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CONTENTS

2019; VOLUME 4, NUMBER 4, 193–226

ORIGINAL ARTICLES

Perception of illness by patients treated with haemodialysis 193
Magdalena Strugała, Dorota Talarska, Mary Kalfoss, Tomasz Niewiadomski, Beata Rozmarynowska, Danuta Dyk

The assessment of usefulness of HE4 and CA125 quantification for the diagnostics of endometrial cancer..... 201
Marta Zalewska-Zacharek, Jolanta Zegarska, Lena Nowak-Łoś, Magdalena Kuligowska-Prusińska, Grażyna Odrowąż-Sypniewska, Marek Grabiec, Karina Chmielarz

Cardiac Biomarker Levels After a Football Match in Professional Versus Amateur Lithuanian Football Players 210
Ali Aldujeli, Kasparas Briedis, Montazar Aldujeili, Auguste Stalmokaite, Ramunas Unikas

REVIEW ARTICLE

Analysis of antibiotic resistance genetic conditioning of Enterobacteriaceae NDM-1 family members and the related epidemiological threat in Poland 216
Wojciech Rogóż, Karolina Kulig, Magdalena Knopik-Koclęga, Agnieszka Szkudlarek, Małgorzata Maciążek-Jurczyk

IMAGES IN MEDICINE

Angioedema of the small intestine in a 28-year-old woman 225
Mikołaj Kamiński, Tomasz Głowacki, Daria Koczara, Zbigniew Fabiszewski

VIA MEDICA

ORIGINAL ARTICLE

Magdalena Strugała[1], Dorota Talarska[1], Mary Kalfoss[2], Tomasz Niewiadomski[3], Beata Rozmarynowska, Danuta Dyk[4]

[1]Department of Preventive Medicine, University of Medical Sciences, Poznan, Poland
[2]Diakonova University College, Oslo, Norway
[3]Orthopaedics-Rehabilitation Clinical Hospital, University of Medical Sciences, Poznan, Poland
[4]Department of Nursing Anaesthesiology and Intensive Care, University of Medical Sciences, Poznan, Poland

Perception of illness by patients treated with haemodialysis

Corresponding author:

Magdalena Strugała,
Department of Preventive Medicine,
University of Medical Sciences,
Święcickiego 6 Str.,
60-781 Poznan, Poland,
e-mail: magdastrugal@ump.edu.pl

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ABSTRACT

Introduction: Perception of illness is the way in which a condition is perceived, which reflects the patient's attitude towards illness and treatment.

Aim: The aim of the research was to understand the perception of illness among patients treated with haemodialysis. The specific goal was to determine the factors affecting the perception of the illness and their interrelationships.

Material and methods: The study included 98 people treated with haemodialysis as part of the international project "Health, coping, and quality of life in people with chronic kidney disease and in their families". As research tools the following were used: the Barthel Index, Instrumental Activities of Daily Living Scale (IADL), Edmonton Symptom Assessment Scale – Revised (ESAS – R), and the Brief Illness Perception Questionnaire (Brief IPQ) Scoring Instructions.

Results: The perception of illness in the study group was significantly influenced by the intensity of physical symptoms ($p = 0.007$), especially dyspnoea or fatigue. Whereas, the following areas of the perception of illness: *Consequences, Treatment control*, *Timeline, Illness concern*, and *Comprehensibility* were mainly affected by functional efficiency, age, and education level. A worse perception of illness was observed with the increase in IADL dependency, younger age, and lower education level.

Conclusions:

1. Perception of illness in the study group was at a moderate level.
2. Perception of illness in the study group was most strongly influenced by the intensity of symptoms, especially dyspnoea and fatigue.
3. Functional efficiency, age, and education significantly affected the perception of illness.

Key words: perception of illness, haemodialysis

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Introduction

The perception of illness by patients with end-stage renal disease (ESRD) is not a topic often addressed in the literature. Researchers mainly focus on various aspects of treatment, transplantation, biochemical test results, etc. while little attention is paid to the struggles of patients in their everyday functioning [1]. Understanding the perception of illness among patients with ESRD facilitates and strengthens the effects of therapy and care [2].

