TOD (n=52)	2 TOD (n=44)	3 TOD (n=30)	*P*
Age, years	43±10.9	58±10.7	57.7±11.4	62.7±11.2	<.001
Men	26 (78.8)	31 (59.6)	31 (70.5)	19 (63.3)	.285
Diabetes mellitus	1 (3)	4 (7.7)	11 (25)	14 (46.7)	<.001
Current smoker	5 (15.2)	13 (25)	11 (25)	15 (50)	<.05
Dyslipidemia	9 (27.3)	26 (50)	20 (45.5)	17 (56.7)	.095
BMI	29.8±4.3	30.6±4.3	31.4±4.8	31.7±5	.312
Waist circumference, cm	101.8±11	102.3±10.9	104.9±11.9	110.7±12.2	<.01
SBP, mmHg	146.3±17.5	151.4±21.1	161±24.6	158.2±27.2	<.05
DBP, mmHg	92±13.1	89.3±11.1	90.8±15.4	87.4±14	.531
Heart rate, bpm	76.3±13.7	72.3±14.6	72.6±15.9	72.2±11.4	.602
Glucose, mg/dL*	101 [95-112]	106 [96-117]	111 [94.3-134]	129 [97.8-148.5]	<.05
Total cholesterol, mg/dL	194.4±30.8	212.4±39.9	203.8±40.5	195.7±52.2	.168
LDL cholesterol, mg/dL	118.9±26.5	136±38.2	126.5±37.3	125.4±47	.252
HDL cholesterol, mg/dL	49.2±11.4	49±14.3	47.8±13.2	44±14.3	.377
Triglycerides, mg/dL*	101 [85-138]	139 [98-173.5]	135 [97-175.5]	141 [108.5-176]	.053
Estimated GF (MDRD)	94.8±18	85±18.7	81.7±23.2	74.7±19	.001
Fibrinogen	3.37±0.52	3.48±0.63	3.61±0.54	3.89±0.73	<.05
C-reactive protein*	0.22 [0.1-0.39]	0.25 [0.12-0.59]	0.33 [0.14-0.54]	0.36 [0.21-0.68]	.1
NT-proBNP*	25.9 [13-54.3]	43.9 [20.8-86.7]	132.8 [46.1-264.4]	228.7 [101.3-406.4]	<.001
24 h MAU, mg*	5 [3-11.6]	7.8 [4-30.1]	17.9 [6.8-66.4]	72.3 [40.9-157.5]	<.001
Left atrium, mm	36.9±4.5	39±4.8	42.7±5	44±4.8	<.001
LVMsa, g/m^2	86.7±15.6	92.4±23.1	123.3±29.4	140.8±35.6	<.001
Carotid IMT, mm	0.60±0.11	0.77±0.17	0.93±0.24	0.93±0.14	<.001
ABI	1.10±0.11	1.05±0.16	1.01±0.14	0.98±0.18	<.01
24 h SBP (ABPM)	126.7±13.1	134.6±15.3	131.7±15.3	130.7±16.5	.141
24 h DBP (ABPM)	78.2±11.6	80.8±10.1	78.1±10.4	75.8±9.2	.228
Years of duration of HT*	6.7 [1.8-16]	7.7 [3.1-18.3]	7.5 [2.2-16.8]	10.5 [2-20.8]	<.01

ABI, ankle-brachial index; ABPM, ambulatory blood pressure monitoring; BMI, body mass index; DBP, diastolic blood pressure; GF, glomerular filtration; HDL, high-density lipoprotein; HT, hypertension; IMT, intima-media thickness; LDL, low-density lipoprotein; LVMsa, left ventricular mass adjusted by surface area; MAU, microalbuminuria; NT-proBNP, N-terminal pro-B-type natriuretic peptide; MDRD, Modification of Diet in Renal Disease; SBP, systolic blood pressure; TOD, target organs damaged.
Unless otherwise indicated, data are expressed as mean±standard deviation or no. (%).
* The Kruskal-Wallis test was used to analyze non-normally distributed variables and results are expressed as median [interquartile range].

Table 2
Comparison of Plasma Markers by Number of Target Organs Damaged

	No TOD (n=33)	1 TOD (n=52)	2 TOD (n=44)	3 TOD (n=30)	*P*
IL-6, pg/mL	1.6 [1.6-1.72]	2.06 [1.6-2.91]	2.33 [1.6-5.16]	3.29 [1.82-5.3]	.001
sTNF-R1, pg/mL	1258.4 [1081.8-1576]	1468 [1173.3-1667.4]	1533.8 [1192.3-1865.4]	1823.7 [1653.4-2036.5]	<.001
sTNF-R2, pg/mL	330.3 [240.6-466.14]	440 [316.5-559.1]	410.2 [324.7-587.5]	562.2 [436.1-808]	<.001
sFas, pg/mL	105.3 [78.1-153.1]	116.5 [84.4-137.4]	110 [83-137.4]	105.8 [78.1-142.5]	.915

