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The usefulness of determining the serum concentrations of vascular endothelial growth factor (VEGF) and its soluble receptor type 2 (sVEGF-2) in the differential diagnosis of adrenal incidentalomas

Przydatność oznaczania stężenia naczyniowo-śródbłonkowego czynnika wzrostu (VEGF) i rozpuszczalnej formy receptora dla tego czynnika (sVEGFR2) w diagnostyce różnicowej przypadkowo wykrytych guzów nadnerczy (*incydentaloma*)

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Abstract

Introduction: Angiogenesis plays an important role in tumour growth, progression and invasiveness. Vascular endothelial growth factor (VEGF) is a recognised angiogenesis-stimulating factor. Soluble VEGF receptors (sVEGFRs) have antiangiogenic properties. Recent studies have indicated that serum concentrations of these factors show a good correlation with the aggressiveness of these tumours in various organs. The aim of this study was to assess the usefulness of determining serum concentrations of VEGF and sVEGFR-2 in patients with adrenal incidentalomas.

Material and methods: The study included 51 patients: 38 women aged 53.57 ± 10.12 years and 13 men aged 54.66 ± 12.73 years without a history of cancer but with non-functioning adrenal tumours incidentally detected on a CT scan. The analysis of the CT images included such morphological features of the tumour as: tumour size, tumour homogenicity, tumour density before and after administration of an intravenous contrast medium, and the value of percentage washout of the contrast medium from the tumour. Based on the above criteria, we identified a group of 40 patients with adrenal tumours who met the CT criteria for benign adenomas (Group 1) and 11 patients whose incidentally discovered tumours did not meet the radiological criteria for benign adenomas, thereby providing grounds for referring these patients for surgery (Group 2). The control group consisted of 20 healthy sex- and age-matched individuals.

Results: The mean serum concentrations of VEGF in the study and control groups were similar, although patients with adrenal tumours had significantly higher concentrations of sVEGFR-2 than healthy individuals. There were no significant differences in the mean concentrations of VEGF and sVEGFR-2 between the patients undergoing surgery (Group 2) and the patients not undergoing surgery (Group 1), or between the patients undergoing surgery (Group 2) and the control group. Postoperative histopathology of the resected adrenal tumours revealed benign adrenocortical adenoma in eight patients and the following in the remaining patients: adrenocortical carcinoma in one patient, phaeochromocytoma in one patient and ganglioneuroma in one patient. The adrenocortical carcinoma patient had the highest concentration of VEGF, while this patient's concentration of sVEGFR-2 was the lowest in the study group. In the patients diagnosed with ganglioneuroma and phaeochromocytoma, VEGF and sVEGFR-2 concentrations did not differ significantly from their mean concentrations in the study group. There were also no relationships between the serum concentrations of VEGF or sVEGFR-2 and the following parameters: tumour size, precontrast and postcontrast tumour densities or the value of percentage washout. Positive correlations were, however, identified between the concentration of VEGF and the concentrations of total cholesterol and LDL-cholesterol.

Conclusions: Determining the serum concentrations of such angiogenesis markers as VEGF and sVEGFR-2 seems useful in the evaluation of the nature of incidentally detected adrenal masses (incidentalomas), especially in the preoperative differential diagnosis of adrenal masses that do not meet the CT criteria for benign tumours. **(Pol J Endocrinol 2012; 63 (1): 22–28)**

Key words: *adrenal incidentaloma, vascular endothelial growth factor (VEGF), soluble vascular endothelial growth factor receptor type 2 (sVEGFR-2)*

Streszczenie

Wstęp: Nowotworzenie naczyń odgrywa istotną rolę w procesie wzrostu guza, jego progresji i inwazyjności. Uznanym czynnikiem stymulującym angiogenezę jest naczyniowo-śródbłonkowy czynnik wzrostu (VEGF, *vascular endothelial growth factor*). Rozpuszczalne formy receptorów dla VEGF (sVEGFR, *soluble vascular endothelial growth factor receptors*) wywierają działanie antyangiogenne. Badania prowadzone w ostatnich latach wskazują, że stężenia tych czynników w surowicy krwi dobrze korelują z agresywnością zmian nowotworowych w różnych narządach. Celem pracy była ocena przydatności oznaczania stężenia naczyniowo-śródbłonkowego czynnika wzrostu VEGF i rozpuszczalnej formy receptora typu 2 dla tego czynnika sVEGFR2 u chorych z przypadkowo wykrytymi guzami nadnerczy (*incydentaloma*)