The perception of illness is defined as the "emotional and cognitive representation of the disease". It contains beliefs about the aetiology of the illness, its symptoms, subjective personal consequences of the illness, and the degree to which the illness can be controlled or treated [3]. It also means that patients create certain mental models of their illness in order to interpret body experiences, reduce symptoms and psychological suffering, and also to seek to understand the illness and the role of its impact on their life [4]. An individual's perception of illness also impacts functional adjustment to disability and affects the way a patient follows recommendations related to the disease [3, 5].

In chronic illnesses, such as ESRD, the severity of symptoms as well as the duration and nature of treatment significantly impact patient quality of life [6]. Patients with ESRD experience the impact of many

interconnected stressors. Work and finances are disrupted, as are family and personal relations, recreation, and general health. There are various symptoms, such as chronic pain, dyspnoea, fatigue, and lowered mood (disease intolerance). It is important to identify the symptoms that are the main cause of psycho-physical discomfort among dialysis patients because they affect the patient's general well-being. They can influence the particular perception of illness, and they are not always part of routine clinical evaluations [7, 8, 9]. More severe symptoms are usually reported by patients who are worried about the course of their illness and experience unpleasant emotions (such as sadness or anger). The form of the treatment, haemodialysis, determines the time of activities undertaken during the day by the patient and is a significant economic burden. Some patients do not adapt well to the treatment procedures and manage their time poorly. This limits their independence and reduces self-esteem and self-confidence. They are more likely to say that the disease significantly affects their lives [10].

Maintaining fitness and independence is essential, not only for general patient well-being and effectiveness of therapy but also as a factor that significantly reduces the number of complications, recurrent hospitalisation, and even mortality for haemodialysed patients [11, 12]. People treated with haemodialysis are characterised by a varied degree of functional capacity when compared with the general population [13]. Currently, both in retrospective and prospective studies [14, 15], increased levels of functional activity in patients with ESRD can be found. Undoubtedly, this is a result of medical advances, including technological solutions that influence the general quality of life and foster improved functioning. Although patients with ESRD do no not usually experience major difficulties in everyday functioning, the most common problems for them are inability to work and disturbed family relations. The intensity of symptoms, functional fitness, and applied therapy have a significant influence on the perception of illness, including ESRD [8].

Aim

The aim of this study was to understand the way that illness is perceived by patients treated with haemodialysis. The specific aim was to determine factors affecting the perception of illness and their interrelationships.

Material and methods

As part of the international project "Health, coping, and quality of life in people with chronic kidney disease and in their families", a group of 98 people treated with haemodialysis in two renal replacement therapy centres in the city of Poznan, Poland were studied.

Organisation of the study

Permission was obtained to conduct research in two of the four haemodialysis centres in the city of Poznan. Each patient was informed in detail about the aim of the study, and the research instruments were discussed. Ultimately, 105 people participated in the study because seven questionnaires were rejected during the study due to not being filled out or patient withdrawal.

The conditions for participation in the study were patient consent, a psychosocial condition enabling independent completion of the questionnaire, and the absence of other debilitating illnesses.

Research instruments

The **Barthel Index** of Activities of Daily Living – a scale of 0 to 100 points. Patients with score above 86 are in good functional condition, while a score below 20 indicates severe impairment.

The **Instrumental Activities of Daily Living** (IADL) **Scale** – the version with nine questions, with three options for answers was used: 1 point indicates full dependence, 2 points — partial dependence, and 3 — independence. The maximum total score is 27 points.

The **Edmonton Symptom Assessment Scale – Revised (ESAS – R)** – the scale contains 10 questions about the degree of perceived symptoms that may accompany haemodialysis: pain, fatigue, somnolence, nausea, appetite, dyspnoea, depression, nervousness, well-being, and other problems (e.g. constipation). The response range is within the range 0 pts (no symptoms) to 10 pts (maximum intensity). Each symptom is analysed separately.

