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Early detection of Contrast-induced nephropathy with novel biomarker Neutrophil Gelatinase-Associated Lipocalin (NGAL) and renoprotective effects of different prophylaxis strategies

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Ранно диагностициране на контраст-индуцирана нефропатия с новия биомаркер Неутрофил Гелатиназа-Асоцииран Липокалин (НГАЛ) и ренопротективните ефекти на различни профилактични стратегии

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РЕЗЮМЕ

Контраст-индуцираната нефропатия (КИН) е термин използван за остра бъбречна недостатъчност, настъпила след вътресъдово приложение на контрастни вещества, обичайно използвани при сърдечна ангиография. Развитието на КИН е свързано с други сериозни последици като големи нежелани сърдечно-съдови събития и смъртност. Същевременно броя на контрастните ангиографии и перкутанни коронарни интервенции нараства непрекъснато всяка година, което води до повишаване честота на КИН.

В основата на превенцията стои подходящата рискова стратификация, интравенозна хидратация с физиологичен серум или натриев бикарбонат, преустановяване на нефротоксичните медикаменти, използването на ниско- или изоосмоларни контрастни вещества и редуциране на приложената доза. Някои проучвания дока-

ABSTRACT

The contrast-induced nephropathy (CIN) is a term applied to acute renal failure associated with intravascular injection of contrast agents typically used for cardiac angiography. The development of CIN is associated with other adverse outcomes including major adverse cardiovascular events (MACE) and death. At the same time, the number of contrast angiographies and percutaneous coronary intervention increase continuously each year, which may possibly result in increased incidence of CIN.

The cornerstone of prevention is appropriate risk stratification, intravenous hydration with normal saline or sodium bicarbonate, withholding of nephrotoxic medications, use of low or isoosmolar contrast, and contrast dose reduction. Some studies have shown that acetylcysteine may have additional preventive capacity administrated before and after contrast angiography.

заха, че ацетилцистеинът може да има допълнителна превантивна сила, ако се приложи преди и след контрастната ангиография.

От изключителна важност е ранното улавяне на КИН и адекватните превантивни мерки за намаляването на неочакваните усложнения. Все още се търсят надежден лабораторен тест или стойност, които да разпознаят бъбречното увреждане преди нарастването на серумния креатинин. Те биха били най-полезния подход за започване на подходящо лечение навреме. Неутрофил Гелатиназа-Асоцииран Липокалин (НГАЛ) е нов биомаркер предсказващ остро бъбречно увреждане и надежден за ранна диагностика на КИН, при пациенти подложени на контрастна ангиография. Освобождаването на НГАЛ настъпва до часове след стимули като исхемия или токсичност, които са свързани с тубулно увреждане и дълго време преди регистриране на увеличаване в серумните нива на креатинина. Нивата на НГАЛ бяха предложени като индикатор в реално време за остро бъбречно увреждане докато нивата на креатинина и скоростта на гломерулна филтрация са маркери за функциониращия брой нефрони.

Ключови думи: Контраст-индуцирана нефропатия, нови биомаркери, Неутрофил Гелатиназа-Асоцииран Липокалин (НГАЛ)

The early detection of CIN as well as adequate preventive measures aiming at reduction of unexpected complication is extremely important. It`s still searching for a reliable laboratory value or test that recognizes renal damage before serum creatinine increase. It would be a most helpful tool to initiate proper treatment on time. Neutrophil Gelatinase-Associated Lipocalin (NGAL), a new biomarker predictive for acute renal injury has been shown to be capable for earlier diagnosis of CIN in patients undergoing contrast angiography. The release of NGAL occurs within hours after the stimulus associated with tubular damage by ischemia or toxicity and long before an increase in serum creatinine level occurs. NGAL level has been proposed to be real-time indicator of active kidney damage whereas creatinine level and GFR are markers of functional nephron number.

Key words: contrast-induced nephropathy; new biomarkers, Neutrophil Gelatinase-Associated Lipocalin (NGAL)

1. INTRODUCTION. EPIDEMIOLOGY

In recent years, the use of contrast agents (CA) has increased steadily in routine medical practice. One of the most important and well-known complications of CA administration is contrast-induced nephropathy (CIN). With the growing complexity of diagnostic and interventional procedures, which require large doses of CA and are performed in an ever-expanding number of elderly patients with a high prevalence of chronic kidney disease (CKD) and other comorbidities, CIN and its clinical management are becoming an increasingly important issue.

In absence of an universally accepted definition, most authors define CIN as a relative (≥25%) or an absolute (≥0.5 mg/dl = 44 μmol/l) increase in serum creatinine (SCr) from baseline. In case of contrast-induced toxicity serum creatinine typically rises within the first 24–48 hours after exposition, peaks at 3–5 days and returns near to baseline within 1–3 weeks.[42]

The incidence rate of contrast-induced nephropathy as a complication of radiographic diagnostic and interventional studies varies markedly, dependent on the adopted definition and on other variables such as the type of radiology procedure performed, the dose and type of contrast agent administered, the differences across patient populations in regard to number and type of risk factors, and the length of patient follow-up. An overall incidence of 14.5% was announced in a large epidemiologic study[39] (defined as > 25% increase in serum creatinine levels over baseline in the first 5 days), however

rates may vary from 0% to 90%, depending on the presence of risk factors, most notably chronic renal insufficiency, diabetes mellitus, and high contrast volume administered. Incidence among patients with diabetes has been reported to be 9–40% in patients with mild-to-moderate chronic renal insufficiency and 50–90% in those with severe chronic renal insufficiency.[37]

The development of CIN has important effects on clinical outcome and may contribute to other post-PCI complications including death, myocardial infarction (MI), and stroke. In the Mayo Clinic PCI Registry, the incidence of in-hospital death, most of which resulted from MI, increases >10-fold in the 254 patients who developed CIN as compared to patients who did not.[55] The patients with CIN also experienced higher rates of access-site bleeding, haematoma formation, pseudoaneurysms, stroke, coma, adult respiratory distress syndrome, pulmonary embolus, and gastrointestinal haemorrhage.[55] Other studies also emphasize the poor short-term outcomes associated with CIN. An analysis of nearly 20 500 patients who underwent percutaneous coronary intervention (PCI) indicated that the 2% of patients who developed CIN had a 15-fold higher rate of major adverse cardiac events (MACE) during hospitalization than those patients without CIN. They had a six-fold increase in risk for MI, an 11-fold increased risk of reocclusion, and a 22-fold higher risk of death when compared with patients without CIN.[8] Long-term outcome is also affected. The Mayo Clinic PCI Registry study showed that only 88% of patients who experienced CIN survived for 1 year and only 55% survived for 5 years when compared with 96% and 85% of patients without CIN who survived for 1 or 5 years, respectively ($P < 0.0001$).[55] In another study, 80% of patients who developed CIN requiring dialysis after coronary intervention did not survive for 2 years.[39] This number was higher for both patients who needed only temporary dialysis and for those who needed it permanently.

In a retrospective study of the Mayo Clinic PCI Registry including about 7600 patients treated between 1996 and 2000 there is examined the incidence and in-hospital consequences of CIN defined as an increase in SCr ≥0.5 mg/dL from pre-procedure values.[55] The incidence of CIN was found to be greater in patients with baseline renal insufficiency (>22% at SCr baseline of 2–2.9 mg/dL), especially if there was co-existent diabetes mellitus (4.5% at SCr baseline of 1.2–1.9 mg/dL). In the absence of these risk factors, the incidence of CIN was ~2%.

It has been recognized that the development of CIN is linked to an increased risk of in-hospital and long-term mortality, probably, as a result of the co-morbidities described. In a large retrospective study including more than 16 000 patients undergoing procedures requiring CA, the risk of death during hospitalization was 34% in patients who developed CIN, compared with 7% in matched controls who received CA but did not develop CIN.[33]

Other studies confirm this observation, with in-hospital mortality rates ranging from 7.1% to 14.9%, and with 1-year mortality rates from 30% to 37%, depending on the patient's baseline risk profile.[19,39]

2. SUMMARY OF THE RISK FACTORS

The risk factors for CIN could be divided into *patient-related* or *intrinsic* risk factors, and *procedure-related* or *extrinsic* risk factors. The most prevalent *intrinsic* risk factors are pre-existence of renal failure, concomitant hypotension, presence of congestive heart failure, older age, anaemia, diabetes mellitus, and concomitant use of nephrotoxic drugs. [1,16,41]

On the other hand, *extrinsic* risk factors comprise the total amount and type of CM used, its route of administration (arterial versus venous) and the time period between two (or iterative) CM expositions.[13,61]

Several scoring systems have been proposed in order to help clinicians to minimise or stratify the risk of CIN. Mehran et al compiled a „simple risk score" to predict CIN occurrence after PCI, with weighted coefficients for independent predictors of CIN. Risk 1 category (≤5

points) is associated with a 7.5% risk of CIN and 0.04% risk of dialysis; risk 2 category (6 to 10 points) with risks of 14% and 0.12%; risk 3 category (11 to 16 points) with risks of 26.1% and 1.09%; and risk 4 category (≥16 points) with risks 57.3% and 12.6% respectively.[41]

The effect of risk factors is additive, and the likelihood of contrast-induced acute kidney injury (AKI) elevates sharply as the number of risk factors increases.