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Materiał i metody: Badaniem objęto 51 chorych, w tym 38 kobiet w wieku 53,47 (SD ± 10,12) i 13 mężczyzn w wieku 54,66 (SD ± 12,73) roku, bez obciążeń onkologicznych, z przypadkowo wykrytymi w badaniu TK, nieczynnymi hormonalnie guzami nadnerczy. Analiza obrazu TK obejmowała cechy morfologiczne guza takie jak: wymiary guza, jego homogenność, gęstość przed i po dożylnym podaniu środka kontrastowego a także wskaźnik procentowego wypłukiwania kontrastu. W oparciu o ww. kryteria wyodrębniono grupę 40 chorych z guzami nadnerczy, które w badaniu TK spełniały kryteria dla łagodnych gruczolaków (grupa I) i 11 chorych, u których przypadkowo wykryte guzy nie spełniały radiologicznych kryteriów dla łagodnych gruczolaków, co stanowiło podstawę do skierowania tych chorych do leczenia operacyjnego (grupa II). Grupę kontrolną stanowiło 20 zdrowych osób, odpowiednio dobranych pod względem płci i wieku. U wszystkich badanych oznaczono stężenie VEGF i sVEGFR2 w surowicy krwi.
Wyniki: Średnie stężenie VEGF w grupie badanej i kontrolnej było podobne, stwierdzono znamiennie wyższe stężenie sVEGFR2 u chorych z guzami nadnerczy w porównaniu ze zdrowymi. Średnie stężenia VEGF i sVEGFR2 w grupie chorych operowanych (grupa II) nie różniły się istotnie od średnich stężeń w grupie chorych nieoperowanych (grupa I) i w grupie kontrolnej. Pooperacyjne badanie histopatologiczne guzów nadnerczy wykazało w 8 przypadkach łagodnego gruczolaka kory nadnerczy, u pozostałych chorych rozpoznano raka nadnerczy (1 pacjentka), guza chromochłonnego (1 pacjentka) i ganglioneuroma (1 pacjentka). U chorej z rakiem kory nadnercza stężenie VEGF było najwyższe, natomiast stężenie sVEGFR2 było najniższe w grupie badanej. U pacjentów z rozpoznaniem ganglioneuroma i pheochromocytoma stężenia VEGF i sVEGFR2 nie różniły się istotnie od średniego stężenia tych czynników w grupie badanej. Nie wykazano zależności pomiędzy stężeniami czynnika VEGF i sVEGFR2 a rozmiarami guza, jego densyjnością przed i po podaniu środka kontrastowego oraz wskaźnikiem procentowego wypłukiwania kontrastu. Stwierdzono natomiast dodatnią korelację pomiędzy stężeniem VEGF i stężeniem cholesterolu całkowitego i LDL-cholesterolu.
Wnioski: Oznaczenie stężenia surowiczych markerów angiogenezy VEGF i sVEGFR2 wydaje się być pomocne w ocenie charakteru przypadkowo wykrytych nieczynnych hormonalnie guzów nadnerczy „incydentaloma", zwłaszcza w przedoperacyjnym różnicowaniu zmian nie spełniających radiologicznych kryteriów łagodności w badaniu tomografii komputerowej. **(Endokrynol Pol 2012; 63 (1): 22–28)**

Słowa kluczowe: *incydentaloma nadnerczy, naczyniowo-śródbłonkowy czynnik wzrostu (VEGF ,vascular endothelial growth factor), rozpuszczalna forma receptora dla VEGF typu 2 (sVEGFR2, soluble vascular endothelial growth factor receptors type 2)*

Introduction

The development of modern imaging techniques, and their increasing accessibility, has resulted in an increased detectability of non-functioning adrenal tumours, also referred to as incidentalomas [1].

Most of these tumours are benign adenomas and are only rarely malignant. Preoperative differentiation between benign and malignant nature of these masses is difficult, and in some cases impossible. Certain diagnostic hints are provided by imaging studies. The radiological criteria that suggest the malignant nature of a tumour include: large tumour size, high baseline tumour density (expressed in Hounsfield units, HU), and poor washout of the contrast medium from the pathological lesion [2, 3]. In many cases, the radiological picture is equivocal, which has prompted researchers to look for other indicators of the malignant nature of incidentally detected adrenal tumours.