The **Brief Illness Perception Questionnaire (Brief IPQ) –** this is a questionnaire that provides a quick assessment of the perception of illness (Weinman et al.). It consists of nine questions about the perception of and attitudes towards illness. The rating for questions 1–8 is based on a Likert scale, ranging from 0 to 10 points. During the analysis in questions 3, 4, and 7 answers must be reversed. Questions 1 to 5 determine the cognitive perception of illness, 6 and 8 the emotional perception, while question 7 is about understanding the illness. Question 9 is open, allowing respondents to give reasons they recognise for the disease to occur. The higher the score on the scale, the more harmful the effect of the illness.

Individual questions are analysed separately, but if the study requires it, then a sum (0–80 pts) can be taken into account that reflects the degree to which the disease is perceived negatively. The higher the point total the more negative the perception of illness [5].

Questions on the Brief IPQ scale have been adapted into three areas:

1. cognitive representation of illnesses perception:	2. emotional representation:
Brief – IPQ 1 — *consequences*	**Brief – IPQ 6** — *concern*
Brief – IPQ 2 — *timeline*	**Brief – IPQ 8** — *emotions*
Brief – IPQ 3 — *personal control*	**3. comprehensibility:**
Brief – IPQ 4 — *treatment control*	**Brief – IPQ 7** — *comprehensibility*
Brief – IPQ 5 — *identity*	**Brief – IPQ 9** — *the causes of disease*
	Brief-IPQ — *score*

Statistical analysis

Analysis was performed using the STATISTICA 10 PL package (StatSoft Inc.). Differences between two independent groups were evaluated using the non-parametric Mann-Whitney test and the parametric Student's t-test. Correlations were assessed using Spearman's r_s rank correlation coefficient. Nominal variables were analysed using Pearson's chi-squared test. To identify factors significantly affecting the risk of falls, a logistic regression model was used. Results obtained were presented as odds ratios with 95% confidence intervals. A two-factor ANOVA AB variance analysis and Levene Test were used for variance error homogeneity. All the tests were considered statistically significant at $p < 0.05$.

Variable analysis

Age was analysed in two groups: up to 60 years and over 60 years. Taking into account the average duration of dialysis, two groups were distinguished: up to 48 months and over 48 months. Marital status was analysed in two groups: married and unmarried.

Results

Demographic and clinical data

The study included 61 (62.24%) men and 37 (37.76%) women, aged 18–85 years. The mean age was 59.65 ± 15.51 years. Married persons dominated (n = 67, 68.37%). Half of the group were retirees (50.00%); the next most numerous group were those on disability (35.71%). Only six (6.12%) persons worked professionally. The mean haemodialysis time for the entire group was 42.67 ± 50.30 months (Tab. 1). The most common reasons for dialysis were chronic glomerular nephritis (21.43%), diabetic nephropathy (18.37%), polycystic kidney disease (12.24%), and hypertensive nephropathy (9.18%).

Functional status of the subjects

Patients included in the study showed a fairly large degree of independence in undertaking basic and instrumental activities (Tab. 2). In the **Barthel Index** 64 (65.31%) patients obtained scores indicating low impairment, while the scores of 34 (34.69%) patients indicated moderately severe impairment.

People aged **under 60 years** were more efficient in basic (ADL) and instrumental (IADL) life activities (Student's t-test: Barthel [$p < 0.000$] and IADL [$p < 0.001$]).