Once contrast induced nephropathy has occurred patients should receive supportive care similar to every patient with acute renal failure: monitoring and correction of serum electrolytes abnormalities and metabolic acidosis, and tight control of fluid balance. Therefore, as no treatment specifically targets CIN, the main goal for clinicians remains prevention and support.

3. PREVENTIVE PROCEDURES

General measures to minimize the incidence of nephropathy include careful consideration whether the contrast examination is absolutely needed, especially in high-risk patients; using the minimal effective dose; and eliminating potentially nephrotoxic drugs (e.g., NSAIDs, aminoglycoside antibiotics, cisplatin, cyclosporin A, and amphotericin B) at least 24 hrs before the study.

One of the easiest and perhaps most effective approach of protecting renal function is adequate hydration. Solomon et al. conducted a prospective trial in 78 patients with chronic renal insufficiency in whom simple fluid therapy (1 mL/kg per hour of 0.45% saline for 12 hr before and after coronary angiography) was shown to be beneficial in reducing renal dysfunction after contrast administration.[59]

Mueller et al. reported a study of 1620 patients who were assigned to receive isotonic (n=809) or half-isotonic (n=811) hydration. Contrast media–associated nephropathy was significantly reduced with isotonic (0.7%, 95% confidence interval, 0.1%-1.4%) vs half-isotonic (2.0%, 95% confidence interval, 1.0%-3.1%) hydration (P=0.04). The authors concluded that isotonic hydration is superior to half-isotonic hydration in the prevention of contrast media–associated nephropathy.[48] This trial and some others recommend that high-risk patients should be administered 0.9% saline by IV infusion at a rate of approximately 1 mL/kg per hour, adjusted appropriately for the patient's current fluid status and cardiovascular condition. This treatment should be commenced 6–12 hr before the procedure and continued for up to 12–24 hr after the radiographic examination, if diuresis is appropriate.[27] Further trials in patients undergoing angiography have demonstrated lower rates of nephropathy in studies using a hydration protocol as compared to studies without mandatory hydration.[17]

Interesting data have been reported by Merten et al. from a prospective, single-center randomized trial of 119 patients. They have suggested that the use of sodium bicarbonate hydration is superior to sodium chloride hydration. The rates of contrast-induced nephropathy were significantly lower in the sodium bicarbonate group (1.7%, n = 1) when compared with the sodium chloride group (13.6%, n = 8) when both cohorts were administered 154 mEq/L of either solution IV.[44]

A meta-analysis of total of 17 trials including 2,633 subjects implicated also that preprocedural hydration with sodium bicarbonate was associated with a significant decrease in the rate of contrast-induced nephropathy (odds ratios 0.52; 95% confidence interval 0.34–0.80, P = 0.003).[43]

Although a clearly emerging concept is that volume expansion is critical in the prevention of CIN, the prognostic impact of hydration is still controversial, and there are no definitive data on the possible advantage of this strategy on CIN-associated cardiovascular complications and mortality rate in high-risk patients. There is still missing evidence from controlled clinical trials to define the most effective hydration period, infusion rate, or hydration volume.

Another widely discussed countermeasure to prevent CIN is the administration of acetyl-

cysteine (NAC). Nine meta-analyses have been published, all documenting the significant heterogeneity between studies and pooled odds ratios for NAC approaching unity.[60] It is worth mentioning that, the renal injury rates appeared to be reduced only in those trials where NAC reduced SCr below baseline values because of decreased skeletal muscle production. Thus, NAC appears to falsely lower SCr and not fundamentally protect against AKI. Differences in CA type and volume, definition of CIN, patient selection, type of intervention, applied hydration regimen, NAC dose (cumulative dosage varied between 1500 mg and > 10000 mg in the different studies) and route of administration (intravenous vs. oral), as well as timing of the cardiovascular procedure (urgent vs. elective) may have contributed to the heterogeneity (i.e., variation of effect across studies greater than can be expected by chance) observed in the pooled analysis.[28]

However, NAC as an antioxidant has been shown to lower rates of AKI and mortality after primary PCI in 1 trial.[38] The published REMEDIAL (Renal Insufficiency Following Contrast Media Administration) trial suggested that the use of volume supplementation with sodium bicarbonate together with NAC was more effective than NAC alone in reducing the risk of AKI.[12] Dosage of NAC varies across the trials; however, the most successful approach has been with 1,200 mg orally twice a day on the day before and after the procedure. Although popular, N-acetylcysteine has not been consistently shown to be effective in prevention of CIN.

Other pharmacological agents such as fenoldopam, dopamine, calcium-channel blockers, atrial natriuretic peptide, and L-arginine have not been proved to be effective in the prevention of contrast-induced AKI. Furosemide, mannitol, and an endothelin receptor antagonist are potentially detrimental.[60]

4. THE ROLE OF CONTRAST AGENT

The topic of contrast agents employed in radiographic studies and their different effects of kidney function is widely discussed and is a part of preventing measures of CIN. On the basis of their chemical and pharmacologic properties, radiographic contrast agents can be classified into ionic or nonionic and as monomers or dimers.The osmotoxic effect of contrast medium is central to the development of contrast-induced nephropathy and is described in terms of the ratio of iodine atoms to dissolved particles. The higher is this ratio, the better is the attenuation of X rays because there are more iodine atoms for fewer particles of contrast agent. Media with a ratio of 1.5:1 are high-osmolal contrast media (HOCM), media with a ratio of 3:1 are low-osmolal (LOCM), and, most recently, agents with a ratio of 6:1 have been developed and are referred to as iso-osmolal contrast media (IOCM).[27] In one study of 1,196 patients, it was shown that patients receiving HOCM (diatrizoate) were 3.3 times as likely to have nephropathy induced as those receiving LOCM (iohexol).[57] Subsequently, a metaanalysis of 31 trials (45 trials included, and 14 had data unavailable) concluded that the use of LOCM rather than HOCM was beneficial to patients with preexisting renal failure.[46]

The unresolved issue is whether the other available CA, either LOCM or IOCM, differ in terms of nephrotoxicity. Several studies have compared LOCM with IOCM. A reduced nephrotoxic effect was demonstrated with non-ionic IOCM (iodixanol) by the NEPHRIC trial.[2] The latter was randomized, double blind, prospective, multicentre study comparing the non-ionic iodixanol with the non-ionic LOCM iohexol. The study included 129 patients with diabetes and SCr > 1.5 mg/dl undergoing coronary or peripheral angiography. The incidence of CIN was 3% in the iodixanol group and 26% in the iohexol group (p=0.002). These results were attributed to the greater osmotic diuresis induced by LOCM, which may increase the work of the medullar tubules, induced ischemia within the renal medulla and volume depletion with activation of vasoregulatory hormones. However, the purported advantage of IOCM has not been sup-

ported by more recent and larger trials. First, other studies with iodixanol in CKD patients have shown higher rates of CIN than that observed in the NEPHRIC study (21% in the RAPPID trial, 12% in the study of Boccalandro et al, 33% and 25% with iodixanol and other CA, respectively, in the CONTRAST trial).[7,10,62] Second, several large-scale randomized controlled trials (PREDICT, ACTIVE trial) have shown no difference in CIN rate when iodixanol was compared with different types of LOCM.[30,63] In addition, a recent meta-analysis comparing iodixanol to a pool of non-ionic LOCM showed no significant reduction in the rates of CIN.[25] In 2009, Reed et al published another meta-analysis in which a total of 16 trials including 2763 patients were pooled.[54] The vast majority (12 out of 16) of these trials included patients with elevated SCr values. Moreover only trials designed to assess CIN as an endpoint, thus ensuring adequate control of confounding variables and minimizing ascertainment bias, were included. The authors did not find any significant difference in CIN incidence between iodixanol and a pool of ionic and non-ionic LOCM. Interestingly, using a stratified analysis that indirectly compared different types of LOCM, it was found that some LOCM (ioxaglate, iohexol) are relatively more likely to cause CIN compared to iodixanol. Finally, the Contrast Media and Nephrotoxicity Following Coronary Revascularization (CONTRAST) trial compared IOCM (iodixanol) and a LOCM (iomeprol) in patients with CKD undergoing PCI.[67] No significant differences were observed between the two groups, when CIN rate, need for dialysis, duration of hospitalization and major adverse cardiac events at 6-month follow-up were considered. These data further support the concept that a wide spectrum of nephrotoxicity may exist among different CA, regardless of their osmolarity.

It has been implicated by another group of studies focused on CA that the volume of contrast medium is a risk factor for CIN. The mean contrast volume is higher in patients with contrast-induced acute kidney injury, and most multivariate analyses have shown that contrast volume is an independent predictor of CIN.[18,34,39,41] However, even small volumes (~30 ml) of contrast medium can have adverse effects on renal function in patients at particularly high risk.[37] As a general rule, the volume of contrast received should not exceed twice the baseline level of eGFR in milliliters.[32] This means that for patients with significant CKD, a diagnostic catheterization should be planned to apply <30 ml of contrast, and if followed by PCI then <100 ml should be a reasonable goal.

Regardless of the countermeasures applied to prevent CIN the most important issue remains the consideration of such complication after contrast angiography, early detection of kidney injury and development of nephropathy. Given that most patients are discharged on the next day after contrast procedure the real incidence and complication of CIN are probably greater and not well recognized. Because of this early diagnosis of CIN and taking prevention measurements are very important.