Previous studies have shown that the serum concentrations of angiogenesis markers, such as vascular endothelial growth factor (VEGF) and soluble vascular endothelial growth factor receptors (sVEGFRs) show a good correlation with the aggressiveness of tumours in various organs and are independent prognostic factors [4–8]. Angiogenesis plays a particularly important role in tumour growth, progression and invasiveness. Tumours exceeding 2–3 mm in size require the presence of blood vessels supplying them with oxygen, and nutrients that allow them to continue to grow. VEGF stimulates the proliferation and migration of endothelial cells [9, 10]. This protein is a potent mitogen for endothelial cells that supports their survival by affecting the suppression of apoptotic pathways. VEGF also increases the permeability of blood vessels, which is a characteristic feature of tumour blood vessels [11]. VEGF exerts its actions by specific membrane receptors (VEGFRs), which belong to the thyrosine kinase family. One of these receptors is VEGFR-2. Activation of the membrane-bound receptor initiates a number of biochemical processes in endothelial cells that lead to cell division and the resulting formation of blood vessels in tumours [12]. The soluble form of this receptor, sVEGFR-2, shows antiangiogenic properties because, by binding with VEGF, it suppresses its effects on membrane-bound receptors [13, 14].

Aims

The aims of our study were to:

— Assess the serum concentrations of VEGF and sVEGFR-2 in patients with incidentally detected non-functioning adrenal tumours (incidentalomas);
— Assess the relationships between the serum concentrations of VEGF, sVEGFR-2 and the radiological criteria for malignant/benign nature of adrenal tumours, such as: tumour size, tumour density and percentage washout on CT.

Material and methods

The study included 51 patients without a history of cancer but with adrenal tumours incidentally detected on CT: 38 women aged 53.57 ± 10.12 years and 13 men aged 54.66 ± 12.73 years. All the patients underwent an assessment of the hormonal function of their tu-

mour, which involved determination of diurnal cortisol concentrations, determination of blood concentrations of androstenedione and dehydroepiandrosterone sulfate (DHEAS) and determination of free cortisol, aldosterone and catecholamine metabolites in 24-hour urine. The patients also underwent determination of adrenocorticotropic hormone (ACTH) in the serum and the lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides). Only patients with non-functioning adrenal tumours were qualified for the study. The analysis of the CT images included such morphological features of the tumours as: tumour size, tumour homogenicity, tumour density before administration of the contrast medium, tumour density 60 seconds, and 15 minutes, after administration of the contrast medium, and the percentage washout of the contrast medium from the tumour. We adopted the commonly used radiological criteria for benign adenomas, i.e. tumour size below 5 cm, homogenous tumour structure, initial tumour density (density before administration of the contrast medium) below 10 HU, and the absolute percentage washout (APW) exceeding 60% [3]. Based on these criteria, we divided the patients into two groups.

Group 1 comprised 40 patients with adrenal tumours that met the CT criteria for benign adenomas. Group 2 consisted of 11 patients whose incidentalomas did not meet the radiological criteria for benign adenomas, thus providing grounds to refer these patients for surgery.

The control group comprised 20 healthy sex- and age-matched individuals. All the patients underwent determination of serum VEGF and sVEGFR-2 by enzyme immunoassay (ELISA) with the use of commercially available kits (Human VEGF, Human sVEG-FR2/KDR/Flk-1, R&D Systems Europe Ltd.).

Statistical analysis

The investigated parameters (expressed as means ± standard deviations) were compared with the *t*-Student test, and the analysis of correlation was performed using the Pearson's method. Differences with p values below 0.05 were considered statistically significant.

Results

The mean serum concentrations of VEGF in the study and control groups were similar, although patients with adrenal tumours had significantly higher concentrations of sVEGFR-2 than healthy individuals (Table I).

There were no significant differences in the mean concentrations of VEGF and sVEGFR-2 between the patients undergoing surgery (Group 2) and the patients not undergoing surgery (Group 1), or between the patients undergoing surgery (Group 2) and the control group (Table II).