There were no differences between **the time of dialysis** (Student's t-test) and the efficiency of patients

Table 1. Demographic factors

Demographic variables	Women n = 53 (53%)		Men n = 47 (47%)		All n = 100 (100%)	
Age						
20–40 years	7	13.2%	4	8.5%	11	11%
41–65 years	25	47.2%	27	57.4%	52	52%
66–90 years	21	39.6%	16	34.1%	37	37%
Educational level						
elementary	19	35.8%	12	25.5%	31	31%
vocational	19	35.8%	23	48.9%	42	42%
secondary	12	22.7%	8	17.0%	20	20%
post–secondary	3	5.7%	4	8.5%	7	7%
Marital status						
single	9	17.0%	8	17,0%	17	17%
married	26	49.0%	33	70.2%	59	59%
divorced	3	5.7%	2	4.3%	5	5%
widowed	15	28.3%	4	8.5%	19	19%

Table 2. Evaluations from the Health Questionnaire Specific for End-Stage Renal Disease with respect to demographic variables

Indexes	Variables							
	Age*		**Sex***		**Educational level***		**Marital status***	
Objective QOL (0–10 pts.)	< 60 5.7 ± 1.5 > 60 4.5 ± 1.5	< 0.001	F 4.6 ± 1.4 M 5.6 ± 1.8	0.006	B 4.8 ± 1.6 H 5.8 ± 1.6	0.009	S 4.5 ± 1.4 M 5.4 ± 1.7	0.006
Subjective QOL (0–100 pts.)	< 60 63.5 ± 12.5 > 60 52.1 ± 13.3	< 0.001	F 54.3 ± 14.1 M 61.1 ± 13.4	0.014	B 54.7 ± 13.8 H 64.8 ± 12.4	0.003	S 55.1 ± 14.4 M 59.2 ± 13.7	0.105
Physical symptom scale (12–60 pts.)	< 60 46.7 ± 5.1 > 60 43.7 ± 5.9	0.005	F 43.6 ± 5.6 M 46.8 ± 5.4	0.004	B 44.5 ± 5.9 H 46.9 ± 5.1	0.084	S 43.9 ± 6.0 M 46.0 ± 5.5	0.105
Affect scale (12–60 pts.)	< 60 41.5 ± 9.7 > 60 38.7 ± 6.4	0.096	F 37.8 ± 5.4 M 42.6 ± 9.9	0.002	B 38.8 ± 5.9 H 43.4 ± 12.0	0.029	S 37.1 ± 6.0 M 42.1 ± 8.9	< 0.001
Satisfaction with life scale (1–7 pts.)	< 60 4.6 ± 0.9 > 60 3.7 ± 1.0	0.000	F 3.8 ± 1.0 M 4.4 ± 0.9	0.004	B 4.0 ± 1.0 H 4.4 ± 1.0	0.051	S 3.8 ± 1.0 M 4.3 ± 0.9	0.018
General affect (1–7 pts.)	< 60 4.5 ± 0.9 > 60 3.8 ± 0.9	< 0.001	F 4.0 ± 1.0 M 4.4 ± 0.9	0.012	B 3.9 ± 0.9 H 4.7 ± 0.9	0.000	S 3.7 ± 1.0 M 4.4 ± 0.9	< 0.000
Well–being (2.1–14.7 pts.)	< 60 9.5 ± 1.7 > 60 7.9 ± 1.9	< 0.001	F 8.2 ± 2.0 M 9.3 ± 1.7	0.004	B 8.3 ± 1.9 H 9.6 ± 1.9	0.005	S 7.9 ± 2.0 M 9.2 ± 1.8	< 0.000

*Test Mann-Whitney, statistically significant p-value $p < 0.05$.
Key: age: < 60 below 60, > 60 above 60; sex: F — female, M — male; education: B — basic (elementary, vocational), H — secondary and post-secondary); marital status: S — single, M — married

for ADL ($p > 0.05$) and IADL ($p > 0.05$). There was no difference in functional status in the study group when **marital status** ($p > 0.05$) and duration of dialysis therapy ($p > 0.05$) were taken into account. A mong women, those under 60 years of age and those with a higher level of education had a higher level of functional efficiency (ADL and IADL).

