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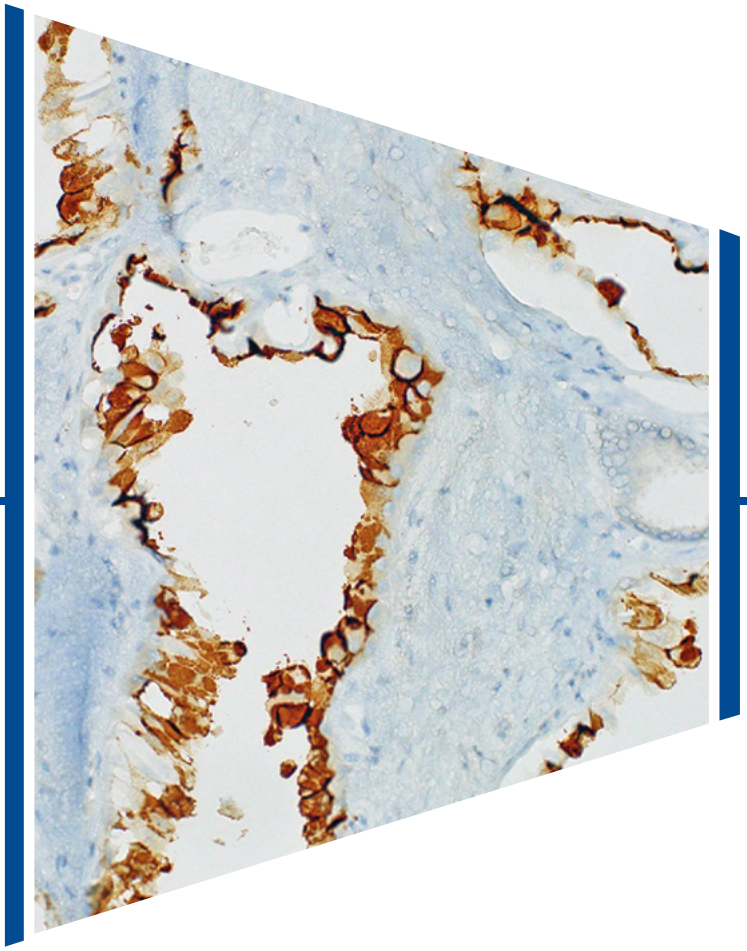

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Editorial Office

Department of Cardiology and Electrotherapy
Medical University of Gdańsk
Dębinki 7
80-211 Gdańsk, Poland

Phone: +48 58 349 39 10
Fax: +48 58 349 39 20
E-mail: ejtcm@gumed.edu.pl
www.ejtcm.gumed.edu.pl

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CONTENTS

INVITED REVIEW ARTICLE

RESEARCH ARTICLES

SHORT COMMUNICATIONS

REVIEW ARTICLES

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2018;1(2):4-10

INVITED REVIEW ARTICLE

Pediatric cardiology emergencies – the role of Long QT Syndrome among arrhythmias

Mariusz Zipser[1], Agnieszka Zienciuk-Krajka[2], Dominika Daria Krenska[1], Joanna Kwiatkowska[1]

[1] Department of Pediatric Cardiology and Congenital Heart Defect, Medical University of Gdańsk, Poland
[2] Department of Cardiology and Electrotherapy, 2nd Department of Cardiology, Medical University of Gdańsk, Poland

Abstract

Long QT syndrome (LQTS) is a hereditary disease with significant mortality, which might be reduced with appropriate management. This cardiac disorder is regarded as rare, but its prevalence remains unknown. The clinical course of LQTS is variable and syncope is a common first manifestation of LQTS. Therefore in each patient after syncope an ECG should be carried out. However, there is no universal QT value applying to all patients (especially infants and children), because it varies depending on age and sex. Genetic testing can be of great importance for the management of families with LQTS and early identification of patient relatives at risk of developing disease. We aimed to show that a very important part of treatment is not only pharmacotherapy, especially beta-blockers, but change of lifestyle plays a significant role.

Keywords: long QT syndrome / torsades de pointes / pediatric arrhythmias

Citation

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Introduction

Syncope is a common complaint of children and young adults visiting emergency departments. In terms of etiology, syncope is classified as reflex syncope (including vasovagal), syncope due to orthostatic hypotension and cardiac syncope [1].

Arrhythmias such as bradyarrhythmias or tachyarrhythmias are the most common cardiogenic causes of syncope. The former includes hemodynamically significant bradycardia in e.g. advanced atrio-ventricular block or Sick Sinus Syndrome. Whereas the latter lead to syncope due to reduced ejection fraction because of fast, hemodynamically insufficient ventricular contractions. Another reason of cardiogenic syncope is

Corresponding author:
Joanna Kwiatkowska, Department of Pediatric Cardiology and Congenital Heart Defect, Medical University of Gdańsk, Poland
e-mail: joanna.kwiatkowska@gumed.edu.pl
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Long QT Syndrome (LQTS) with high risk of fatal ventricular arrhythmias such as ventricular fibrillation (VF) and torsade de pointes (TdP). However, LQTS is treatable and therefore it needs to be excluded early in the diagnostic process of patients with recurring syncope or with family history of sudden cardiac death (particularly of an infant or a child).

In this article we describe the pathomechanism and clinical picture of LQTS as well as the diagnostic pitfalls associated with it.

Definition and genetic basis

LQTS is a hereditary arrhythmia syndrome caused by mutations of genes coding for ion channels and their accessory proteins in cardiomyocytes. Abnormal ion transport prolongs the repolarization of cardiac muscle, thus increasing the risk of fatal ventricular arrhythmias (VF and TdP). At rest, the patient's ECG might reveal a prolonged QT interval, abnormal T waves and a tendency for bradycardia [2]. Therefore, a more precise name would be "prolonged QT and abnormal T-wave syndrome" [3]. It is proven that the T-wave patterns in ECGs of LQTS patients not only present genotype dependence, but also vary dynamically on exercise stress test or due to epinephrine, b-blockers or glucose.

Clinically, LQTS may present as syncope, tremor or sudden cardiac death, particularly in situations with increased sympathetic nervous system activity (stress, physical effort, intense emotions). Mutations of 19 different genes were linked with sodium/potassium channel dysfunction that prolongs cardiomyocyte repolarization [4]. Three of them are the basis of ~90% of all genetically confirmed cases of LQTS: LQT1 (50%), LQT2 (35%) and LQT3 (8%). Despite the advanced diagnostic methods, 15-20% of patients with long QT interval have no abnormalities in their genetic tests [5].

Epidemiology

The incidence of LQTS varies according to the source of information. A prospective analysis of the QT interval in ECGs revealed LQTS in 1 out of 2500 Italian newborns, which is more frequent than previously reported [4]. It is noteworthy that about 20-25% of patients with genetically confirmed LQTS have a normal ECG tracing at rest, thus all studies based on ECG analysis underestimate its incidence. Autopsy studies reveal mutations of genes responsible for sodium/potassium channels in 5-10% of patients who died of Sudden Infant Death Syndrome (SIDS).

Secondary LQTS

Although LQTS by definition is caused by a gene mutation, clinicians should not forget about the secondary causes of LQTS which are treatable: severe bradycardia, electrolyte deficiencies (hypokalemia, hypomagnesemia, hypocalcemia) and drugs (anesthetics, antiarrhythmics, antibiotics, antihistamines, CNS drugs and prokinetic agents) (Table 1). For more details see www.crediblemeds.org.

Amiodarone deserves attention due to its wide and often long-term use as the antiarrhythmic drug. Despite causing a significant prolongation of QT interval, amiodarone only slightly increases the risk of TdP, due to its ability to inhibit early after depolarizations (EADs). The occurrence of TdP in patients on long-term amiodarone therapy is probably due to an electrolyte deficiency or concurrent treatment with other QT-prolonging drugs [6]. Regardless, patients with long-term amiodarone therapy they all should have their QT interval measured at every follow-up visit.

Table 1. Drugs causing secondary LQTS

Drug class	Examples
antiarrhythmics	amiodarone, dronedarone, sotalol
antibiotics	diphenhydramine
antihistamines	makrolids, ciprofloxacin, levofloxacin
anesthetics	sevoflurane
CNS drugs	sertraline, paroxetine, amitryptiline, aripiprazole, citalopram, escitalopram, clozapine
prokinetic agents	cisapride

Clinical picture of LQTS

LQTS is often asymptomatic and the first clinical manifestation can be cardiac arrest due to TdP progressing to VF. Symptoms of LQTS can be either clinical (due to experiencing an episode of arrhythmia) or electrocardiographic (based on ECG at rest).

Patients with LQTS most often experience episodes of TdP, a polymorphic ventricular tachycardia named by French cardiologist Dessertenne in 1966 after the alternating electric axis of the ventricular complexes. TdP typically involves short episodes of tachycardia 200-250/min with either spontaneous return of sinus rhythm or progression to VF. During a TdP episode, the work of the ventricles is hemodynamically insufficient and the intensity of symptoms is related to the duration of the episode. Patients with short episodes of TdP might complain of palpitations, dizziness and weakness. Longer episodes may cause syncope, convulsions and cardiac arrest. There are many factors known to provoke episodes of arrhythmia, however typical for LQTS are situations in which loss of consciousness (TdP) provokes: physical exertion, emotional stress and sleep. Of note is the increased risk of sudden cardiac death during swimming and exposure to loud sounds. Symptoms might appear as early as the first months of life, with peak at 10-30 years of age [7].

Diagnostic tests

The first and critical step in diagnosing LQTS is taking a detailed history from the patient. The physician must focus on the history of episodes of pre/syncope and their circumstances (was there a transient loss of consciousness? how quick was the recovery? any concurrent injuries or convulsions?). The physician should ask detailed questions in order to confirm or rule out vasovagal syncope (most frequent cause of syncope in children and young adults). Another critical subject is family history of sudden and/or unexplained death at a young age (car accidents, drownings, miscarriages).

In daily practice, the cornerstone of LQTS diagnosis continues to be the QT and QTc assessment in a 12-lead ECG taken at rest (Figure 1) [8-11]. ECG tracings of LQTS patients at rest may reveal a rather wide spectrum of changes in the heart rate and repolarization phase (Figure 2-4: LQT 1-3 types). Specific changes in the repolarization phase are as follows: prolonged QT interval, abnormal T waves (wide, notched, low or high voltage) or alternating T waves (indicating significant electrical instability of the heart) (Figure 5). The repolarization phase (QT interval measured from the beginning of Q wave to the end of the T wave) depends on the patient's heart rate, age and sex. Therefore, we rely on the so-called corrected QT interval (QTc) calculated using Bazett's formula and it should be used optimally for heart rate between 60-120(100)/min: $QTc=QT/(\sqrt{RR})$.

Newborns and infants with QTc of 440-470ms in repeated ECG need further diagnostics especially taking detailed family history including LQTS and SCD, Holter ECG. For older children of both sexes, QTc of 450ms is currently considered normal and is slightly affected by growing. QTc >460ms for adult females and 450ms for adult males should be investigated. In case of noting QTc >500ms and excluding possible secondary causes, a diagnosis of LQTS can be made. In case of borderline QTc values (460-480ms), it is necessary to take into account the entire clinical picture and to assess the probability of LQTS based on Schwartz's diagnostic criteria.

The Schwartz diagnostic criteria published in 1993 continue to be the tool of choice for stratifying the risk of LQTS. Included are clinical symptoms, family history and ECG changes. According to current guidelines, LQTS should be recognised in case of >3 points in Schwartz scale [12].

Multicenter studies revealed correlations between the LQT genotype and the specific clinical picture of LQTS (arrhythmia-provoking factors, benefits of beta-blocker therapy and age groups at greatest risk of SCD) [13]. Such correlations highlight the usefulness of genetic testing, even in cases when there are doubts

Figure 1. Diagram shows the QT interval measurements rules [2]

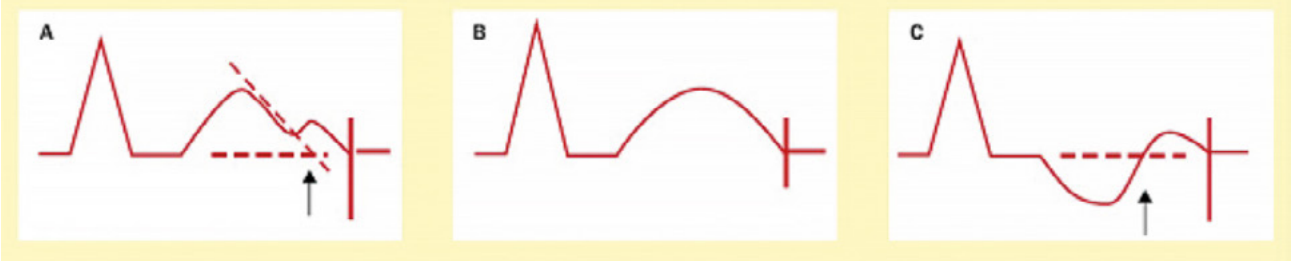
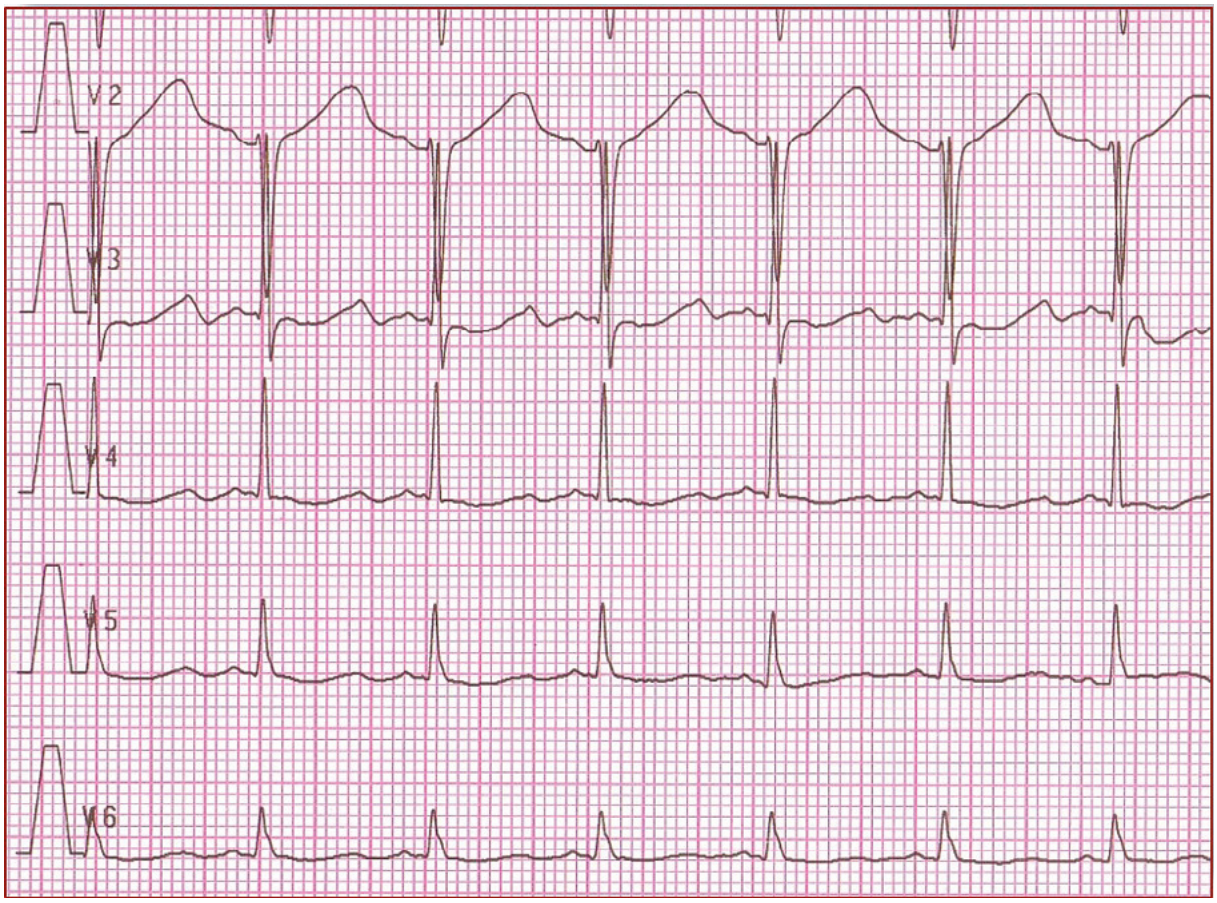


Figure 2. It shows 'early onset' broad based, slowly generated T waves. LQT1 (R591H)



about the clinical diagnosis. Confirming the patient's precise genotype allows a more precise therapy e.g. adding sodium channel blockers in therapy of patients with LQT3 genotype [12]. On the other hand patients with LQT2 are more sensitive to hypokalemia. In addition, genetic testing allows making the diagnosis in the asymptomatic family members of LQTS patients.

Due to the incomplete penetrance of LQTS genes, some of the patients with genetically confirmed LQTS actually have a normal QT interval in resting ECG. Diagnosing this subset of patients is particularly challenging because they are clinically and electrocardiographically asymptomatic. Often these patients have family members who either survived cardiac arrest or have definite ECG changes. These patients must avoid QT-prolonging drugs. Other tests such as Holter ECG, exercise stress test and provocative tests play less important role [14].

The next step in diagnostic process should be stratification for the risk of Sudden Cardiac Death. Traditional SCD risk factors are as follows: aborted cardiac arrest (ACA) at < 1 year of age, prior syncope, torsade de Pointes, T-wave alternans, QTc>500ms, males <14 years old, females 18-40 years old, congenital deafness (JLN).

Figure 3. It shows small late T waves. Sometimes these T waves will be notched or double peaked in lead V4. LQT2 (G601S)

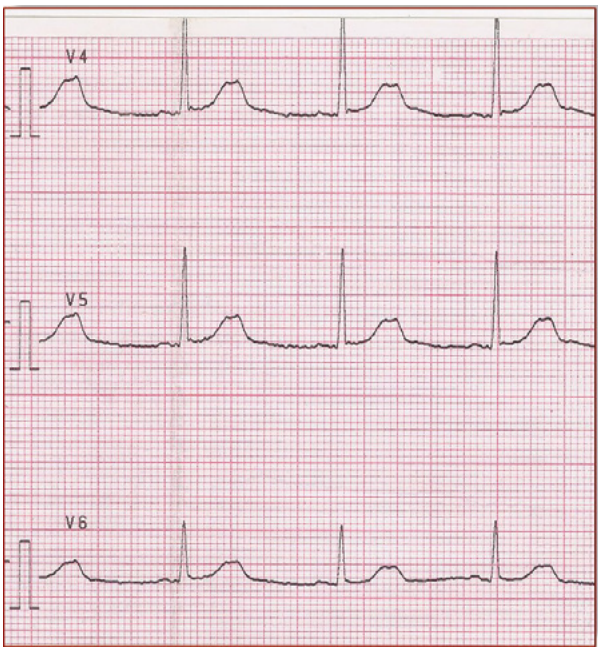
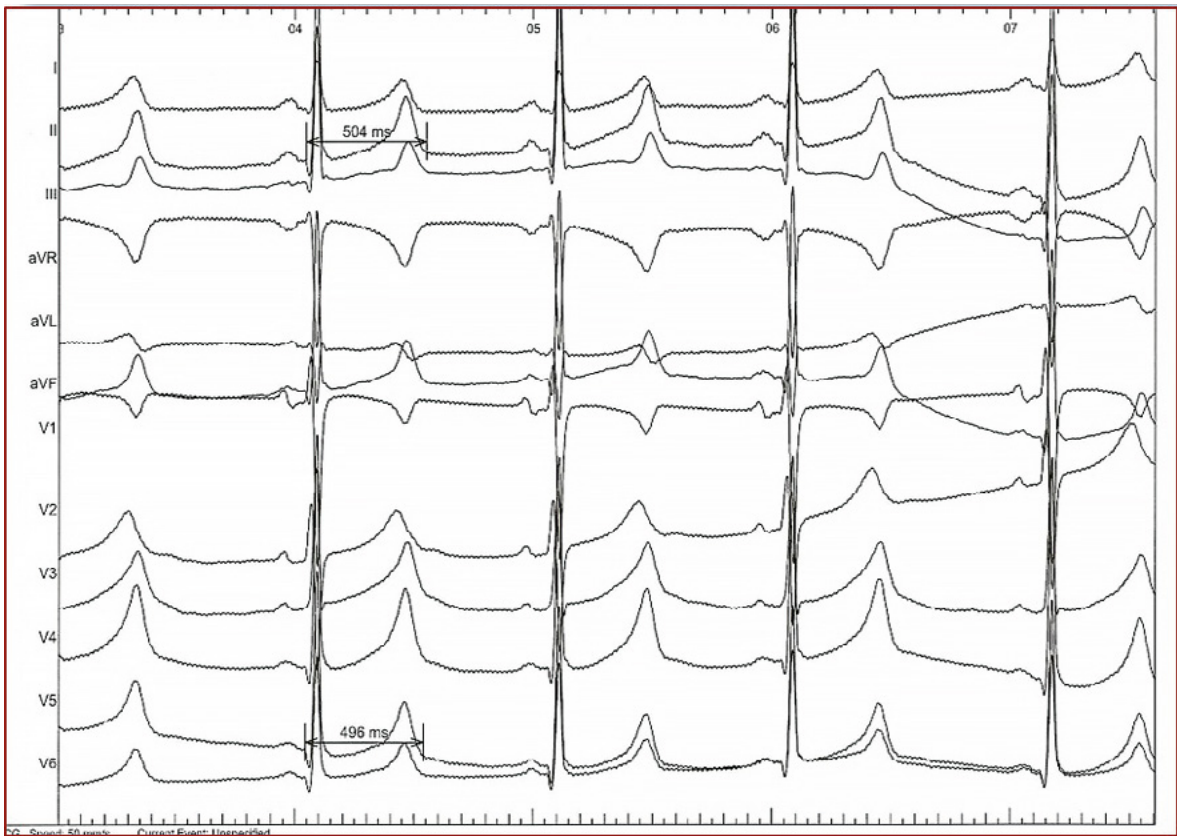


Figure 4. It shows a flat and prolonged ST segment with a 'late onset' normal-shaped T wave. LQT3 (K1477N)



Treatment

The actual recommendations for LQTS management include genetic-specific treatment and lifestyle advice [15]. Specifically, this means chronic treatment with propranolol or mexiletine (currently not sold in Poland and available only upon special request for import), avoidance of QT-prolonging drugs, electrolyte/hydration replenishment, aggressive fever reduction, genetic testing whenever possible, access to AED, BLS training for the entire family, psychological support and family screening.

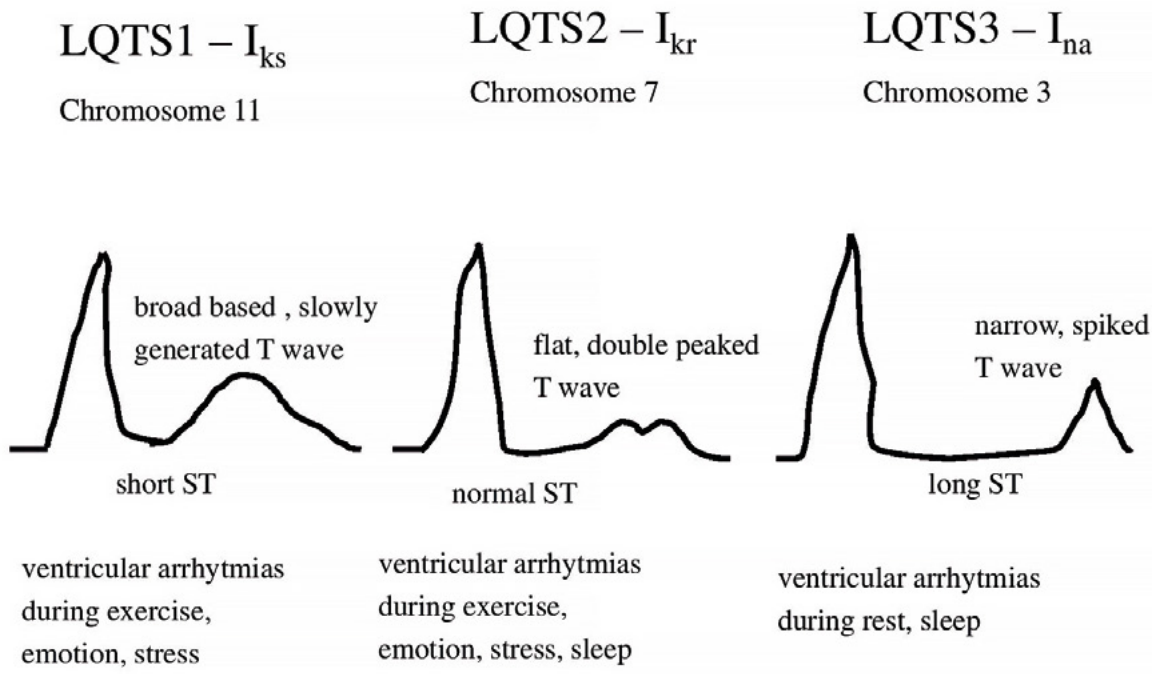
During an episode of TdP, the management is identical regardless if the etiology is primary/genetic LQTS or secondary. The drug of choice is magnesium sulphate administered intravenously either in fast boluses children: 3-12mg/kg in 1-2 minutes; adults: 1-2g in 30-60 seconds) or continuous infusion (children: 0.5-1mg/kg/h; adults: 3-10mg/min). The suggested magnesium sulphate concentration in pediatric dosing is 3-5mg/dl [16]. Electric cardioversion should be reserved for the hemodynamically unstable patients because TdP frequently recurrrent after electric discharges. Next steps in management should focus on treating the cause, if possible (e.g. correcting the electrolyte imbalance, discontinuing the QT-prolonging drug).

Beta-blockers propranolol and nadolol have been the mainstay of LQTS treatment for years. They are able to prevent syncope and SCD in up to 70% of LQTS patients [15]. The particularly poor effectiveness of beta-blockers is noted in patients with LQT3 genotype, in whom ventricular tachycardias occur during sleep or at rest. Most authors point to propranolol (3mg/kg 3 times/day in children) and nadolol (1mg/kg 1-2 times daily). Atenolol should be avoided [17]. Despite their lower effectiveness, beta-blockers are also recommended in LQT3 therapy.

In case of lack of response to drug treatment or cardiac arrest, the next step is implantation of a cardioverter-defibrillator or left stellate ganglioectomy (LSG) [18]. Side effects of LSG are: intermittent temperature changes, facial flush, decreased sweating on one side and pain.

The literature describes numerous cases of LQTS patients misdiagnosed with seizure disorders. This is

Figure 5. Abnormal types of T waves in LQTS (LQTS1, LQTS2, LQTS3)



particularly common among patients whose symptoms include muscle tremors or recurrent syncope. We suggest all patients treated for recurring seizures should have an ECG performed before increasing doses or adding another drug for epilepsy resistant to treatment. MacCormick et al. noted that a delay in diagnosis of LQTS "for those who were misdiagnosed as having epilepsy, the median time for diagnostic delay was 11.8 years, with a range of 9.5 to 23 years" [19]. Considering the high mortality due to LQTS (20% in the first year and 50% within 5 years after an episode of transient unconsciousness), there is little time for making the correct diagnosis [19-20]. It is worth underlining that symptomatic LQTS is the only opportunity for excluding it in his/her closest family members, therefore lack of correct diagnosis is not just one patient's risk.

Conclusions

Long QT syndrome is a rare cardiac cause of transient loss of consciousness. However, its high mortality and high effectiveness of preventive treatment means that every patient presenting with syncope or convulsions should have an assessment of the QT interval and T waves on a 12-lead ECG. This is especially true of patients with a family history of sudden or unexplained death. Due to an increasing number of prescription drugs which prolong the repolarization phase in cardiac muscle (thus increasing the risk of TdP), physicians of all specialties should be trained in QT assessment and when in doubt should have an easy access to a cardiology consultation. It appears that information about the patient's QT interval is just as important in the planning of treatment, as information allergies and adverse reactions to drugs. Some authors suggest that an ECG screening program for newborns is justified, as it would reduce the frequency of sudden death in the entire pediatric population and would at the same time meet the criteria of cost-effectiveness accepted in European healthcare systems [21].

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RESEARCH ARTICLE

The use of over-the-counter analgesics in patients with chronic kidney disease

Marzena Jakimowicz-Tylicka, Michał Chmielewski, Izabella Kuźmiuk-Glembin, Piotr Skonieczny, Grażyna Dijakiewicz, Grażyna Zdrojewska, Bolesław Rutkowski, Leszek Tylicki, Alicja Dębska-Ślizień

Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Poland

Abstract

Background: Analgesics can be sold following medical prescription, but also as over-the-counter (OTC) medications. In patients with chronic kidney disease (CKD), their use could potentially be associated with increased risk of side-effects, due to impaired renal elimination. The aim was to evaluate the epidemiology and indications for the use of OTC analgesics, and the knowledge of their side-effects in patients with CKD.
Materials and methods: A cross-sectional, controlled survey on the use of OTC analgesic drugs was conducted among 180 CKD patients (stage 1-5, dialysis, kidney transplant), compared to 60 controls.
Results: The proportion of patients using OTC analgesics on a regular basis was higher in the CKD group, compared to controls (18.9% vs. 10.0%, p<0.02). The major indications included musculoskeletal issues, followed by headaches and other. Subgroup analysis revealed that analgesic use was lowest among transplanted patients, in comparison to CKD stage 1-5, and dialysis subjects (10%, 20%, 26%, respectively, p=0.06). Less than half of CKD patients and controls declared any knowledge on potential side-effects of analgesic drugs (45.6% vs. 40.0%, NS).
Conclusions: The use of OTC analgesics among patients with CKD is higher than in subjects without CKD, with the exception of transplanted patients. The knowledge on the potential side-effect of analgesics is limited.
Keywords: chronic kidney disease / analgesics / over-the-counter

Citation

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Corresponding author:
Michał Chmielewski, Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Dębinki 7, 80-211, Gdańsk, Poland
e-mail: michal.chmielewski@gumed.edu.pl
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Introduction

Analgesics are drugs administered to achieve relief from pain. They are typically prescription-only but many of them are also sold as over-the-counter (OTC) medications, i.e. without a prescription from a healthcare professional. Analgesics are among the most popular and best-selling OTC drugs, as according to some studies they account for 40-50% of all the medications sold without prescription [1-2]. Their consumption is constantly growing among the general population due to widespread availability and intense advertising of OTC analgesics as safe products with proven therapeutic potential. OTC analgesics are often effective for self-management of pain. However, they are also associated with a low but important rate of gastrointestinal and hepatic events, as well as a risk of intentional and non-intentional overdose [3].