Postoperative histopathology of the resected adrenal tumours that did not meet the radiological criteria for benign nature revealed benign adrenocortical adenoma in eight patients, and the following in the remaining three patients: adrenocortical carcinoma in one patient, phaeochromocytoma in one patient and ganglioneuroma in one patient. The concentration of VEGF in the adrenocortical carcinoma patient equalled

Table I. *Comparison of the concentrations of VEGF and sVEGFR-2 in the study and control groups*
Tabela I. *Porównanie stężeń VEGF i sVEGFR 2 w grupie badanej i grupie kontrolnej*

	Study group				Control group				p
	n	Mean	SD	SEM	n	Mean	SD	SEM	
VEGF	51	300.369	218.8228	30.6413	22	328.909	234.5201	38.9806	NS
sVEGFR-2		7,566.553	1,674.8084	234.5201		6,577.591	1,413.0904	301.2719	0.018

Table II. *Comparison of the mean concentrations of VEGF and sVEGFR-2 between the group of patients meeting the radiological criteria for benign tumours on CT (group 1) and the group of patients not meeting these criteria (group 2)*
Tabela II. *Porównanie średnich stężeń VEGF i sVEGFR2 pomiędzy grupą chorych z guzami nadnerczy, które spełniają radiologiczne kryteria łagodności guza w badaniu TK (Grupa I) i nie spełniają tych kryteriów (Grupa II)*

	Group 1				Group 2				p
	n	Mean	SD	SEM	n	Mean	SD	SEM	
VEGF	40	292.018	212.9189	33.6654	11	330.736	247.6272	74.6624	NS
sVEGFR-2		7,603.715	1,730.9524	273.6876		7,431.418	1,521.2293	458.6679	NS

766.1 pg/ml and was the highest in the study group, while the concentration of sVEGFR-2 in this patient equalled 6,843.5 pg/ml and was the lowest in the study group. In the patients diagnosed with ganglioneuroma and phaeochromocytoma, VEGF and sVEGFR-2 concentrations did not differ significantly from their mean concentrations in the study group.

There was no correlation between the concentrations of VEGF and sVEGFR-2 and the following parameters: tumour size, tumour density before administration of the contrast medium, tumour density after administration of the contrast medium and percentage washout (Table III). A positive correlation was, however, observed between the concentration of VEGF and the concentration of total cholesterol (Figure 1) and between the concentration of VEGF and the concentration of LDL-cholesterol (Figure 2).

Discussion

Incidentally discovered adrenal mass, first described by Geelhoed and Spiegel in 1981, continue to pose a considerable diagnostic challenge for clinicians. Adrenal tumours are discovered in 4–6% of patients who undergo imaging studies that involve these organs [15, 16]. They are mainly benign tumours with the predominant type being adenomas (non-functioning adenomas (94%), cortisol-secreting adenomas (5%) and aldosterone-secreting adenomas (1%). The other benign tumours include myelolipoma (9%), cysts, ganglioneuroma, haemangioma, neuroblastoma, haemorrhagic changes and granulomatous diseases, which account for 1–2% of all benign lesions. Among the lesions of the incidentaloma type, which is much less frequent, are: primary adrenocortical carcinoma

Table III. *Correlations between the concentrations of VEGF and sVEGFR-2 and age, tumour size, precontrast tumour density and percentage washout*

Tabela III. *Korelacja pomiędzy stężeniem VEGF i sVEGFR2 a wiekiem, rozmiarami guza, densyjnością guza przed podaniem kontrastu i wskaźnikiem procentowego wypłukiwania kontrastu*

		Age	Maximum tumour size	Precontrast density	Percentage washout
VEGF	r	0.10	–0.07	0.18	–0.002
	p	0.36	0.59	0.19	0.985
	n	73	51	51	51
sVEGFR-2	r	0.06	0.12	0.13	0.15
	p	0.60	0.38	0.34	0.28
	n	73	51	51	51

r — correlation coefficient; n — number of subjects; p — statistical significance level

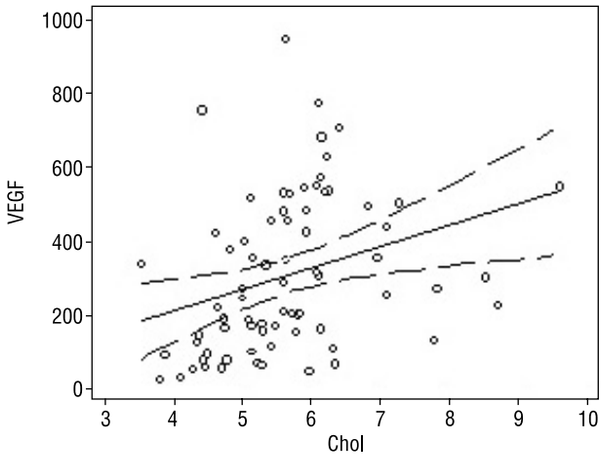

Figure 1. *Correlation between the concentration of VEGF and that of total cholesterol (p = 0.008)*