Severity of ESAS-R symptoms

On the ESAS-R symptom severity scale the mean score in the study group was 16.72 ± 14.09 out of 100 possible points, which means that most of the patients' symptoms occurred at a low level of intensity. Out of 10 analysed symptoms, the most severe (7–10 pts) was fatigue and drowsiness, which occurred at a significant intensity in 18.37% of those surveyed. The least severe (0–4 pts) were nausea (92.86%), depression (84.69%), dyspnoea (83.67%), and other problems such as constipation (76.53%). The mean score on the entire scale for women was 17.41 ± 13.92 and for men 16.31 ± 14.30. Women most often reported feeling unwell (54.10%; 62.16%) while men reported fatigue (65.57%; 59.46%) and drowsiness (63.93, 59.46%). When the **sex** of the patients is considered, only a difference between the feeling of discomfort and loss of appetite was found (Student's t-test 42.97; $p < 0.05$); more often this disorder occurred in women (M 24.59%; W 32.43%). Patients aged under 60 years scored 15.80 ± 12.56 points, while for those over 60 years of age the score was 17.69 ± 15.61 points. The **age** of respondents correlated with the feeling of pain (Spearman's test r_s $p < 0.008$), loss of appetite ($p < 0.046$), and other occurring problems ($p < 0.002$). There was no correlation between **education** and severity of symptoms. **The duration of** renal replacement **therapy** correlated (test r_s) with loss of appetite ($p < 0.0322$), dyspnoea ($p < 0.0479$), and nervousness ($p < 0.0115$). A greater severity of symptoms affected patients aged over 60 years.

Brief IPQ

The perception of illness in the study group was at a moderate level. Scoring was in the middle of the scale and was 46.68 ± 8.80 points (Tab. 3). The highest score, i.e. the most negative responses, was found in the question about Timeline (9.21 points) and Consequences (7.36 points). A positive element was the patients' perception of the low impact of disease symptoms on their lives (Identity – 5.57 points) and worrying (Illness concern – 5.65 poits).

There was no significant correlation between gender and perception of illness. The statistically significant difference (Mann-Whitney test p = 0.027) was only found in the question about worrying about own illness (How concerned are you about your illness?). Women answered this question more negatively (W 6.24; M 5.29).

Age differed perceptions of the disease in the Timeline area (< 60 years 8.72; > 60 years 9.60 Mann-Whitney test p = 0.000) and Emotional representation (< 60 years 5.93; > 60 years 5.05 points, p = 0.016). Older people were more often convinced that the disease would last for the rest of their lives, whereas younger people more often experienced discomfort due to disease onset (Fig. 1).

The perception of illness was also different depending on education. People with lower **education** level more often worried about their illness and experienced it emotionally (Fig. 2).

Table 3. Correlation between sub-scales of the Health Questionnaire Specific for End-Stage Renal Disease*

Sub-scales	PS	AS	IOLS	IGA	IW	SQOLS	OQOLS
The physical symptom scale – PS	–	0.62	0.63	0.56	0.61	0.66	0.63
The Affect scale –AS	0.62	–	0.68	0.66	0.70	0.63	0.69
Index of overall life satisfaction – IOLS	0.63	0.68	–	0.85	0.94	0.84	0.82
Index of general affect – IGA	0.56	0.66	0.85	–	0.96	0.79	0.85
Index of well-being – IW	0.61	0.70	0.94	0.96	–	0.84	0.87
Subjective QOL scale – SQOLS	0.66	0.63	0.84	0.79	0.84	–	0.83
Objective QOL scale – OQOLS	0.63	0.69	0.82	0.85	0.87	0.83	–

*R — Spearman correlation co-efficient, p < 0.05; PS — The physical symptom scale; AS — The affect scale; IOLS — Index of overall life satisfaction; IGA — Index of general affect; IW — Index of well-being; SQOLS — Subjective QOL Scale; OQOLS — Objective QOL Scale