Chronic kidney disease (CKD) is a prevalent disorder, estimated in Poland at ~6% of the general population [4]. Irrespective of the cause, it often leads to a state of renal insufficiency with impaired excretion of numerous metabolites and waste products. The use of OTC analgesics in this patient group could potentially be associated with an increased risk of side-effects and/or drug toxicity, due to impaired renal metabolism and/or elimination. Taking into account the above-mentioned prevalence of CKD, and the widespread use of OTC drugs in the general population, the issue of OTC analgesic use in this specific patient group seems to be of clinical importance. Therefore, the aim of this study was to evaluate the epidemiology and indications for the use of OTC analgesics in patients at various stages of CKD, in comparison to controls with preserved kidney function.

Materials and methods

A cross-sectional controlled survey study of CKD patients was conducted to evaluate their analgesic self-medication practices. The study cohort included 180 patients with CKD treated in the Department of Nephrology Transplantology and Internal Medicine of the Medical University of Gdansk. CKD was diagnosed according to KDOQI definition [5]. Inclusion criteria were restricted to the presence of CKD, age >18, and willingness to participate in the survey. Patients were divided into subgroups: CKD stage 1-5 (CKDND; n=80), end-stage renal failure patients treated with hemodialysis or peritoneal dialysis (CKDD; n=50), and patients after renal transplantation (CKDT; n=50). Glomerular filtration rate (GFR) was estimated based on the CKD-EPI equation [6]. The control group comprised of 60 age- and gender-matched subjects without CKD, selected from a cohort of the Family Medicine Centre of the same hospital.

The study was based on a written anonymous survey. The questions referred to analgesic self-medication practices: frequency of analgesic use (never, sometimes, regularly i.e. >1 time/week), major indications, the source (advised by the doctor, by the pharmacist, by the mass media, etc.), as well as the patient's awareness of the potential side-effects of the analgesics used. Protocol of the study received approval from the Local Bioethics Committee. Results were expressed as percentages (for categorical variables), mean and standard deviation or median and interquartile range. The assumption of normality was verified with the Kolmogorov-Smirnov test. The quantitative variables' differences were assessed by t-test, analysis of variance (ANOVA) or non-parametric Kruskal-Wallis test. Differences in prevalence between selected categories were measured using the two-sample t-test test. A p-value <0.05 was considered to be statistically significant. Statistical processing of the results was performed with the use of the statistical software STATISTICA PL v 12.0 (Statsoft, Poland).

Results

The general demographic data of the studied groups is presented in Table 1. Patients with CKD, in general, declared greater comorbidity in comparison to subjects from the control group. Renal transplant recipients (CKDT) were, on average, younger and lived in less populated places than CKDND and CKDD patients (Table 2). Regular use of OTC analgesic drugs was reported by 35 CKD patients (18.9%), and by 6 patients (10.0%) from the control group; p=0.01. Subgroup analysis revealed that among CKD patients, CKDT reported the least frequent use of OTC analgesics (10.0%), in comparison to CKDND (20.0%) and CKDD subjects (26.0%); p=0.06. The patients from the studied groups took usually one analgesic drug at a time. However, 14 subjects (7.8%) admitted to use at least two analgesics simultaneously. In the control group, three subjects (5.0%) reported taking at least two analgesic drugs at the same time; p=0.46. The major indications for the use of OTC analgesics are presented in Table 3. In the CKDND group, musculoskeletal issues prevailed, while headaches were the main indications in transplanted patients. Most of the patients (67.2%) kept their doctor informed about the OTC medications used. In the control group, this percentage was slightly lower (53.3%); p=0.06. Less than half of CKD patients and controls declared any knowledge on the potential side-effects of OTC analgesic drugs (45.6% vs. 40.0%, NS).

Table 1. Demographic data of the study groups

	All CKD patients	Control group	p-value
Number of patients (n)	180	60	
Male gender (%)	101 (56.1%)	32 (53.3%)	NS
Age (years)	58.0 ± 15.1	57.3 ± 14.3	NS
Living conditions			**NS**
Good	62 (34.4%)	24 (40.0%)	
Average	108 (60.0%)	34 (56.7%)	
Poor	10 (5.6%)	2 (3.3%)	
Education			**NS**
Basic / Professional	22 (12.2%)	8 (13.3%)	
Secondary	106 (58.9%)	34 (56.7%)	
Higher	52 (28.9%)	19 (31.3%)	
Place of residence			**NS**
Large city >100,000	123 (68.3%)	43 (71.7%)	
Small city <100,000	36 (20.0%)	12 (20.0%)	
Village	21 (11.7%)	5 (8.3%)	
Co-morbidities			**NS**
Hypertension Diabetes mellitus	123 (68.3 %)	32 (53.3%)*	<0.05
Cardiovascular disease	43 (23.9%)	5 (8.3%)**	<0.01
Higher	24 (13.3%)	19 (15.0%)	NS

CKD – chronic kidney disease

NS – non-significant

Table 2. Demographic data of the study subgroups

	CKDND	CKDD	CKDT	p-value
Number of patients (n)	80	50	50	
Age (years)	64.2 ± 14.7	59.3 ± 14.0	50.1 ± 12.5	<0.001
Gender M: n (%)	41 (51%)	28 (56%)	32 (64%)	0.37
Other diseases: n (%)				
Diabetes mellitus	29 (36)	7 (14)	7 (14)	0.001
Hypertension	56 (70)	28 (56)	39 (78)	0.06
CVD	14 (17)	7 (14)	3 (6)	0.17
Education: n (%)				
Basic	7 (9)	7 (14)	8 (16)	0.62
Secondary	45 (56)	31 (62)	31 (62)	0.88
Higher	28 (35)	12 (24)	11 (22)	0.47
Place of residence: n (%)				
City >100,000	66 (82)	38 (76)	20 (40)	0.001
City <100,000	7 (9)	10 (20)	20 (40)	0.001
Village	7 (9)	2 (4)	10 (20)	0.16
Living conditions				
Good	22 (27)	24 (48)	16 (32)	0.18
Average	55 (69)	20 (40)	33 (66)	0.008
Poor	3 (4)	6 (12)	1 (2)	0

CKDND – non-dialysis dependent CKD patients (CKD stage 1-5); CKDD – dialysis patients; CKDT – patients after kidney transplantation; CVD – cardiovascular disease

Table 3. Indications for analgetics use in CKD subgroups

	CKDND	CKDD	CKDT	p-value
Musculoskeletal	35%	30%	20%	0.19
Headaches	12%	30%	26%	0.04
Abdominal pain relief	9%	10%	10%	0.96

Discussion

The present study demonstrates that the use of OTC analgesics is higher in CKD patients, as compared to controls. This finding seems of clinical significance, given the potential toxicity of these drugs in subjects with impaired kidney function.

Intense advertising and OTC availability results in a growing consumption of analgesics worldwide. In a Canadian study, OTC non-steroidal anti-inflammatory drugs (NSAIDs) were taken by 24% of responders [7]. In Czech Republic, consumption of ibuprofen is estimated to be as high as 30 doses/1000 inhabitants/day and in Denmark consumption of paracetamol reaches 60 doses/1000 inhabitants/day [8]. The most consumed analgesic in France is paracetamol and its use increased by 140% during 2006-2015 [9]. The doses of OTC analgesics are relatively small, and therefore the risk of side-effects is believed to be negligible. However it still exists, mainly in a form of gastrointestinal and hepatic complications [3,10].

Chronic pain is a common feature of CKD [11]. Calcium-phosphate disorders leading to renal osteodystrophy, rapid changes in blood pressure during dialysis leading to headaches, and an all-together lower quality of life of CKD patients, are among the major culprits. Indeed, our study demonstrates that OTC analgesic consumption is almost twice as high in CKD patients in comparison to controls. Analysis within the CKD population revealed that there were some differences among particular subgroups of patients. While in the CKDND subjects, analgesics were taken mainly for musculoskeletal issues, in transplanted patients headache was the most prevalent indication. In CKDD patients, both these indications were similarly common. In general, analgesic use in transplanted patients was comparable to the control group, while it was twice as prevalent in CKDND and CKDD subjects. Again, the above-mentioned reasons for chronic pain in CKD could, at least in part, explain these differences in the prevalence of analgesic use and the major indications for their consumption among the studied CKD subgroups.

CKD patients differ from people with preserved kidney function. Impaired glomerular filtration rate can lead to accumulation of drugs and/or their metabolites for which the urinary tract is the primary path of elimination. Therefore, the use of OTC analgesics in this patient group might be potentially associated with a greater risk of complications, as compared to the general population. Moreover, analgesics may contribute to the progress of CKD itself. Indeed, in the XX century, analgesic nephropathy was among the major causes of end-stage renal disease (ESRD) in many countries. In the US, as much as 7% of ESRD cases were classified as a result of analgesic nephropathy [12], and in Australia pathological changes attributable to analgesics were observed in 5% of all the kidneys in the post mortem surveys in the general population [13]. However, based on numerous studies, it was proven that the sole medicine responsible for analgesic nephropathy was phenacetine. Either through direct nephrotoxicity or through decreasing prostaglandin synthesis, phenacetine led to chronic kidney insufficiency with erythrocyturia, increased blood pressure, and papillary necrosis found in kidney specimens [14]. Phenacetine was banned throughout the world in the second half of XX century. At present, analgesic nephropathy is not a major clinical issue any more.

Although the classic form of analgesic nephropathy is slowly fading into history, it does not mean that analgesics are completely safe for the CKD population. Manufacturers inform about the recommended and maximum doses associated with impaired kidney function in the leaflets enclosed in every drug package. For instance: metamizole dose should be decreased in case of renal insufficiency because of prolonged elimination time of the drug, and should be totally avoided in patients with GFR <30 ml/min. Severe kidney dysfunction constitutes a contraindication for the use of ibuprofen, diclofenac and paracetamol. Furthermore, NSAID use is associated with increased risk of gastrointestinal bleeding, especially in case of concurrent use of anticoagulants, as in CKDD patients who receive heparin during hemodialysis sessions. The patients are usually unaware of these limitations. In our survey, less than half declared any knowledge on the potential side-effects of OTC analgesic drugs. By using them without control, they put themselves at risk of serious and sometimes life-threatening complications.

It is not to be said that OTC analgesics should be totally avoided in CKD patients. Taking into account the chronic pain commonly experienced by CKD patients, analgesics are often the only way to improve their quality of life. However, given the scarce knowledge on potential side-effects of analgesic medications among CKD patients, it is of utmost importance to meticulously elicit the medical history of analgesic use, and keep the patients informed on the advised doses, and potential side-effects associated with their use. In our study, as much as one third of the patients did not inform their doctor about the OTC analgesic use.

The major limitations of the study are its cross-sectional character and a relatively small sample size. However, even with these groups of patients, significant differences in analgesic use were demonstrated.

Conclusion

In conclusion, the prevalence of OTC analgesic use is higher in CKD patients than in controls, except for the transplanted subjects in whom it is similar to controls from the general population. The CKD patients' knowledge of the side-effects of OTC analgesics appears to be very limited. Taking into account the potential threats associated with the accumulation of analgesic drugs and their metabolites in the course of CKD, meticulous history-taking regarding OTC analgesics use and comprehensive patient education seems of crucial importance.

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RESEARCH ARTICLE

The impact of renal function on clinical and biochemical characteristics in advanced heart failure patients

Michał Bohdan[1,2], **Anna Frankiewicz**[1], **Marcin Gruchała**[1,2]

[1] First Department of Cardiology, Medical University of Gdańsk, Poland
[2] Clinical Centre of Cardiology, University Clinical Centre, Gdańsk, Poland

Abstract

Background: Coexistence of heart failure with reduced ejection fraction (HFrEF) and chronic kidney disease is associated with poor prognosis. We assessed the effect of renal function on exercise capacity and clinical parameters of patients with HF. **Materials and methods:** Forty five patients aged 58.2 ± 10.6 years with stable severe HFrEF were recruited. Patients were divided into 3 groups: group 1 - eGFR: 30-59 ml/min/1.73 m2; group 2 - eGFR: 60-89 ml/min/1.73 m2 and group 3 - eGFR: ≥90 ml/min/1.73 m2. Biochemical analysis, echocardiography, 6-minute walking test and cardiopulmonary stress testing were performed. **Results:** Patients in group 1 were significantly older than patients in group 3 (60.4 ± 11.1 years vs. 49.25 ± 11.2 years, respectively, $p<0.05$). Patients in group 2 had significantly higher BMI in comparison to group 3 (29.8 ± 4.4 vs. 25.1 ± 4.2; $p<0.05$). Interestingly, patients in group 1 had significantly lower peak oxygen uptake (10.2 ± 3.1 ml/kg/min vs. 16.1 ± 3.5 ml/kg/min, $p<0.05$) and oxygen uptake at anaerobic treshold (7.9 ± 2.4 ml/kg/min vs. 10.7 ± 1.9 ml/kg/min, $p<0.05$). **Conclusions:** Diminished renal function in patients with stable, advanced HFrEF may be associated with significantly worse peak VO2 and VO2 in AT.

Keywords: heart failure / kidney disease / exercise capacity

Citation

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Background

Heart failure with reduced left ventricular ejection fraction (HFrEF) is becoming a common disease and its prevalence is rising due to ageing of the population and improvements in cardiovascular disease management [1]. In the last decades there has been a significant progress in pharmacotherapy (β-blockers, angiotensin con-

Corresponding author:
Michał Bohdan, First Department of Cardiology, Medical University of Gdańsk, Poland
e-mail: michal.bohdan@gumed.edu.pl
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verting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs), angiotensin receptor blocker in combination with neprylisin inhibitor (ARNI), ivabradine) and cardiac devices. Despite these advancements the prognosis in HFrEF is still poor. In addition, HFrEF may be associated with various comorbidities which also affect the morbidity and mortality and reduce the quality of life [3]. Chronic kidney disease is regarded as one of the comorbidities of a particular importance and its relationship with HFrEF has been recognized as cardio-renal syndrome [4-5]. Impaired renal function not only affects the HF prognosis but also may have an impact on the pharmacotherapy especially in advanced stages of renal failure. Furthermore, some studies suggested that even small increase in serum creatinine levels in HF patients is associated with worse outcome [6-7].

The aim of the study was to investigate the potential effect of decreased estimated glomerular filtration rate (eGFR) on exercise capacity and clinical and biochemical parameters in stable advanced chronic HFrEF.

Material and methods

This retrospective, single-centre study was performed 45 patients with severe chronic heart failure with reduced left ventricular ejection fraction evaluated for potential heart transplantation. All patients received pharmacotherapy for at least 6 months with β-blocker, ACE-I or ARB and aldosteron blocker and also ivabradine, diuretics and digoxin if indicated. The inclusion criteria were as follows: ischaemic or nonischaemic HFrEF for at least 1 year, NYHA II or III, stable course of the disease defined as no increase in symptoms severity and/or no modification of treatment for at least 3 months prior to inclusion, LVEF ≤35% in two-dimensional echocardiography during evaluation, at least one HFrEF decompensation within 12 months prior to inclusion and age ≥18 years old. Whereas valvular heart disease as a main cause of HF, eGFR ≤30 ml/kg/1.73 m2, neurological disorders, inability to perform exercise tests, and frailty were the exclusion criteria.

Patients were divided into 3 groups based on their initial renal function: group 1: eGFR 30-59 ml/kg/1.73 m2, n = 18; group 2: eGFR 60-89 ml/kg/1.73 m2, n=19; and group 3: eGFR ≥90 ml/kg/1.73 m2, n = 8. In each group the following tests were performed: laboratory analysis, electrocardiogram, echocardiography, 6-minute walking test, cardiopulmonary stress testing. In addition, prognosis was assessed using the Seattle Heart Failure Risk Model [1].

GFR was estimated using the Modification of Diet in Renal Disease (MDRD) formula. Anaemia was diagnosed according to the World Health Organization criteria (hemoglobin <13 g/dL in males and <12 g/dL in females).

The 6-minute walking test (6MWT) was performed in each patient. The patients were allowed to self-pace and rest as needed during 6 minutes walking along a marked walkway.

Cardiopulmonary stress testing (CPET) was performed in each patient using Medisoft Ergocard (Belgium). Patients underwent maximal, symptom-limited test with exercise bicycle using the ramp protocol (10W/min). Patients were asked to fast for 4 hours before the test. The gas exchange data were collected throughout the test with a metabolic cart. The following parameters were assessed during CPET: blood pressure, heart rate, respiratory rate, electrocardiogram monitoring, symptoms, and arrhythmias. Gas exchange variables assessment included measurement of: CO2 production (VCO2), oxygen consumption (VO2), and minute ventilation.

The study protocol was approved by the local bioethics committee.

All data are presented as mean ± SD. Kolmonogrov-Smirnov test was used to assess the normal distribution. Analysis of variance (ANOVA) with Tukey's post hoc and Chi-squared test were performed. Statistical analysis was done using Statistica 13 software. P value <0.05 was considered statistically significant.

Results

The mean age of the study group was 58.2 ± 10.6 years and mean LVEF was: 23.1 ± 6.3%. Patients in group 1 and 2 were significantly older than in group 3 (60.4 ± 11.1 years and 59.8 ± 8.2 vs. 49.3 ± 11.2 years respectively, p<0.05 for both interactions). The majority of patients in all sub-groups were male. Concomitant disease prevalence is presented in Table 1. There were no significant differences in pharmacotherapy between the study groups.

Patients in group 2 had significantly higher BMI in comparison to group 3 (29.8 ± 4.4 kg/m2 vs. 25.1 ± 4.2 kg/m2, p<0.05). No differences in BMI were found between group 1 and 3. In addition, the prognosis assessed using the Seattle Heart Failure Risk Model was similar in the analysed groups (Table 1).

Moreover, hemoglobin level was significantly lower in group 1 in comparison to group 2 and 3 (13.5 ± 1.7 g/l vs. 14.6 ± 1.4 g/l and 14.9 ± 0.8 g/l, respectively, p<0.05 for both interactions). Furthermore, there was a tendency towards higher prevalence of anaemia in group 1 in comparison to other 2 groups (38.9% vs. 15.8% and 0% respectively, p=0.059). In addition, uric acid level was significantly higher in group 1 in comparison to group 3 (10.2 ± 2.3 mg/dl vs. 7.4 ± 2.2 mg/dl, p<0.05). The other laboratory, clinical and echocardiographic parameters are summarized in Table 2.

Table 1. Characteristics of patients based on renal function

	All	GFR 30-59 (n=18)	GFR 60-89 (n=19)	GFR ≥90 (n=8)	P
Age (years)	58.2 ± 10.6	60.4 ± 11.1	59.8 ± 8.2	49.25 ± 11.2	1:3 p<0.05; 2:3 p<0.05)
Male n (%)	43 (95.6%)	17 (94%)	19 (100%)	7 (87.5%)	NS
HFrEF duration (years)	3.3 ± 2.5	3.6 ± 3.1	3.6 ± 2.2	2.3 ± 1.5	NS
Concomitant diseases					
Coronary artery disease n (%)	30 (66.7%)	13 (72%)	13 (68%)	4 (50%)	NS
DM or prediabetes n (%)	13 (29.9%)	4 (22.2%)	8 (42%)	1 (12.5%)	NS
Hypertension n (%)	23 (51.1%)	9 (50%)	9 (47.4%)	5 (62.5%)	NS
COPD n (%)	5 (11.1%)	3 (16.7%)	1 (5.3%)	1 (12.5%)	NS
CRT-D n (%)	10 (22.2%)	7 (38.9%)	2 (10.5%)	1 (12.5%)	p=0.08
Optimal medical therapy n (%)	22 (48.9%)	7 (38.9%)	12 (63.2%)	3 (37.5%)	NS
Maximum dose of β-blocker n (%)	11 (24.4%)	3 (16.7%)	6 (31.6%)	2 (25%)	NS
Maximum dose of ACE-I/ARB n (%)	6 (13.3%)	1 (5.6%)	4 (21.1%)	1 (12.5%)	NS
Maximum dose of AB n (%)	14 (31.1%)	5 (27.8%)	8 (42.1%)	1 (12.5%)	NS
BMI (kg/m2)	28.2 ± 4.4	27.8 ± 3.9	29.8 ± 4.4	25.1 ± 4.2	2:3 p<0.05)
SBP (mmHg)	116.8 ± 14.7	116.4 ± 15.9	115.8 ± 13.8	119.8 ± 15.5	NS
DBP (mmHg)	73.5 ± 9.9	73.7 ± 11.4	72.8 ± 10.5	74.6 ± 4.6	NS
HR (bpm)	74.4 ± 11.3	74.6 ± 8.9	74.5 ± 13.9	73.6 ± 9.9	NS
NYHA	2.4 ± 0.5	2.3 ± 0.5	2.3 ± 0.5	2.5 ± 0.5	NS
SHFRM					
Mortality 1 year (%)	6.2 ± 5.3	8.3 ± 6.2	5 ± 4.2	4.5 ± 3.9	NS
Mortality 2 years (%)	11.9 ± 9.7	15.7 ± 11.2	9.7 ± 7.9	8.9 ± 7.6	NS
Mortality 5 years (%)	28.4 ± 19.7	35.8 ± 22	23.9 ± 16.8	22.3 ± 17.4	NS
Mean life expectancy (years)	11.7 ± 6.5	9.9 ± 6.7	12.2 ± 5.2	14.3 ± 8.3	NS

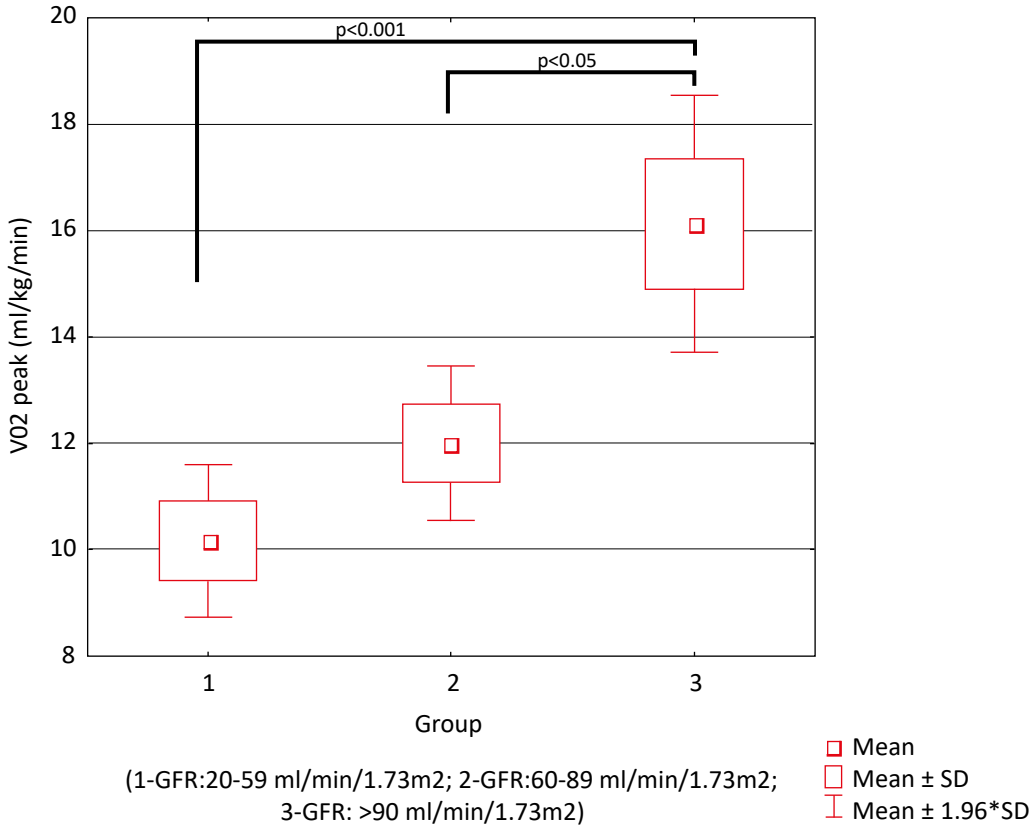
AB = aldosteron blocker; BMI = body mass index; CRT-D = cardiac resynchronizing therapy with defibrillatior; COPD = chronic obstructive pulmonary disease; DBP = diastolic blood pressure; DM = diabetes mellitus; HFrEF = heart failure with reduced left ventrivular ejection fraction; HR = heart rate; SBP = systolic blood pressure; NYHA = New York Heart Association; SHFRM = Seattle Heart Failure Risk Model

Table 2. Biochemical and clinical characteristics of patients based on renal function

	All	GFR 30-59	GFR 60-89	GFR ≥90	P
HgB (g/dL)	14.2 ± 1.5	13.5 ± 1.7	14.6 ± 1.4	14.9 ± 0.8	1:2 and 1:3 p<0.05
Hct (%)	41.7 ± 4	40.2 ± 4.4	42.5 ± 3.9	43.4 ± 2	NS
RBC (mln/µl)	4.7 ± 0.5	4.5 ± 0.6	4.8 ± 0.5	4.9 ± 0.4	NS
RDW (%)	15.2 ± 1.9	15.6 ± 1.6	14.4 ± 1.6	16.1 ± 2.5	1:2 and 1:3 p<0.05
TC (mg/dL)	166.4 ± 41.6	167.8 ± 43.2	159.3 ± 41.3	183.7 ± 39.1	NS
HDL (mg/dL)	43.8 ± 15.4	41.8 ± 13.5	42.2 ± 13.6	54 ± 22.7	NS
LDL (mg/dL)	98.6 ± 30.3	104.2 ± 32.1	92.4 ± 31.9	102 ± 15	NS
TG (mg/dL)	134.3 ± 110.5	109.4 ± 32.3	145 ± 134.2	168.3 ± 164.9	NS
Na (mmol/L)	137.9 ± 2.8	138.2 ± 3	138.1 ± 2.8	136.8 ± 2.6	NS
Glc (mg/dL)	117.5 ± 52	107 ± 18.9	132.8 ± 72.8	98 ± 15.1	NS
BNP (pg/mL)	631.9 ± 626.3	828.7 ± 748.9	480.6 ± 491.9	529.6 ± 537.8	NS
CRP (mg/dL)	3.1 ± 2.2	3.5 ± 2.3	2.7 ± 1.8	3.2 ± 3.2	NS
Uric acid (mg/dL)	8.9 ± 2.8	10.2 ± 2.3	8.5 ± 3.1	7.4 ± 2.2	1:3 p<0.05
Creat (mg/dL)	1.2 ± 0.3	1.4 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	p<0.001
BUN (mg/dL)	22.4 ± 9.3	28.9 ± 10.7	19.7 ± 3.7	14 ± 4	1:2 and 1:3 p<0.001
Fe (ug/dL)	106.6 ± 47.2	82.6 ± 48.9	127 ± 42.7	129.7 ± 23	NS
LVEDD (mm)	72.3 ± 8.3	75.2 ± 9.3	70.7 ± 8.1	70 ± 4.7	NS
LVESD (mm)	61.6 ± 9.2	63.6 ± 10.2	60.6 ± 8.9	59.8 ± 7.8	NS
IVS (mm)	9.5 ± 1.7	9.6 ± 1.6	9.9 ± 1.7	8.6 ± 1.8	NS
PWD (mm)	8.9 ± 1.6	8.8 ± 1.6	9.3 ± 1.5	8.3 ± 1.8	NS
LA (mm)	51.7 ± 6.5	53.1 ± 5.6	51.9 ± 6.3	47.6 ± 7.8	NS
LVEF (%)	23.1 ± 6.3	22.6 ± 6.5	23.9 ± 5.7	22.3 ± 7.9	NS
PH n (%)	9 (22.5%)	5 (31.3%)	3 (17.7%)	1 (14.3%)	NS
VO2 peak ml/kg/min	12 ± 3.8	10.2 ± 3.1	12 ± 3.2	16.1 ± 3.5	1:3 p<0.001, 2:3 p<0.05
VO2 (AT) ml/kg/min	9 ± 2.2	7.9 ± 2.4	9 ± 1.7	10.7 ± 1.9	1:3 p<0.05
VE/VCO2 slope	43.5 ± 12.2	48.9 ± 15.3	40.4 ± 6.2	39.5 ± 13.3	p = 0.09
6MWT (m)	385 ± 99.1	361.1 ± 119.5	406.1 ± 83.8	388.9 ± 79.9	NS

BNP = brain natriuretic peptide; BUN = blood urea nitrogen; creat = creatinine; CRP = C-reactive protein; glc = glucose; Htc = hematocrit; HgB = hemoglobin; IVS = interventricular septum; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter Na = sodium; PH = pulmonary hypertension; PWT = posterior wall diameter; RBC = red blood count; 6MWT = 6-minute walk test

Figure 1. VO2 peak in 3 groups



Interestingly, patients in group 1 and 2 had significantly lower peak VO2 in comparison to group 3 (10.2 ml/kg/min ± 3.1 and 12 ± 3.2 ml/kg/min vs. 16.1 ± 3.5 ml/kg/min, p<0.001 and p<0.05, respectively). Similarly, VO2 in anaerobic threshold (AT) was significantly lower in group 1 in comparison to group 3 (7.9 ± 2.4 ml/kg/min, vs. 10.7 ± 1.9 ml/kg/min, p<0.05) (Figure 1). No changes in 6MWT distance or VE/VCO2 slope were observed.