5. DIAGNOSTIC BIOMARKERS

In everyday practice, changes in SCr are used to estimate acute modifications in renal function and SCr monitoring remains the cornerstone for diagnosis of CIN. Unfortunately creatinine is a late and insensitive indicator of acute changes in renal function as there is a 24–48 hours delay between renal insult and SCr changes.[22]

Most physicians are aware of the limitations of serum creatinine for the detection of early CKD, which are related to patient-dependent factors such as sex, race, age, and muscular mass, as well as standardization of laboratory measurement of the biomarker. In addition serum creatinine concentrations do not reflect the true decrease in glomerular filtration rate (GFR) in the acute setting, since several hours or days must elapse before a new equilibrium between the presumably steady-state production and the decreased excretion of creatinine is established. Hence an increase of serum creatinine represents a late indication of a functional

change in GFR that underlies important structural changes which occur in the kidney during the early-damage stage of AKI.

Since levels of current biomarkers of kidney disease become elevated relatively late in the injury process, they do not allow early interventions that could be successful at preventing propagation of the injury. Early identification can motivate injury-specific interventions that currently are not possible. This could be particularly useful in AKI when a defined insult, for example, after contrast administration or cardiac surgery, may determine more appropriate risk stratification and treatment. Thus, novel biomarkers have a number of potential roles in nephrology (1) to more accurately diagnose AKI early in the course of the disease, (2) to correlate better with GFR in patients with CKD, (3) to determine the anatomical location of injury in patients with AKI (ie, glomerular, tubular, interstitial, or vascular), (4) to identify the cause of AKI and CKD, (5) to monitor the effectiveness of interventions, and (6) to provide information regarding the prognosis of AKI and CKD.

In interventional cardiology, early detection of CIN may suggest pre-discharge selection of outpatients needing hospitalisation for closer renal, metabolic and volaemic control. Recently, several promising biomarkers of tubular insult have been under investigation and the following plasmatic or urinary ones are of special interest. Table 1 summarises some of their characteristics relevant for the diagnosis of CIN.[52]

Neutrophil gelatinase-associated lipocalin (NGAL) is a small (25 kDa) protein expressed in renal tubular cells and released into the blood and urine after exposure to harmful stimuli such as ischemia or toxicity. The release of NGAL occurs within hours after the stimulus and long before an increase in serum creatinine level occurs (figure1). NGAL level has therefore been proposed to be real-time indicator of active kidney damage whereas creatinine level and GFR are markers of functional nephron number.[47]

Urinary and plasma NGAL levels have been shown to correlate with degree of kidney injury, and levels return to baseline on restitution after AKI. Interestingly, the source of plasma and urinary NGAL appears to be different. Most urinary NGAL is produced in tubules in response to injury, whereas most plasma NGAL originates from distant organs, where its production is upregulated under the setting of AKI. Commercial assays are available for NGAL measurement. Elevated NGAL level in the absence of elevated serum creatinine level may be prognostically significant and suggests that creatinine level may misclassify individuals with currently currently subclinical kidney disease. This is implicated by a meta-analysis that found that elevations in NGAL level even in the absence of an elevated creatinine level predicted worse outcomes. This finding suggests that further reappraisal in the way that we define AKI to incorporate these biomarkers may be necessary. Along with the potential role of NGAL in the diagnosis of AKI

Table 1.
Relevant characteristics of biomarkers for the diagnosis of CIN after PCI

Biomarkers	Molecular weight (kDa)	Site of lesion	Significant increase in CIN-patients	AUC for CIN-prediction (cut-off)
pNGAL	25	Distal and collector tubules	2 hours	0.92 (≥100 ng/ml at 2 hours)
pCysC	13	Glomerules and proximal tubule	8 hours	0.92 (≥ 10% increase at 24 hours)
uNGAL	25	Distal and collector tubules	2 hours	0.92 (≥100 ng/ml at 2 hours)
uIL-8	18	Distal tubule	8 hours	0.75 (≥25% increase at 24 hours)
uL-FABP	14	Proximal tubule	24 hours	NA
uβNAG	130	Proximal tubule	24 hours	NA

NGAL = neutrophil gelatinase-associated lipocalin; CysC = cystatin C; IL-18 = interleukin 18; L-FABP = liver fatty acid-binding protein; βNAG = n-acetyl-β-glucosaminidase; p = plasma; u = urinary[52]

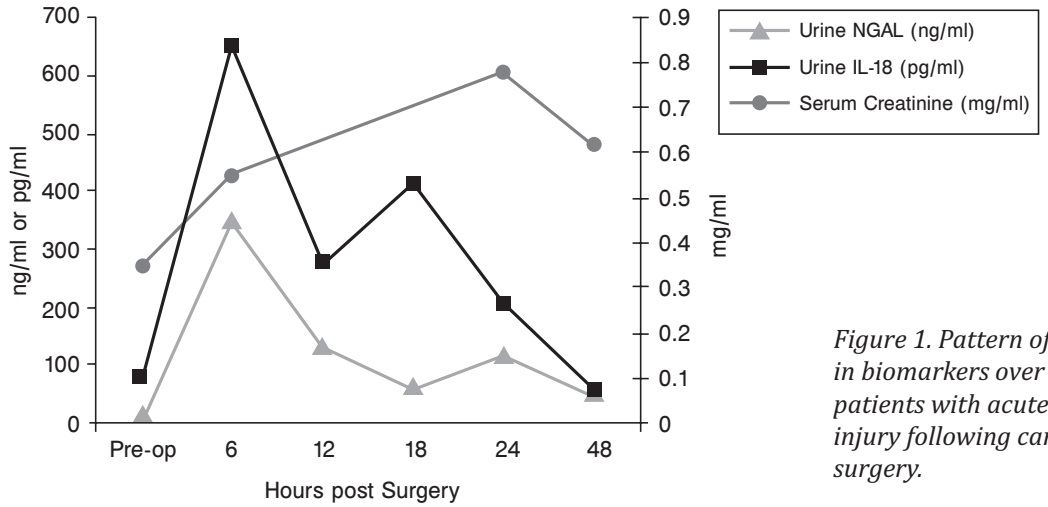

Figure 1. Pattern of change in biomarkers over time in patients with acute kidney injury following cardiac surgery.

Abbreviation: NGAL, neutrophil gelatinase- associated lipocalin. Conversion factor for units: serum creatinine in mg/dL to μmol/L, x 88.4. Data from Parikh CR, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am Soc Nephrol. 2011;22:1737–1747

it may also be considered a useful biomarker in patients with CKD, particularly for identifying patients at risk of a significant decrease in GFR, because of its ability to detect subtle changes in tubular function. Urinary and serum NGAL levels are elevated in a wide range of kidney diseases, including IgA nephropathy, autosomal dominant polycystic kidney disease, and diabetic nephropathy. Urinary NGAL level has been demonstrated to differentiate HIV (human immunodeficiency virus) nephropathy from other forms of kidney disease, whereas higher levels were associated with increased risk of progression in a diverse group of patients with CKD. AKI itself is a risk factor for future CKD, but there is no reliable means of determining who will recover entirely and who will be left with some kidney impairment after an episode of AKI. NGAL is a potential marker of future progression of kidney insufficiency. [40]

The role of NGAL as a specific, sensitive and early predictor of AKI after contrast media administration, septic shock, kidney transplantation and cardiac surgery has been investigated extensively in different clinical studies.[11,47] In several prospective studies in children who underwent elective cardiac surgery, AKI (defined as a 50% increase in serum creatinine) occurred 1–3 days after surgery.[45,51,53] By contrast, NGAL measurements by ELISA revealed a tenfold or more increase in the urine and plasma, within 2–6 h of the surgery, in those who subsequently developed AKI. Both urine and plasma NGAL were excellent independent predictors of AKI, with an area under the curve (AUC) of the ROC of over 0.9 for the 2–6 h urine and plasma NGAL measurements. These findings have now been confirmed in prospective studies of adults who developed AKI after cardiac surgery, and in whom urinary and/or plasma NGAL was significantly elevated by 1–3 h after the operation. [21,23,24,29, 64–66, 68]

In adults undergoing cardiopulmonary bypass, those who subsequently required renal replacement therapy were found to have the highest urine NGAL values soon after surgery.[21,23,24,29,64–66,68] Similar results were documented in the adult critical care setting.[6,14, 15,36,49,50,58] Collectively, the published studies revealed an overall AUC-ROC of 0.78 for the prediction of subsequent dialysis requirement, when NGAL was measured within 6 h of clinical contact.[20]

Furthermore, a number of studies conducted in the cardiac surgery and critical care populations have identified early NGAL measurements as a very good mortality marker [15,29,49,58,65,66] , with

an overall AUC-ROC of 0.71 in these heterogeneous populations.[9]

In addition, there is current evidence for the utility of subsequent NGAL measurements in critically ill adults with established AKI. Serum NGAL measured at the inception of renal replacement therapy was an independent predictor of 28-day mortality, with an AUC of 0.74.[31]

In prospective studies of adults or children receiving radiocontrast, urine and plasma NGAL predicted radiocontrast- induced nephropathy within 2 to 4 hours after radiocontrast administration, with an AUC of 0.91 to 0.92. [3–5, 26,35]

Several investigators have examined the role of NGAL as a predictive biomarker of AKI following contrast administration.[3–5, 26,35]