Rycina 1. *Korelacja pomiędzy stężeniem VEGF i cholesterolu całkowitego (p = 0,008)*

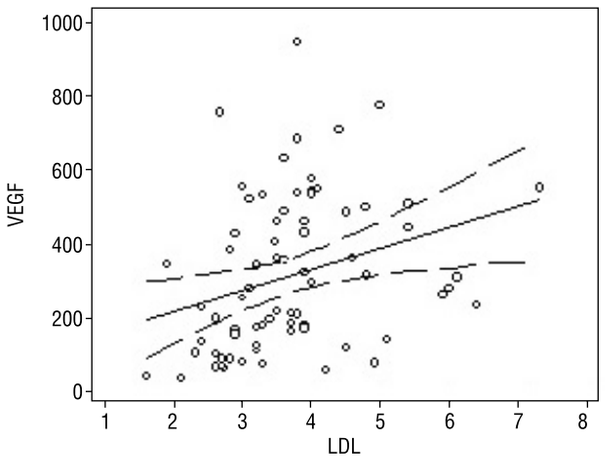

Figure 2. *Correlation between the concentration of VEGF and that of LDL-cholesterol (p = 0.011)*

Rycina 2. *Korelacja pomiędzy stężeniem VEGF i LDL-cholesterolu (p = 0,011)*

(2–10%), phaeochromocytoma (5–8%) and metastatic foci of other cancers [17, 18].

A Polish study of 1,790 patients with accidentally discovered adrenal tumours revealed adrenal carcinoma in 7.5% of the patients [19]. Due to the different management and prognosis in functioning and non-functioning adenomas and in adrenal carcinomas, a thorough differential diagnosis in the preoperative period is of the utmost importance, with imaging studies, mainly computed tomography, playing the key role. Large tumours (exceeding 5–6 cm), irregular outlines and the presence of regressive lesions such as extravasation, necrosis, fibrosis and calcification within the tumour, all point to its malignant nature. An additional indicator of malignancy is a rapid tumour growth dynamic in subsequent imaging studies [16, 20]. Another radiological feature of the malignant nature of tumours is a high density of the tumour on CT without contrast (> 10 HU), which indicates a low content of fat. In contrast to malignant lesions, most benign adenomas (about 70%) contain intracellular fat (mainly cholesterol and fatty acids) and are therefore characterised by a low density (< 10 HU) [2, 21]. Dynamic studies using contrast provide additional information that makes differentiation between benign and malignant lesions easier. Adrenocortical carcinoma is characterised by a high baseline density, a strong contrast enhancement during the enhancement phase, and a low washout of the contrast in the delayed phase. The percentage washout of the contrast in adrenocortical carcinoma is lower than 50%, while in benign tumours it usually exceeds 60% [22]. Assuming a threshold value of 10 HU for tumour density before administration of the contrast medium, and 60% for the percentage washout of the contrast medium, the sensitivity and specificity of computed tomography in diagnosing benign adenomas is 98% and 92%, respectively [23].

Based on the above criteria, we qualified for surgery 11 patients with adrenal tumours of the incidentaloma type. Adrenal tumours in this group of patients were characterised, on CT scans, by large sizes ranging from 2 cm to 12 cm (mean 7 cm), non-homogenous structure, high baseline density (> 10 HU), and a percentage washout below 50%.

However, in one case, histopathology revealed an adrenocortical carcinoma. It was a tumour measuring 5.2 × 5.0 cm, with a non-homogenous structure with calcifications, high density before and after contrast, and a percentage washout of 22%.

In one patient, a diagnosis of clinically asymptomatic phaeochromocytoma was made with normal concentrations of methoxycatecholamines in 24-hour urine.

In one case, histopathological examination of the resected adrenal gland revealed a benign lesion, namely ganglioneuroma.

In the remaining eight patients who had undergone unilateral adrenalectomy, benign adenomas were diagnosed, although in the preoperative CT scans these tumours had not met the criteria for benign lesions. They were most probably lipid-poor adenomas, which account for 10–40% of all benign adrenal adenomas. These tumours are characterised by a higher density before and after administration of the contrast medium compared to lipid-rich adenomas, although the percentage washout in both these adenoma types is usually similar [2]. In our study, baseline tumour density in the preoperative period exceeded 10 HU and the percentage washout was lower than 50%, which raised concerns that these tumours might be malignant. In some cases, preoperative differentiation of lipid-poor adenomas, carcinomas and non-adenomas of the adrenal gland is difficult, and additional diagnostic studies are required.