Discussion

Renal dysfunction is becoming a common abnormality in cardiovascular diseases and its burden may be often seen in HFrEF patients [8-9]. In our study, patients with chronic kidney disease with eGFR <60ml/min/1.73 m2 had more advanced age in comparison to other 2 groups. Katsanos et al. observed that there was a strong association between increasing age and comorbidities that affected the short-term survival [10]. In addition, in their study on HFrEF patients aged >65 years old Braunstein et al. found that nearly 40% of them had >5 non-cardiac comorbidities [11]. Furthermore, older patients with many comorbities experienced much more adverse events requiring hospitalizations than the younger HFrEF patients [11]. In our study only non-sinus rhythm prevalence was significantly higher in group 1 than in other groups. However, no statistically significant differences in the prevalence of other comorbidities were noted.

In addition, we found significantly lower hemoglobin levels in group 1 (with more advanced renal disease) in comparison to the other two groups. This observation is supported by literature [12-14]. The so-called „renal anaemia" is in the majority of cases a consequence of endogenous erythropoetin deficiency [12]. Furthermore, anaemia in HFrEF is also common and its origin is multifactoral [15]. In our study we observed a tendency towards higher anaemia prevalence in group 1. Since data concerning anaemia etiology were not assessed in our study, we cannot provide further details on this issue. We also observed that patients in group 1 had significantly higher concentration of uric acid when compared to group 3. Fillipatos and al. reported that hyperuricaemia is associated with poor outcomes in HFrEF patients with normal renal function but has no effect in patients with chronic kidney disease [16]. High concentration of uric acid in HFreEF patients with kidney disease is a consequence of impaired uric acid excretion in kidneys [16].

In our study we found that patients in group 1 had significantly lower mean peak VO2 and VO2 in AT in comparison to other two groups. No significant differences in VE/VCO2 slope and 6MWT were noted. Ebner et al. observed a significant reduction in exercise capacity cor-

relating with decreasing hemoglobin levels (r=0.24, p < 0.001) [17]. In addition, our results were similar to the study by Hotta's et al. in which HF patients who attained an exercise capacity of ≥5 METs were younger and had higher hemoglobin and eGFR level in comparison to patients with exercise capacity <5 METs. [18]. However the patients enrolled in Hotta's study had less advanced HFrEF with higher LVEF and lower BNP level than our study group. Moreover, Scrutinio et al. recruited 2,938 HFrEF patients who underwent cardiopulmonary stress test [19]. They observed that renal disease significantly correlated with peak VO2. Patients with renal dysfunction (eGFR <45 ml/min/1.73 m2) were older and had a more advanced NYHA class, lower SBP and hemoglobin values, and higher BNP concentrations in comparison to patients with better renal function [19].

Our study has some limitations. The study group was relatively small and was recruited in one clinical centre. On the other hand, we recruited only patients with advanced, stable HFrEF – a population of patients with less evidence in terms of renal function and exercise capacity in comparison to other HFrEF patients. Moreover, we observed only 3 cardiovascular deaths during a 8-month follow-up. Therefore, it was not possible to perform any survival analysis in the study group. Our findings must be interpreted with caution and need further evaluation in large clinical trials focusing on advanced heart failure patients.

Conclusions

Chronic kidney disease in advanced HFrEF may have a significant impact on peak VO2 and VO2 in AT but not on VE/VCO2 slope and 6MWT.

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RESEARCH ARTICLE

Significance of the *PIK3CA* mutations in the differential diagnosis of ovarian epithelial carcinoma

Monika Żuk[1,2], Magdalena Koczkowska[1], Adam Gorczyński[3], Aleksandra Sejda[3], Wojciech Biernat[3], Bartosz Wasąg[1,2]

[1] Department of Biology and Medical Genetics, Medical University of Gdańsk, Poland
[2] Laboratory of Clinical Genetics, University Clinical Centre, Gdańsk, Poland
[3] Department of Pathomorphology, Medical University of Gdańsk, Poland

Abstract

Background: Ovarian carcinoma, one of the most common gynecological malignancies in Central and Eastern Europe, is characterized by a clinical and genetic heterogeneity with a distinct molecular signature for each histologic subtype. **Materials and methods:** Here, we established the frequency of the *PIK3CA* mutations and amplifications in 100 FFPE tissues with the initial diagnosis of serous ovarian carcinoma. Accordingly, the diagnostic value of combining morphology with genetic and immunohistochemical testing was estimated in this cohort. **Results:** The *PIK3CA* mutations and amplifications were found in 4.1% (4/97) and 7.2% (7/97) of samples, respectively with a higher prevalence in low-grade tumors (p=0.0121). All identified variants were classified as pathogenic missense mutations, located within the *PIK3CA* mutational hotspots. In the light of the molecular and immunohistochemical results, two tumors with the somatic *PIK3CA* mutations and strongly positive expression for PI3K and hNF1β were eventually re-classified from serous to clear cell carcinomas after pathological re-evaluation. **Conclusions:** These findings demonstrate that the *PIK3CA* mutational screening facilitated establishing an accurate diagnosis of ovarian carcinomas and, more importantly, might allow for personalized treatment optimization. As the *PIK3CA* mutations result in the PI3K/AKT pathway deregulation, the individuals with the somatic PIK3CA variants may be eligible for personalized targeted therapies with PI3K inhibitors.

Keywords: *PIK3CA* / PI3K inhibitor / clear cell ovarian carcinoma / serous ovarian carcinoma

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Corresponding author:
Bartosz Wasąg, Medical University of Gdańsk, Dębinki 7, 80-211, Gdańsk, Poland
e-mail: bartosz.wasag@gumed.edu.pl

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Introduction

Besides the tumor suppressor genes *BRCA1* (MIM: 113705) and *BRCA2* (MIM: 600185) other cancer-related genes have been associated with pathogenesis and progression of ovarian cancer (OC) [1-2]. Indeed, each histological subtype of OC is reflected by the specific tumor molecular signature what enables establishing a widely accepted classification of OC into type I and type II tumors developing through different pathways [3]. Besides the *BRCA1/2* susceptibility genes, high-grade serous ovarian tumors (type II) are characterized by genomic instability, including frequent mutations in *TP53* (MIM: 191170), *NF1* (MIM: 613113) and/or *CDK12* (MIM: 615514), while clear cell and endometrioid carcinomas (type I) mostly harbor alterations in the *ARID1A* (MIM: 603024), *PIK3CA* (MIM: 171834) or *PTEN* (MIM: 601728) genes [4].

The *PIK3CA* gene encoding the catalytic subunit p110α of phosphatidylinositol 3-kinase (PI3K) is mutated or amplified in a broad spectrum of neoplasms including colon, breast, ovarian, endometrial, gastric, thyroid, cervical cancer as well as brain tumors, resulting in the deregulation of the PI3K/AKT signaling pathway [5]. The over-activation of the PI3K/AKT pathway contributes to the progression and tumorigenesis in OC and, importantly, may be followed by resistance to chemotherapy [6-9]. However, recent studies demonstrated that this pathway may play an attractive therapeutic role in OC with several agents, i.e. PI3K inhibitors that are currently being explored in pre- and clinical trials [4,10].

In this study, we established the spectrum and frequency of the *PIK3CA* mutations and amplifications in 100 formalin-fixed paraffin-embedded (FFPE) tissues with the initial diagnosis of serous OC. In addition, the diagnostic value of combining morphology with genetic and immunohistochemical testing was estimated in the group of individuals with OC.

Materials and methods

Samples selection and histopathological review

A total of 100 FFPE OC samples were enrolled to the study. All tissue samples were collected between 2008 and 2012 and have been stored in the archives of the Department of Pathomorphology at the Medical University of Gdansk. Hematoxylin-eosin (H&E)-stained tumor tissue sections were reviewed independently by two pathologists (A.G. and W.B.). The initial histological diagnosis of serous OC and the tumor tissue content were evaluated for all samples. To obtain a concentration of at least 50% of cancer cells, tissue macrodissection was performed. Briefly, 84% of individuals (84/100) were diagnosed with high-grade tumors, mostly with IIIB (25%) and IIIC (54%) tumor's stage. The mean individual age at diagnosis was 60 years (range: 36-81).

The study was approved by the local ethics committee at the Medical University of Gdansk. All individuals provided informed written consent prior to study enrollment.

DNA extraction

Genomic DNA was extracted from the macrodissected FFPE tissues using Cobas DNA Sample Preparation Kit (Roche) following the manufacturer's protocol. NanoDrop 1000 UV Spectrophotometer and Qubit Fluorometer (ThermoFisher Scientific) were used to check the quantity and quality of isolated DNA.

Mutational analysis

The *PIK3CA* gene mutation screening of exons 1, 4, 7, 9 and 20 was performed using cobas® *PIK3CA* mutation kit (Roche) in DNA derived from all FFPE tissue samples following the manufacturer's protocol. The presence of the pathogenic *PIK3CA* variants was confirmed by next-generation sequencing (NGS) analysis with Tumor Hotspot MASTR plus kit (Agilent). The somatic origin of the alterations, i.e. the paucity of the mutations in the normal tissues, was determined by bidirectional Sanger sequencing (ABI PRISM 3130, Life Technologies) and by cobas® *PIK3CA* mutation kit (Roche). In all samples, the comprehensive analysis of the *BRCA1/2* genes testing was done as part of our previous study [11].

To explore the presence of the *PIK3CA* amplification and/or the ploidy, samples were tested by dual in situ hybridization (DISH) method using *PIK3CA*-specific and centromer 3-specific (CEP3) probes on the BenchMark GX automated system (Ventana Medical Systems, Inc.) according to the manufacturer's protocol. A quantitative scoring algorithm was applied to 50 representative non-overlapping nuclei within the invasive region per specimen. To assess gene copy number, *PIK3CA* and CEP3 signals were counted and used for the resulting ratio calculation. The *PIK3CA* gene status was classified as non-amplified (*PIK3CA*/CEP3 ratio <2.0) or amplified (*PIK3CA*/CEP3 ratio ≥2.0).

The nomenclature of the mutations was based on *PIK3CA* mRNA sequence NM_006218.2, *BRCA1* mRNA sequence NM_007294.3 and *BRCA2* mRNA sequence NM_000059.3 according to the recommendations of the Human Genome Variation Society (HGVS).

Immunohistochemical staining

Immunostaining was performed on tissue microarrays (TMAs) of the representative tumor site from FFPE tissue sections. With Manual Tissue Arrayer MTA1 (Beecher Instruments Inc., Sun Prairie, USA), two cylindrical cores of 1.0 mm diameter were removed from each donor paraffin block and transferred to pre-molded recipient paraffin blocks at defined array positions. The presence of PI3K protein in TMAs was assessed by immunohistochemical staining with monoclonal rabbit antibody against p110α PI3K, clone C73F8 (Cell Signaling Technology Inc., Danvers, USA) with dilution 1:50, using the Novolink Polymer Detection System (Novocastra, Wetzlar, Germany) with appropriate positive and negative controls included. Each core was individually evaluated according to the H-score system [12]. To exclude the diagnosis of clear cell carcinoma, all TMAs samples were immunostained with the polyclonal rabbit hNF1- β antibody against human HNF1 homeobox B (Sigma-Aldrich, St. Louis, USA) of 1:100 dilution, and in cases showing any nuclear positivity the staining was repeated on the whole slides.

Statistical analysis

For univariate analysis, two-tailed Fisher's exact test was used to compare categorical variables with a p-value < 0.05 considered as statistically significant. All statistical analyses were performed with GraphPad software.

Pathogenic variants in BRCA1/2

Of the 11 tumors with the *PIK3CA* mutation or amplification, two samples (#13 and #36) had an additional variant in the *BRCA2* gene: c.5328dupT (p.Leu1777Glufs*4) and c.5993_5997dupAAGTG (p.Phe2000Lysfs*6), respectively (as detailed in Table S2). Due to the lack of normal tissue for case subject #13, the germline origin of the aforementioned alteration was confirmed only in sample #36. The comprehensive *BRCA1/2* analysis in the studied material was performed by NGS as part of our previous study [11].

Immunohistochemical analysis and diagnosis refinement

Based on the routine histopathological examination, initially all samples were classified as serous OC (Figure 2A). Additional immunohistochemical analysis revealed a high expression (+++) for PI3K in all tumors carrying the *PIK3CA* mutations or amplifications (Figure 2C-D). Moreover, two samples (#22 and #43) were strongly positive (+++) for hNF1β (Figure 2E). In the light of immunohistochemical and genetic results, these samples were eventually diagnosed as clear cell carcinomas after independent re-evaluation by two expert pathologists. The remaining tumors were characterized by negative (-) or weak (+) PI3K and hNF1β expression (Figure 2F). The algorithm of the study approach is shown in Figure 3.

Results

PIK3CA *alterations*

The pathogenic *PIK3CA* mutations and amplifications were identified in 4/97 (4.1%) and 7/97 (7.2%) samples, respectively (Figure 1 and Table 1). Three samples (#8, #34 and #95) were excluded from the molecular analysis due to the low quality of DNA. Among the positive *PIK3CA* missense variants, c.1634A>C (p.Glu545Ala) and c.1624G>A (p.Glu542Lys) in exon 10 as well as c.3140A>G (p.His1047Arg) and c.3140A>T (p.His1047Leu) in exon 21 were found; each observed in a single tumor as a somatic alteration (samples #22, #44, #20 and #43, respectively). The *PIK3CA* mutations or amplifications were more common in low-grade tumors compared to high-grade samples (33.3% versus 7.3%; p=0.0121; Table S1). We did not observe the presence of the gene amplification in combination with the *PIK3CA* point mutation.

Discussion

As OC are characterized by a clinical and genetic heterogeneity with different treatment sensitivity and prognosis, it is critical to make a definitive histological diagnosis. In the current study, the spectrum and frequency of the *PIK3CA* mutations and amplifications in 100 FFPE samples with the initial diagnosis of serous OC was established. Additionally, we estimated the efficacy of implementation the expanded genetic testing panel, comprising *PIK3CA* mutation screening, into the routine diagnostic procedures in OC.

The spectrum of the *PIK3CA* mutations has been widely studied in numerous neoplasms, including breast and ovarian cancer [5]. Although *PIK3CA* is one of the most commonly mutated oncogenes in human malignancies with over 13,000 tissue samples carrying the *PIK3CA* pathogenic variants (as reported in the COSMIC database as of November 2018), the prevalence of the somatic *PIK3CA* mutations in OC is moderate. As the overall frequency is estimated at 4-12%, depending on the histological subtype, the finding of the *PIK3CA* variants in 4.1% (4/97) of the samples in our

Table 1: Spectrum of the *PIK3CA* alterations in ovarian carcinomas

Case no.	Age	Mutation	Exon	RS number	Classification [a]	Immunohistochemistry	FIGO stage	Diagnosis [b]
#44	42	c.1624G>A (p.Glu-542Lys)	10	rs121913273	Pathogenic	PI3K (+++), hNF1B (-)	IIA	serous
#22	58	c.1634A>C (p.Glu-545Ala)	10	rs121913274	Pathogenic	PI3K (+++), hNF1B (+++)	IA	clear cell
#20	79	c.3140A>G (p.His1047Arg)	21	rs121913279	Pathogenic	PI3K (+++), hNF1B (-)	IIA	serous
#43	54	c.3140A>T(p.His1047Leu)	21	rs121913279	Pathogenic	PI3K (+++), hNF1B (+++)	IA	clear cell
#9	44	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIB	serous
#11	46	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIC	serous
#13[c]	51	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIB	serous
#36[c]	76	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIC	serous
#49	58	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIA	serous
#83	40	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIC	serous
#85	58	Amplification	N/A	N/A	N/A	PI3K (+++), hNF1B (-)	IIIA	serous

[a] Variants' classification reported in the publicly available databases, including ClinVar and HGMD.

[b] Based on the molecular and immunohistochemical analysis, in two cases (#22 and #43) the initial diagnosis of serous ovarian carcino ma was refined to the clear cell ovarian carcinoma (in italics) after histological re-evaluation by two independent pathologists.

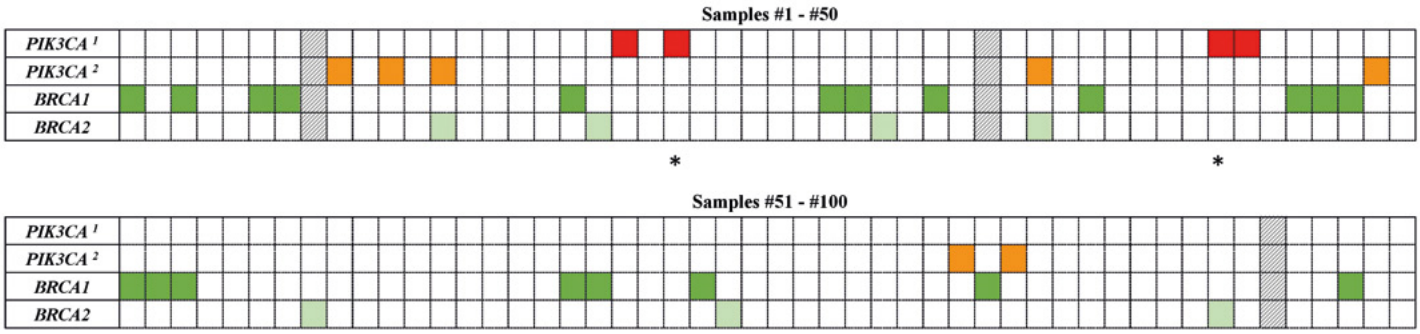
[c] Tumors with presence of an additional pathogenic variant in the *BRCA2* gene (#13 and #36).

N/A: not applicable

cohort is in line with expectations [13-16]. Campbell et al. (2005) and Wang et al. (2005) reported that the PIK-3CA variants predominantly clustered among clear cell and endometrioid OC compared to serous carcinomas (20% versus 2.3%), suggesting different mechanisms of their pathogenesis [14,16]. In this study, the presence of the *PIK3CA* mutations was observed in tumors with the initial diagnosis of serous carcinomas that may likely explain such a low detection rate. Moreover, two *PIK3CA* mutational hotspots locations have been described in the highly conserved regions within the helical and kinase domains of the PI3K subunits (exon 10 and 21, respectively) with the estimated frequency of over 80% of all somatic missense mutations [5]. Functional studies demonstrated that these mutations affect the cellular transformation in vivo and in vitro by increasing the kinase activity [17-18]. In this study, the *PIK3CA* variants were identified exclusively within these hotspots, suggesting functional importance.

The *PIK3CA* amplification, another mechanism activating PI3K/AKT pathway, has been reported in OC more frequently than point mutations (16%-25% ver-

Figure 1. A heat map with the mutation pattern of the PIK3CA and BRCA1/2 genes in ovarian carcinomas



[a] ***PIC3CA* mutation; [b] *PIC3CA* ampflification**

The column represents each tumor sample, while each row represents a gene (red and orange –*PIK3CA*; dark green – *BRCA1*; light green – *BRCA2*). Asterisks (*) indicate the tumor samples with diagnosis refinement based on the molecular and immunohistochemical results (#22 and #43). Three samples (#8, #34 and #95), excluded from the study due to the low DNA quality, are shown as the black and white pattern.

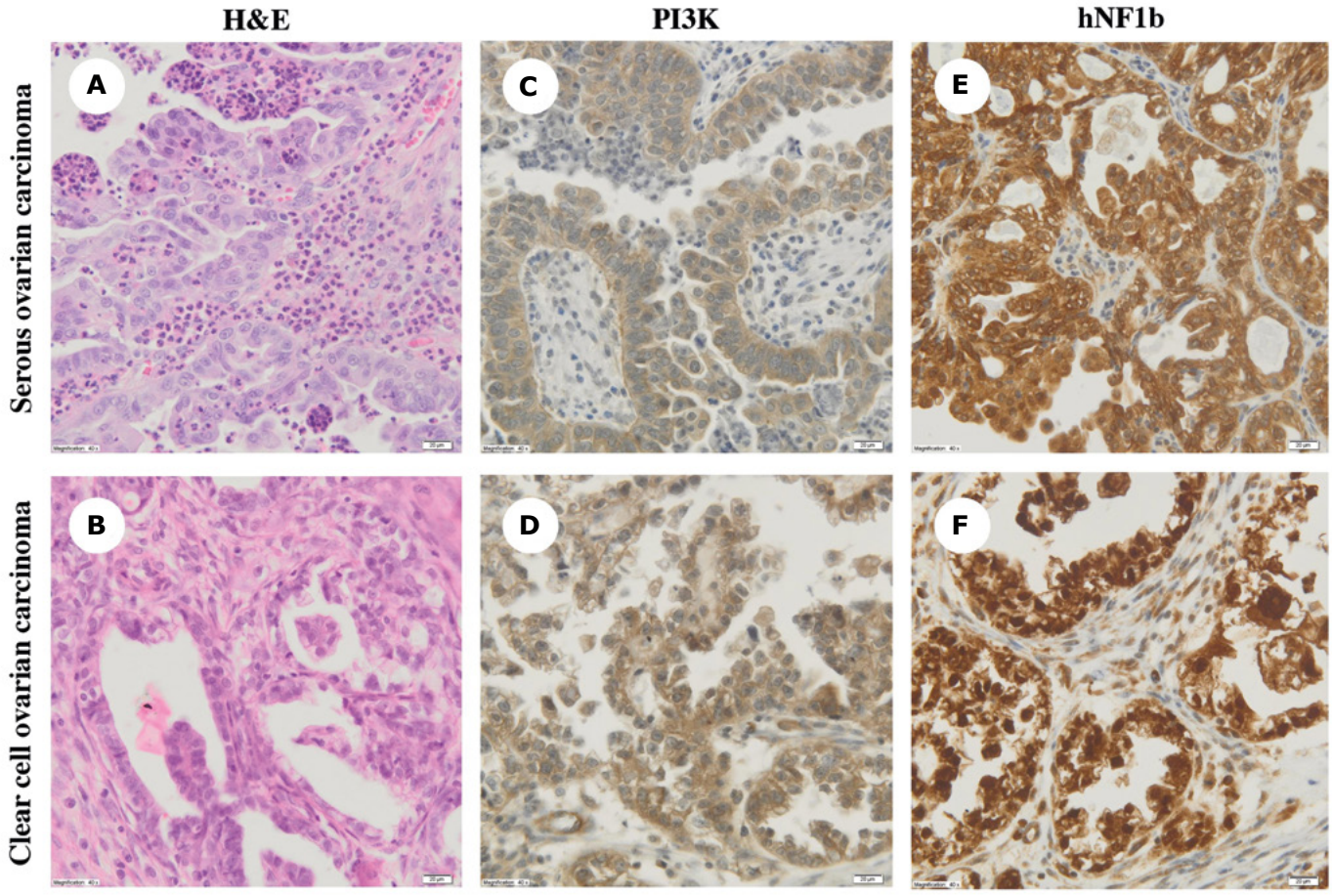
sus 4-12%), but no clear bias towards any particular histological subtype was observed [14,19-21]. Taken together, the overall frequency of the *PIK3CA* mutations and amplifications in the studied cohort was 11.3% (11/97) that is slightly lower compared to previous reports [14,21]. Indeed, the PIK3/AKT pathway is reported to be activated in approximately one third of OC cohort, but comprising mostly of samples with the clear cell histological subtype [4]. In this study, all tumors represented serous OC, mostly high-grade (84% with the stage III), except for two cases with the diagnosis refinement to clear cell OC (Table 1 and Figure 3). That likely explains the differences in the overall frequency of the *PIK3CA* alterations between our studied group and the previously described cohorts.

With the rapid development and accessibility of genomic technology, genetic testing is becoming increasingly important in the clinical management of cancer patients and expecting to be widely implemented in routine oncological practice. That facilitates not only establishing an accurate diagnosis, but, more importantly, allows for personalized treatment optimization. As demonstrated by Italiano et al. (2016), the molecular genetic results enabled for the diagnosis refinement in ~14% (53/384) of soft tissue sarcomas samples, suggesting that genetic testing should be a mandatory step in soft tissue sarcomas diagnostic approach even if the histological diagnosis is made by the expert pathologists in the field [22]. In this study, two tumors (#22 and #43) were wrongly classified as serous OC prior to molecular testing (Figure 3). Only the identification of the PIK3CA mutations, commonly present in endometrioid and clear cell OC, casted a shadow of doubt on the accuracy of the initial histological diagnosis in these samples [3-4]. Therefore, we believe that genomic profiling of tumors, including at least mutational screening of the cancer-related genes specific for each histological subtype (i.e. *PIK3CA, PTEN, ARID1A, NF1* and/or *CDK12*) might offer benefits also in the differentiation of OC.

Although the *BRCA1/2* mutation-positive tumors represent the most common group of OC tumors and these women are eligible for the poly(ADP-ribose) polymerase 1 inhibitors (iPARP1) targeted therapy [23], there is a need to develop new effective treatment alternatives. Another advantage of the *PIK3CA* mutation screening in OC is the possibility of the application the targeted therapies in individuals with the *PIK3CA* mutation-positive tumors. As the PI3K/AKT pathway is frequently deregulated in selected histological subtypes of OC, inhibition of PI3K seems to be a worthwhile treatment strategy in these individuals. To date, several PI3K inhibitors have been tested in pre- and clinical trials and the most promising results are observed in combination with other targeted therapies, such as MEK inhibitors, anti-angiogenic therapy and hormonal therapy in selected OC individuals [24-25]. Moreover, the *PIK3CA* alterations are reported to play a significant role in acquiring chemoresistance in OC cells, all the more so, it is critical to diagnose those tumors early enough to implement an appropriate treatment in these individuals and consequently improve their clinical prognosis [7-8,21].

As effective cancer treatment starts with the correct pathological diagnosis, genetic testing may play a complementary role in this critical diagnostic process.

Figure 2 A-F. Hematoxylin and eosin (H&E) staining (panels A-B) and immunohistochemical analysis of phosphatidylinositol 3-kinase (PI3K) and hNF1b expression (panels C-D and E-F, respectively) in serous and clear cell ovarian carcinomas (magnification, ×40)



In the current study, the initial misdiagnosis of two OC samples was re-evaluated after providing the additional molecular and immunohistochemical results. Moreover, we confirmed the frequency of the *PIK3CA* mutations and amplifications in OC samples, and more importantly, demonstrated that the genomic profiling along with the detailed histological and immunohistochemical examinations may resolve the diagnostic dilemma. Although the *PIK3CA* alterations affect only a small percentage of OC tumors, it is still important to evaluate their mutational status because these individuals may be eligible for the personalized targeted therapies.

Abbreviations

OC: ovarian carcinoma; FFPE: formalin-fixed paraffin-embedded tissue sample; PI3K: phosphatidylinositol 3-kinase; NGS: next-generation sequencing; H&E: hematoxylin & eosin; DISH: dual in situ hybridization; TMA: tissue microarray

Online databases

ClinVar: https://www.ncbi.nlm.nih.gov/

Cosmic: http://cancer.sanger.ac.uk/cosmic

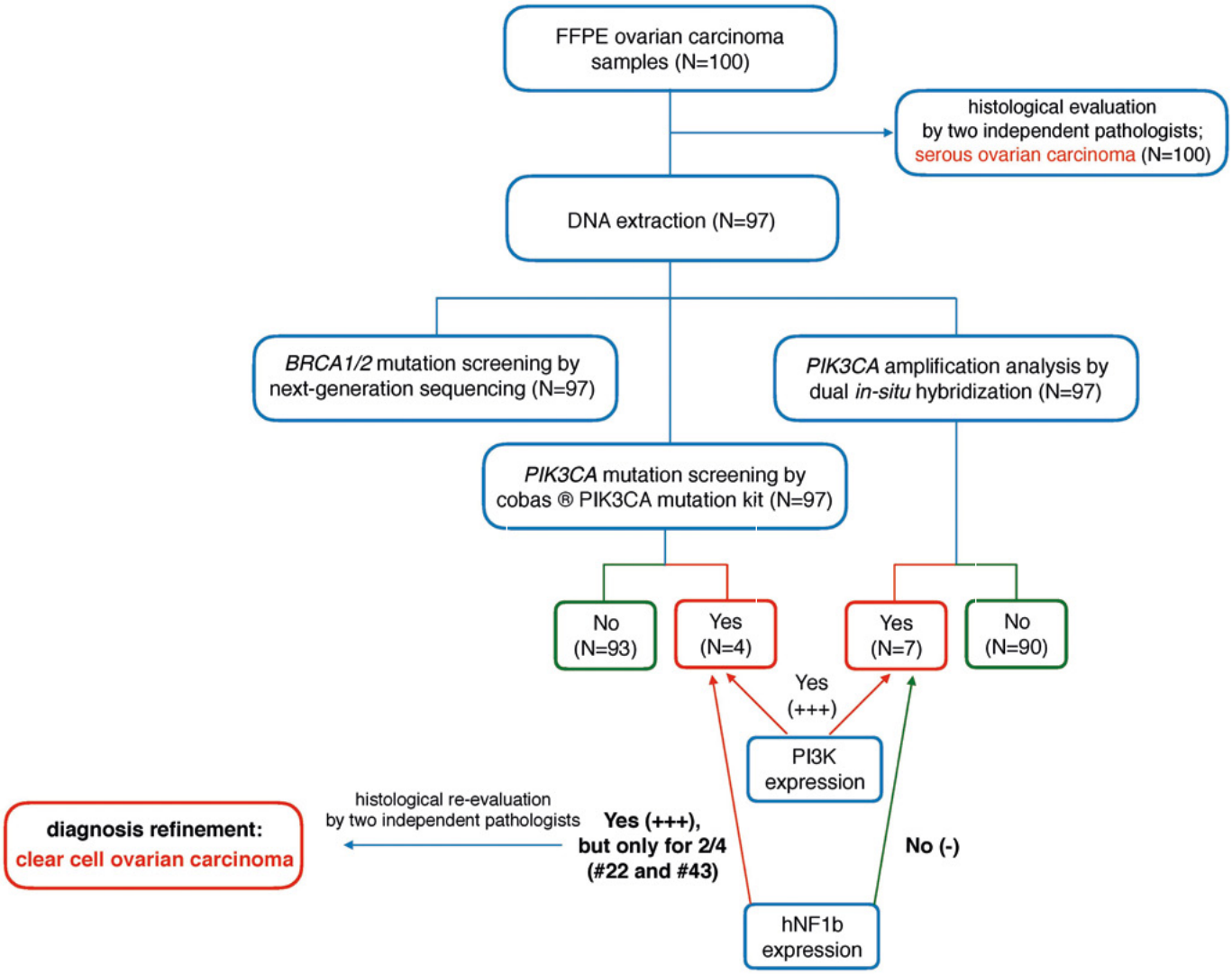
GraphPad: https://www.graphpad.com/

HGMD: http://www.hgmd.cf.ac.uk/ac/index.php

HGVS: http://varnomen.hgvs.org/

OMIM: https://www.omim.org/

Figure 3. Algorithm of the study approach in the differential diagnosis of epithelial ovarian carcinomas



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RESEARCH ARTICLE

The topographic relationship between the maxillary teeth roots and the maxillary sinus floor, assessed using panoramic radiographs

Anna Starzyńska , Paulina Adamska , Łukasz Adamski

Department of Oral Surgery, Medical University of Gdańsk, Poland

Abstract

Introduction: The topographic relation between the maxillary teeth roots and the maxillary sinus floor is important for diagnosis and planning of many surgical procedures. The aim of the study was to assess the topographic relationship between posterior maxillary teeth and the sinus floor.
Materials and methods: 50 pantomographs of patients aged 18-72, treated at the Medical University of Gdańsk, were analysed. We analysed only maxillary molars and premolars. Teeth were assessed using Kwak classification. Statistical analysis was carried out using STATISTICA 13.3.
Results: We analysed 180 molars and 181 premolars (total 361). According to the root-sinus classification, most of the first molars were type V (55.96%), most of the second molars – type III (32.29%), most of the first premolars – type I (76.09%) and most of the second premolars – type III (35.96%). Non-parametric Spearman's rank-order correlation coefficient revealed significant correlation between right and left molars and between right and left premolars ($p<0.05$).
Conclusions: Proper assessment of the pantomograph allows for correct pre-operative planning, which may affect the course of the procedure and enables to avoid possible complications. These findings may have clinical applications.
Keywords: Maxillary sinus / dental imaging / pantomographic radiographs

Citation

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Corresponding author:
Anna Starzyńska, Department of Oral Surgery, Medical University of Gdańsk, Dębinki 7, 80-211, Gdansk, Poland
e-mail: anna.starzynska@gumed.edu.pl
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Introduction

The maxillary sinus (antrum of Highmore) is the largest of paranasal sinuses. It is the first of the sinuses to develop and is present at birth. It is pyramid-shaped and is comprised of four walls: nasal (base), posterior, anterior and floor of the orbit (roof) [1-2]. The sinus communicates with the nose through an opening into the semilunar hiatus. An additional hiatus is sometimes present [2-3]. This sinus has seven recesses: zygomatic (in the zygomatic bone), alveolar (in the alveolar process of maxilla), orbital process, infraorbital, lacrimal, palatal and a recess formed after tooth extraction [3-4]. The relation of maxillary teeth roots to the maxillary sinus floor is important for diagnosis and planning of many surgical procedures, e.g. extraction, dental or orthodontic implant placement or sinus lift [2,5-6].