In a prospective study of children undergoing elective cardiac catheterization with contrast administration, both urine and plasma NGAL predicted contrast-induced nephropathy (defined as a 50% increase in serum creatinine from baseline) within 2 h after contrast administration, with an AUC-ROC of 0.91–0.92.[26] In several studies of adults administered contrast, an early rise in both urine (4 h) and plasma (2 h) NGAL were documented, in comparison with a much later increase in plasma cystatin C levels (8–24 h after contrast administration), providing further support for NGAL as an early biomarker of contrast nephropathy. [3–5,35] A recent meta-analysis revealed an overall AUC-ROC of 0.894 for prediction of AKI, when NGAL was measured within 6 h after contrast administration and AKI was defined as an increase in serum creatinine of over 25%. [20]

Haase et al. conducted meta-analysis of 19 studies involving 2538 patients who investigated prognostic value of NGAL to predict AKI initiation of renal replacement therapy (RRT), and in-hospital mortality. The best predictive performance of NGAL level was found in the setting of AKI after exposure to contrast agents (Diagnostic Odds Ratio (DOR), 92.0; AUC-ROC, 0.894). The specificity of NGAL level for prediction of AKI after exposure to contrast media was >95%, whereas it was ~75% after cardiac surgery and in critically ill patients at sensitivity of ~70%-75% in all AKI settings. The DOR and AUC-ROC of plasma/serum NGAL level (DOR, 17.9; AUC-ROC, 0.775) were similar to those of urine NGAL level (DOR, 18.6; AUC-ROC, 0.837) in the prediction ofAKI, with a slightly higher cutoff value for urine NGAL.[20] A recent meta-analysis found that elevated urinary or plasma NGAL level predicted the future need for renal replacement therapy and in-hospital mortality in critically ill patients in the intensive care unit, even in the absence of a clinically significant increase in creatinine level. This suggests that there is a spectrum of subclinical but significant AKI that is not being detected by the traditional gold-standard methods.

Haase et al. conducted a multicenter analysis of pooled data from 2,322 critically ill patients with predominantly cardiorenal syndrome from 10 prospective observational studies of NGAL to explore the prognostic value of AKI detected by NGAL. The authors found that a positive NGAL finding carried a similar risk of adverse outcome than a positive creatinine finding. They also found that NGAL(+)/sCREA(–) tests identified approximately 40% more AKI cases than sCREA(+) alone and that these patients were at greater risk of longer ICU and hospital stay, renal replacement therapy, and death compared with control subjects. As expected, NGAL(+)/sCREA(+) patients had the greatest risk of adverse outcomes. A smaller group of patients were NGAL(–)/sCREA(+), implying loss of renal function without evidence of acute tubular injury. Outcome of these patients was intermediate in severity. Their study suggests that acute tubular damage might occur without detectable loss of excretory function (and vice versa) and might predict worse clinical outcome and that NGAL and serum creatinine reflect distinct pathophysiological events.[22]

Finally according mentioned above meta-analysis, NGAL detected approximately 40% of patients with probable AKI who were missed by consensus criteria. This proportion is similar to that

identified by troponin in subjects with myocardial injury missed by conventional biomarkers.[56]

A changed view of AKI might change clinical practice and its treatment. Detection of elevated NGAL might enable more rapid conventional interventions or introduction of novel therapies to prevent or effectively treat such otherwise undetected AKI. [56]

Novel renal biomarkers might facilitate standardization of early diagnosis and treatment. By contrast, a normal NGAL result might inform clinical decisionmaking and lead to improved use of hospital resources.

The approach of using NGAL as a trigger to initiate and monitor novel therapies, and as a safety biomarker when using potentially nephrotoxic agents, is expected to increase. It is also hoped that the use of predictive and sensitive biomarkers, such as NGAL, as end points in clinical trials will result in a reduction in required sample sizes, and hence, the cost incurred.

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Estrogen receptor alpha polymorphismS in bulgarian patients with pOLICYSTIC OVARY SYNDROM and healthy controls

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Полиморфизми на естрогенния рецептор алфа при български пациентки със синдром на поликистозните яйчници и здрави жени

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РЕЗЮМЕ

Синдромът на поликистозните яйчници (СПЯ) е комплексно ендокринно заболяване с висока честота сред жените в репродуктивна възраст. Генетичните фактори имат съществено значение за етиологията на синдрома, но влиянието на полиморфизмите на стероидните рецептори не е изяснено до момента. Естрогенният рецептор алфа (ЕРα) е от изключителна важност за нормалната репродуктивна функция, поради което настоящото проучване си постави за цел да изследва връзката между полиморфизмите на ЕРα *Pvu*II и *Xba*I и развитието на СПЯ сред български паци-

ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex endocrine condition with very high prevalence. Genetic factors play a crucial role in the aetiology of PCOS, but the influence of the steroid receptor polymorphisms remains unknown. Considering the potential important effects of the ERα polymorphisms on the reproduction, the present study aimed to investigate the relationships between PvuII and XbaI and the PCOS development and phenotype expression among Bulgarian women.

The PvuII and XbaI polymorphisms were investigated in 58 patients with polycystic ovarian syn-

ентки. Полиморфизмите *Pvu*II (rs2234693) и *Xba*I (rs9340799) бяха изследвани при 58 пациентки със синдром на поликистозните яйчници и 56 клинично здрави жени с редовен менструален цикъл.

Честотното разпределение на двата полиморфизма не се различаваше значимо между групите на болните със СПЯ и здравите контроли, въпреки че *Pvu*II PP носители се срещаха по-рядко сред изследваните пациентки. Носителите на PvuII pp генотип бяха със значително по-високи нива на дехидроепиандростерон сулфат в сравнение с пациентките с Pp генотип (p=0.033). Комбинираният ppxx генотип беше свързан със значимо по-високи концентрации на лутеинизиращия хормон в сравнение с PpXx генотипа (p=0.043). В заключение проучването показа, че XbaI и PvuII полиморфизмите на ERα могат да модулират фенотипната изява на синдрома на поликистозните яйчници.

Ключови думи: синдром на поликистозните яйчници, естрогенен рецептор алфа, PvuII, XbaI полиморфизъм.

drome according to the ESHRE/ASRM criteria. The control group consisted of 56 clinically healthy women with regular menstrual cycle.

The distribution of estrogen receptor alpha PvuII and XbaI polymorphisms did not differ significantly between patients and controls, although the presence of PP genotype was lower in the PCOS group. The PvuII pp genotype carriers had significantly higher levels of DHEAS in comparison to Pp carriers (p=0.033). The X allele carriers were significantly taller than women with xx genotype (p=0.042). Combined ppxx genotype was related with significantly higher levels of LH (p=0.043) in comparison to PpXx genotype.

The study showed that XbaI и PvuII polymorphisms could modulate the height, gonadotropin and androgen levels in PCOS patients. Further studies are needed to reveal the potential mechanisms and the precise role of the ERα polymorphisms for the development, clinical characteristics and treatment of women with PCOS.

Key words: PCOS, estrogen receptor, PvuII, XbaI polymorphism.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine condition with high prevalence among women of reproductive age. Despite the serious reproductive and metabolic consequences of the syndrome, the aetiology of PCOS remains uncertain (4). Most investigations have been focused on ovarian disturbances, hypothalamic-pituitary disorders and primary defect of insulin action (17). However, large epidemiological twin-family studies have demonstrated a strong influence of inherited factors on the pathogenesis of PCOS (24). Numerous predisposing genes have been identified as important modulators that could interact with environmental and lifestyle factors to produce the phenotype expression of PCOS (10). The genes encoding steroid receptors are logical target of interest, but their role for the development of PCOS is still not clarified.

Estrogens exert crucial effects on the female reproductive system through nuclear estrogen receptors alpha and beta (ERα and ERβ, respectively). They belong to the steroid/thyroid hormone superfamily and are composed of three independent functional domains: the regulating amino-terminal, the DNA-binding, and the ligand-binding domain (19). Both estrogen receptors are expressed in ovary but in different cell types, e.g. ERα is mainly expressed in theca cells, while ERβ is found in granulosa cells (7). The functional significance of the ERα has been proved by the ERα knockout mice models (αERKO). The lack of ERα does not affect substantially the ovarian differentiation, but the ovaries of adult ERα knockout mice are characterized by hemorrhagic, cystic follicles with no signs of ovulation (25). Moreover, significant alterations in the expression of ERα and ERβ protein in PCOS patients compared to healthy controls have been established (15). These data show the importance of ERα for the normal ovarian function and its possible role for the development of polycystic ovaries and chronic anovulation. However, it is still not clear, if the polymorphisms of the estrogen receptor alpha could modulate the susceptibility to PCOS.

Several single nucleotide polymorphisms (SNPs) have been identified on ERS1 gene and some of them are associated with an increased or decreased risk of various diseases. The best characterized SNPs are the rs2234693 (c.453–397T>C) and rs9340799 (c.453–351A>G) – both located in the first intron of the gene, 397 and 351 bp upstream of exon 2, respectively. These polymorphisms are more popular with the names of detecting restriction enzyme – PvuII and XbaI, respectively and have been associated with different estrogen related diseases such as breast cancer, endometriosis and leiomyoma, as well as osteoporosis (3, 13, 19). Moreover, PvuII and XbaI polymorphisms have been related with fertility in populations with different reproductive patterns (5). Considering the potential modulating effects of the ERα polymorphisms on female reproduction the present study aimed to investigate the relationships between *Pvu*II and *Xba*I and the PCOS development and phenotype expression among Bulgarian women.