IL-6, interleukin 6; sFas, soluble Fas receptor; sTNF-R1, soluble tumor necrosis factor-alpha receptor type 1; sTNF-R2, soluble tumor necrosis factor-alpha receptor type 2; TOD, target organs damaged.
Because of their non-normal distribution these variables were analyzed using the Kruskal-Wallis test and results are expressed as median [interquartile range].

of inflammation and apoptosis in patients with essential HT, using well-established plasma markers such as sTNF-R1, sTNF-R2, and IL-6.[15]

It has been suggested that the organ damage induced by HT can result from a complex multifactorial process involving leukocytes, procoagulant parameters, transcription and growth factors, as well as inflammatory molecules.[16] Several studies have examined the relationship between various inflammatory molecules and the presence of TOD in hypertensive patients. Pedrinelli et al.[17] studied the correlation between C-reactive protein (CRP) and microalbuminuria in hypertensive men without complications. Yasmin et al.[18] demonstrated a significant association between CRP and pulse wave velocity, a measure of aortic distensibility in apparently healthy subjects (mainly hypertensive). Recently, CRP and specifically tumor necrosis factor alpha (TNF-α), have been correlated with markers of early cardiac (Cornell product as a measure of left ventricular hypertrophy) and kidney (microalbuminuria) TOD.[6] Our group also showed that plasma concentrations of soluble TNF-α receptor, IL-6 receptor antagonist, and IL-1 were significantly correlated with LVMsa in hypertensive patients.[9] The

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Table 3
Univariate Correlation Between Plasma Markers and Different Hypertensive Target Organ Damage (Spearman's Test)

	LVMsa		Microalbuminuria		ABI		Mean IMT*		Glomerular filtration	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-6	0.239	<.005	0.213	<.01	−0.087	.278	0.286	<.001	−0.126	.114
sTNF-R1	0.282	<.001	0.226	<.005	−0.106	.185	0.276	<.001	−0.479	<.001
sTNF-R2	0.227	<.005	0.147	.066	−0.135	.091	0.301	<.001	−0.493	<.001
sFas	−0.015	.850	−0.059	.457	0.021	.792	0.079	.323	−0.245	<.01

ABI, ankle-brachial index; IL-6, interleukin 6; IMT, intima-media thickness; LVMsa, left ventricular mass adjusted for body surface area; sFas, soluble Fas receptor; sTNF-R1, soluble tumor necrosis factor-alpha receptor type 1; sTNF-R2, soluble tumor necrosis factor-alpha receptor type 2.
*The larger IMT for both carotid arteries.

Table 4
Multinomial Logistic Regression Analysis Showing Variables Significantly Associated With the Number of Target Organs Damaged, Using the Absence of Target Organ Damage as the Reference Category

Factors	1 TOD		2 TOD		3 TOD	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.12 (1.06-1.19)	<.001	1.12 (1.05-1.19)	<.001	1.19 (1.10-1.29)	<.001
Diabetes mellitus	3.29 (0.26-42.40)	.361	11.98 (0.99-144.7)	.051	27.8 (1.72-449.1)*	<.05
Current smoker	2.75 (0.61-12.38)	.188	2.93 (0.6-14.42)	.186	27.06 (3.56-205.78)	.001
Waist circumference	1.003 (0.95-1.06)	.923	1.02 (0.96-1.09)	.535	1.1 (1.02-1.19)	.016
IL-6	1.39 (0.77-2.51)	.271	1.81 (1-3.26)	<.05	1.93 (1.05-3.53)	<.05
sTNF-R1	1.001 (0.99-1)	.451	1.001 (0.99-1)	.311	1.003 (1-1.01)	<.05

95%CI, 95% confidence interval; IL-6, interleukin 6; OR, odds ratio; sTNF-R1, soluble receptor of tumor necrosis factor-alpha type 1; TOD, target organ damage.
Variables included in the model: age, diabetes mellitus, dyslipidemia, current smoking, treatment with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, diuretics, calcium channel blockers, statins and antiplatelet agents, systolic blood pressure, duration of hypertension, plasma glucose, fibrinogen, IL-6, sTNF-R1 and soluble receptor of tumor necrosis factor-alpha type 2 and soluble Fas receptor.
*The possibility that a diabetic patient will suffer injury in 3 target organs is 27.8 (95%C, 1.72 to 449.1) times greater compared to having no target organ damage (reference group).

present study goes further and shows a linear association between plasma levels of different inflammatory molecules and the severity of hypertensive disease, whereby patients with generalized injury presented significantly higher values of these inflammatory parameters. Moreover, in multivariate analysis, plasma concentrations of IL-6 and sTNF-R1 were associated independently and significantly with the condition of 3 target organs. The lack of significance for the other TNF-α receptor (sTNF-R2) emphasizes the structural and functional differences between these two receptors.[5]