The aim of this study was to assess the pantomographic images and to evaluate topographic relationship of the posterior maxillary teeth to the maxillary sinus floor.

Materials and methods

Fifty pantomographic radiographs of patients aged 18-72, treated at the Department of Oral Surgery of the Medical University of Gdańsk (Poland), were analysed. Inclusion criteria: presences of at least one first or second permanent molar in maxilla, fully erupted posterior teeth, complete development of the maxillary sinus and not damaged by any disease, fully developed root apices without resorptions, pantomograph of good quality. Patients were generally healthy, not suffering from chronic diseases, not taking any medications and antibiotics. We analysed first molars, second molars, first premolars and second premolars, because the roots of these teeth are in the closest to the sinus floor. Exclusion criteria were: lack of one first or one second molar in the maxilla, not fully erupted teeth, not fully developed maxillary sinus, root apices with resorption, bad quality of pantomograph, chronic diseases, taking medications. Patients' full anonymity was preserved during this study.

Teeth were identified using the Fédération Dentaire Internationale (FDI) classification. Radiological examinations were assessed by two independent researchers and then critically evaluated by the third author.

Teeth were assessed using Kwak et al. classification (root-sinus classification; Figure 1). This classification is based on the root-sinus relationship:

- type I – the roots of the posterior tooth are not in contact with inferior wall of the sinus;
- type II – the root apex/apices is/are located in contact with maxillary sinus floor;
- type III – the buccal root apex/apices is/are observed over inferior wall of the sinus;
- type IV – the palatal root apex is located over maxillary sinus floor;
- type V – the buccal and palatal apices are observed over inferior wall of the sinus [7].

Statistical analysis was carried out using STATISTICA 13.3 (StatSoft Inc. Tulsa, United States) licensed by the Gdańsk Medical University. Normal distribution of variables characterizing objects was verified using the Shapiro-Wilk test. Non-parametric Spearman's rank correlation coefficient was used. P value of ≤0.05 was considered statistically significant.

Results

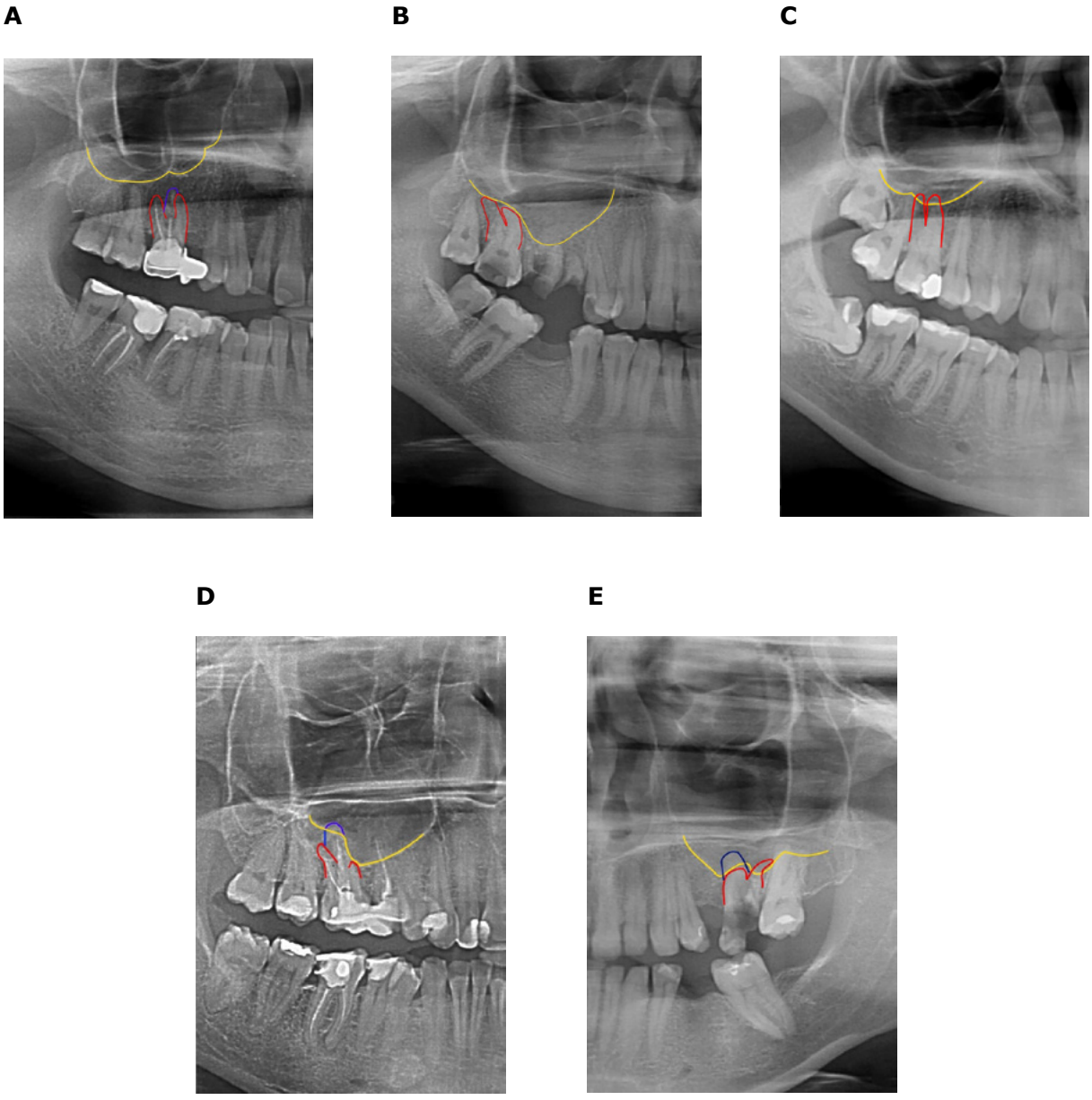
We analysed 180 permanent molars and 181 premolars (total 361 teeth): 44 right first molars, 48 second molars, 40 left first molars and 48 left second molars in the maxilla; 46 right first premolars, 43 second premolars, 46 left first premolars and 46 left second premolars in the maxilla.

According to the root-sinus classification, most of the first molars were type V (47; 55.96%), the second molars were type III (31; 32.29%), the first premolars were type I (70; 76.09%) and the second premolars were type III (32; 35.96%) (Table 1). Non-parametric Spearman's rank-order correlation coefficient revealed statistically significant correlation between right and left first molars ($p=0.030$), second molars ($p<0.010$), first premolars ($p<0.010$) and second premolars ($p=0.003$).

Discussion

The root apices of the molars are generally located closer to the maxillary sinus floor than the premolar apices. The evaluation of radiographs is very important in dental treatment. Endodontic treatment of teeth with root apices above the inferior wall of the sinus (types III, IV and V) can contribute to the perforation of the mucous membrane. Dentists should be careful when extracting teeth, because an oroantral communication may arise in such cases. Removal of the teeth should be carried out atraumatically, with separation of roots [8-11].

Figure 1. Kwak HH, et al. classification: A) type I; B) type II; C) type III; D) type IV; E) type V (yellow line – the maxillary sinus floor; red line – the buccal root; blue line – the palatal root)



Panoramic radiograph is one of the most frequently used dental imaging. This imaging shows a 2D view of maxilla and mandible. It is helpful in the assessment of teeth and bone. Sometimes ghost images form in the pantomographs, which results in the image being unclear and inaccurate. Panoramic radiography is relatively safe as the radiation dose is very low [12-13].

The relationship between tooth root apices and sinus floor plays a vital role in periodontal surgery. Huang et al. and Brunsvold et al. described that maxillary sinusitis resulted from periodontal treatment of the first permanent molars having deep periodontal and bony pockets [14-16]. Fry et al. and Shokri et al. described that most of the first premolars are not in contact with inferior wall of the sinus. In our study the results were the same [10,16]. The most common type observed in the first molars was the one with the buccal and palatal apices over the floor of the sinus. Shokri et al. assessed teeth on cone beam computed tomography. The difference between left and right side was not statistically significant in Fryet al. study, but in our study it proved significant [10,16].

Conclusions

Radiological examination should be routinely performed to assess the stomatognathic system against all treatments in this localisation. This study demonstrated various relationships between the root apices and the floor of the maxillary sinus. These findings may have clinical applications. Proper assessment of the pantomographs allow for correct pre-operative planning, which may affect the course of the procedure, and enables to avoid avoid possible complications.

Table 1. The relationship between the maxillary posterior teeth and the sinus floor (N - number of teeth; type I – the roots of the posterior tooth are not in contact with inferior wall of the sinus; type II – the root apex/apices is/are located in contact with maxillary sinus floor; type III – the buccal root apex/apices is/are observed over inferior wall of the sinus; type IV – the palatal root apex is located over maxillary sinus floor; type V – the buccal and palatal apices are observed over inferior wall of the sinus)

Tooth number according to FDI classification	Root-sinus classification										p value
	Type I		Type II		Type III		Type IV		Type V		
	N	%	N	%	N	%	N	%	N	%	
14	36	78.26	5	10.87	1	2.17	0	0	4	8.70	<0.010
24	34	79.91	7	15.22	0	0	0	0	5	10.87	
15	15	34.09	11	25	15	34.09	0	0	3	5.82	0.030
25	13	28.26	13	28.26	17	36.96	1	2.17	2	4.35	
16	5	11.36	8	18.18	6	13.64	6	13.64	19	43.18	0.030
26	2	5.13	1	2.56	5	12.82	3	7.69	28	71.79	
17	8	16.67	12	25	17	35.42	1	2.08	10	20.83	<0.010
27	2	4.17	14	29.17	14	29.17	1	2.08	17	35.42	
Total number of teeth (each type)	115		71		75		12		88		—
Total number of teeth	361										

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RESEARCH ARTICLE

A novel approach to visualization of the right ventricular outflow tract

Agata Ewa Kaczyńska, Adam Kosiński, Rafał Kamiński, Miłosz Andrzej Zajączkowski, Marta Gleinert-Rożek, Ewa Nowicka

Department of Clinical Anatomy, Medical University of Gdańsk, Poland

Abstract

Introduction: Nowadays, heart is one of the most demanding organs for imaging procedures. This is related to its irregular shape and complex internal structure. Increased demand for imaging complex cardiac structures has resulted in the development of novel 3D modeling techniques. Not only did the methods of imaging the organs of the living patients developed in recent years, but also new methods of post-mortem analysis. Acquired 3D models have a number of applications, both clinical and educational.

Detailed knowledge of the morphology of right ventricular outflow tract (RVOT) is extremely important in terms of cardio-invasive therapeutic procedures. Its significance was noticed during the exploration of the optimal pacing sites in the area of right ventricle. What is more, accurate analysis of the RVOT morphology and spatial structure is also the basis for the treatment of ventricular arrhythmias which foci are located within the outflow tract.

The aim of this study was to elaborate the most accurate technique of preparing interior models of the right ventricle and digitizing them to the 3D form. For this purpose we used a silicone molding of the heart cavities with digital photogrammetry.

Keywords: 3D models / cardiac imaging / silicone molding / photogrammetry / right ventricular outflow tract

Citation

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Introduction

The progress of technologies that enable the imaging of complex anatomical structures has resulted in the increased interest in these techniques, in particular in connection with cardiac visualization. Its imaging poses a great challenge, e.g. due to the unique functioning of this organ, the diversity of dimensions and

Corresponding author:
Agata Ewa Kaczyńska, Department of Clinical Anatomy, Medical University of Gdansk, Poland
e-mail: agata.kaczynska@gumed.edu.pl
No external funds.
Available online: www.ejtcm.gumed.edu.pl

shapes depending on the phase of the heart cycle. The visualization of the inside of the heart is extremely demanding due to the complex internal structure of the atria and the chambers [1-3].

Despite obtaining a large amount of the information about the internal structure of the heart, thanks to the use of commonly used imaging techniques, including magnetic resonance, computed tomography and echocardiography [4-6], there is still little attention paid to the structure that is a part of the right ventricle - the outflow tract.

The right ventricular outflow tract (RVOT) is the space located between the supraventricular crest and the pulmonary valve. Exploration of the exact morphological architecture of this structure is extremely important in terms of the cardio-invasive therapeutic procedures. During the search for the optimal stimulation sites from the right ventricle area, it was proved that the most appropriate location for an electrode placement is the RVOT region. It was shown that the stimulation of this area is characterized by a fewer complications compared to the often used stimulation of the apex of the right ventricle [7-8].

Analysis of RVOT morphology is also extremely important in the context of treatment of the ventricular arrhythmias, whose source is located within this area. Accurate assessment of the arrhythmia and the precise location of its foci allows to increase the efficiency of ablation and shorten its duration [9].

Knowledge of the RVOT morphology and its closest neighborhood allows proper optimization of both the implantation of the stimulation system and the ablation of arrhythmogenic foci in this area, as well as limiting the risk of complications. For this purpose, it is extremely important to examine the internal structure of the right ventricle, taking into account the thickness of its muscular wall, the location of vessels and elements of the conductive system, as well as the position of heart valves and cavities in close proximity. Acquiring such information may appear crucial, due to the lack of precise literature data in the subject of the morphological structure of RVOT and its surroundings. Proposing an adequate anatomical nomenclature of this area and determining the exact boundaries of RVOT is a very important aspect of the research, which would significantly improve the work of clinicians.

The aim of this study was to develop an optimal method of obtaining a model of the right ventricle cavity and to transform its physical form into a virtual form that would give the possibility to perform significant measurements of the RVOT area.

Material and methods

30 hearts fixed in a formalin solution were used for the study. Hearts, without macroscopically visible pathological changes, belonged to adults of both sexes.

The first stage of the research was to construct right ventricle models using a silicone molding compound (Xiameter 4250 S Green). The applied technique has already been used in the case of modeling the heart's appendages [10]. Before modeling, the hearts were thoroughly rinsed with cold water to remove blood clots from the inside. The silicone was poured into the heart cavity from the apex of the right ventricle, because filling RVOT with silicone was more advantageous when it was deposited inside the heart in a gravitational manner, for instance, with the apex pointing upwards. For this purpose, the apex of the right and left ventricles was cut to pour the plastic mass (Figure 1A). Next, the venous outlets of the right atrium were sutured and a catheter balloon was inserted into the pulmonary trunk above the level of the valve. The catheter balloon was filled with air so as not to distort the surface of the free wall of the RVOT and the pulmonary trunk (Figure 1B, Figure 1C). Thus prepared and dried hearts were placed in a glass vessel with the atria directed towards its bottom and with the apex towards the top (Figure 1D). After preparing the molding mass by adding an activator, the syringe was filled with silicone (Janet 100 ml), and then the mass was poured into the heart. The tip of the syringe was placed in the incised part of the right ventricle and the silicone was poured inside. Occasionally, the wall of the right ventricle and atrium was slightly pressed to remove air bubbles from among the trabeculations (Figure 1E). When the right part of the heart was completely filled with silicone, the heart was covered tightly with foil. The silicone-filled heart was left to harden for 24 hours. After this time, the ventricular wall was incised along its edge, starting with the incision of the apex of the right ventricle to the base of the right appendage of the heart, including the wall of the right atrium (Figure 1F). After making the incision, the model of the interior of the heart was carefully removed and it was left to dry for 24 hours (Figure 2A, Figure 2B).

The obtained 30 silicone models of the interior of the right heart, including the right atrium and the right ventricle, were subjected to the virtual visualization procedure to form the 3D model. A photogrammetry technique was used which uses serial images of the object in order to transform the physical model into virtual form. A scale was applied to every model, which allows for precise spatial measurements.

With the use of silicone modeling and photogrammetry techniques, 30 RVOT models were obtained.

Exemplary models and their modifications, as well as exemplary measurements that can be made on the models, are shown in Figure 3A – Figure 3E.

Discussion

Intensive development of heart imaging techniques, including spatial modeling, allows its more accurate virtual reproduction. Such a model can be subjected to various computer analyses, e.g. specific measurements and simulations. In addition, the 3D model that has been subjected to the appropriate virtual processing can be successfully duplicated to the physical form thanks to the use of the 3D printing technology. Such created 3D heart models, both virtual and those obtained using silicone molding, have a number of applications in many areas of medicine. Computer simulations give the opportunity to explore the exact anatomy of the heart and help to understand the structural defects of this organ. What is more, the virtual heart model makes it possible to analyze some physiological aspects without carrying out complicated in vivo tests [11–13].

According to some reports 3D models of the hearts of patients with complex cardiac disease were constructed in order to plan the optimal surgical approach [14]. Commonly used methods of cardiac imaging were used for this purpose: computed tomography and magnetic resonance. Next, a 3D model was printed which was based on the virtual heart model that accurately reflected the patient's heart and defect. Such a model helped during the search for the most advantageous method of the resection of the aneurysm of the ventricle and the tumor in the right ventricle, as well as the reconstruction of the area after excision of the lesion. What is more, the surgeon was also able to estimate the risk of complications of the planned surgery and to choose the technique with the least risk of failure.

Figure 1 A-F. Stages of RVOT modeling with silicone mass. A – incision of the ventricles apex, B,C – placement of a catheter in the pulmonary trunk, D – placement of the heart in the glass vessel, E – pouring the molding mass, F – incision of the ventricle and atrium in order to take out done model

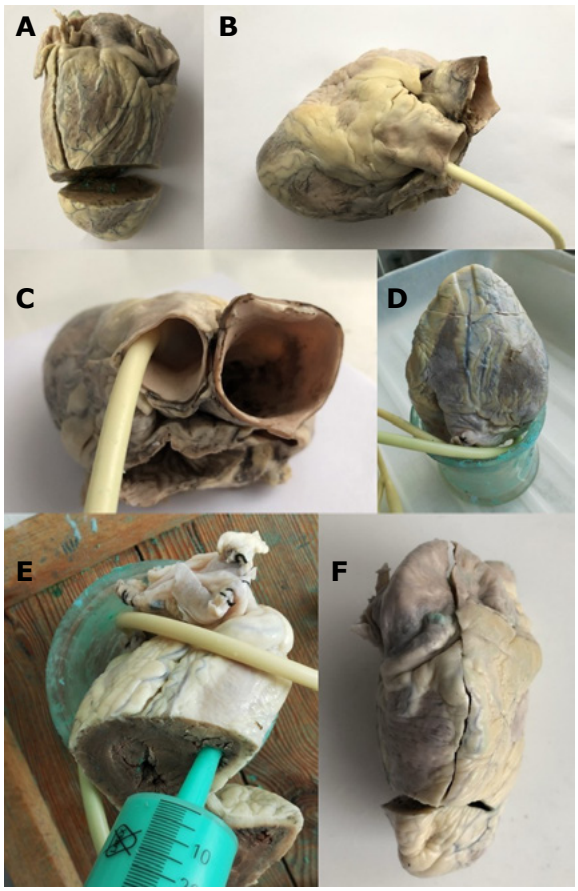


Figure 2 A, B. Finished model of the interior of right atrium and right ventricle

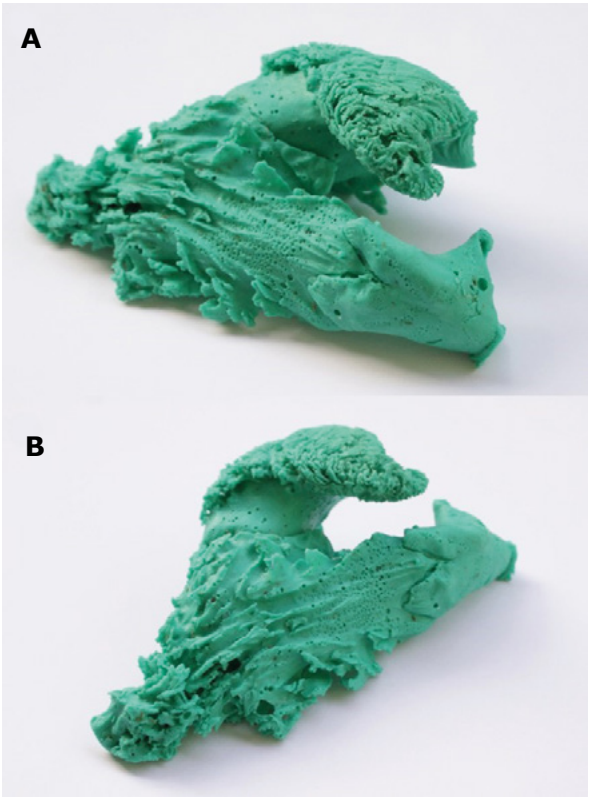
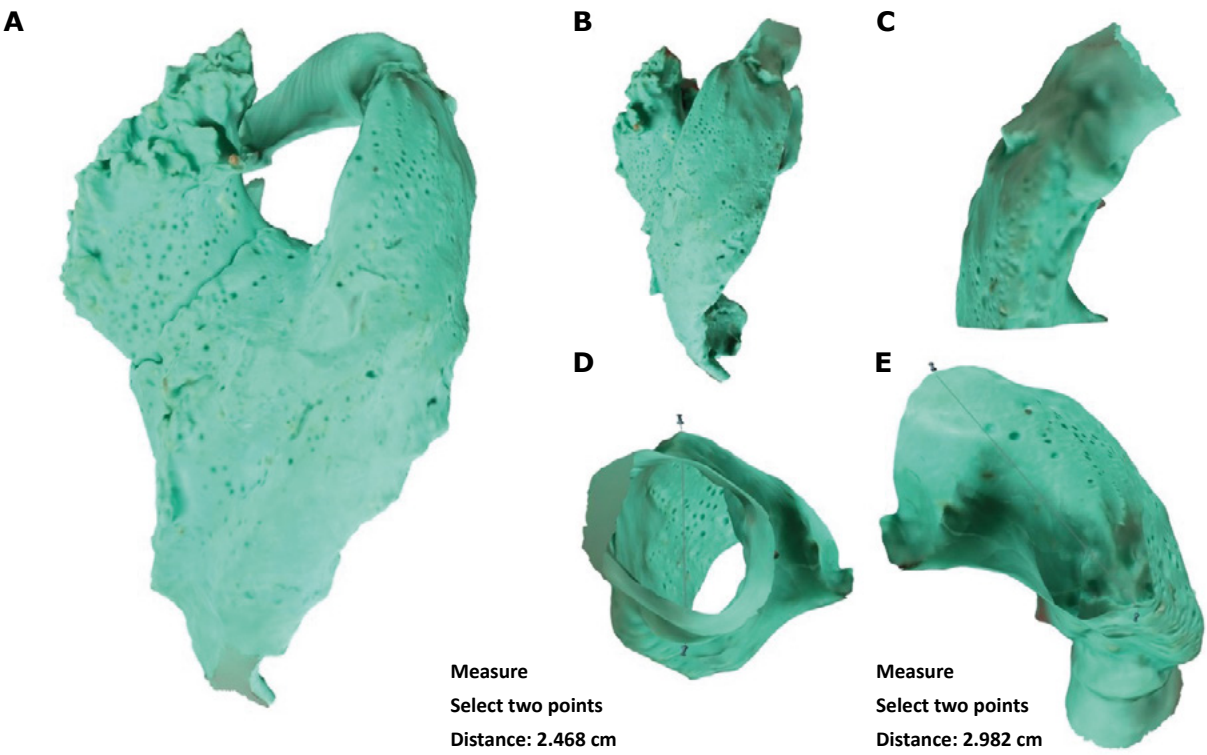


Figure 3 A-E



A – 3D model of the right ventricle and right atrium, B – view of the right ventricle and RVOT, C – separated view of RVOT and pulmonary valve, D – section through RVOT and exemplary measurement, E – exemplary measurement of RVOT

Modeling the heart with the use of 3D technology is also widely used in the case of valve defects. In Vukicevic et al., a mitral valve replacement was performed - specific for a given patient's case - using images obtained by transesophageal 3D echocardiography and computed tomography [15]. In Maragiannis et al., also on the basis of the images from computed tomography, a copy of the patient's severely stenotic aortic valve was constructed [16]. It was also proven that such models are not only a source of information about the structure of the valve defect, but also retain the functional properties of a given defect. Such models are an ideal material for planning valve reconstruction and for designing the most beneficial tools for in this type of surgery. This was also proven in the work of Schievano et al., where on the basis of the images obtained from magnetic resonance imaging, the RVOT model and the pulmonary trunk were made to analyze the possibility of percutaneous implantation of the pulmonary valve [17].

The wide range of applications of in vivo heart modeling with the use of imaging methods is an extremely important element of cardiac diagnostics. However, possibility of mapping post-mortem structures should be also mentioned, both in the case of normal hearts and those with defects. Grabherr et al. compared the post-mortem imaging techniques, taking into account their advantages and disadvantages [18]. The photogrammetry method was mentioned as one that does not require a large financial investment, in particular if other methods of post-mortem imaging of structures are not available, for example, scanners that use a beam of light. The authors also emphasized that in addition to its low cost, the most important advantages of the photogrammetry are the simplicity of this method and the availability of various computer software that support this technique. In addition, a model made with the use of the photogrammetry retains its natural color, which is extremely important in forensic medicine.

The procedure of the photogrammetry and the characteristics of its accuracy were described by Bobkowska et al. [19]. That study showed that the silicone model of the right ventricular interior, which was virtualized using photogrammetry and then scaled, is suitable for analyzing the shape and size of the structure. The use of imaging and visualization techniques has also become more important in these areas of medical sciences which deal with biometric analysis in the context of face modeling and its correct identification

[20]. An accurate reflection of the dimensions and spatial structure of a given object is undoubtedly a great advantage of modern virtualization methods, thanks to which they are widely used, particularly in medical sciences.

Conclusions

Post-mortem heart modeling with the use of the molding mass and then transforming a given model into a virtual form combines methods that are primarily characterized by low cost and ease of performing a 3D heart model. The obtained virtual models are widely used because they can be used for measurement analysis and to modify a given spatial model. In addition, the acquired heart model can be reproduced using 3D printing.

The models of the interior of the heart provide an ideal source of information on the morphological structure and spatial architecture of the interior of the ventricles and atria. The combination of both techniques, molding the heart with silicone and photogrammetry are the original approach to obtaining information about the internal structure of the right ventricle, in particular the RVOT, which still attracted too little attention. Due to existing inconsistencies in the morphology of this area, boundaries and spatial structure, RVOT is a very interesting cardiac component for anatomical research. The results of such studies may be useful for many clinicians in their daily work. The use of 3D RVOT modeling will contribute to the acquisition of additional information about the morphology of this area and may be the basis for further research related to, for example, determining the location of other structures in the close vicinity of the RVOT.