MATERIALS AND METHODS

Subjects and study protocol

One hundred and fourteen women (31.05±9.27 years [29]) were included in the study. Fifty-eight patients with polycystic ovarian syndrome were recruited from the Clinical center of endocrinology and gerontology. They fulfilled the ESHRE/ASRM (European Society of Human Reproduction and Endocrinology and the American Society for Reproductive Medicine) criteria for PCOS by having two of the following three features: i) oligo- or anovulation, ii) clinical and/or biochemical signs of hyperandrogenism, or iii) polycystic ovaries (8). Prolactinoma, untreated thyroid diseases, Cushing's syndrome, adrenal hyperplasia or androgen-producing tumor were excluded in all of them.

The patients underwent a complete general assessment, including height, weight, BMI and presence of hirsutism according to the modified Ferriman-Gallwey score. Hormonal values (total testosterone – T, luteinizing hormone – LH, follicle-stimulating hormone – FSH, dehydroepiandrosterone-sulphate – DHEAS) as well as pelvic ultrasonography data were obtained from the patients' files.

The control group consisted of 56 clinically healthy women with history of regular menstrual cycle. The study was approved by the institutional ethic commission and written informed consent was obtained from all participants.

Genetic analysis

All patients and controls provided blood samples for genetic analysis. The genomic DNA was extracted from venous blood by salt extraction method. The patients and controls were genotyped for estrogen receptor alpha polymorphisms PvuII T/C (rs2234693) and XbaI A/G (rs9340799) through restriction fragment length polymorphism /PCR-RFLP/ analysis according to the protocol described by Jakimiuk et al. (14). The absence of PvuII and XbaI restriction sites was described as „P" and „X" and represented C and G allele respectively. The presence of restriction sites was marked as „p" and „x" determining T and A alleles, respectively. The genotype distribution of both polymorphisms in patients and controls was in agreement with the Hardy-Weinberg equilibrium ($p>0.05$).

Statistical analysis

All results were expressed as mean ± S.D [median]. Differences between dichotomous variables were tested with Chi-square and Fisher's exact test. Differences between two groups were established with independent sample t-test or Mann-Whitney test after the Kolmogorov–Smirnov test for normality of the distribution. Binary logistic regression was used where appropriate. All results were considered significant at the 0.05 level. Statistical analysis was conducted through SPSS v. 11 for Windows (SPSS, Chicago, IL, USA).

RESULTS

The distribution of estrogen receptor alpha PvuII polymorphism did not differ significantly between patients and controls (Table 1), although the presence of PP genotype was lower in the PCOS group. The prevalence of estrogen receptor alpha XbaI polymorphism also did not differ between patients and controls (Table 2).

Patients with PCOS were significantly younger than controls (26.21±5.26 [26.0] years vs. 36.07±9.87 years [34]; p<0.001). After adjustment for age the PP genotype was negatively related with the development of PCOS (OR 0.169, 95%CI 0.043–0.671; p=0.011), while no association with the XX genotype was found (OR 0.289, 95%CI 0.080–1.037; p=0.057).

Both polymorphisms were significantly related (r=+0.826, p<0.001) and the distribution of the combined genotypes was shown on figure 1. No significant differences in the genotype prevalence between patients and controls were established (p=0.262).

The role of PvuII and XbaI polymorphisms was investigated in the PCOS group. The PvuII pp genotype carriers had significantly higher levels of DHEAS in comparison to Pp carriers (11.39±5.29 [10.20] μmol/l vs. 8.53±3.99 μmol/l

Table 1. ER alpha PvuII polymorphism in patients with PCOS (n=58) and healthy controls (n=56).

	Healthy women		Women with PCOS		P
	N	%	N	%	
Pp	22	39.3	24	41.4	0.334
Pp	24	42.9	29	50.0	
PP	10	17.9	5	8.6	
pp vs. Pp and PP	22	39.3	24	41.4	0.850
PP vs. Pp and pp	10	17.9	5	8.6	0.173
Total	56		58		

Table 2. ER alpha XbaI polymorphism in patients with PCOS (n=58) and healthy controls (n=56).

	Healthy women		Women with PCOS		P
	N	%	N	%	
Xx	18	32.1	27	46.6	0.230
Xx	28	50.0	25	43.1	
XX	10	17.9	6	10.3	
xx vs. Xx and XX	18	52.1	27	46.6	0.289
XX vs. Xx and xx	10	17.9	6	10.3	0.434
Total	56		58		

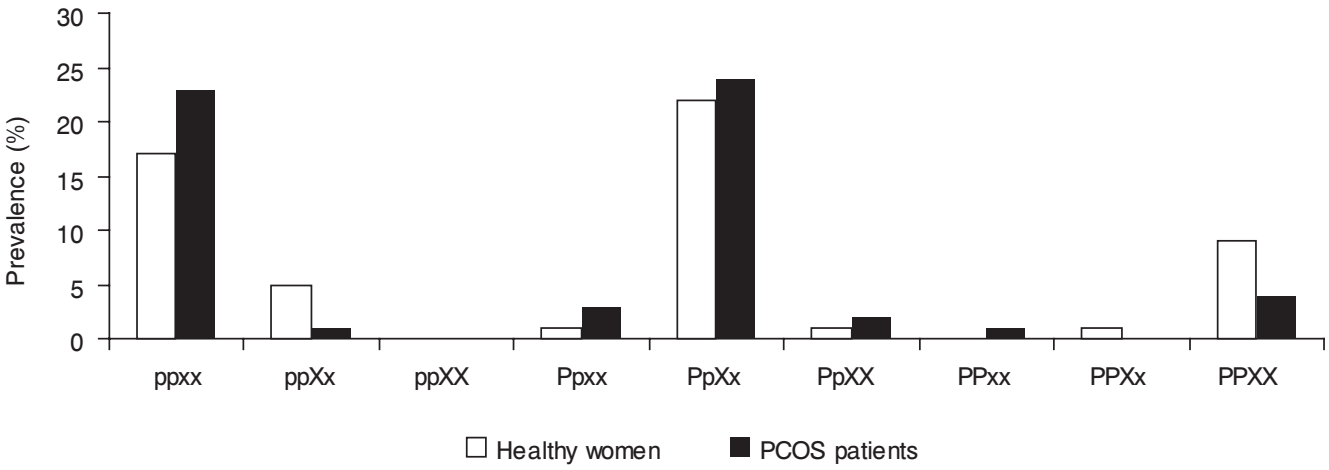

Figure 1.
ER alpha PvuII and XbaI polymorphism in patients with PCOS (n=58) and healthy controls (n=56).

[7.55], p=0.033), while no significant differences in the age, BMI, FSH, testosterone and the presence of hirsutism were established (p>0.05). The LH levels tended to be lower in Pp carriers compared to women with genotype pp (p=0.068). The XbaI xx genotype carriers had higher levels of LH (7.17±6.30 [4.70] U/l vs. 3.73±1.90 U/l [3.25], p=0.058) and LH to FSH ratio (1.35±1.47 [0.98] vs. 0.74±0.41 [0.63], p=0.050) in comparison to Xx carriers, but the differences did not reach statistical significance. The XbaI polymorphism was not related to changes in other investigated hormones (p>0.05). X allele carriers were significantly taller than women with xx genotype (167.39±5.54 cm [168.00] vs. 164.22±6.07 cm [163.00], p=0.042).

The combined ppxx genotype was related with significantly higher levels of LH (7.54±6.62 [5.00] U/l vs. 3.77±1.93 U/l [3.30], p=0.043) in comparison to PpXx genotype despite the similar age and BMI of the patients (p>0.05). Differences in DHEAS levels (11.54±5.37 [10.60] vs. 8.69±4.15 [7.60], p=0.052) and LH to FSH ratio (1.47±1.63 [0.98] vs. 0.74±0.42 [0.63], p=0.055) were not statistically significant. FSH or testosterone levels were similar in the investigated groups. The PP, XX and PPXX genotypes were not separately investigated in the PCOS group because of their low prevalence.

DISCUSSION

No significant differences in PvuII and XbaI polymorphisms between Bulgarian patients with PCOS and healthy controls were found, as in previous studies conducted in other populations (18, 23). In a large study, Valkenburg et al. compared 518 PCOS patients (median age 27.8 years) with significantly older group of 2996 population based controls (all over 55 years). They did not found significant differences in the PvuII and XbaI genotype distribution in both groups. The age difference was not regarded as a possible confounder, because the presence of ERα polymorphisms could not shorten life-span leading to a stable prevalence of PvuII and XbaI in the subsequent generations (23). Nevertheless, after adjustment for age PP genotype was associated with decreased risk for PCOS development in the investigated group. Definitive conclusions could not be made, because of the low prevalence of PP carriers, but future larger studies would be of clinical importance. Women carrying px haplotype were shown to be at an increased risk of reproductive loss (20). However, recent study of tissue samples revealed that ppxx genotype could be protective against miscarriage for the fetus (2). Thus, is still not clear, whether the prevalence of ER polymorphisms could differ in the subsequent generations.

In our study the XbaI X allele was significantly related with the height of PCOS patients. Similar associations were already obtained in healthy adolescents as well as in large population-based studies of pre- and postmenopausal women (16, 22).