It seems that the assessment of neoangiogenesis in these cases may play a significant role. VEGF, discovered in 1989, is a key angiogenic cytokine [11]. Exerting its action through specific membrane-bound VEGFRs, it stimulates the development of new blood vessels necessary for the growth of solid tumours in various organs, including endocrine glands [24–27]. An important angiogenesis regulatory role is played by the soluble form of type 2 VEGF receptor (sVEGFR-2). This receptor, as a result of VEGF binding, decreases its effects on membrane-bound receptors in endothelial cells, contributing to the suppression of angiogenesis [13, 14]. In our study, we attempted to establish whether determination of serum VEGF and sVEGFR-2 concentrations could be useful in the preoperative differential diagnosis of accidentally detected adrenal tumours. We found no differences in mean VEGF concentration between the study and control groups.

It should be noted, however, that the study group mainly comprised patients with the diagnosis of benign adenoma (48 patients): in 40 of these patients, the diagnosis had been made on the basis of the radiological criteria for the benign nature of the tumour on CT, while in the other eight the diagnosis had been based on postoperative histopathological examination. The lack of difference in the concentration of VEGF between the group of patients with benign adenomas and healthy individuals, which we observed, is consistent with other studies [27, 28].

We also found no differences in the concentrations of VEGF and sVEGFR-2 between the group of 11 patients undergoing adrenalectomy due to failure to meet the radiological criteria for the benign nature of their tumours, and the group of patients whose CT scans unequivocally revealed benign adrenal adenomas. Most of the resected adrenal tumours (eight out of 11) were benign lipid-poor adenomas.

Some authors have demonstrated a significantly higher serum concentration of VEGF and, at the same time, a significantly lower serum concentration of sVEGFR-2, in patients with adrenal cancer compared to patients with benign adenomas [24, 25]. Likewise, in our study, the patient with adrenocortical carcinoma had the highest concentration of VEGF and the lowest concentration of sVEGFR-2 in the study group. In the cases of phaeochromocytoma and ganglioneuroma, the concentrations of VEGF and sVEGFR-2 did not differ significantly from the study group. According to other authors, the concentration of VEGF is significantly higher in patients with functioning adrenal adenomas, including phaeochromocytoma, compared to healthy individuals [24, 29].

Our results indicate that the concentrations of VEGF and sVEGFR-2 are similar in both lipid-rich and lipid-poor benign adenomas. Hence, the determination of these factors may be helpful in the differential diagnosis of adrenal tumours that do not meet the radiological criteria for benign character on CT.

These results should, however, be interpreted with caution due to the small number of subjects. In addition, lipid-rich adrenal tumours were not verified histopathologically and the diagnosis of benign adenoma in these cases was based solely on radiological criteria.

We found no correlation between VEGF or sVEGFR-2 and tumour size, which is consistent with other studies [26]. We also found no relationship between the concentration of these factors and tumour density or the percentage washout of the contrast medium from the tumour on dynamic CT imaging. This could result from the fact that the study group was quite homogenous in terms of diagnosis (in 94% of the patients, the diagnosis of benign adrenal adenoma was made). It would be interesting to look for a relationship between serum markers of angiogenesis and the radiological criteria for malignant nature of the tumours in the group of patients with histopathologically confirmed adrenocortical carcinoma.

The results of the determination of sVEGFR-2 were quite surprising. The mean concentration of this antiangiogenic molecule in the group of patients with adrenal incidentalomas was significantly higher than that in the control group, while other authors have observed significantly lower mean sVEGFR-2 concentrations in patients with benign adrenal adenomas [27].

The positive correlation between the concentration of VEGF and the concentration of total cholesterol, and between the concentration of VEGF and the concentration of LDL-cholesterol, probably results from overexpression of VEGF in endothelial cells secondary to hypoxia and blood vessel wall inflammation in the early period of atherogenesis. The atherogenic effects of VEGF result from increased permeability of the endothelium, increased migration of neutrophils, and stimulation of endothelial cell proliferation, which initiates atherogenesis [30–32].

In conclusion, determining serum markers of angiogenesis, VEGF and sVEGFR-2 would seem to be helpful in assessing the nature of accidentally detected non-functioning adrenal tumours of the incidentaloma type, especially in the preoperative differentiation of lesions that do not meet the radiological criteria for benign lesions on computed tomography.

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