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RESEARCH ARTICLE

Clinical and epidemiological aspects of acute pancreatitis – 10 years of single-center experience

Mateusz Jagielski, Filip Kijowski, Monika Fruczek,

Magdalena Sternau-Kuklińska, Maria Janiak, Krystian Adrych

Department of Gastroenterology and Hepatology, Medical University of Gdańsk, Poland

Abstract

Background: We can observe an increase in acute pancreatitis (AP) incidence in the recent years.
Materials and methods: Retrospective clinical data analysis of 370 patients with AP, hospitalized between 2007 and 2016 at our Department.
Results: AP was diagnosed during 406 hospitalisation in 370 patients [average age 52.15 (21-93), 237(64.05%) male]. AP of high clinical severity was diagnosed in 60/370 (16.22%) patients. Average time of hospitalisation was 16.13 (1-121) days. Mortality was 12/406 (2.96%). The after effect of AP in form of parapancreatic fluid reservoirs was diagnosed in 202/406 (54.59%) cases. Comparing the early phase of the study (2007-2011) and the later one (2012-2016) a shorter time of hospitalisation was proven and a lower mortality of the patients in the later phase of the study. Analysis of patients' blood tests revealed that patients with severe AP have significantly elevated levels of inflammatory parameters and amylase comparing to group with mild and moderate AP, during the first days of hospitalisation.
Conclusion: The development of conservative treatment options for AP, especially in early stages of the illness, has significantly shortened the duration of hospitalisation of patients with AP at our Department.
Keywords: acute pancreatitis / pancreatic necrosis / pancreatic fluid collection

Citation

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Corresponding author:

Mateusz Jagielski, Clinical Centre of Cardiology, University Clinical Centre, Smoluchowskiego 17, 80-214 Gdansk, Poland

e-mail: mateusz.jagielski97@gumed.edu.pl

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Introduction

Acute pancreatitis (AP) is an acute inflammatory state of the pancreas, often involving peripancreatic tissues and organs during progression of the illness [1-2]. Pathogenesis of AP involves premature activation of pancreatic proenzymes, which cause damage to the gland [1-4]. The diagnosis of AP is based on fulfilling two out of three criteria: abdominal pain of characteristic location, blood levels of pancreatic enzyme activity elevated threefold over the norm and a typical image of pancreas in radiological examinations [1-2]. According to the 2012 Revision of Atlanta classification there are 3 clinical forms of AP (mild, moderate, severe) diagnosed depending on the occurrence and duration of organ failure, which is classified by modified Marshall scale [1-2]. The 2012 Atlanta classification also suggests a division of AP: interstitial edematous AP and necrotizing AP, which are diagnosed based on imaging (mainly abdominal contrast-enhanced computed tomography [CECT]) [1-2]. Usually cases of mild and moderate AP are described by pathomorphologists as interstitial edematous, while necrotizing AP is often clinically classified as severe.

The incidence of AP is rising worldwide, which greatly increases the costs of hospitalization and treatment of the patients [5-8]. AP is the most common pancreatic illness and one of the most common acute conditions in gastroenterology [7]. The few existing epidemiological studies of AP in Poland suggest that Poland is among the countries with the highest rates of incidence of AP in the world which is 72/100000/year (worldwide 33.74/100000/year) [5-9]. The total annual cost of AP patients' treatment depends on etiology, severity, treatment in intensive care units and infection complications [8].

The aim of our study was retrospectively analyze the trends in clinical data of patients with AP, who were hospitalized at our Department in the years 2007-2016.

Materials and methods

A retrospective analysis of all patients treated at our department between 2007 and 2016 with AP was conducted. Patients who began treatment at another hospital and were referred to our department in order to treat the complications of AP were excluded from the study. Patients with a prior diagnosis of chronic pancreatitis were also excluded.

All the definitions contained herein are based on the revised Atlanta classification from 2012 [1-2]. AP was diagnosed based on revised Atlanta 2012 classification if two out of three criteria were met: (1) amylase and/or lipase levels ≥ three times above the norm, (2) typical abdominal pain radiating from front to back, (3) typical appearance on imaging [1-2]. All patients had blood tests conducted [blood morphology, amylase and lipase activity level, liver parameters (AST, ALT), bilirubin, cholestasis parameters (GGTP, ALP), C-reactive proteins, arterial blood gas]. All patients had an abdominal ultrasound upon admission. Abdominal CECT was conducted, when the diagnosis of AP was doubtful.

After diagnosing AP in the first day of hospitalization, a prognosis was made based on relevant scales. In the majority of cases the Bedside Index for Severity in Acute Pancreatitis (BISAP) score was used [1-2]. Furthermore, the clinical state during hospitalization was assessed continuously with particular attention being paid to the amount of intravenous fluids administered in the first 72 hours from admission. The type and duration of organ failure was assessed using Marshall scale [1-2]. Organ failure was deemed as transient (under 48 hours) and persistent (over 48 hours) [1-2].

Endoscopic retrograde cholangiopancreatography (ERCP) with sphincterotomy and gallstone removal with prosthesis introduction and/or nasal drain introduction was performed in all cases with AP and coexisting acute cholangitis within the first 24 hours. Furthermore, in cases of acute biliary pancreatitis with clinical symptoms of persistent biliary blockage ERCP was also conducted within the first 72 hours from admission.

In order to find out the etiology of AP, after excluding alcohol, biliary, trauma, drugs and iatrogenic cause, blood tests involving calcium and triglycerides concentration were done. If hypercalcemia and hypertriglicerydemia were ruled out as AP cause there were two pathways. Once the inflammatory process has seized, CECT and additional blood tests of carcinoembryonic antigen (CEA) and cancer antigen 19-9 (Ca19-9) were conducted in all patients over 40 years of age in order to rule out a tumor. If the patient was under 40 years old, endoscopic ultrasonography (EUS) or magnetic resonance cholangiopancreatography (MRCP) was made. Only after these conditions were met we looked for genetic and autoimmune causes of AP. If all the above steps were completed and the etiology was not established, then the case was diagnosed as idiopathic AP.

The standards of conservative treatment of AP at our Department are not significantly different from the international guidelines [10-11]. Nutritional therapy is the basis of treatment of patients with AP. In

case of mild and moderate AP, transient starvation diet and oral fat-restricted diet was introduced after gastric syndromes such as: nausea, vomiting, abdominal pain were alleviated. Enteral nutrition via flocare was used in severe form of AP. Some of the patients had to be fed intravenously. An intensive liquid therapy is applied together with analgesic treatment of AP. Additional treatment was applied in patients with organ failure if deemed necessary.

In accordance with international guidelines [10-11] a prophylactic antibiotic therapy was not administered in patients with necrotizing AP or severe form of AP. Intravenous antibiotic therapy was administered to patients with infected necrosis in AP, proven by culture growth from necrotic tissue. Intravenous antibiotic therapy was also administered to patients in whom radiological imaging revealed signs of infection such as: gas bubble external to gastrointestinal tract imaged in CECT scans or likelihood of infection shown as appearance of new symptoms of systemic inflammatory response after a minimum of 7 days since the inception of the illness. Intravenous antibiotic therapy was also used in patients who presented symptoms of infection localized externally to the pancreas. We used the following antibiotics: ceftriaxone with metronidazole, ciprofloxacin with metronidazole, tazobactam with piperacillin or imipenem.

Abdominal CECT was performed in every patient with severe AP, with suspicion of necrosis infection or when there was no improvement within the first 48 hours of treatment. CECT scans were evaluated in accordance with computed tomography severity index (CTSI) [1-2].

The majority of AP cases (medical documentation and radiological imaging) were discussed in detail during interdisciplinary weekly clinical meetings. The professionals consisted of radiologists, gastroenterologists and surgeons. Treatment decisions were mostly dictated by the outcomes of these meetings.

All statistical calculations were performed using the Statistica software (v 13.0 StatSoft Inc. Tulsa, USA). Quantitative variables were characterized by arithmetic means and standard deviation, along with minimal and maximal values (range). Qualitative data are presented as means of numbers and percentage. Raw data were checked for normality using the Shapiro-Wilk test. Multivariate comparisons were performed using a t-test/Mann-Whitney test for quantitative variables. Comparisons between percentage values in groups were conducted using Chi Square test for Percentage values. Two-tailed tests were carried out after setting a significance level of $p \leq 0.05$.

Results

AP was diagnosed in 406 hospitalizations and in 370 consecutive patients [133 (35.95%) females, 237 (64.05%) males]. The average age was 52.15 (21-93) years. Detailed characteristics of patients with AP are presented in Table 1. The most common etiology of AP was alcohol 182/406 (44.83%), followed by biliary 135/406 (33.25%). The other etiologies were: idiopathic 45/406 (11.08%), iatrogenic 12/406 (2.96%), hyperlipidemia 11/406 (2.71%), pancreatic cancer 10/406 (2.46%), hypercalcemia 5/406 (1.23%), anatomical variant 3/406 (0.74%), drugs 2/406 (0.49%), trauma 1/406 (0.25%). The average duration of hospitalization of AP patients was 16.13 (1-121) days. Death was noted in 12/406 (2.96%) patients. The number of re-hospitalizations due to AP was 36/406 (8.87%). Only 23/370 (6.22%) patients were admitted ≥2 (2-5) times due to AP. The most common reason for readmission was alcohol 32/36 (88.89%).

Table 1. The characteristics of all hospitalized patients with acute pancreatitis

Number of hospitalizations	406
Age (years), mean±SD, [range]	52.1±16.3 [21-93]
Male sex (%)	262 (64.53%)
Time of hospitalization (days), mean±SD, [range]	16.1±16.3 [1-121]
Etiology	
Alcoholic, amount (%)	182 (44.83%)
Non-alcoholic, amount (%)	224 (55.17%)
Clinical forms of AP	
Mild, amount (%)	269 (66.26%)
Moderate, amount (%)	77 (18.97%)
Severe, amount (%)	60 (14.77%)
Pathomorphological forms of AP	
Interstitial edematous, amount (%)	333 (82.02%)
Necrotizing, amount (%)	73 (17.98%)

Of the 135 patients with biliary AP, 89 (65.93%) underwent ERCP with gallstone removal. Acute cholangitis was diagnosed in 42/135 (31.11%) patients. CECT was performed in 242/406 (59.61%) cases. The average CTSI in a group of 242 subjects was 6.17 (1-10). Necrotizing AP was diagnosed in 71/370 (19.19%) patients in 73/406 (17.98%) hospitalizations. In other cases 299/370 (80.81%) (333/406, 82.02% of hospitalizations) interstitial edematous AP was diagnosed.

Mild case of AP was diagnosed in 235/370 (63.51%) patients [average age 51.58±16.43 (21-92) years, 154 males] during 269/406 (66.26%) hospitalizations. Whereas moderate AP was diagnosed in 75/370 (20.27%) patients [average age 53.75±16.88 (21-93) years, 39 males] during 77/406 (18.97%) hospitalizations. The most common transient organ failure (<48 hours) with moderate cases of AP was kidney failure, diagnosed in 48/77 (62.34%) hospitalizations. The second most common organ failure was respiratory insufficiency (23/77, 29.87% hospitalizations).

Severe AP was diagnosed in 60/370 (16.22%) patients [average age 52.95±15.14 (25-90) years, 44 males] during 60/406 (14.77%) hospitalizations. In this group the most common persistent (>48 hours) organ failure was also kidney failure in 51/60 (85%) patients. All patients with severe AP had morphologically classified as necrotizing AP based on radiological imaging evidence. Fatal complications were only recorded in the severe AP patient group 12/60 (20%). Clinical data of all patients with AP in regards to their age were presented in table 2.

The complications of AP taking form in pancreatic and peripancreatic fluid collections (PFCs) in early phase of disease were noted in 202/406 (54.59%) of cases. In the early phases of AP (<4 weeks since the onset of symptoms) 59 (29.21%) patients were diagnosed with acute necrotic collection (ANC) (Figure 1a-c.). Acute peripancreatic fluid collection (APFC) was documented in remaining 143 (70.79%) patients. In the group with ANC, 40/59 (67.8%) of the patients were

Table 2. Clinical characteristics of < 60 and ≥ 60 HCM patients

	Under 50 years	Over 50 years	P-value
Number of hospitalizations (%)	182 (44.83%)	224 (55.17%)	-
Age (years), mean±SD, [range]	37.4±7.4 [21-49]	64.1±10.9 [50-93]	-
Male sex (%)	129 (70.88%)	134 (59.82%)	0.0205
Etiology			
Alcoholic, amount (%)	105 (57.69%)	77 (34.38%)	< 0.0001
Non-alcoholic, amount (%)	77 (42.31%)	147 (65.68%)	< 0.0001
Clinical forms of AP			
Mild, amount (%)	121 (66.48%)	147 (65.63%)	0.8575
Moderate, amount (%)	35 (19.23%)	42 (18.75%)	0.9025
Severe, amount (%)	26 (14.29%)	35 (15.63%)	0.7074
Pathomorphological forms of AP			
Interstitial edematous, amount (%)	151 (82.97%)	182 (81.25%)	0.6315
Necrotizing, amount (%)	31 (17.03%)	42 (18.75%)	0.5097

later diagnosed (after 4 weeks of illness) with walled-off pancreatic necrosis (WOPN) (Figure 2A-C.). Alternatively the patients with APFC, only 24/143 (16.78%) of the patients were diagnosed with a persistent form of fluid collection, namely a pancreatic pseudocyst. 46/202 (22.77%) of patients with PFCs due to AP were requiring interventional treatment. Moreover, 51/370 (13.78%) of patients treated for AP for the first time went on to develop chronic pancreatitis.

Figure 1 A-C. A 53-year-old patient with necrotizing AP.
An acute necrotic collection wasvisible in the abdominal CECT performed on the 13rd day of illness

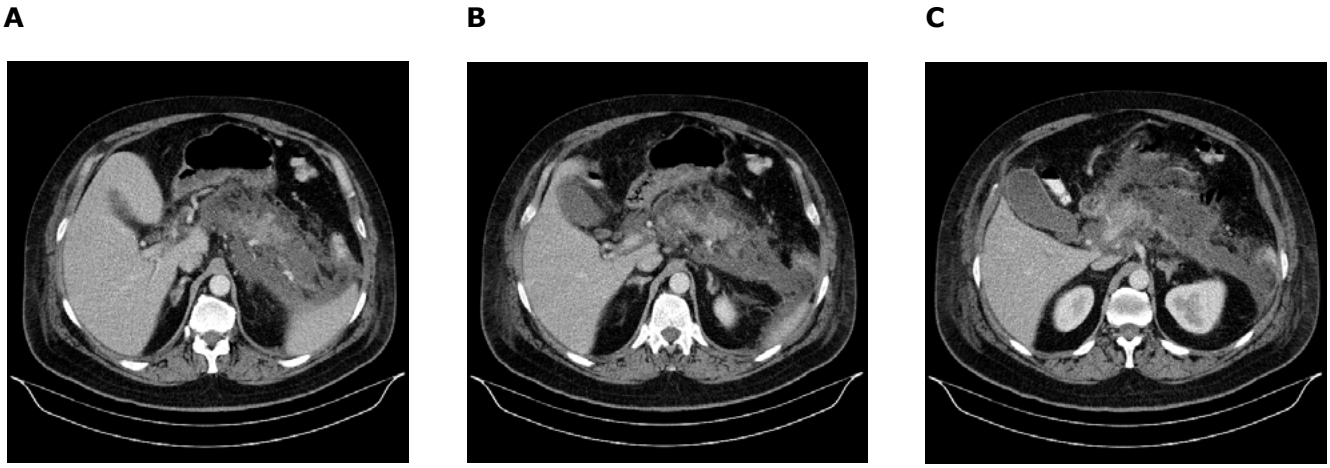
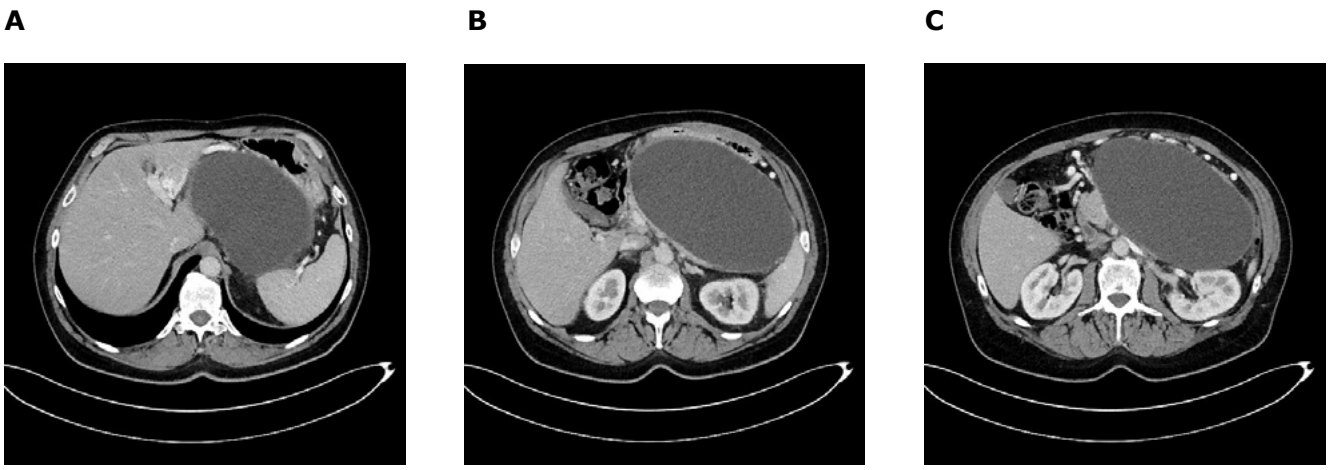


Figure 2 A-C. A 53-year-old patient with ANP. A walled-off pancreatic necrosis collection 145x220x180 mm in size, which was pressing upon the lumen of the gastrointestinal tract, was subsequently identified by abdominal CECT



Comparing the early phase of the study (2007-2011) with the late phase (2012-2016) (Table 3) a shorter time of hospitalization (20.8 days vs 10.7 days, p<0.05) and a lower mortality rate (4.1% vs 1.6%, p<0.05) were documented in the later phase of the study.

Table 3. The characteristics of all hospitalized patients depending on the study period

	2007-2011	2012-2016	P-value
Number of hospitalizations (%)	217 (53.45%)	188 (46.55%)	-
Age (years), mean±SD, [range]	50.5±13.4 [21-86]	54.0±19.0 [21-93]	0.0969
Male sex (%)	162 (74.65%)	100 (53.19%)	< 0.0001
Time of hospitalization (days), mean±SD, [range]	20.8±17.8 [4-112]	10.7±12.5 [1-121]	< 0.0001
Etiology			
Alcoholic, amount (%)	129 (59.45%)	53 (28.19%)	< 0.0001
Non-alcoholic, amount (%)	88 (40.55%)	135 (71.81%)	< 0.0001
Clinical forms of AP			
Mild, amount (%)	135 (62.21%)	132 (70.21%)	0.0414
Moderate, amount (%)	41 (18.89%)	37 (19.68%)	0.8428
Severe, amount (%)	41 (18.89%)	19 (10.11%)	0.0131
Pathomorphological forms of AP			
Interstitial edematous, amount (%)	152 (70.05%)	180 (95.74%)	0.1382
Necrotizing, amount (%)	65 (29.95%)	8 (4.26%)	< 0.0001

Furthermore, the laboratory blood test results revealed that patients with severe AP have a significantly higher levels of CRP (124.18 ± 95.93 mg/l vs 45.22 ± 58.73 mg/l, p<0.05) and amylase (3996.6 ± 15267.03 U/l vs 1206.84 ± 2523.43 U/l, p<0.05) in comparison to patients with mild and moderate AP within the first days of hospitalization (Table 4).

Table 4. Comparison of patients with mild and moderate acute pancreatitis versus severe acute pancreatitis

	Mild and moderate AP	Severe AP	P-value
Number of hospitalizations (%)	346 (85.22%)	60 (14.78%)	-
Age (years), mean±SD, [range]	52.1±16.5 [21-93]	53±15.1 [25-90]	0.0969
Male sex (%)	217 (74.65%)	43 (71.67%)	< 0.0001
Time of hospitalization (days), mean±SD, [range]	11.9±7.6 [1-59]	40.5±28.1 [2-121]	< 0.0001
Etiology			
Alcoholic, amount (%)	155 (44.80%)	26 (43.33%)	0.8183
Non-alcoholic, amount (%)	191 (55.20%)	34 (46.67%)	0.3739
Pathomorphological forms of AP			
Interstitial edematous, amount (%)	320 (92.49%)	13 (21.67%)	0.0009
Necrotizing, amount (%)	26 (7.51%)	47 (78.33%)	< 0.0001
The laboratory blood test results			
C-reactive proteins (mg/l), mean±SD, [range]	45.2 ±58.7 [0.55-401.8]	124.2±95.93 [2.15-398.0]	< 0.0001
Leukocytes (G/l), mean±SD, [range]	10.6±4.09 [0.42-28.17]	14.7±5.42 [1.83-30.1]	< 0.0001
Bilirubin level (mg/dl), mean±SD, [range]	2,5±2,96 [0.15-17.26]	3.1±3.32 [0.4-16.5]	0.22
Amylase activity level (U/l), mean±SD, [range]	1206.9±2523.5 [27-24506]	3996.6±15267 [67-113020]	0.0003

Discussion

In recent years major changes occurred in the treatment strategy of patients with AP. Due to lack of large scale population studies it is difficult to accurately and scrupulously obtain viable data of incidence of AP in the Polish population. This is mainly caused by a [5]. There are 2 epidemiological studies containing clinical data of patients with AP in Poland [6, 9]. Bogdan et al. presented results of analysis of 441 hospitalizations in 298 patients admitted to a single hospital in the years 2005-2010 [9]. Whereas Głuszek et al. conducted a prospective observation of 1044 hospitalized patients with AP in all departments of surgery of the Świętokrzyskie province [6]. The results of both of the above studies suggest an average AP incidence in Poland of 64.4-99.96/100000 [6,9].

The second problem connected with hardships in determining the realistic incidence of AP is a lack of proper diagnosis. In a 2016 article it was proven, that in a group of 694 patients with AP, 143 (20.6%) were previously admitted to the hospital for that illness without

an accurate diagnosis or treatment [12]. In the same study it was determined that nearly half of the cases the cause was gallstones (46.5%) whereas in our study it accounted for just 33.35% of cases [12]. It is noteworthy that biliary AP was also a significant reason for re-hospitalization due to AP [40/132 (30.3%) cases] [12]. In our study the situation is completely opposite as alcohol was the major cause for re-hospitalization. It needs to be pointed out that the above-mentioned study was conducted in United Kingdom (excluding Scotland) [12]. As most authors highlight in different values regarding incidence and other clinical and epidemiological data and most importantly etiology differs significantly depending on the population of the country or region [5-6,9,12-13]. Furthermore, even within the same population, the incidence of AP differs depending on the time of year (e.g. the incidence often rises around the New Year's Day) [13].

The most common reasons for AP are alcohol and gallstones [6,9,13-17]. The other etiologies are much less frequent [6, 9, 13, 16-18]. Biliary AP is the most common cause in the Mediterranean countries (Italy, Greece, Spain) [5,17]. Whereas alcohol AP is dominant in the Northern and Eastern Europe and Russia [5,17]. In our sample the alcohol etiology of AP was defined in 44.83% of cases and biliary etiology in 33.25%. In other Polish studies there are discrepancies regarding the dominating etiology [6, 9] According to Bogdan et al. the most common etiology was alcohol (49% cases), whereas biliary AP was diagnosed in 80/298 (27%) patients [9]. Whereas according to Głuszek et al. the most common etiology was gallstones (30.1%) and alcohol was second (24.1%) [6]. In the same article a coexistence of gallstones and alcohol abuse was diagnosed in 2.9% patients [6]. Similar results have been reported in a Swedish study, which also highlights that alcohol was also the dominant reason for AP recurrences [19]. In the conclusion of the Głuszek et al. [6] we find that alcohol is the most prevailing reason for AP in young Poles (mainly males), while biliary disease is dominant among older females. It is worth mentioning that the mechanism of AP in the two dominating causes of AP has not been thoroughly explained.

In the majority of cases AP is mild and without organ failure or local complications [1-2,5-6,9]. We do however observe an increase in incidence of severe AP, which is strongly associated with a higher mortality rate [17]. In our study, severe AP was diagnosed in 60/370 (16.22%) patients with 60/406 (14.77%) hospitalizations. The overall mortality was 12/406 (2.96%). Fatalities were noted exclusively in severe AP, which accounted for 12/60 (20%) patients. Similar results were reported by Bogdan et al., where total mortality due to AP stands at 3%, and in severe AP 15% [9]. Głuszek et al. declares severe AP in 7% cases with total mortality at 3.9% [6]. Mortality in severe AP was rated at 52.9% patients [6]. Within the same study 80.7% of AP cases were mild [6].

The recent years brought developments in the conservative treatment methods of the early phase of AP [20-27], particularly in the first week of illness, when the mortality is mostly connected to organ failure [20, 25, 28]. In our study, comparing the early phase of the study (2007-2011) with the late phase (2012-2016) a shorter duration of hospitalization and a lower mortality rate were documented in the later phase of the study. At our Department we have also noticed an improvement in the effectiveness of conservative treatment of early phase AP, which has led to significantly shorter duration of hospitalization with AP alongside with a decline in mortality rates. Differences between the populations (sex and etiology of AP) were noticed between the two phases of this study. Above-mentioned differences make it difficult to compare both groups. However, we noted an improvement in the effectiveness of conservative treatment of early phase AP, which has led to significantly shorter durations of hospitalization at our Department.

AP usually leads to a local inflammatory response, which involves many cytokines and inflammatory cells partake [3-4]. The inflammatory reaction can be so strong, that it can lead to a systemic inflammatory response syndrome (SIRS) and multi-organ failure [4,28-29]. Depending on where will the organ failure manifest itself and depending on its duration, we can differentiate between three types of clinical AP (mild, moderate, severe) [1-2]. We demonstrated that in the first days since admission the inflammatory state parameters are statistically significantly higher in patients with severe AP than in those with moderate and mild AP. This seems to suggest a direct correlation with the severity of inflammation which is significantly increased in severe AP. Furthermore, in the severe AP all of the patients were diagnosed with necrotic pancreatitis which had an exacerbating effect on the inflammation in that group of patients.

One of the criteria to correctly diagnose AP is an increase of pancreatic enzymes (amylase, lipase) activity in the blood [1-2]. While it is widely thought that the direct levels of enzyme activity in the blood do not have a prognostic value, we have noted that amylase levels do correlate with a clinical type of AP [30-34]. In the first days of illness, the amylase activity levels are significantly higher in severe AP than in mild or moderate AP.

According to the 2012 Revision of Atlanta classification, depending on the morphological type we can

differentiate four types of pancreatic fluid structures in the progression of AP, all of which are complications of AP [1-2]. In interstitial edematous AP, an acute peripancreatic fluid collection (APFC) may form within the first four weeks, which can then evolve into a pancreatic pseudocyst [1-2,35-37]. In the necrotizing form of AP, the pancreatic fluid collections within the first four weeks are called acute necrotic collection (ANC), whereas after four weeks we are dealing with a walled-off pancreatic necrosis (WOPN) [1-2,35-37]. According to multiple authors, the natural progression of AP varies [1-2,35-37]. We have noted that pancreatic fluid collections were diagnosed in 54.59% of hospitalizations due to AP. Within four weeks of the illness, ANC was diagnosed in 29.21% patients with AP. In 70.79% of patients an APFC was diagnosed. Most of ANC (67.8%) transformed into WOPN. In contrast, the majority of APFC were resorbed (83.22%) and a persistent fluid collection in the form of a pancreatic pseudocyst was formed in as little as 16.78% patients. Sarathi Patra et al. presented that in all of the patients (100%) with necrotic AP an ANC was diagnosed, whereas 48.75% of cases transformed into WOPN in the latter phases of illness [35]. In the same study the authors have declared a presence of APFC in 37.93% of interstitial edematous AP and it is important to note that all of the APFC spontaneously regressed and no pseudocysts were found [35]. In a study by Manrai et al. [36] ANC was diagnosed in 93.4% cases of necrotizing AP, of which 58.74% transformed into WOPN. 22.22% of patients with interstitial edematous AP an AFPC was diagnosed. In 2.77% a pseudocyst was diagnosed [36].

The main condition that must be met to start interventional treatment of AP complications in the form of pancreatic and peripancreatic fluid collection is an infection of that collection [10-11,38-40]. Interventional treatment is also reserved for patients with clinical symptoms of the presence of fluid collection [10-11,38-39]. They are compression symptoms such as obstructive jaundice or ileus [10-11,39-40]. Patients with pancreatic fluid collections without clinical symptoms should not be treated interventionally [10-11,41], because as we have presented in our study, these collections are susceptible to spontaneous regression during the hospitalization.

In the last decades we can observe an intensive development of minimally invasive treatment methods of consequences of AP [22-24,26-27,38-45]. The application of interventional methods of treatment was presented in our previous publications [38-42]. In the presented sample interventional treatment was needed in 46/202 (22.77%) patients with pancreatic and peripancreatic fluid collections, because of persistent symptoms related with fluid collection.

Summarizing, in this study a large group of cases were analyzed and the natural progression of acute pancreatitis was studied, including its complications, mainly pancreatic and peripancreatic fluid collections, with strong emphasis being placed on clinical and epidemiological data. In this study we demonstrated that intensive conservative treatment in the early phase of acute pancreatitis and delaying interventional treatment of acute pancreatitis complications significantly improves the treatment results and decreases mortality.