A significant association between ERα PvuII polymorphism and DHEAS levels in PCOS women was found, while the testosterone levels did not differ between groups. In a large population-based study of postmenopausal women no associations between PvuII-XbaI haplotype and androgen levels were established (21), while in Greek PCOS patients the presence of PP genotype was related to slightly increased testosterone levels in comparison to pp genotype carriers, although the difference did not reach statistical significance (18).

The ppxx genotype carriers had significantly higher levels of LH and increased LH to FSH ratio in comparison to PpXx heterozygous patients despite the similar age and BMI, while no differences in other reproductive hormones between two groups were found. Our results showed that ERα polymorphism could modulate the gonadotrophin secretion in PCOS patients. Studies with αERKO mice models have shown that disruption of ERα could lead to significantly elevated LH levels due to impaired central negative feedback. Increased LH concentrations have been considered as a major cause of the observed polycystic phenotype and enhanced steroidogenic capacity in the αERKO mice ovary (6, 25). In infertile

women undergoing controlled ovarian hyperstimulation the ERα polymorphism have been related to different number of mature follicles, different number of obtained oocytes, and different FSH doses required to get gut-quality embryo (1). A Greek study has shown that XbaI polymorphism could modulate the FSH levels in PCOS women (18). In opposite, Valkenburg et al. did not find any associations between PCOS features and ERα polymorphisms (23). The differences between the studies could be explained with different inclusion criteria, different ethnicity of the participants or other unknown reasons. Ethnic affiliation is an important factor that could modulate the phenotype of the PCOS because of genetic or environmental causes. For instance, androstenedione levels were higher, and LH levels were lower in Caucasian Icelandic compared with Boston women with PCOS (26). Significant differences in the androgen levels were found even in healthy Caucasian women from different ethnic groups (11). Consequently, the conclusions about the relationships between PCOS phenotype and genetic polymorphisms should not be directly transmitted from one ethnic population to another, but should be based on the appropriate studies.

Pathophysiological mechanisms modulating the activity of ERα are still unknown. According to Herrington et al. the presence of PvuII P allele could produce a transcription factor binding site amplifying estrogen receptor transcription. Thus, PvuII polymorphism could be related with different estrogen response in cells expressing B-myb or other related transcription factors (12). Additionally, the ERα polymorphisms in intron I may be in linkage disequilibrium with causal polymorphisms elsewhere in the same gene (9).

Here we report the results from a study focused on the role of two estrogen receptor alpha polymorphisms in a cohort of Bulgarian PCOS patients. The main limitation of the study was the relatively small number of participants. Nevertheless, our preliminary data showed that XbaI and PvuII could modulate the LH and androgen levels in PCOS patients. Further studies are needed to reveal the potential mechanisms and the precise role of the ERα polymorphisms for the development, clinical characteristics and treatment of women with PCOS.

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Erectile and endothelial dysfunction in men treated with statins

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Еректилна и ендотелна дисфункция при мъже, лекувани със статини

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РЕЗЮМЕ

Еректилната дисфункция (ЕД) е резултат от сложни взаимодействия между съдови, неврологични и хормонални фактори. Рисков фактор за ЕД са ранните съдово-ендотелни увреждания от атеросклеротичен тип. Нарушената синтеза на азотен оксид (NO) и последващото увреждане на ендотел-зависимата вазодилатация е общият етиопатогенетичен механизъм на еректилната и ендотелната дисфункция. Затова ЕД се разглежда като предиктор на все още неизявила се съдова болест. ЕД може да предшества клиничните признаци на коронарните и каротидните съдови увреждания, защото диаметърът на артериите на пениса е по-малък (1–2 mm) от диаметъра на коронарните (3–4mm) и каротидните артерии (5–7 mm), което означава, че симптомите на атеросклероза ще се появят най-рано в пенилните артерии.

Все още няма надежден лабораторен тест за ранна оценка на ЕД. Маркерите, използвани за определяне на ендотелната дисфункция, биха могли да се използват като ранни предиктори и

ABSTRACT

Erectile dysfunction (ED) is a result of complex interactions between vascular, neurological and hormonal factors. Endothelial dysfunction is a risk factor for ED. Both erectile and endothelial dysfunction share common ethiopathogenic mechanism of impaired nitric oxide (NO) synthesis, which leads to damage of endothelium-dependent vasodilation . So the ED can be discussed as a predictor of cardiovascular disease. ED can occur before any other clinical signs of cardiovascular diseases because the diameter of the penile arteries is a smaller (1–2 mm) than the diameter of the coronaries (3–4mm) and the carotid artery (5–7 mm), which means that the symptoms of atherosclerosis will occur earlier in penile arteries.

There are still no reliable laboratory tests for early assessment of ED.The markers used for assessment of endothelial dysfunction, may be used as an early predictor of ED. Measurement of endothelin-1,as the most powerful endogenous vasoconstrictor, asymmetric dimethyl L-arginine as an endogenous competitive antagonist of NO-synthase,C

на ЕД. Измерването на ендотелин-1, който е най-мощният ендогенен вазоконстриктор, на асиметричният диметил L-аргинин, който е ендогенен конкурентен антагонист на ензима NO-синтаза, на С реактивният протеин (CRP), като възпалителен маркер, на инхибиторът на плазминоген активатора (PAI-I), като протромботичен маркер при мъже с ЕД би дало информация за наличие на скрито съдово-ендотелно увреждане.

Положителният ефект на статините върху съдовия ендотел, чрез увеличаване на нивото на NO и чрез намаляване на равнището на възпалителните медиатори, би намерил място и за лечение на ЕД. Съществува научен спор относно ползите и рисковете на статините върху ЕД. Статините, блокирайки действието на ключов ензим в синтезата на холестерола, биха довели до промяна в концентрацията на тестостерона. Дали след лечение със статини ще се намали концентрацията на тестостерона и това ще влоши еректилната функция или подобряването на ендотелната дисфункция ще подобри и ЕД е въпрос без точен отговор.

Този обзор обобщава ползите и някой спорни проблеми на статиновата терапия при мъже с ЕД.

Ключови думи: Еректилна дисфунция, ендотелна дисфункция,статини,тестостерон

reactive protein(CRP), as an inflammatory marker, and plasminogen activator proliferator(PAI-I), as a prothrombotic marker in ED patients, would provide an information for existence of latent vascular endothelial damage.

The positive effect of the statins on endothelial dysfunction by increasing the level of NO and by reducing the level of inflammatory mediators, could find a place for treatment of ED. There is a scientific debate about risks and benefits of statins on ED.Statins,by blocking a key enzyme in cholesterol synthesis, could lead to a change in testosterone concentration.Whether statin therapy will reduce testosterone concentration and impair erectile function or improvement of endothelial dysfunction will cure ED, is still a question without an accurate answer.

This review summarizes the benefits and some controversial points of statin therapy in men with ED.

Key words: Erectile dysfunction,endothelial dysf unction,statins,testosterone

INTRODUCTION

Erectile dysfunction (ED) is an inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse. It is estimated that approximately 5 % of men at 40 years age and 15 % of men at 70 years age have ED. Some degree of ED have approximately 50% of all men between the ages 40–70 years [15].

From the etiological point of view ED has psychogenic and organic causes. Among the main organic etiologic factors are: diabetes mellitus, hypertension, hypercholesterolemia, atherosclerosis, cardiovascular disease, and ect. ED is most often the final result of complex interactions between vascular, neurological and hormonal factors. Achieving and maintaining an erection requires an adequate arterial blood flow and venous outflow.

It seems that a vascular endothelium is a focus for understanding pathophysiological mechanisms of ED. Vascular endothelial dysfunction (VED) is a state of impaired balance between vasoconstriction and vasodilation, evidenced by the reduced production of nitric oxide (NO) and increased levels of free oxygen radicals.

PHYSIOLOGY OF THE ERECTION

In the absence of sexual stimulation penis is in a relaxed state, which is determined by the sympathetic part of thoracic – lumbar spinal cord. Under the influence of norepinephrine released by adrenergic nerve fibers occurs tonic contraction of the cavernosal smooth muscle and vasculature, resulting in a relaxed state of the penis. Norepinephrine also inhibits the release of

nitric oxide (NO), stopping further mechanism of erection. In the presence of sexual stimulation there is an activation of parasympathetic nerves from the lumbar spinal cord. The prevalence of this parasympathetic activity leads to erection. The primary neurotransmitter for excitation of the male penis is NO. NO is formed from the amino acid L- arginine in the participation of androgen dependent enzyme NO synthase (NO-S). NO is a main mediator released from neurons innervating the corpus cavernosum and basic vasorelaxing mediator released from the endothelium. NO has a vasodilating activity, leading to relaxation of the arteries and arterioles supplying blood to erectile tissue and increased blood flow in the penis [3]. NO as a first messenger activates membrane bound enzyme guanylate cyclase, causing conformational changes. Under the action of this enzyme from guanosine triphosphate cyclic guanosine monophosphate (GMP) is formed. Cyclical GMP as a second messenger activates a specific protein kinase, which closes the calcium channels and stops the intracellular calcium influx [37]. Increased intracellular level of cyclical GMP results in a decrease in intracellular calcium concentration and relaxation of smooth muscle in the corpus cavernosum, active dilatation of penile arteries, arterioles and sinusoids, enhancing the arterial flow and enhancing passive compression of the venous outflow.