We know that HT can promote the activation of inflammation in endothelial cells, as well as the recruitment and activation of inflammatory cells characterized by increased expression of inflammatory parameters, such as CRP and inflammatory cytokines.[1] Mechanical stress and the proinflammatory effects of humoral factors, with angiotensin playing a fundamental role, may be involved in the development of this inflammatory response.[19] The amplification of this response in target organs may explain the increase in inflammatory parameters observed in our hypertensive patients with TOD. These inflammatory molecules can lead to detrimental effects in target organs and vessels and thus contribute to functional and structural changes. The lack of correlation between CRP and the TOD observed in our study may be explained by the low discriminatory power of the molecule (in contrast to CRP) and its specificity compared to other, more sophisticated markers used in this study.

In addition, we know that cell death by apoptosis induced by cytokines activated by mechanical stress plays an important role in the pathogenesis of various cardiovascular diseases including essential HT.[2] Given the abundant evidence showing that the growth of various cell types is altered in the target organs of human and experimental HT, it is unsurprising that alterations of apoptosis in the kidneys and brain of hypertensive rats have also been found, in addition to the heart.[20] These studies underscore the importance of altered regulation of cell death in HT, indicate new directions for research into the pathogenesis of this disease and TOD, and point to new targets for therapeutic intervention.

Several single-center studies have observed that sFas is elevated in patients with myocarditis, chronic heart failure and coronary artery disease, indicating that the Fas/FasL system may contribute to the pathogenesis of cardiovascular disease.[21–23] A study in patients with high levels of cardiovascular risk showed higher concentrations of sFas in this population, especially in hypertensive patients, than in healthy subjects, indicating that this protein could be a new marker of vascular damage.[24] The prognostic impact of sFas in several cardiovascular diseases has recently been demonstrated, both in patients with carotid atherosclerosis[25] and in those with heart failure,[26] whereby patients with higher levels of this protein had worse outcomes. The fact that we found no association between sFas and TOD in this study may indicate that we are dealing with early HT-related subclinical lesions in our patients, with a lesser degree of apoptotic activation. We also cannot rule out the possibility that apoptosis plays a different role in each cardiovascular disease. One difference observed was the relationship found between the decreased glomerular filtration rate, which implies greater functional and probably structural impairment of the kidneys, with cell loss and fibrosis. In fact, several studies have observed a correlation between apoptosis and the degree of renal dysfunction.[27] Furthermore, pretreatment with renin-angiotensin system blockers may modify the concentrations of apoptosis molecules[28] and explain the lack of correlation between sFas and LVMsa found in other studies but not here. In contrast, we did find a correlation between values of sTNF-R1 (an apoptoptic molecule with

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inflammatory action)[29] and the extent of TOD. This finding is important, since concentrations of TNF-α and its receptors are predictive of mortality in patients with heart failure[30] and, specifically, because sTNF-R1 is a major predictor of death and heart failure, over both the short and long term, in patients with acute myocardial infarction.[5]

Limitations

This study has some limitations which should be taken into account when interpreting the results. Due to the small number of patients, the results cannot be extrapolated to the entire population of hypertensive patients. In addition, because of the cross-sectional design of the study, we cannot establish a causal relationship between inflammation/apoptosis and TOD, but merely reflect an association. Furthermore, the possibility that the values of markers of inflammation and apoptosis were influenced by differences in the clinical profiles and risks of the groups analyzed cannot be ruled out. Finally, a common limitation in these studies is that patients are receiving conventional therapy, and it is well-established that several drugs can decrease the concentrations of the parameters analyzed. Specifically, inhibitors of angiotensin converting enzymes and beta blockers decrease the plasma concentrations of inflammatory and anti-inflammatory cytokines,[31,32] although the *in vivo* effects of these drugs in the cytokines analyzed have not yet been evaluated. However, this study confirms that a high degree of immune activation and inflammation persists in hypertensive patients even during treatment with standard therapy, and that this activation is also detectable in plasma.

CONCLUSIONS

This study shows that, in hypertensive patients, more widespread hypertensive disease and a higher number of affected target organs was associated with higher levels of inflammation and apoptosis, characterized by a significant increase in plasma concentrations of IL-6, sTNF-R1 and sTNF-R2, specifically in patients with simultaneous heart, renal, and vascular disease. Further research is required to fully understand the role of cytokines in the development and progression of TOD in essential HT. Cytokines could come to be considered as therapeutic targets, which would change the prognosis for hypertensive patients, in whom the development of TOD is associated with high cardiovascular mortality.

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CONFLICTS OF INTEREST

None declared.

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