Diagram 1. The distribution of sex in the two age groups (under 50 and over 50). Each one of the four bars represents one sex in one age group. The first group of two bars is age group under 50 and the second group is over 50. Blue is assigned to males and the red to females

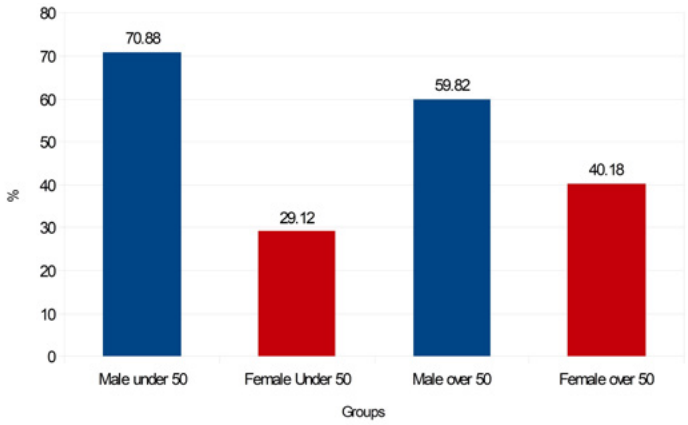


Diagram 2. The decreasing number of days of hospitalization trend in the years 2007 to 2016. The line follows a trend representing a decrease of average number of days of patient hospitalization. Values at either end represent the average number of days of hospitalization in two phases of the study (2007-2011 and 2012-2016). Each point represents the average number of days of hospitalization in that specific year

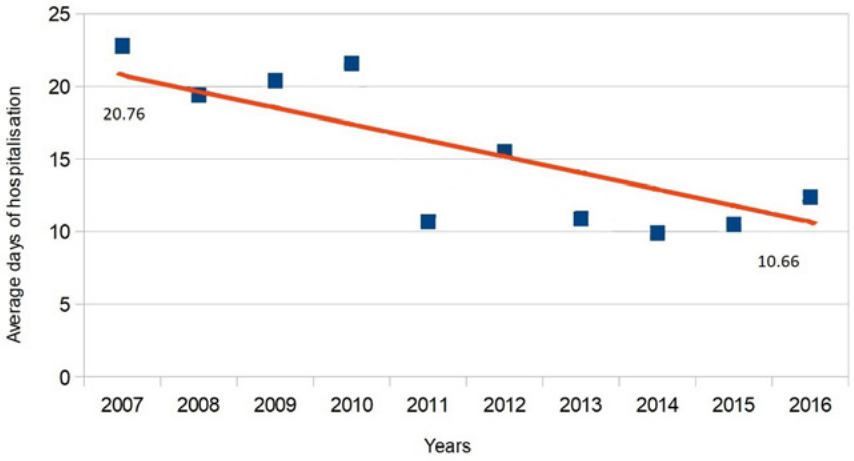
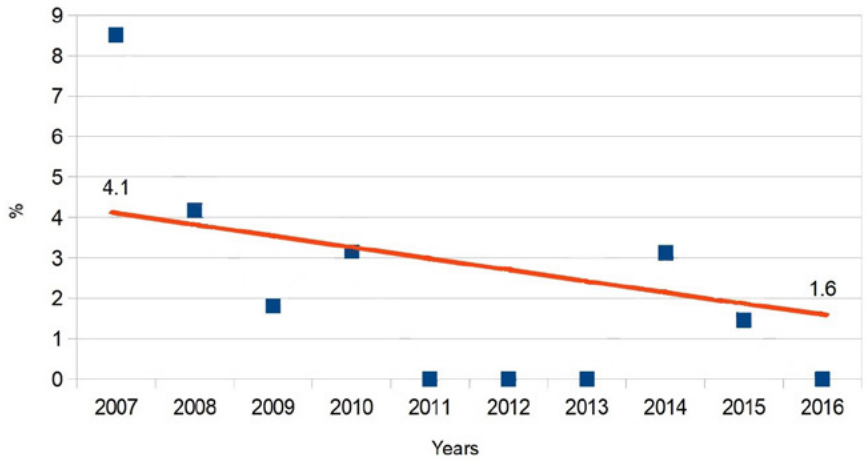


Diagram 3. The decreasing mortality trend in our Department in the years 2007-2016. The line follows a trend of decreasing mortality with values at either end representing the average percentage of mortalities in two phases of the study (2007-2011 and 2012-2016). Each point in every year represents the mortality percentage that specific year



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SHORT COMMUNICATION

Expression profile of circulating microRNAs in patients with ischemic heart failure with moderately reduced left ventricular ejection fraction – pilot study

Damian Kaufmann[1], Ludmiła Daniłowicz-Szymanowicz[1], Monika Sakowicz-Burkiewicz[2], Małgorzata Szwoch[1], Tadeusz Pawełczyk[2], Grzegorz Raczak[1]

[1] Department of Cardiology and Electrotherapy, Medical University of Gdańsk, Poland
[2] Department of Molecular Medicine, Medical University of Gdańsk, Poland

Abstract

Introduction: Heart failure (HF) is a growing global pandemic that affects millions of people around the world. Despite the progress in medicine, diagnosis and treatment of HF remains problematic. Recently, noncoding micro ribonucleic acids called miRNAs have become significant in the diagnosis and stratification of HF risk.
Aim: The aim of this study was the attempt to identify the profile of circulating miRNAs specific for ischemic HF with moderately reduced left ventricular ejection fraction (HFmrEF).
Methods and Results: A number of changes in the miRNA profile can characterise patients with ischemic HFmrEF. This is a pilot study before further research on a larger group of patients.
Conclusions: Using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR), serum levels of 84 miRNA were measured and compared between a patient with ischemic HFmrEF and a healthy volunteer. Analysis reveals a down-regulation of let-7f-5p and miR-1-3p, as well as up-regulation of miR-100-5p, miR-10b-5p, miR-125a-5p, miR-140-5p, miR-144-3p, miR-149-5p, miR-15b-5p, miR-183-5p, miR-208b-3p, miR-224-5p, miR--26b-5p, miR-27b-3p, miR-302a-3p, miR-320a, miR-7-5p, miR-99a-5p.
Keywords: heart failure / circulating microRNAs / biomarkers

Citation

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Corresponding author:
Damian Kaufmann, Department of Cardiology and Electrotherapy, Medical University of Gdańsk, Poland
e-mail: damian.kaufmann@gumed.edu.pl
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Introduction

Heart failure (HF) remains a growing global pandemic that affects at least 37 million people worldwide and counting [1]. Despite significant expenditures on HF treatment, advances in therapy and prevention, mortality remains high and about 50% of patients with HF die within 5 years of diagnosis [2], which means the need to continually aim at improving HF diagnosis and treatment. Therefore, new diagnostic and therapeutic methods are being sought for the early identification of high-risk patients and to improve treatment efficacy.

In the last few years, special attention has been paid to microRNA (miRNA). These molecules, usually 21-25 nucleotides in length, are considered to be one of the major post-transcriptional regulators of gene expression. This process is achieved by binding miRNA with the target mRNA in the base pairing mechanism, followed by the induction or repression of translation or mRNA degradation. It is estimated that from 1% to 5% of the total genetic material in both humans and animals are genes encoding miRNA [3]. Many reports in the literature confirm that miRNA plays a key role in important processes such as angiogenesis, oncogenesis, fibrosis, apoptosis, cell division or cell differentiation. MiRNA can act intracellularly or can be actively secreted by cells and be involved in cell-cell or cell-tissue communication. Despite high extracellular RNase activity, circulating miRNAs are very stable due to their combination with proteins, lipoproteins, or by packing inside microvesicles [4]. Most miRNAs are located intracellularly, however, they can also be found in various body fluids such as plasma, urine, tears and saliva [5].

Numerous studies have shown that changes in the expression of both intracellular and extracellular miRNAs correlate with various cardiovascular conditions such as myocardial infarction, left ventricular hypertrophy, cardiomyopathy, arrhythmias or HF [6-10]. The unique miRNA expression profile can be a promising marker of various cardiovascular diseases.

MicroRNA biogenesis cellular process

The miRNA biogenesis cellular process is multistage and includes both nuclear and cytoplasmic processes [11]. The first stage in the cell nucleus is transcription leading to the creation of the original miRNA (pri-miRNA). This process occurs with the participation of RNA polymerase II, which transcribes from the chromosomal DNA pri-miRNA with a length of several thousand base pairs. Thereafter, the microprocessor complex consisting of the DGCR8 nuclear protein and Drosh ribonuclease joins the pri-miRNA and processes them into about a 70-nucleotide long double-stranded pinhead structure called a precursor miRNA (pre-miRNA). Subsequently, pre-miRNA by exportin-5 transferase is actively transported from the nucleus to the cytoplasm where it combines with the Dicer enzyme, which catalyses the reaction leading to the creation of a miRNA-miRNA* duplex with a length of about 21-25 nucleotides. In the last stage, the duplex is separated, and one of the strands (the lead strand) is connected via the Argonaut protein to the RNA-induced silencing complex (RISC). This mature miRNA strand built into RISC has the ability to inhibit gene expression by binding to complementary mRNAs and their subsequent degradation or inhibition of translation [12].

Aim

The aim of this study was the attempt to identify the profile of circulating miRNAs specific for ischemic HFmrEF.

Materials and methodology

Comparison of the patient with a healthy volunteer

Both the patient and the control group were matched in terms of age and sex. The healthy volunteer did not suffer from chronic diseases and did not use any medications. The patient with HFmrEF has a history of 3 percutaneous coronary interventions in the area of the left coronary artery. The last coronary angiography was performed in 2010 where balloon angioplasty of the left anterior descending coronary artery was performed. There were no haemodynamically significant changes in the remaining coronary arteries. While participating in the study he was in a stable clinical condition, NYHA II with LVEF of 42%. Apart from coronary disease, the patient was burdened with arterial hypertension and hypercholesterolemia. The patient was treated according to the latest guidelines and received a bisoprolol 2,5 mg q.d, ramipril 2,5 mg q.d, aspirin 75 mg q.d and rosuvastatin 10 mg q.d.

Using the miScript™ miRNA Array Human Cardiovascular Disease miRNA, the full profile of 84 miRNA (let-7a-5p, let-7b-5p, let-7c-5p, let-7d-5p, let-7e-5p, let-7f-5p, miR-1-3p, miR-100-5p, miR-103a-3p, miR-107, miR-10b-5p, miR-122-5p, miR-124-3p, miR-125a-5p, miR-125b-5p, miR-126-3p, miR-130a-3p, miR-133a-3p, miR-133b, miR-140-5p, miR-142-3p,

miR-143-3p, miR-144-3p, miR-145-5p, miR-146a-5p, miR-149-5p, miR-150-5p, miR-155-5p, miR-15b-5p, miR-16-5p, miR-17-5p, miR-181a-5p, miR-181b-5p. miR-182-5p, miR-183-5p, miR-185-5p, miR-18b-5p, miR-195-5p, miR-199a-5p, miR-206, miR-208a-3p, miR-208b-3p, miR-21-5p, miR-210-3p, miR-214-3p, miR-22-3p, miR-221-3p, miR-222-3p, miR-223-3p, miR--224-5p, miR-23a-3p, miR-23b-3p, miR-24-3p, miR-25-3p, miR-26a-5p, miR-26b-5p, miR-27a-3p, miR-27b-3p, miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-302a-3p, miR-302b-3p, miR-30a-5p, miR-30c-5p, miR-30d-5p, miR-30e-5p, miR-31-5p, miR-320a, miR-328-3p, miR--342-3p, miR-365b-3p, miR-378a-3p, miR-423-3p, miR-424-5p, miR-451a, miR-486-5p, miR-494-3p, miR--499a-5p, miR-7-5p, miR-92a-3p, miR-93-5p, miR-98-5p, miR-99a-5p) was evaluated from blood serum of the patient and healthy volunteer. For the purpose of the study, written informed consent was obtained from all participants and the study protocol was approved by the local Ethics Committee at the Medical University of Gdańsk.

Molecular methods

Serum samples. Blood samples were left at room temperature for 30 minutes to allow complete coagulation. Coagulated blood samples were centrifuged at 1,500 g for 15 minutes at 4 °C to separate serum. Serum was transferred to a cryotube with care to not disturb the buffy coat and was immediately frozen at –80°C until miRNA extraction.

RNA isolation. Small RNAs was isolated from the serum using the kit the miRNeasy Serum/Plasma kit (Qiagen). Before RNA isolation, all serum samples were thawed completely on ice followed by centrifugation once at 15,000 g for 15 minutes at 4°C to remove remaining cell debris. Then, 200 µl of serum sample was carefully transferred to a new 2 ml tube. Serum was mixed with QIAzol lysis reagent in the volumes described within the manufacturer's protocols. Next, 18 fmol of synthetic microRNA - C. elegans miR-39 miRNA was spiked into the all isolation mixtures. Further stages of RNA isolation were carried out in accordance with the manufacturer's protocols. The RNA concentration was measured using a Qubit fluorometer (Invitrogen).

Reverse Transcription. Isolated small RNAs were reverse-transcribed using commercial primers and the miScript II RT kit (Qiagen) according to the manufacturer's protocol. 5 µL of RNA (~30-40 ng) was reverse--transcribed in a 10 µL reaction volume for each assay.

MiRNA expression by quantitative real-time PCR. Preamplification was performed using miScript PreAMP PCR Kit and miScript PreAMPPrimer Mix Human Cardiovascular Disease (Qiagen) according to the manufacturer's protocol. Quantitative amplification reaction was performed using the miScript miRNA PCR ArrayHuman Cardiovascular Disease MIHS-113ZF (Qiagen) with miScript SYBR Green PCRkit (Qiagen) in a RightCycler 480 II system (Roche).

Statistical analysis

Data were normalised by scaling with the mean Ct of the samples: for each sample the average Ct of all miRNAs measured in the sample was subtracted from the Ct of each miRNA. In order to return the signals to a scale which is easier to interpret, a constant (the average Ct over the entire sample set) was added back. Normalised signals were compared between groups in order to find miRNAs which can be used to differentiate between the groups. Fold change was calculated as 2Δ, where Δ is the absolute difference in median values of the normalised Ct in the two groups. The p values are calculated based on a Student's t-test of the replicate 2Δ (Δ Ct) values for each gene in the control and treatment group.).

Results

The conducted analyses showed statistically significant differences in the expression of the following miRNAs: down-regulation of let-7f-5p, miR-1-3p, and up--regulation of miR-100-5p, miR-10b-5p, miR-125a-5p, miR-140-5p, miR-144-3p, miR-149-5p, miR-15b-5p, miR-183-5p, miR-208b-3p, miR-224-5p, miR-26b-5p, miR-27b-3p, miR-302a-3p, miR-320a, miR-7-5p, miR--99a-5p (Table 1). However, there were no differences in the expression of the remaining miRNAs.

Discussion

The significant development of molecular biology in recent years has led circulating miRNAs to gain an increasing diagnostic and prognostic significance in cardiovascular diseases. Resistance to degradation in the extracellular environment and relatively simple molecular biology methods allowing the detection of miRNA in a quantitative manner in plasma have made them a valuable clinical biomarker. In the available literature, there can be found papers investigating the usefulness of miRNA in diagnosing and stratifying the risk of a wide spectrum of cardiovascular diseases.

Table 1. MicroRNA (miRNA) with increased level in sera of patients with mid-range ejection fraction

miRNA	p-value	Fold change
let-7f-5p	0,04505	-1,98620
miR-1-3p	0,01057	-4,95880
miR-100-5p	0,00009	3,94490
miR-10b-5p	0,00015	3,58010
miR-125a-5p	0,00000	8,16810
miR-140-5p	0,00000	7,94470
miR-144-3p	0,00050	2,90790
miR-149-5p	0,00001	6,36430
miR-15b-5p	0,00000	9,06310
miR-183-5p	0,00041	3,01050
miR-208b-3p	0,00053	2,88790
miR-224-5p	0,00000	15,77970
miR-26b-5p	0,00001	7,21000
miR-27b-3p	0,00000	7,26020
miR-302a-3p	0,00000	14,52030
miR-320a	0,00000	592,22440
miR-7-5p	0,00149	2,44530
miR-99a-5p	0,00149	2,44530

The list includes all miRNAs with an increase of at least 1.2 fold in serum of the mid-range ejection fraction patient vs. serum of the healthy control, sorted by p-value.

Devaux et al proved that miRNA-208b and miR-499 levels are significantly elevated in patients with acute coronary syndrome [7]. A study conducted by Rhodes et al, showed that the decreased level of miR-150 is associated with poor prognosis in patients with pulmonary arterial hypertension [13]. Whereas, in the study of Liu et al, the reduction in miR-150 level was statistically significant among the group of patients with atrial fibrillation, in comparison with the healthy control group [9]. Chen et al, comparing the miRNA expression profile of a healthy control group with patients with HF (both with preserved systolic function (HFPEF) and with impaired left ventricular systolic function) showed that plasma levels of three miRNAs (miR-3135b, miR-3908, and miR-5571-5p) can be used as biomarkers [6].

In our study, the identified profile of 84 circulating miRNAs could be characteristic for patients with ischemic HFmrEF. From among the 84 examined circulating miRNAs, 18 of them showed significant differences in expression compared to the healthy control (Table 1). According to the author's knowledge, this is the first study that characterizes the miRNA profile for such patients. Wong et al, who compared the miRNA expression profile in HF patients with preserved and reduced left ventricular ejection fraction in relation to healthy subjects, showed miR-1233, miR-183-3p, miR-190a, miR-193b levels -3p, miR-193b-5p, miR-211-5p, miR-494, and miR-671-5p were significantly different between patients with HF and the control group, while miR-125a-5p, miR-183-3p levels, miR-193b-3p, miR-211-5p, miR-494, miR-638 and miR-671-5p differentiated the group of patients with reduced left ventricular ejection fraction and the control group [14]. Comparing Wong's results and ours one can notice that there is compliance in the up-regulation of only two miRNA's: miR-183 and miR-125a-5p. There may be several reasons for the low compliance of these two studies. In above mentioned paper the expression profile of more miRNAs (806 miRNA vs. 84 miRNA) was determined, as well as the aetiology of HF was not homogeneous. In Wong's et al the expression profile of more miRNAs (806 miRNA vs. 84 miRNA) was determined, as well as the aetiology of HF was not homogeneous. Another study conducted by Goren et al, comparing serum miRNA concentrations between patients with HF and left ventricular ejection fraction 30% (23-33%) and a healthy control group showed a significant increase in miR-423-5p, miR-320a, miR-22 and miR-92b in people with HF [15]. Likewise, our study also showed an increase in miR-320a expression in serum. Compliance has not been shown in the expression of other miRNA, which most likely was due to the selection of the stu-

died group as well as the fact that only some of them had an ischemic HF aetiology. Additionally, in our study we did not assess the concentrations of miR-92b and mi-423-5b, which in the referenced study showed statistically significant differences.

Much has been published about miRNA in HF. However, the obtained results are heterogeneous, and more HF-related miRNAs are being constantly identified. This is most likely the result of complex pathophysiological processes leading ultimately to the clinical manifestation of the disease. Therefore, it seems important to conduct further research, including careful selection of the studied population, as well as standardisation of analytical processes in order to obtain more reproducible results.

Limitations of the study

There are several limitations of our study. First of all, it has been conducted on a small number of patients and further research is needed in this area. Second, although the 84 miRNA panel was used for analysis, it should be emphasized that there are considerably more of these molecules and many have not been included in the study.

Conclusions

Our study identified the profile of circulating miRNAs that distinguished a patient with HFmrEF from a healthy person. In patients with HFmrEF there is a down-regulation in let-7f-5p and miR-1-3p, as well as an up-regulation in expression of miR-100-5p, miR-10b-5p, miR-125a-5p, miR-140-5p, miR-144-3p, miR-149-5p, miR-15b-5p, miR-183-5p, miR-208b -3p, miR-224-5p, miR-26b-5p, miR-27b-3p, miR-302a-3p, miR-320a, miR-7-5p, miR-99a-5p. Further studies of circulating miRNAs in patients with HFmrEF may offer additional diagnostic and prognostic applications that may be used in clinical practice. This is the first pilot study conducted on a small group of participants, which requires research on a larger group of people in order to confirm the results.

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SHORT COMMUNICATION

Patient satisfaction with the perioperative care by anesthesiologists: pilot study at four surgical departments

Janusz Springer[1], Karolina Dudzińska[1], Monika Nurkowska[1], Liudmila Hasak[2], Anna Dylczyk-Sommer[2]

[1]Student Scientific Club at the Department of Anesthesiology & Intensive Therapy, Medical University of Gdańsk, Poland
[2]Department of Anesthesiology & Intensive Therapy, Medical University of Gdańsk, Poland

Abstract

Background: The aim of this pilot study was to measure the outcomes of perioperative care by anesthesiologists and patient satisfaction at four surgical departments. **Methods:** We designed an original 25−item questionnaire and used it to complete structured interviews of 80 consenting, alert, adult surgical patients during their 1st to 3rd post−operative day. **Results:** Although >70% were satisfied with the information sharing, 43% patients were unsure or not informed about the possible complications of anesthesia. Similarly, >75% positively rated the anesthesiologists' bedside manner; however 69% were either unsure or sure that an anesthesiologist did not visit them after surgery. Interestingly, this lack of continued care had no overall effect on patient satisfaction. Majority reported receiving immediate post−operative analgesia (65%). The Oncological Surgery patients reported highest (and the Orthopedic patients the lowest) satisfaction with their postoperative nausea and pain management. Majority of responders were overall satisfied with their care. **Conclusions:** Our data indicate a high level of patient satisfaction with nearly all aspects of perioperative anesthesiology care. However, anesthesiologists need to more thoroughly inform patients about possible complications of anesthesia. A larger survey is needed to fully assess the patient care and satisfaction trends discussed above.
Keywords: patient / satisfaction / surgical / perioperative care / anesthesiologist

Citation

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Background

Due to the technological and pharmacological advancements, major negative clinical outcomes such as mortality, myocardial infarction or brain injury became too rare in anesthesia practice to be analyzed in

Corresponding author:
Janusz Springer, Department of Emergency Medicine, Medical University of Gdańsk, Poland
e-mail: janusz.springer@gumed.edu.pl
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multicenter studies [1]. Furthermore, the clinical outcomes are increasingly being evaluated regarding the patient's quality of life, instead of the technical success of the intervention [2]. Increasing attention is also paid to the patient's informed consent, which according to some authors includes not just consent for the particular procedure but also for specific complications and discomfort associated with the suggested treatment method [3]. Another patient-centered outcome is satisfaction, defined as the degree of compatibility between the patient's expectation and the health care provider's achievement [4]. Therefore, a growing number of anesthesiology research and principles of healthcare management focus on the patient-doctor information sharing, patient patients' quality of life and satisfaction from care received with the goal of increasing the quality of perioperative care.

Aim

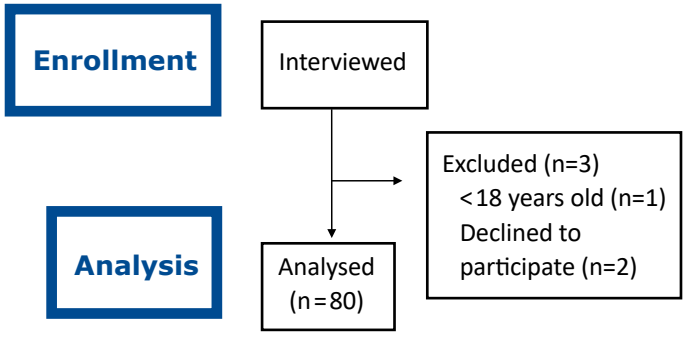
The aim of this pilot study was to measure the outcomes of perioperative care by anesthesiologists and patient satisfaction at four surgical departments of the University Clinical Centre (UCC) in Gdańsk.

Methods

After a review of literature on anesthesiology satisfaction questionnaires we developed a 25-item questionnaire. It covered 5 dimensions: preoperative consultation (9 questions), bedside manner (5), pre-operative fear & discomfort (4), pre & post-operative care (5) and the overall patient satisfaction (2). To ensure clarity, all questions were neutrally worded and did not contain medical jargon. Except for questions about demographics and specific fear/s, the responses were rated using a 5-point Likert scale [5]. Scores were assigned on the scale as follows: Yes or Very Satisfied (4), Rather Yes or Satisfied (3), Hard to say (2), Rather Not or Dissatisfied (1), No or Very Dissatisfied (0).

After obtaining the permission of the local Bioethics Commission (NKBBN/61/2012), we used the questionnaire to complete systematic, in-person interviews at the bedside of consenting and alert adult patients during their 1st-3rd post-operative day at 4 in-patient surgical departments of the UCC. The interviews were conducted by medical student volunteers, who were not directly involved in the interviewed patient care. A total of 80 interviews were analyzed, twenty from each of the following four departments: General, Endocrine and Transplant Surgery, Oncological Surgery, Orthopedic and Trauma Surgery, Plastic Surgery (Figure 1).

Figure 1. Inclusion of interviews



Results

The majority of our respondents were women (57%), age 18-65 (78%), with high-school education (46%), after general anesthesia (93%) and with 1-2 previous operations under anesthesia (51%). Although >70% gave high ratings in the information sharing dimension, as many as 43% (31% of them were Orthopedic patients) were either unsure or not informed about the possible complications of anesthesia (Figure 1). Similarly, >75% of the patients positively rated the bedside manner (Figure 2); however the majority (69%) were either unsure or sure that an anesthesiologist did not visit them in the post-operative period (Figure 3). Interestingly, this lack of continued care had little overall effect on satisfaction scores (56% of the patients were "satisfied" and 38% "very satisfied"). Almost 1/3 of the responders did not report any pre-operative anxiety and majority of them (38%) were General surgery patients.

Overall, the most commonly reported pre-operative concerns were: fear of post-operative pain (37%) and fear of not waking up after the procedure (25%). Interestingly, our patients were 3 times more likely to worry about a surgeon's error than an anesthesiologist's error (18% and 6% respectively, Figure 3). Majority of patients reported no discomfort due to physical factors such as pre-operative thirst or hunger (62%), positioning on the operating table (72%), post-operative nausea or emesis (58%, Figure 7) and majority received immediate post-operative analgesia (65%, Figure 5). The Oncological Surgery patients reported highest satisfaction with management of their postoperative nausea and pain, while the Orthopedic patients were the least satisfied in this area (Figure 6). Majority of responders were overall satisfied with their care, with those at Plastic surgery clinic slightly in the lead.

Discussion

When reading any literature on patient satisfaction with any type of care or medical procedure, one automatically starts to wonder „what exactly is patient satisfaction?" According to an often-cited definition, patient's satisfaction consists of his/her cognitive assessment of the received care and the emotional reactions related to it [6]. Other authors indicated that patient satisfaction depends on the consistency between the patient's expectations and actual care s/he received [7]. Fung et al have dissected these concepts down to their elements and accurately described the methodological challenges, if not frustrations, with

Figure 2. Bedside manner

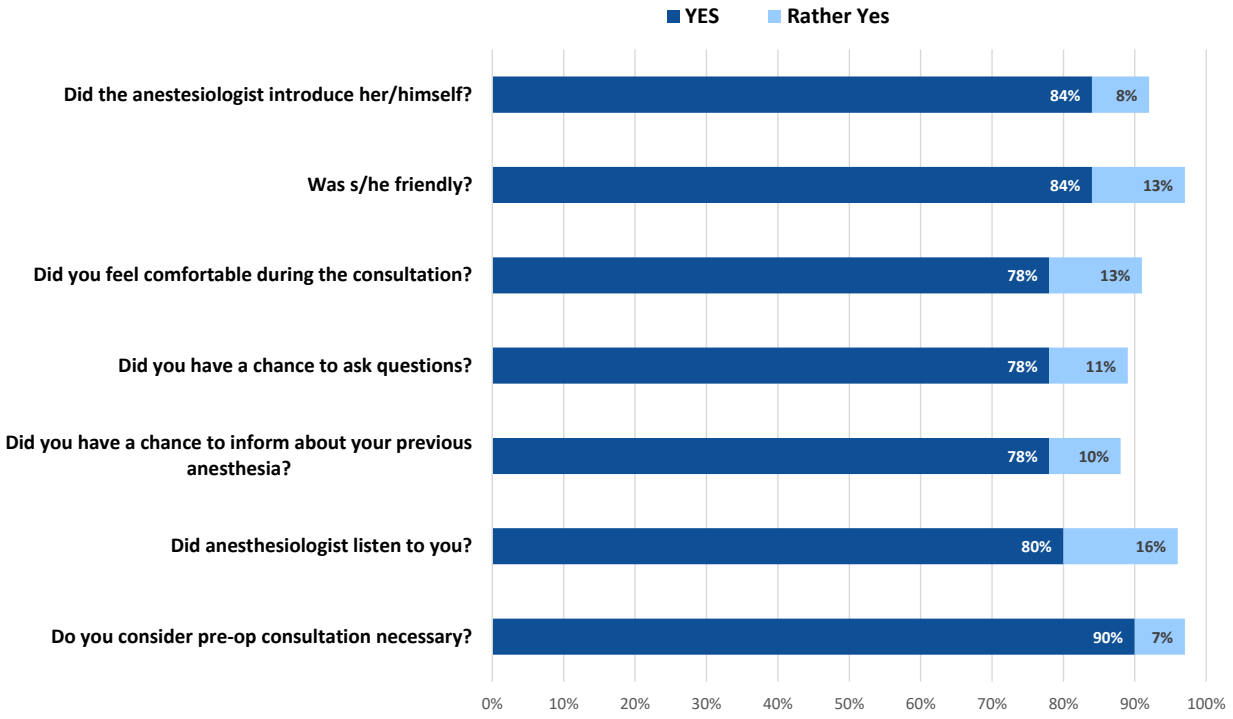
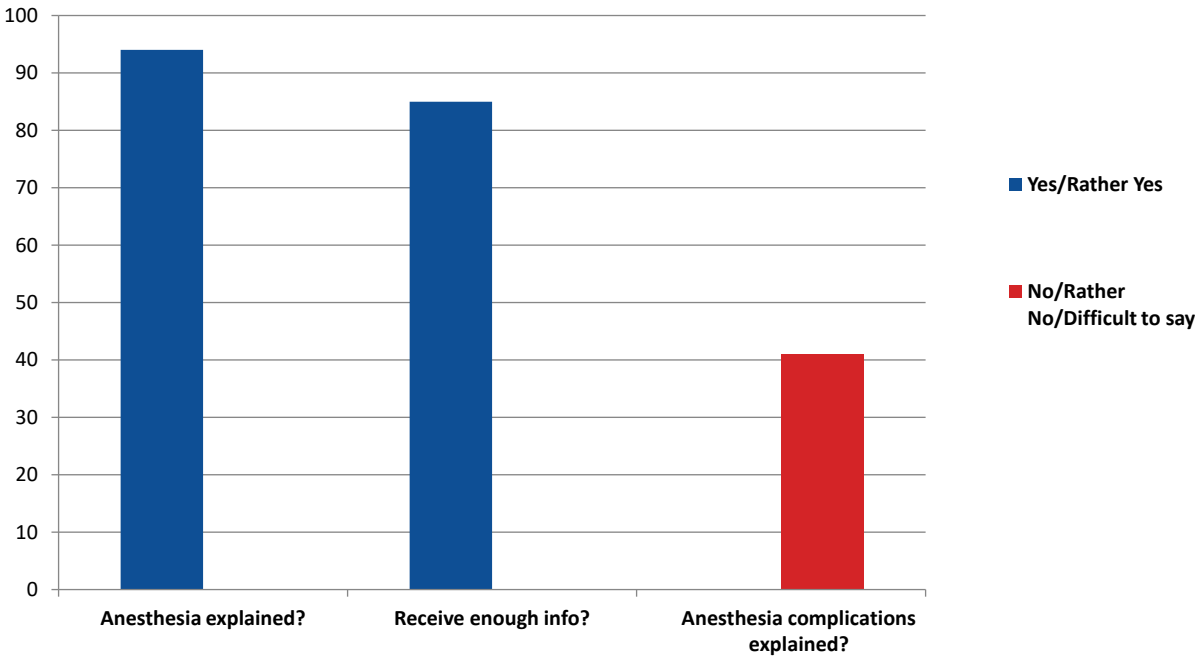


Figure 3. Pre-operative consultation



measuring expectations, ability to accept difference between expectations and reality, mental state during satisfaction etc [1]. Clearly satisfaction is not a one-dimensional concept, thus measuring it is not simple and requires appropriate tools. In the past, researchers relied on psychometric methods to design robust questionnaires [8].