Cessation of sexual stimulation leads to recovery of sympathetic tone and degradation of cyclical GMP, mainly from PDE- 5 in trabecular smooth muscle.

ERECTILE AND VASCULAR ENDOTHELIAL DYSFUNCTION

*Erectile and endothelial dysfunction **s**hare common ethiopathogenic mechanism of impaired NO synthesis, which leads to damage of endothelium-dependent vasodilation. The first step of this process is characterised by inability of arterial vessel to relax by NO. Although, all arterial vessels are included in this process, the impaired NO-mediated vasodilatation occurs in smaller arteries earlier [22**]**. ED appears when the atherosclerotic process in the relatively larger coronaries, carotid as well as femoral arteries is still silent **[**32].*

Some studies in the past concluded that ED was common in male populations with coronary heart disease, diabetes and hypertension, and is a late consequence of atherosclerotic arterial disease [2,18]. In contrast, current evidence demonstrated that ED is an early predictor of cardiovascular coronary disease [21, 36]. If there are cardiovascular risk factors such as diabetes, dyslipidemia, hypertension, or oxidative stress, the initial phase of atherosclerotic process starts earlier in the smaller blood vessels [35]. The first step is characterized by low levels of vasodilators and impairment of endothelium-dependent vasodilatation. In this early phase there is normal anatomy of the vascular wall. Then in the preclinical phase, an atherosclerotic plaque is formed. At this stage there is a compensatory enlargement of the lumen of the vessel. Positive remodeling continues until obstruction of the lumen exceeds 40%. After then plaque narrows the lumen of the artery. This is the beginning of the late phase of atherosclerosis [4]. Focusing on these facts it is easily to understand why ED can occur before any other clinical signs of cardiovascular diseases. The diameter of the penile arteries is smaller (1–2 mm) than the diameter of the coronaries (3–4mm) and the carotid artery (5–7 mm), which means that the symptoms of atherosclerosis will occur earlier in the penis. Plaque, which causes the stenosis only 30–40% of the diameter of the artery in the penis is sufficient to cause ED [32]. Stabilization of nonobstructive atherosclerotic plaques and improvement of endothelial vasomotor response is an essential part of treatment of endothelial dysfunction [1].

Some studies reported that the severity of ED increases with increasing number of risk factors [28]. All these risk factors are characterised by impaired endothelial function, indicating a key meaning of endothelial dysfunction in the pathogenesis of systemic vascular disease including ED [36].

Several non-invasive diagnostic tools for investigating endothelial dysfunction are used. Flow mediated dilation of the brachial artery by ultrasounds is the most widely used vascular test to assess endothelium- dependent vasodilatation [21]. Systemic markers for measurement of NO biology, inflammatory cytokines, adhesion molecules have a limited role in the assessment of endothelium dysfunction [16].

Endothelin-1, as endogenous vasoconstrictor, is the best predictor of ED [5]. Asymmetric dimethyl L-arginine (ADMA) is an endogenous competitive antagonist of NO-synthase. Elevated plasma ADMA levels potentiate the mechanism of endothelial dysfunction. Patients with ED have significantly higher ADMA levels than men with normal endothelial function [9,13]. Low-grade subclinical inflammation is an additional risk factor to an endothelial damage in all stages of the atherosclerotic process. High sensitivity C reactive protein, as an inflammatory marker, and plasminogen activator inhibitor-I (PAI-I), as a prothrombotic marker in ED patients show significant elevations [20].

Erectile function is evaluated through International Index of Erectile Function (IIEF) questionnaire [24].

STATINS AND TESTOSTERONE LEVELS

One interesting and controversial question is the effect of statin therapy to ED[23]. The first aspect is the change of the testosterone concentration as a result of statin' therapy [35]. Statins inhibit the rate-limiting step of cholesterol synthesis (HMG-CoA reductase) and probably may inhibit the synthesis of steroid hormones derived from cholesterol, including tetosterone. There is evidence that simvastatin causes minor changes in circulating androgen concentrations in asymptomatic men[30]. Stanworth et al.compared testosterone levels and hypogonadal symptoms with statins use in a study of 355 men with type 2 diabetes mellitus. They found that the decrease in LDL-cholesterol, which is a major substrate for the synthesis of steroid hormones, is followed by a significant reduction in serum concentrations of testosterone in males treated with atorvastatin while the therapy with simvastatin is not followed by a significant reduction of total testosterone. Men treated with simvastatin did not have significantly different total bioavailable or free testosterone, or SHBG levels than untreated men. In contrast, men treated with atorvastatin had an average total testosterone level 1,96 nmol/l less than that in untreated men. Lowering the testosterone level in the course of statin therapy leads to ED [29]. The authors found that statins were one of the reasons for a high prevalence of hypogonadism in men with type 2 diabetes. Men treated with more than 20 mg / day atorvastatin had an average total testosterone level of only 9,6 nmol/l, which is 3,8nmol/l lower than that of men not treated with statins. International guidelines have suggested that symptomatic men with total testosterone levels below 8 nmol/l are hypogonadal [18].

Corona and colleagues evaluated 3,484 men average age 51, with complaints of sexual disfunction[10]. Seven percent of them were being treated with statins for their high cholesterol levels. Most often used statin was simvastatin or atorvastatin. The researchers calculate the men's total cholesterol as well as free testosterone. When they compared men on statins to those not, the men on statins were twice as likely to have low testosterone levels. The reason could be that, statins' inhibition of cholesterol synthesis may interfere with the production of testosterone, which depends on a supply of cholesterol [10].

Studies in rodents have shown that the effect of statins on testosterone production is dose-dependent [6]. Clinical studies in humans have found conflicting results [8, 12]. The majority of them show a lack of effect on testosterone level, but one study shows reduction of testosterone during the statin therapy [25]. In the efforts to explain this phenomenon prevails the hypothesis that statins therapy reduces total testosterone without reducing free testosterone. This decrease is due to reduced synthesis of sex hormone binding globulin (SHBG) [25].

Sniderman et al. evaluate 501 men with hypercholesterolemia and 368 women with polycystic ovary syndrome in meta-analysis[26]. The authors note that statins reduce androgens, by – 0,66 nmol/l for men and by -0,4 nmol/l for women. Overall statins lowered testosterone by -0,44nmol/l. The average changes are small, the range of normal values for testosterone wide , and there is no clear relation between testosterone concentration and sexual drive and function [26]. While the average changes may be small, the range of changes in individuals, potentially, might be much more substantial.

In a meta-analysis Schooling et al. compare simvastatin 80 mg day with 40 mg day among 640 men , and found median testosterone lower by 10,3 % and 7,5% after 48 weeks, suggesting a possible dose response of statins on testosterone [25]. ED is a rare side effect of statins, perhaps because statins' have beneficial effects on cardiovascular function that would counteract changes on magnitude in testosterone. But if erectile dysfunction occur with statins use, it is reversible by statins withdrawal.

In Spain and France, seventy five cases of impotence associated with statins were identified. About 90 % of men recovered potency on statins withdrawal, and in some cases impotence appeared again on re-challenge [7].

In Holland eight men were identified with decreased libido shortly after starting statins, and in two in those in whom testosterone was measured there was a large decrease in serum testosterone while on statins, with recovery of testosterone levels on stopping statins [11].

STATINS AND ERECTILE DYSFUNCTION

Solomon et al recruit 80 men for completing an IIEF before starting statins[27]. Their mean age was 61 years, and they had high rates of smoking, ischemic heart disease, diabetes, and most of them were receiving antiplatelet therapy. Before staring statins, the median IIEF score was 21out of maximum of 25, and 43 %of men had scores above 21, indicating no erectile dysfunction. After starting statins the median IIEF score fell to 6,5 and the percentage without ED fell to 21 %.Over half of men had a fall in 5 or more points on IIEF with statins , and 22 % experienced new onset ED [27].

ED is more common in men with cardiovascular risk factors, and some small studies indicate that statins can help[34]. One was small , but randomized and double blind comparison of atorvastatin and placebo in 12 men with ED and who were taking sildenafil [17]. After six weeks of statin, but not placebo therapy, the IIEF score increased with sildenafil, and all men on atorvastatin had improved confidence in obtaining and keeping an erection.

El-Sisi et al. recruited to their study 60 men between 40 and 60 years old from Sexual Health Inventory for Men, who had all had ED for at least one year[14]. They also had normal cholesterol levels. The men were randomly split into three groups consisting of 20 men in each group and assigned to the following treatment for six weeks: the first group: 80 mg atorvastatin daily, the second group: 400 IU Vitamin E daily and the third group: placebo capsules daily. Several assessment: IIEF, Rigi Scan and some biochemical markers-nitric oxide; C-reactive protein; intrleukin -6 (IL-6); endothelial nitric oxide syntetaze activity (e-NOS) were done before treatment, after treatment and every two weeks during treatment. After six weeks of treatment, only the group receiving atorvastatin showed a significant improvement from baseline in subjective and some objective assessments on Rigi Scan of erectile function. The subjective score in atorvastain group increased from a baseline average of 11,75 to 18,15 (IIEF) after six weeks. However, despite this improvement none of the men in atorvastatin group had an erectile function score within the normal range at the six –week mark (according IIEF a score less than 22 was considered to indicate erectile dysfunction). Atorvastatin has anti-inflammatory activity, decreases CRP and IL-6 and enhances e-NO S activity, increasing NO production and improving erectile function. Testosterone level for hypogonadal participants was also evaluated at the end of the study, and testosterone deficiency was still present. Andro-

gen replacement therapy was not starting during the study period. This ensured that any improvement was directly related to the improvement of endothelial function by drug therapy and not by androgen therapy [14].