Although there are several validated, previously published anesthesia satisfaction questionnaires, we designed our own instrument. The Iowa Satisfaction with Anesthesia Scale is a validated psychometric questionnaire, however it was not appropriate for our study because it was designed to measure the satisfaction from monitored anesthesia care only [9]. We decided not to

Figure 4. Pre-operative anxiety. The 4 most commonly mentioned causes of anxiety are listed

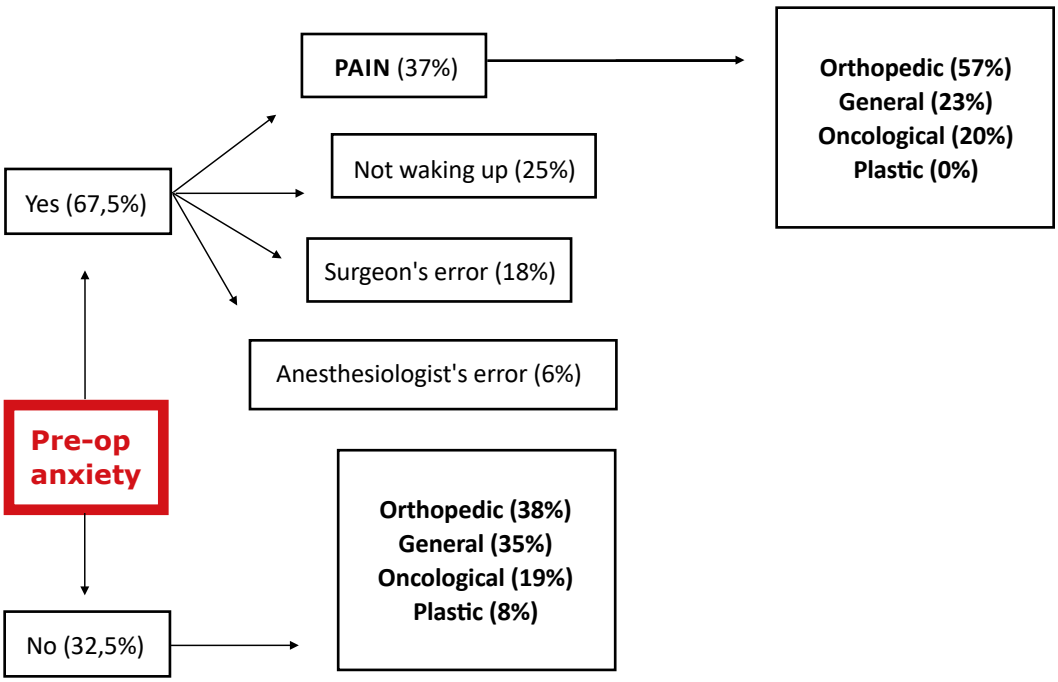
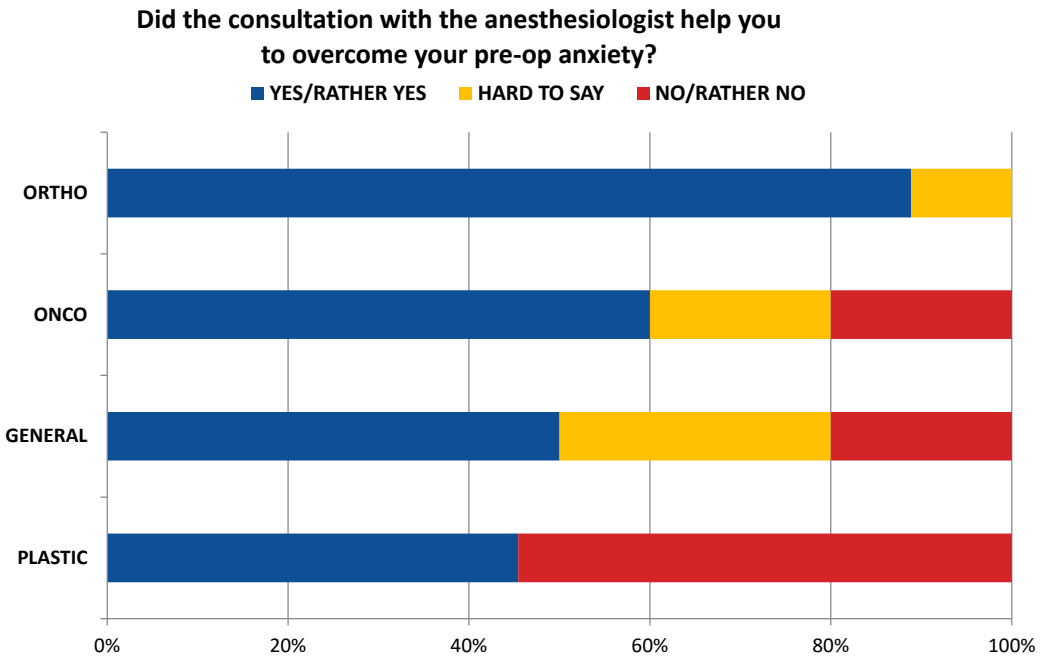


Figure 5. Pre-operative anxiety



use the Leiden Perioperative care Patient Satisfaction questionnaire because in our opinion it was too lengthy to use during a structured interview [10].

Many of the previously published studies involved staff distributing the questionnaires to the patients at the ward, resulting in response rates between 80 and 100% [10-11]. To improve the response rate, we conducted systematic, structured interviews using our questionnaire. We feel that the so-called 'halo effect' and underreporting of dissatisfaction has been minimized because the interviews were conducted by medical students who were not involved in the patient care at their respondents' wards. Although this approach has been viewed with some suspicion, we did not obtain the near-100% response rates that make the validity of prior studies suspect [1].

Figure 6. Post-operative pain management

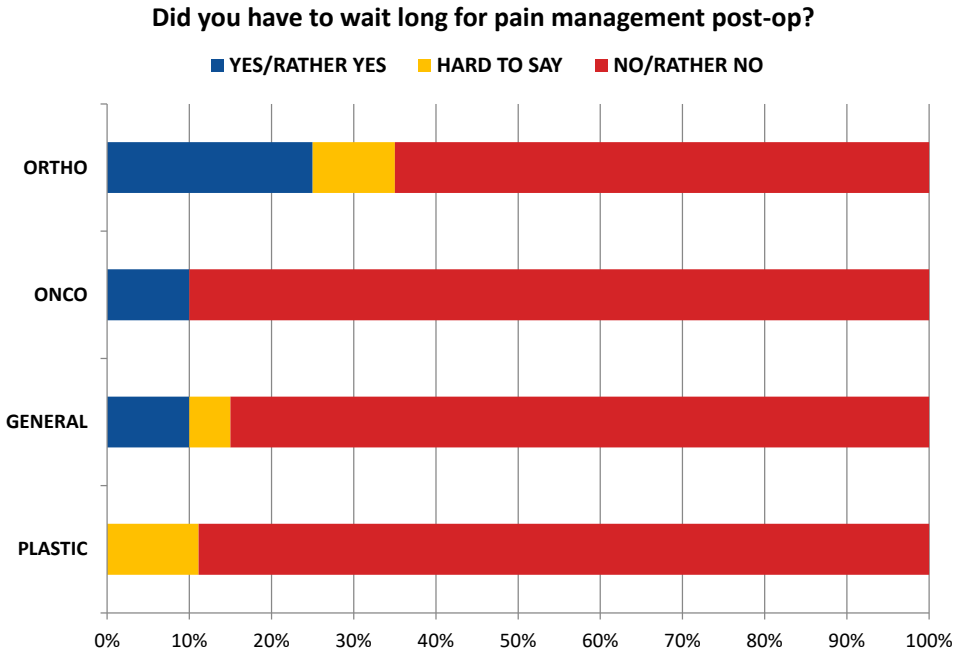
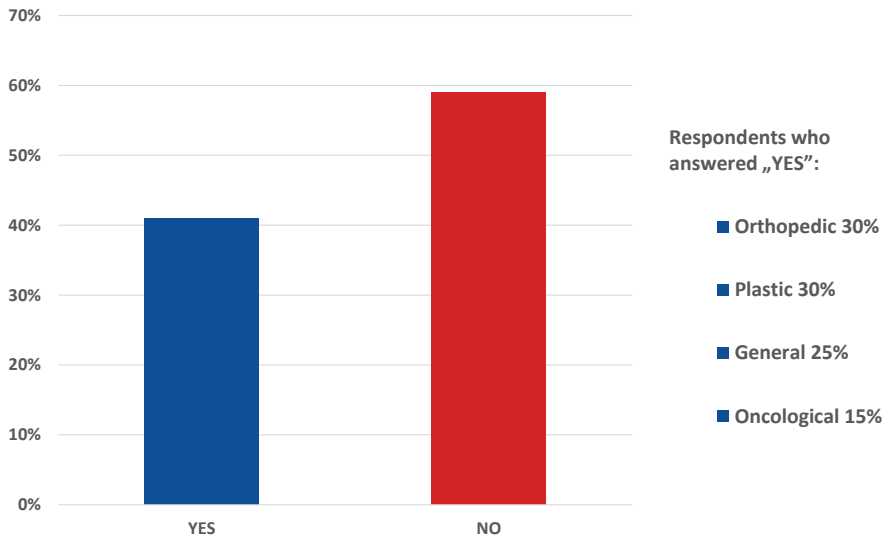


Figure 7. Post-operative discomfort due to nausea & emesis



In terms of methodology, a somewhat similar study was performed by Gaszyński et al, who surveyed 42 patients at a general surgery department [12]. Comparing with our results from a general surgery department, Gaszyński et al had significantly more respondents claiming to be uninformed about the possible adverse effects of anesthesia (52,4% vs. 10%) and fearing not waking up after the procedure (58,8% vs. 30%) [12]. On the contrary, our general surgery patients much more frequently reported post-operative nausea and emesis than those surveyed by Gaszyński et al (40% vs 20,6%) [12].

Our study has several limitations. First of all, our questionnaire was not designed by psychologist. Second, we did not perform a test-retest reliability check or a validity check (no comparison with responses predicted by anesthesiologists or anesthesia nurses). Third, we did not review medical documentation to compare what doses of analgesia our patients received. Fourth, the inclusion of patients who underwent regional (intravenous) anesthesia (7%) might have confounded our results. Finally, despite our best efforts to minimize response bias (interviews conducted by volunteers, who were not directly involved in patient care), it is possible that some of the patients told us what we wanted to hear. However, we performed this pilot study not only to establish a general trend in responses but specifically to test the methods of data collection and analysis.

Our preliminary results confirm earlier observations that the patient-anesthesiologist communication is a critical element of patient satisfaction with peri-operative care and the pre-operative visit is particularly important [13-14]. However, a larger survey at more surgical departments combined with a review of medical documentation is needed to fully assess the patient care and satisfaction trends discussed above.

Conclusion

Our preliminary data suggest a high level of patient satisfaction with nearly all aspects of perioperative anesthesiology care at our institution. However, anesthesiologists need to more thoroughly inform patients about possible complications of anesthesia. It is critical to respect the patient's right to information about the procedure and its complications. Surveys are a simple and useful tool to measure quality of care indicators at an anesthesiology department and can help in training residents. A larger survey at more surgical departments is needed to fully assess the patient care and satisfaction trends discussed above.

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REVIEW ARTICLE

Rare histological subtypes of renal cell carcinoma in everyday diagnostic practice

Małgorzata Hypszer[1], Katarzyna Czarnota[1], Grażyna Kobierska-Gulida[1,2], Adam Nałęcz[1], Wojciech Wesołowski[3], Ewa Iżycka-Świeszewska[1,4]

[1] Department of Pathomorphology, Copernicus Hospitals, Gdańsk, Poland
[2] Department of Pathomorphology, Medical University of Gdańsk, Gdańsk, Poland
[3] Department of Pathology ELPAT, Elbląg, Poland
[4] Department of Pathology & Neuropathology, Medical University of Gdańsk, Gdańsk, Poland

Abstract

Introduction: The fourth edition of the WHO Classification of Tumours of the Urinary System and Male Genital Organs (2016) contains new renal tumour entities. These new subtypes of renal cell carcinoma (RCC) were introduced based on morphological criteria, some genetic features, and clinical characteristics with prognostic implications.

We present three patients with rare renal tumours belonging to newly recognized or still emerging categories of RCC. All cases were diagnosed based on careful morphological examination with immunophenotyping, and patho- clinical correlation. The first case is an example of acquired cystic disease – associated renal cell carcinoma with heterogeneous architecture as well as specific intra- and intercytoplasmic microlumens. The second tumour – a tubulocystic renal cell carcinoma - was composed of multiple, various-sized cysts divided by fibrovascular septa and tubules lined focally with hobnail cells. The third case presents very rare sporadic eosinophilic, solid, and cystic RCC. This tumour contained macro et microcystic, areas intermixed with solid fields composed of large, in part multinucleated eosinophilic cells. Inflammatory infiltrations accompanied the neoplastic stroma.

New subtypes of RCC, although rare, can be encountered in everyday practice. It is important to perform careful differential diagnosis and classify such tumours according to the recent guidelines.

Keywords: renal cancer / tubulocystic RCC / acquired cystic disease-associated RCC / eosinophilic solid and cystic RCC / WHO classification

Citation

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Corresponding author:
Małgorzata Hypszer, Department of Pathomorphology Copernicus Hospitals, Gdańsk, Poland
e-mail: mhypszer@gmail.com

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Introduction

The classification of renal neoplasia is still based on morphology but it has been evolving dynamically over the past years due to advances in the understanding of molecular pathogenesis of these tumours [1]. Correct diagnosis of renal tumours carries significant clinical implications for patients such as prognostic risk stratification, selection of targeted therapeutics and identification of cases for further genetic testing [1-3].

The last WHO Classification of Tumours of the Urinary System and Male Genital Organs (2016) recognizes several distinct RCC subtypes. New tumour entities include: hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cell carcinoma, Succinate dehydrogenase-deficient renal cell carcinoma, tubulocystic renal cell carcinoma, acquired cystic disease-associated renal cell carcinoma, and clear cell papillary renal cell carcinoma [1, 4-5]. In addition, the classification recognizes also tumour types described as emerging, which have distinct histological and genetic pattern but due to their rarity there is yet not enough data to include them as separate subtypes [1, 4-5].

The incidence and mortality due to renal cell carcinomas (RCC) have been increasing over the last years and it the 9th most common cancer in men and 14th in women worldwide [5]. The majority of cases occur in countries with high socioeconomic status [5]. The established risk factors for RCC are cigarette smoking, obesity and certain occupational exposures [3]. Hypertension or its treatment, especially using diuretics has also been associated with increased risk of these tumours [3, 5]. The incidence of renal cell cancer is increased 3-6 times in patients with acquired cystic kidney disease [3, 5]. Most renal cell carcinomas are sporadic but 2-4 % have familial causes [3]. The risk of renal cancer for a first degree relative of a patient with renal cancer is double [5]. There are several genetic disorders associated with RCC such as von Hippel-Lindau syndrome (clear cell RCC), hereditary papillary RCC, hereditary leiomyomatosis and RCC, familial papillary thyroid carcinoma (papillary RCC), hyperparathyroidism-jaw tumour syndrome, Birt-Hogg-Dube syndrome, tuberous sclerosis, and constitutional chromosome 3 translocations [3, 5].

In this article we present three cases of renal cell carcinomas, that disclose distinct histological and clinical features, and belong either to newly recognized or emerging entities.

Materials and methods

Three out of 47 cases of renal tumours diagnosed in routine practice in 2017 in the El-Pat Laboratory of Pathology and the Department of Pathomorphology at the Copernicus Hospital. The cases included in the article were the ones that needed consultation and second opinion. The clinical data included patients' age, sex, basic medical history, radiological imaging results.

All tumours underwent routine pathological procedures- gross examination with adequate tissue sampling and histological examination with wide panel immunophenotyping. Immunohistochemistry was performed based on DAKO auto-stainer antibodies against: CK AE 1/3, CK 7, CK 8/18, CK 19, CK 20, CD10, CD117, AMACR, CA IX, EMA, S100, PAX 8, inhibin, estrogen receptor, vimentin and Ki67; with appropriate producer recommendations.

Literature review was performed in PubMed; the references used in the article were published in the years 2006-2019.

Results

Patient 1

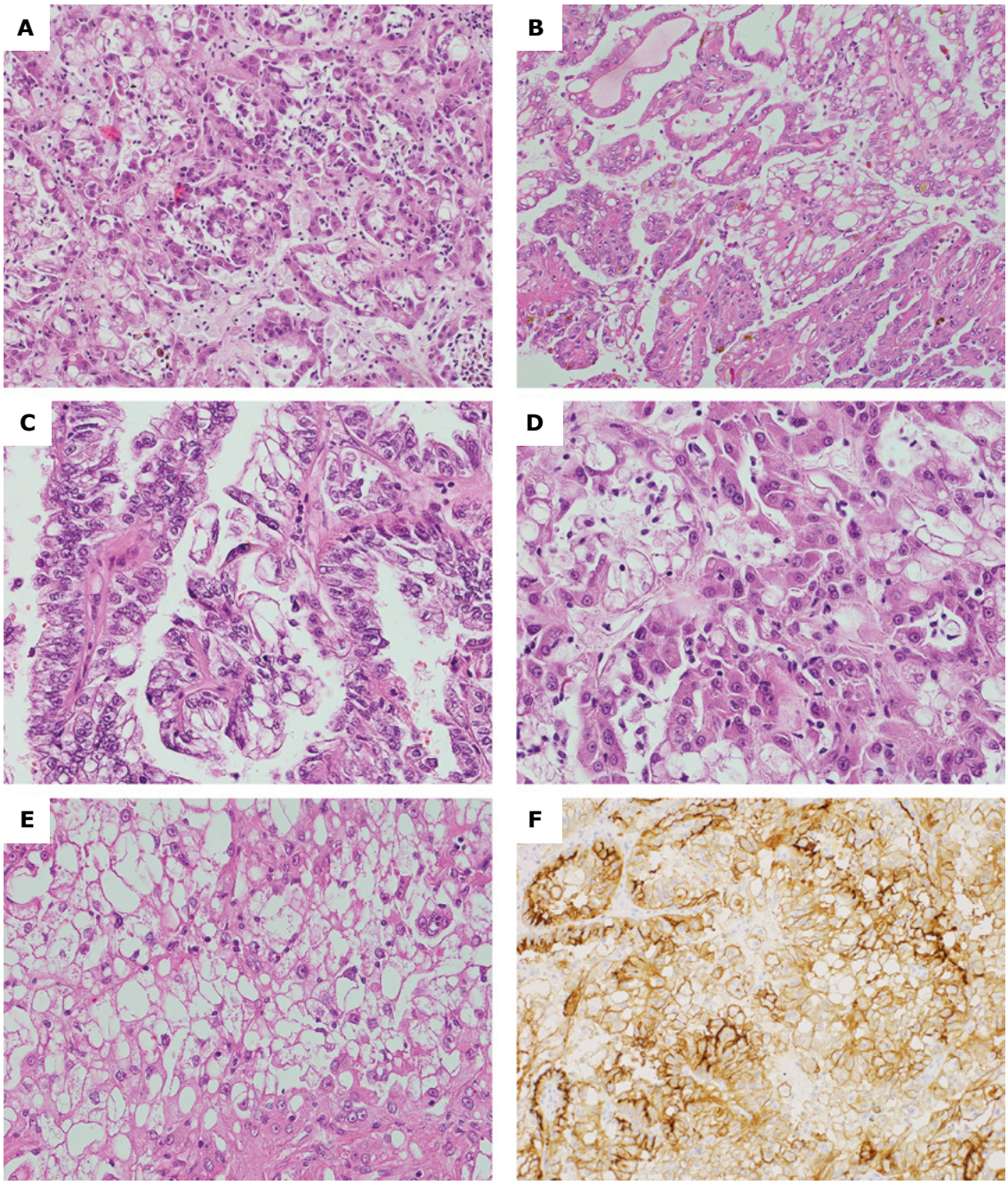
A 58-year old man was admitted to the hospital for a scheduled dialysis. For 6 years he has been suffering from end-stage renal disease caused by poorly managed type 2 diabetes. The patient was asymptomatic. A routine abdominal ultrasound examination revealed a mass in the left kidney. CT scan confirmed a hypodense tumour in the superior pole of the kidney, 6.0 x 5.0 x 4.5 cm. The cortex of both kidneys was thin, and there was also a 1.5 cm cyst in the cortex of the left kidney. The patient underwent radical left nephrectomy.

On macroscopic examination, the kidney measured 12 x 7 x 7 cm. Underneath the capsule of the superior pole, there was a well-circumscribed soft tumour (5.5 cm in its greatest diameter) with a grey cut surface. The tumour didn't break the renal capsule and didn't invade the renal pelvis (pT1b) [6].

Histological examination showed well-circumscribed neoplasm with heterogeneous architecture, mainly composed of solid and papillary areas as well as cribriform/sieve-like pattern (Figure 1A, B, C). The neoplastic cells were large, partially columnar, with eosinophilic granular cytoplasm showing intracytoplasmatic and intercytoplasmatic microlumens (Fig. 1D, E) with rare oxalate crystals – clear to slightly opaque, polarizable structures seen in intracytoplasmic spaces. The nuclei were vesicular with variably conspicuous

nucleoli (International Society of Urological Pathology (ISUP) scale grade 2-4, and overall showed intermediate atypia. Deposits of hemosiderin were present as well as foci of chronic inflammatory infiltrate. The neoplastic cells showed immunopositivity for CD10 (Figure 1F), AMACR, vimentin and were negative for CK7, CD117 and CAIX. Proliferation index measured with Ki67 was around 2%. The kidney tissue surrounding tumour presented chronic inflammatory changes with vascular changes, parenchymal fibrosis, as well as small cysts deriving from renal tubules. Based on tumour morphology, renal pathology, and supplied patient clinical history, acquired cystic disease (ACD)-associated renal cell carcinoma was diagnosed.

Figure 1 A-F. Microscopic findings in Acquired cystic disease-RCC: A – Various architectural patterns (solid, tubular and papillary) (HE, 200x); B – Solid and cystic architectural patterns (HE, 200x); C – Papillary and macrocystic architectural patterns (HE, 400x); D, E – Distinctive intra – and intercytoplasmic microlumens; nucleoli are very prominent (HE, 400x); F – immunoreactivity for CD10 (200x)



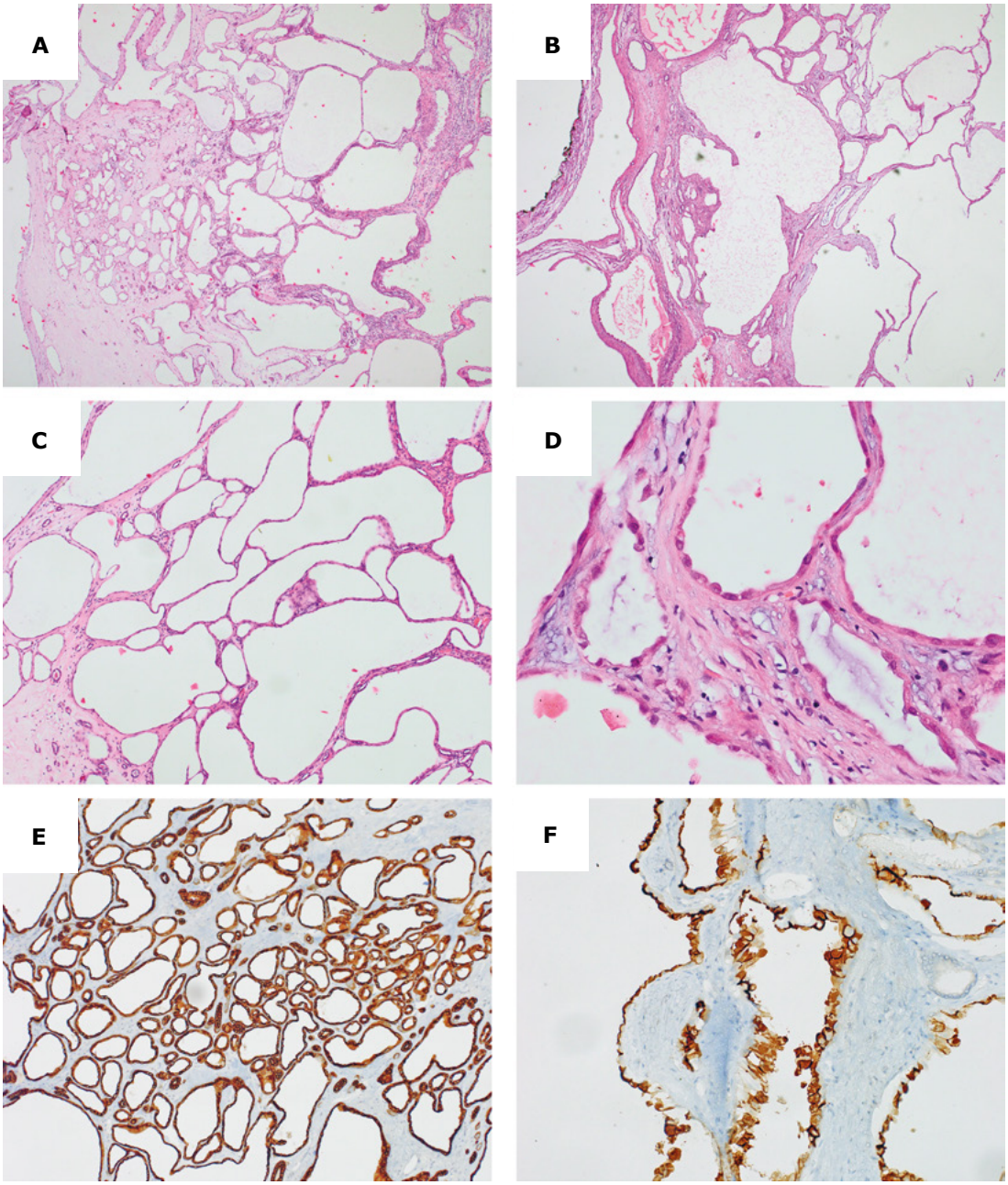
Patient 2

A 65-year old woman was incidentally diagnosed with a 2 cm mass in the left kidney, found on CT-scan and confirmed on MRI examination. In the next weeks, the patient underwent partial nephrectomy.

Gross examination showed a multicystic tumour measuring 2.2 x 1.5 x 2.2 cm (pT1a) [6]. On microscopic examination the tumour was composed of tubules (Figure 2A) and multiple cysts of various size (Figure 2B, C), focally lined with cells with distinctive hobnail pattern (Figure 2D); other cells were flattened. The cysts were separated by fibrovascular septa. The cytoplasm of neoplastic cells was eosinophilic, nuclei were round with mild atypia, some had visible nucleoli (ISUP grade 2, singular cells with ISUP grade 3 nucleoli). Cells were immunopositive for AMACR, CD10, vimentin, CAIX (weak staining), CK8/18, CK19 (Figure 2F) and focally for CK7 (Figure 2E), while negative for inhibin and estrogen receptors. Proliferation Ki67 index was <1%.

The tumour was diagnosed as tubulocystic renal cell carcinoma.

Figure 2 A-F. Microscopic findings in Tubulocystic-RCC: A – Predominately micro- and macrocystic architectural patterns (HE, 40x); B – Outermost part of the tumour (HE, 40x); C – Cystic part of the tumour (HE, 100x); D – Flattened, cuboidal and hobnail cells lining tumour cysts (HE, 400x); E – CK7 highlighting tubular part of the tumour (100x); F – CK19 immunoreactivity prominent in the hobnail cells lining the lumens (200x)



Patient 3

A 77-year old woman with history of arterial hypertension complained of abdominal pain. The ultrasound revealed a pathological mass up to 2.5 cm in the upper pole of her left kidney. A CT scan confirmed a tumour in the upper half of the left kidney, with no clear margins, without signs of breaking renal capsule or involving renal pelvis. The patient underwent partial nephrectomy.

On macroscopic examination, the tumour measured 2.0 x 2.5 x 2.0 cm and was partially solid with cystic spaces, white-grey on cut surface with small red spots (pT1a).

Histologically, macrocystic spaces were lined with cells with eosinophilic cytoplasm (Figure 3A) and solid parts (Figure 3 B, C) were composed of large round cells with abundant eosinophilic cytoplasm, hyperchromatic nuclei with mild nuclear pleomorphism and discrete nucleoli (ISUP grade 2). In addition, there was an admixture of lymphocytes and histiocytes and multinucleated giant eosinophilic cells within the solid areas. The neoplastic cells were positive for AMACR (patchy cytoplasmic staining), vimentin, CKAE1/AE3 (Figure 3D), PAX8 (strong nuclear reaction, Figure 3E), CD10 (Figure 3F), CK20 staining showed focal positivity. In parallel, the tumour was negative for CK7, CD117, EMA, S-100, CAIX. Proliferation Ki67 index was 2%. Based on careful differential diagnosis, and after literature review the tumour was finally diagnosed as a renal cell carcinoma, subtype eosinophilic solid and cystic [7].

Discussion

In 2016 the 4th edition of the WHO Classification of Tumours of the Urinary System and Male Genital Organs introduced important changes into histological approach to renal tumours [5, 8]. As mentioned before, genetics play an increased role in new classifications challenging and sometimes changing our understanding of tumour pathology. While clear cell carcinoma and papillary carcinoma remain the most common renal cancers, careful examination combined with exact clinical data can lead to an exact diagnosis of even rare subtypes and therefore better clinical management. Furthermore, the latest WHO classification recommends using the ISUP scale for tumour grading based on nucleoli appearance (grade 1-3) and cell pleomorphism (grade 4) instead of the traditional Fuhrman scale [5]. The latter, due to its methodology problems (e.g. an overly complicated grading based on 3 seperate parameters, which can cause discordance, imprecise criteria regarding nuclear pleomorphism) and unsatisfactory intraobserver reproducibility, did not meet the criteria of a reliable prognostic factor. Still, the ISUP/WHO scale has only been validated for clear cell RCC and papillary RCC because there have not been enough cases of other subtypes. It is important to mention that there are subtypes of RCC in the appearance of nucleoli does not correlate with the overall grade and prognosis and should be used for descriptive purposes only [4, 8].

Other changes in current classification include an increase of the maximal diameter of papillary adenoma up to 1,5 cm (and increasing the pool of possible kidney donors). Adult cystic nephroma was shifted to mixed epithelial-stromal tumours as a part of their spectrum. Moreover, the term “renal carcinoid” was replaced with “well-differentiated neuroendocrine tumour of the kidney” in order to emphasize the metastatic changes after nephrectomy and poor prognosis. Similar principles led to changing the name of “multilocular cystic renal cell carcinoma” to “multilocular cystic renal neoplasm of low malignant potential” to highlight the lack of malignant potential. Papillary RCC type 2 is now believed to be more than one type of tumour as its subtypes contain different molecular changes [7].

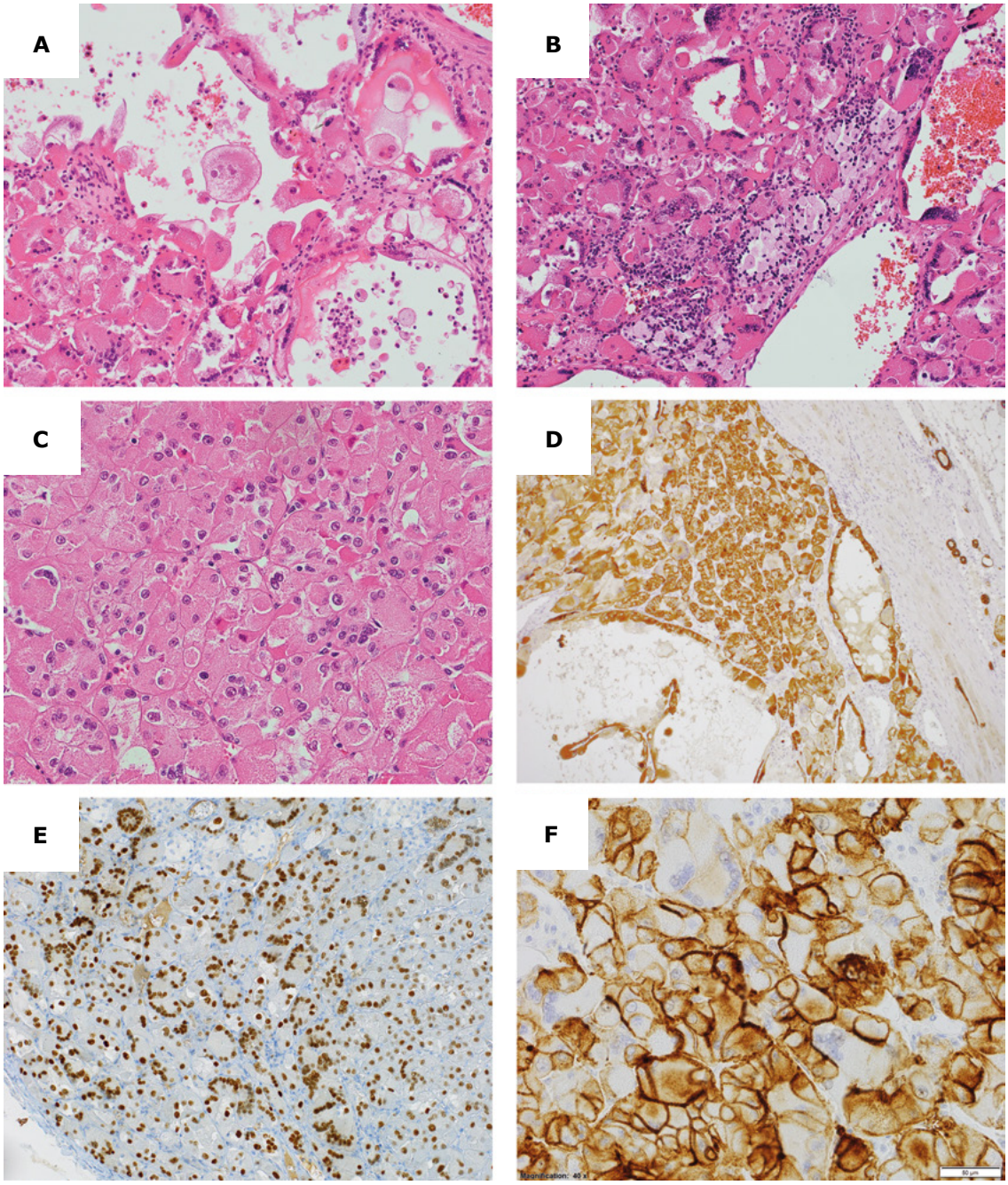
Presented cases

Acquired cystic disease (ACD)-associated RCC is a subtype newly included in the 2016 WHO classification. It was first described in 2005 and it was recognized in the 2013 ISUP Vancouver classification of renal neoplasia [9]. This tumour arises in patients with acquired cystic kidney disease due to end-stage renal disease, often in association with long-term haemodialysis or peritoneal dialysis [4, 9]. It is now the most common subtype of RCC in patients with end-stage renal disease and ACD and accounts for over 1/3 of renal tumours in these patients [4].

Patients are usually asymptomatic and the tumour is found accidentally during routine check-up and imaging [9, 10]. This tumour occurs predominantly in male patients with ACD of younger age but the incidence of carcinoma increases with duration of dialysis [9-11], recent studies report that it is most likely to be diagnosed between 10 to 20 years after the first dialysis [9, 12]. The risk is also increased for individuals with acquired cystic kidney disease who underwent renal transplantation [5].

The ACD-associated RCC tumour is usually well-circumscribed, cystic and/or solid. The cut surface varies from yellow-tan to brown, focal necrosis and haemorrhage may be present [9, 13]. Histologically, these tumours are composed of cells with abundant eosinophilic cytoplasm and large irregular vesicular nuclei with prominent nucleoli. They show a broad spectrum of architectural intermixed patterns including papillary,

Figure 3 A-F. Microscopic findings in Eosinophilic, solid and cystic-RCC: A – More cystic part of the tumour, note bright pink cytoplasm and the various shape of cells lining the cysts (HE, 200x); B – Predominately solid part of the tumour, with multiple giant cells, lymphocytes, and histiocytes (HE, 200x); C – Solid part of the tumour, cells are bright pink with easily noticeable nucleoli (HE, 400x); D – Positive staining for CK AE1/3; also highlighting pre-existing tubules in the healthy part of the kidney on the right side of the photo (100x); E – Nuclear expression of PAX8, note the highlighted giant-cells nuclei (200x); F – Strong membranous staining for CD10; here the multinucleated giant cells are negative (400x)



tubular, acinar and solid, however sarcomatoid and/or rhabdoid features have also been described [13-15]. Another common feature characteristic of this RCC is the presence of intracytoplasmatic and/or intercytopasmatic lumina and intratumoral calcium oxalate crystal deposition [1, 4, 9-10]. Due to its unique morphological features, immunohistochemistry is not required to diagnose ACD-RCC. The most common pattern is positivity for CD10, RCC marker, AMACR. CK 7 is typically not expressed or expressed focally and CAIX is not detected [1, 8-9]. The genetic alterations in ACD-associated RCC are quite complex and even samples taken from the same tumour may not be genetically identical. The most common abnormalities are gains in chromosomes 3, 7, 16 [10]. Mutations in von Hippel-Lindau (VHL) gene have not been reported [5]. In general, ACD-associated RCC seems to be less aggressive than sporadically occurring RCC and has

a better prognosis [9]. Most tumours have indolent behaviour, although some patients may develop metastatic disease [4, 8, 10]. However, it is worth remembering that almost any histological variant of RCC can occur in patients with an acquired cystic disease, but the most common types are ACD-associated RCC and papillary RCC [9]. ACD-RCC might be misdiagnosed as type 2 papillary RCC because its papillary architectural pattern and the presence of clear cell areas may lead to confusion with classical clear cell RCC [3]. The morphology of the presented case, especially combination of multiple architectural patterns, characteristic lumina, as well pathological changes in surrounding kidney together with patient's clinical history formed our diagnosis.

Tubulocystic renal cell carcinoma is an uncommon (<1% of all RCC) renal epithelial malignancy [16]. It has male predominance (7:1), patients' age ranges from 30 to 94 years [17-18]. Majority of tubulocystic RCCs are discovered incidentally, but patients may present symptoms such as abdominal pain and hematuria [19]. In rare instances, it may occur in patients with existing end-stage renal disease [1, 17-18]. On macroscopic examination, this tumours tends to be a solitary, well-circumscribed multicystic renal mass, with a mean diameter of about 4 cm [20]. Cysts are numerous, small to intermediate in size with spongy cut surface [17-18]. Cysts are lined by single layer of flattened, cuboidal or columnar as well as hobnail epithelium. The neoplastic cells nuclei are enlarged and irregular with intermediate to large nucleoli [19, 21]. Enlarged nucleoli are one of the diagnostic features of tubulocystic RCC, but nuclear grading should not be applied as it does not correlate with the outcome [18, 20]. The cytoplasm is abundant and sometimes eosinophilic. Cyst and tubules are separated by thin fibrovascular septa. There may be also components similar to papillary RCC.

Immunohistochemically, tubulocystic carcinoma has similar features to papillary RCC and shows positive staining for racemase, CK7, CD10, and RCC antigen [1, 16-17]. The genetic studies of tubulocystic RCC ale limited and they show some overlapping features with papillary RCC [15]. The most commonly reported were gains of chromosomes 7 and 17 and loss of the Y chromosome [1, 5]. Majority of tubulocystic RCC have indolent behaviour with very few recurrences and unusual metastases [5, 11, 20]. The differential diagnosis includes other tumours with a multiloculated gross appearance: multilocular cystic RCC (multilocular cystic renal neoplasm of low malignant potential), cystic nephroma, mixed epithelial and stromal tumours, cystic oncocytoma [16, 18]. The diagnosis in our case was quite problematic as it demanded a careful examination of tumour architectural patterns and cytology. Its cells exhibited ISUP grade 2 nucleoli, whereas according to WHO recommendations they should be ISUP grade 3. Therefore, immunohistochemistry and especially the tumor's overall benign appearance were helpful in making the final diagnosis.

The last of our cases presented the biggest diagnostic challenges. There are only several published cases of the unique renal neoplasm characterized by eosinophilic cytoplasm and solid and cystic growth reported in patients with tuberous sclerosis complex (TSC) [7, 22]. TSC is an autosomal dominant disorder with characteristic tumours and tumor-like conditions involving multiple organs, while in the kidney the most common tumour is angiomyolipoma [23]. RCC is less frequently reported in this syndrome, but may have very distinct morphology. Some of the RCCs coexisting with TSC show features similar to chromophobe RCC or are described as RCC with smooth muscle stroma, but there are several reports of RCC with a granular eosinophilic-macrocystic morphology [23]. These tumours showed female predominance and occurred at a younger age. Recently, eosinophilic solid and cystic RCC has been documented also in the series of female patients without clinical features of TSC [7]. These tumours, usually asymptomatic, well-defined, were located in the medulla. Cut surface was tan with typically large macrocytic spaces, variable in size, interspersed with solid nodules [7].

Microscopically they contain solid areas admixed with variably sized macrocysts and microcysts lined by cells with abundant eosinophilic cytoplasm and hobnail arrangement. Nuclei are round to oval with prominent nucleoli. There is often chronic inflammatory infiltrate with multinucleated cells within the neoplastic stroma. Some cases have a predominantly microcystic arrangement or septa compressed between solid nodules and therefore difficult to spot [7, 22]. Immunoprofile shows nuclear PAX8 expression, predominant CK20-positive/CK7-negative phenotype (but CK20-positive/CK7-positive and CK20-negative/CK7-negative phenotypes exist), patchy AMACR staining, vimentin usually positive, CD10 focally positive and CAIX negative in most cases [7]. This tumour is believed to have indolent behaviour, but data is still lacking. There is a discussion whether to even label it as "of uncertain malignant potential" or "renal cell carcinoma." So far these types of tumours were described mainly as „unclassified," however it is important to recognize them in order to determine their true biology [7]. It was those peculiar cytological features, especially eosinophilia and multinuclearity, that caught our attention and pushed toward correct diagnosis after the literature review.

Renal cell carcinoma, subtype eosinophilic solid and cystic are among the emerging entities, not included in the current classification, but candidates for the future because of their distinctive morphology and immunohistochemical features [7].

Conclusion

This report shows that new entities recognised by 2016 WHO Classification of Tumors of the Urinary System can be found in everyday practice based on careful morphological assessment [5]. Therefore it is important to be able to diagnose such tumours and then classify them according to the latest guidelines and in correlation to patient's history [24]. It is also important to remember that classification of renal cell carcinoma is still evolving and new tumours with unique morphological, immunohistochemical and molecular patterns are constantly reported and may emerge as a distinctive subtype in future.

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REVIEW ARTICLE

Endocrine disorders in patients with hereditary hemochromatosis

Katarzyna Banaszkiewicz[1], Katarzyna Sikorska[1], Krzysztof Sworczak[2]

[1] Department of Tropical Medicine and Epidemiology, Medical University of Gdańsk, Gdynia, Poland
[2] Department of Endocrinology and Internal Medicine, Medical University of Gdansk, Poland

Abstract

Hereditary hemochromatosis (HH) is a rare genetic disorder, developing secondary to the accumulation of iron in tissues, which may lead to multiple organ failure. If untreated, it may result in liver cirrhosis or cardiomyopathy. The damage to the pancreas and the anterior pituitary, on the other hand, leads to a decreased production and secretion of hormones that are essential to life.
Common symptoms of HH, that are distressing for patients, include joint pain, particularly involving hands and wrists, as well as the chronic fatigue syndrome. Iron overload affects the skeletal system, leading to osteoporosis. The pathological accumulation of iron in the anterior pituitary impairs the gonadotropin synthesis, resulting in reduced serum levels of testosterone in men and estrogens in women. This, however, contributes to lower bone mass. In vivo tests have also revealed that abnormal iron accumulation is related to an increased activity and number of osteoclasts, as well as the influence on the differentiation and activity of osteoblast-lineage cells.
Based on a systematic review of literature, hereditary hemochromatosis (HH) will be presented as a chronic disease, affecting most of the endocrine glands.
Keywords: diabetes mellitus / osteoporosis / endocrinology / iron overload / hemochromatosis

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Introduction

HFE (human hemochromatosis protein)-associated hereditary hemochromatosis (HH) is an autosomal recessive a genetic disorder. Among the Caucasian population, mutations of the HFE gene account for 60-96% of cases in which the symptoms of iron overload develop. Classic HH-Hereditary Hemochromatosis develops in homozygotic carriers of the C282Y mutation in the HFE gene – as a result of a point mutation in the nucle-

Corresponding author:
Katarzyna Banaszkiewicz, Department of Tropical Medicine and Epidemiology, Gdańsk, Poland
e-mail: katarzyna.banaszkiewicz@gumed.edu.pl
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Available online: www.ejtcm.gumed.edu.pl

otide (845G>A), cysteine is substituted with tyrosine in the HFE protein. The prevalence of this mutation among the Caucasian population has been estimated at 3-10 cases per 1000 people, which means that HH is one of the most prevalent genetic metabolic diseases [1].

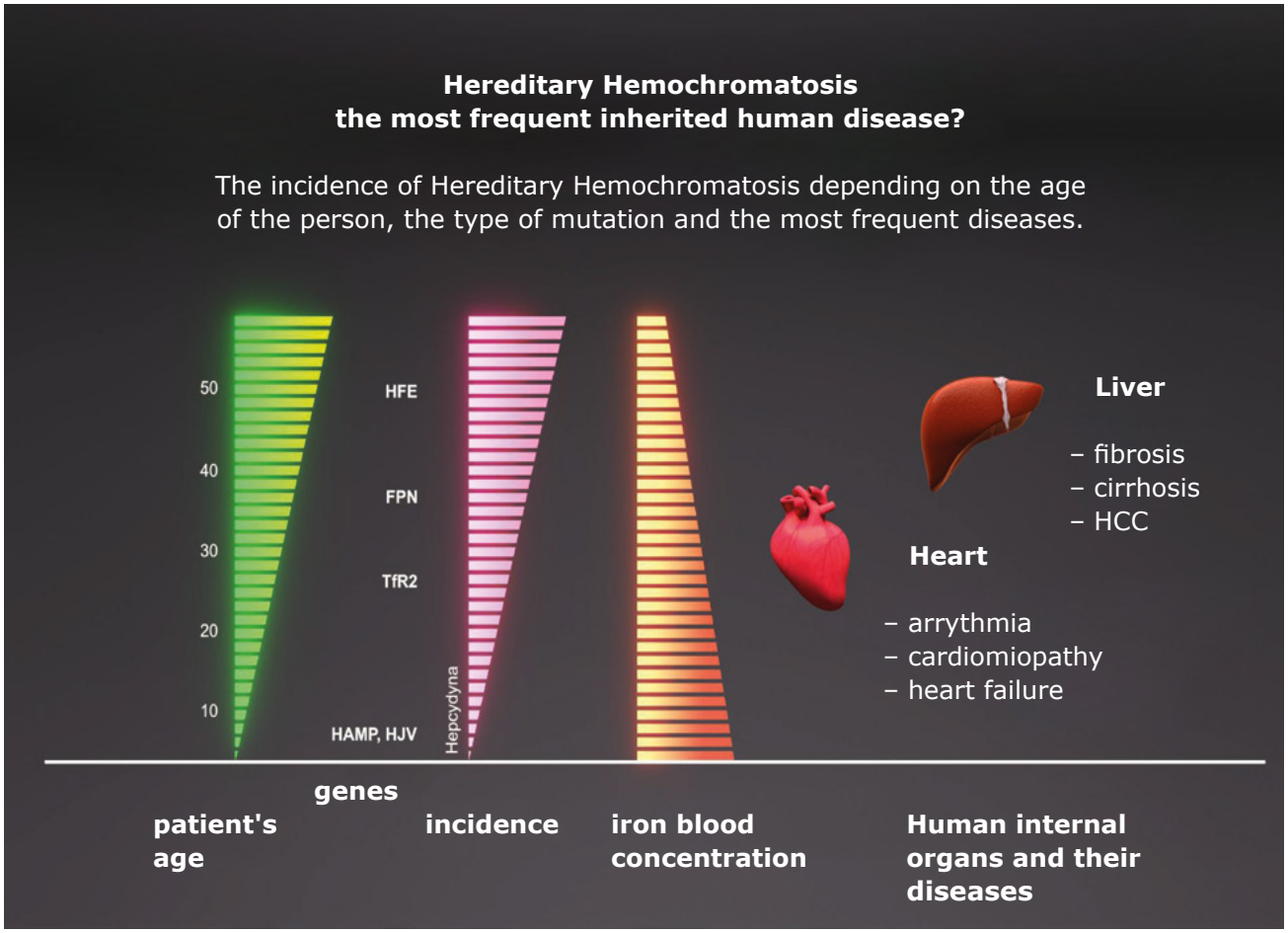
Other polymorphisms in the HFE gene (H63D – substitution of histidine with aspartic acid, S65C – substitution of serine with cysteine), which are frequently detected, do not cause such serious alterations within the spatial structure of the HFE protein and such dynamic symptoms of iron overload [2].

Most commonly, the first clinical manifestation of the disease involves symptoms of liver damage, which in most cases of untreated primary HH leads to liver cirrhosis with significant risk of hepatocarcinogenesis [3]. Iron deposition in other organs can also lead to: diabetes mellitus, cardiomyopathy, bone and joint pathology, hypopituitarism and easily noticeable (brownish) skin discoloration. Early diagnosis and treatment aimed at reducing the tissue iron concentration (e.g. bloodletting as a first-line therapy, iron chelation therapy) are of undeniable importance in preventing irreversible organ damage [4-7].

Any pathological changes in endocrine glands may develop secondary to pathological iron accumulation of a different etiology. In classic HH, these changes are usually diagnosed concomitantly with the main liver pathology. In juvenile hemochromatosis, which is a very rare disorder developing secondary to underlying mutations in the hepcidin gene or the hemojuvelin gene with rapidly progressive iron accumulation, hormonal imbalance manifests itself at a younger age in the form of severe endocrinological dysfunctions (Figure 1) [8].

A juvenile hemochromatosis type 2, a disease with severe symptoms of iron accumulation and an early onset, is diagnosed in patients with abnormal synthesis of hepcidin or hemojuvelin. It is a rare disease characterized by a dynamic cycle of iron deposition in tissues, quick depletion of liver's compensating capacity for storing iron and development of symptoms associated with the failure of the heart muscle and endocrine glands. Mutations in the TFR2 (transferrin receptor 2 gene) encoding gene that cause the dys-

Figure 1. Clinical picture of hemochromatosis depending on mutation of genes encoding HFE, hepcidin (HAMP), hemojuvelin (HJV), transferrin 2 receptor (TfR2), ferroportin (FPN). Organ changes may concern: liver, heart, endocrine organs. Image modification from: Pietrangelo A.: Hereditary Hemochromatosis: Pathogenesis, Diagnosis and Treatment. Gastroenterology 2010; 139: 393-408



function of this protein rarely lead to hereditary hemochromatosis classified as hemochromatosis type 3. The clinical manifestation of hemochromatosis type 3 is similar to that of hemochromatosis type 1, but the symptoms may develop at a young age [9].

Adrenal gland dysfunction

Hemochromatosis, along with sarcoidosis, amyloidosis and non-Hodgkin lymphomas, is listed as one of the causes of primary adrenal insufficiency, which supposedly develops due to the so-called infiltration of the glandular tissue. According to this hypothesis, excessive accumulation of iron may lead to adrenal damage due to oxidative stress, induced by high oxidation-reduction potential of iron [10].

Numerous data concerning the etiopathogenetic relationship between iron overload and endocrine disease are based on observations of patients with secondary hemochromatosis, e.g. patients diagnosed with thalassemia. Adrenal insufficiency affects 13-46% of such patients. Researchers confirmed the dysfunction of the pituitary gland with a decreased ACTH (adrenocoticotropic hormone) production, but also the dysfunction of the adrenal cortex demonstrated in ACTH stimulation tests [11].

Among few publications devoted to juvenile hemochromatosis, there is a case study from 2000, describing a patient with juvenile hemochromatosis, whose only endocrine disorder was isolated adrenal insufficiency without the presence of anti-adrenal antibodies. The authors suggested that the dysfunction of the adrenal glands in this patient is due to the advanced stage of iron overload in tissues [12]. In 2018 a clinical case was published in which the patient's adrenal cortex was damaged in the course of hemochromatosis with homozygous H63D mutation, which was confirmed by endocrinological diagnostics and iron deposits in the adrenal MRI examination [13].

Adrenal insufficiency also develops secondary to the dysfunction of the pituitary gland. In the case of hemochromatosis, the increased deposition of iron in the cells of the pituitary gland may lead to a decreased secretion of tropic hormones [14]. The most common endocrine disorder in patients with hereditary hemochromatosis is hypogonadotropic hypogonadism, which causes low libido (26% of patients) and impotence in men (45% of men). Hypogonadism in patients with hemochromatosis is supposedly caused by both the dysfunction of the hypothalamic-pituitary axis and the destruction of Leydig cells [15].

Hypothyroidism

Hypothyroidism is another disorder that can occur among people with congenital hemochromatosis. Published data referring to the incidence of thyroid dysfunctions among patients diagnosed with congenital hemochromatosis are incoherent and inconclusive. In a 1983 study, Edwards and co-authors demonstrated an increased incidence of primary hypothyroidism in patients with congenital hemochromatosis, but more recent reports from 2004 do not confirm this. The explanation for the relatively low incidence of thyroid disease among patients with hereditary hemochromatosis may be the fact that pituitary glandular cells are more sensitive than thyroid cells to the damaging effects of iron deposits [16-17].

Hypogonadism and osteoporosis

Hypogonadism may lead to a decreased bone mass in men, which is a frequent finding in patients with HH. In a study from 2008 conducted on a group of 87 patients with hemochromatosis who were tested for reduced bone mineral density (BMD), it was found that 25% of them suffered from osteoporosis, and 41% from osteopenia. Moreover, it was stated that BMD was dependent on BMI (body mass index), alkaline phosphatase level, hypogonadism/menopause and the intensity of iron deposition in the liver. However, there was no correlation with liver cirrhosis. In a study from 2005 osteopenia was found in 30 out of 38 patients (78.9%), while osteoporosis in 13 out of 38 patients (34%). The decrease in BMD was greater in the femoral neck than in the lumbar region of the spine [18-19].

In vivo tests suggested that there is a relationship between excessive iron accumulation and an increase in the activity and number of osteoclasts. When analyzing the problem, it is also important to remember about the calcium and phosphate homeostasis, which is disturbed in patients with liver impairment. In chronic liver disease, 20-100% of patients develop the so-called hepatic osteodystrophy. It has also been confirmed that iron has a negative influence on bones. In vivo tests revealed that iron overload suppresses osteoblastic differentiation and activity, which is associated with increased apoptosis of osteoblasts [20]. Iron has also a negative impact on bones through BMP2 (Bone Morphogenetic Protein 2) and osteoblastogenesis. Study results from 2009 suggest that iron overload may contribute to the development of osteoporosis as a result of inhibited osteoblastic proliferation and differentiation [21].

In research from 2008, the HFE gene was mutated in rats causing excessive iron accumulation in the kidneys and liver. The research showed that there was a decrease in connections between cells in the trabecular bone of the femur. Recently, the results of the studies analyzing the influence of iron on the microarchitecture of bones have been published. It was found that animals with iron disorders have a reduced BFR

(bone formation rate), which suggests that iron has a direct influence on the percentage of active osteoblasts. Based on the bone mass analysis, it was found that animals with iron overload have an impaired microarchitecture of bones due to the compromised formation of the trabecular bone and a decreased number of trabeculae. This mechanism is probably the main cause of the harmful effect of iron on bones [22].

Diabetes mellitus

Decreased insulin secretion is considered the main cause of diabetes in HH patients, with insulin resistance playing the secondary role. Research suggest that bloodletting may delay the development of diabetes type 2 in patients diagnosed with HH. According to the recently published studies, 13-23% of patients with hereditary hemochromatosis suffer from diabetes, a rate much lower than several decades ago [23].

Dubois et al demonstrated that the frequency of the Cys282Tyr and His63Aps mutations of the HFE gene in patients with diabetes type 2 is higher in comparison to the general population. In another article, the same research team investigated whether diabetes type 2 constitutes sufficient evidence to suspect HH and suggested genetic screening only for patients with concomitant liver cirrhosis [24-25].

The strongest correlation between the presence of the HFE gene mutation and the incidence of diabetes type 2 was determined by Moczulski et al. In research from 2001 conducted on a Polish population, the presence of the H63D mutation was found to be a risk factor for diabetic neuropathy, and the prevalence of the C282Y mutation was greater in patients with diabetes type 2 than in the general population [26].

The results of the Hemochromatosis and Iron Overload Screening (HEIRS) study published in 2006 are quite different: in a large group of patients (223 homozygotic carriers of the C282Y mutation and 449 controls; all with no previous history of diabetes), the percentage of patients with undiagnosed diabetes or an abnormal fasting blood glucose level was similar for C282Y homozygotes and the general population [25].

In a study published by Davis et al. in 2008, 1245 patients with diabetes type 2 were evaluated and no correlation was found between increased iron levels, the presence of the HFE gene mutation and diabetes type 2. Based on their results, screening for HH in patients with diabetes is not required [27].

In a meta-analysis of 23 studies conducted between 1997 and 2011, the prevalence of diabetes type 2 in carriers of the C282Y mutation, both homo- and heterozygotic, was comparable to the prevalence of diabetes in the general population. Carriers of the H63D mutation in the HFE gene had a slightly higher risk for developing diabetes type 2 [28].

Conclusions

Taking into consideration the latest research, it is not diabetes that seems to be the most common endocrinopathy in hemochromatosis, but hypogonadism – it does not only lower the quality of life, but may also lead to insidious and gradually progressive osteoporosis, fractures and progressing disability. Patients with HH should be monitored by both a hepatologist and endocrinologist, and a special emphasis should be put on the necessity of performing screening tests for decreased bone mineral density. The treatment should not be reduced only to decreasing the amount of iron in the organism, but it should also include vitamin D3 supplementation, gonadal hormone replacement therapy and the use of bisphosphonates.

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