CONCLUSION

Statin therapy may inhibit the synthesis of steroid hormones derived from cholesterol, including tetosterone.

Whether statin therapy will reduce the concentration of testosterone and will worsen erectile function is still unknown. Justify the statistical significance of the two hypothesis whether statins improve erectile dysfunction by improving endothelial function or statins impair erectile function by reducing the testosterone level will clarify one social problem.

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НАУЧНО-ИЗСЛЕДОВАТЕЛСКИ ПРОЕКТ № 10/2014,
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Omental hydatid cyst misdiagnosed as uterus myoma

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Оментална ехинококова киста, погрешно диагностицирана като тумор на матката

Евг. Аструков
Сити Клиник, София

РЕЗЮМЕ

Ехинококът, това най-често паризитно заболяване, при хора развива кисти разположени в различни части на тялото. При деца само в 1% от случаите е ангажиран оментума. Не намерих данни за това колко често се ангажира само оментума при възрастни. В тази статия се разглежда случай на солитарно ангажиране на голямото було от ехинококови кисти погрешно диагностицирани като тумор на матката. Прави се предложение за начина за поставяне на точна диагноза предоперативно.

ABSTRACT

Echinococcosis, one of the most frequent parasitic diseases, gives rise to cysts localized in different parts of the body in humans. In children only in 1% of cases the omentum is involved. I found no data about solitary involvement of the great epiploon in adults. In the article we discuss a case of hydatid cysts affecting only the omentum misdiagnosed as tumor of the uterus. Ways for correct preoperative diagnose are proposed.

Echinococcosis, one of the most frequent parasitic diseases, is still socially significant. After it went down for more than a decade we meet it more and more frequently with the political, social and economic crisis. In humans, as an intermediate host, it gives rise to cysts localized in different parts of the body. The most common site is the liver (59–75%), followed in frequency by lung (27%), kidney (3%), bone (1–4%) and brain (1–2%). [by Yuksel M. et al (1)]. Very rarely affected are the heart, spleen, pancreas and muscles. There are only a few cases when the cyst(s) are localized only in the omentum, adrenal, ovary, peritoneal cavity, mediastinum or retro peritoneum. According to the data of Durakbasa CU. et al (2) in children only in 1% of cases the omentum is involved. The authors do not specify whether or

not it was only the omentum affected or it was a multilocalised desease. When the lesions are into the peritoneal cavity the omentum is more often affected. Hydatid cysts of the omentum are complicated by rupture (4, 5), primary torsion (3, 6) or fistula in the bladder (7). Diagnose is made by ultrasound and/or CT. The lesion is not looked for because it is very rare and doctors/surgeons do not think about it. Here we discuss a case of hydatid cysts affecting only the omentum misdiagnosed as tumor of the uterus.

N.A.M., a 50 years old woman was admitted to the hospital with abdominal pain, more intense in the part distally of the umbilicus, not well localized. She suffered this pain for 2–3 months and was treated with none steroid anti-inflammatory drugs, antibiotics combined in different ways with no relieve. At a routine gynecological checkup was diagnosed a nodule in the fundus of her uterus. An ultrasound with vaginal transducer confirmed the diagnoses. A suspicion of necrosis in the middle of the tumor was expressed. No free liquid inside the peritoneal cavity was found. At laparotomy no gynecological pathology was found. There were two tumors in the omentum sized 7/8 cm. and 5/5 cm. The bigger was localized very close to the uterus. Thorough checkup of the peritoneal cavity revealed no other pathology. The two tumors were extirpated. Macroscopically it was judged to be hydatid cysts (Fig. 1 and 2).

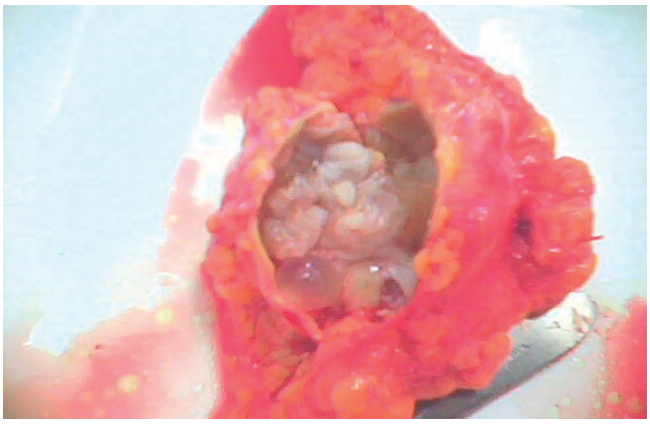

Fig 1

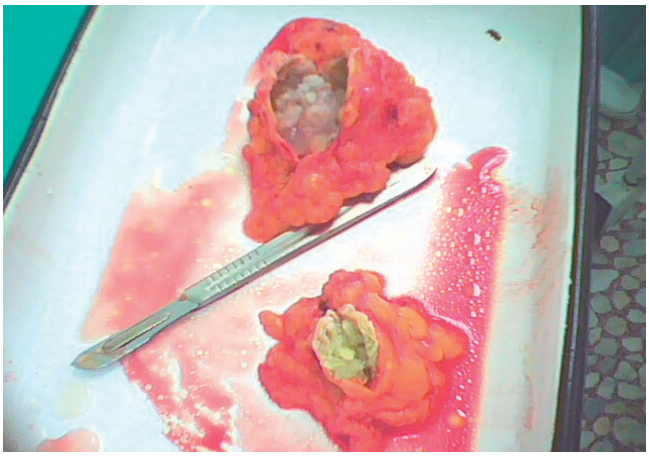

Fig 2

Light microscopy confirmed this diagnose (fig. 3).

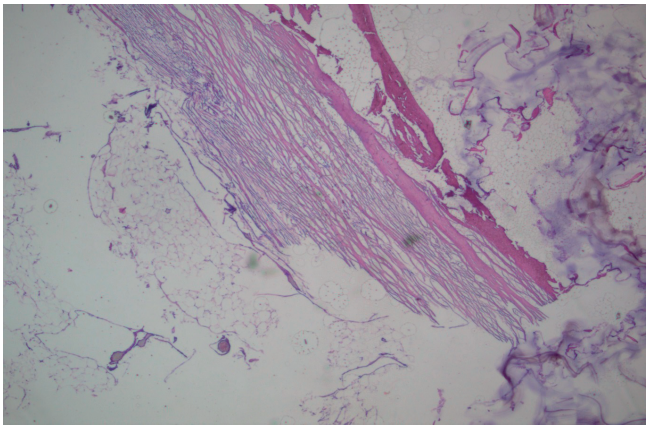

Fig 3

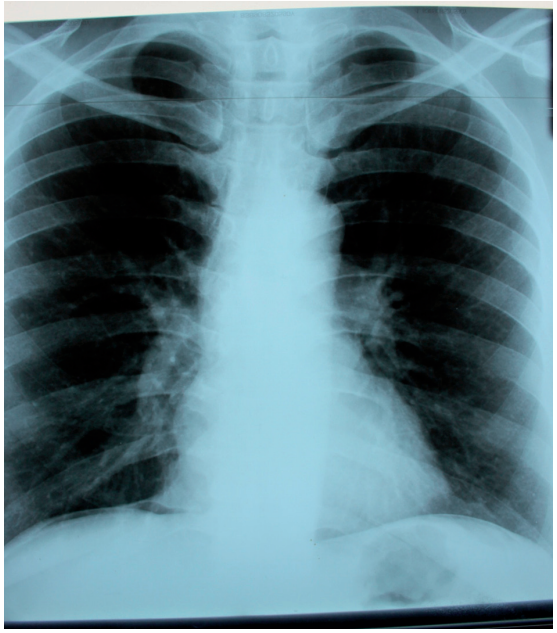

Fig. 4

In the postoperative period we attempted to find primary cysts in other organs of the body. X-ray of the lungs (Fig. 4) and ultrasound of the liver, spleen and kidneys revealed no pathology. We accepted the theses of primary cysts of the omentum and transferred the patient for medical anthelminthic treatment.

With this article I would like to draw the attention of doctors/surgeons to that rare localization of the disease. Because if someone of the team had suspected such a possibility we could do at least two things: first an Elisa test. Being positive we would have known that we are facing a parasitic lesion. Second we would have not trusted 100% the ultrasound even done with vaginal transducer. A CT should have proved that there were two lesions, not one and none of them is connected to the uterus. Another benefit of the CT is that before the operation we would have known that the lesions are localized only to the omentum.

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2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942–955.

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The original and one copy of the complete manuscript are submitted together with a covering letter granting the consent of all authors for the publication of the article as well as a statement that it has not been published previously elsewhere and signed by the first author. The procedure should be complemented via electronic submission. Manuscripts of articles accepted

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ПРИМЕРИ:

Статия от списание:

1. McLachlan, S., M. F.Prumel, B. Rapoport. Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? J. Clin. Endocrinol. Metab., 78, 1994, 5, 1070–1074.

Глава (раздел) от книга:

2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942–955.

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