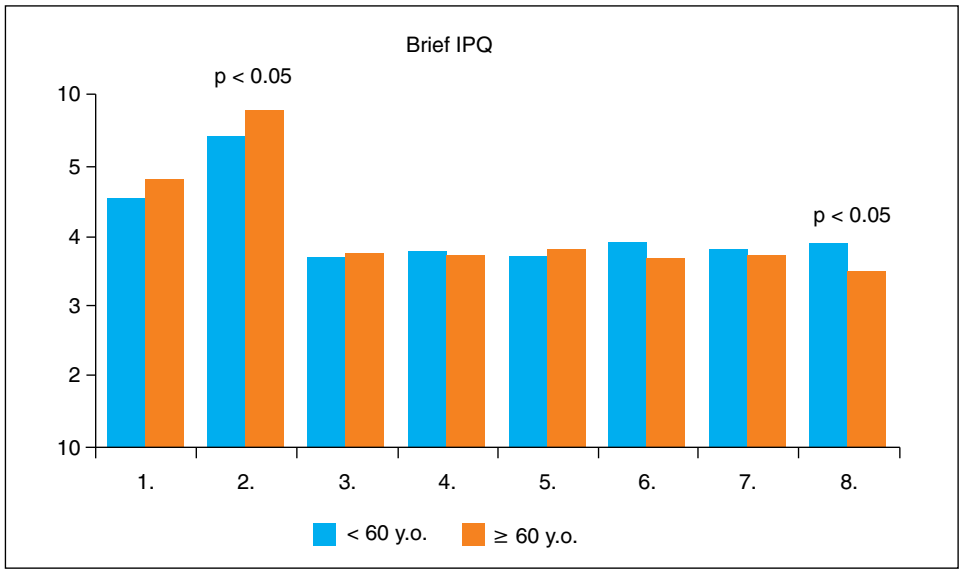

Figure 1. Age *vs.* Brief IPQ

1. Consequences, 2. Timeline, 3. Personal control, 4. Treatment control, 5. Identity, 6. Illness concern, 7. Comprehensibility, 8. Emotions

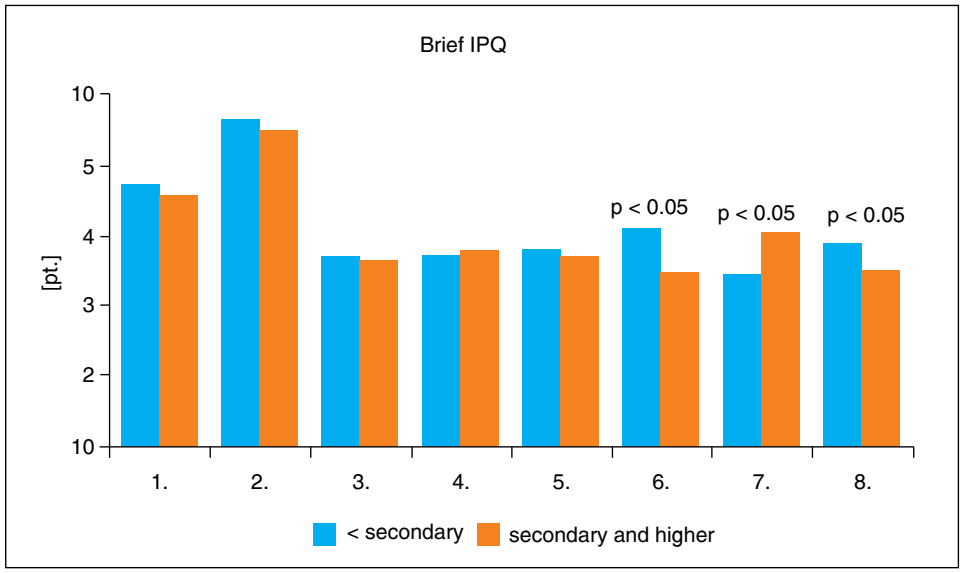

Figure 2. Education *vs.* Brief IPQ

1. Consequences, 2. Timeline, 3. Personal control, 4. Treatment control, 5. Identity, 6. Illness concern, 7. Comprehensibility, 8. Emotions

Analysis of the **duration of dialysis** showed, with the use of Pearson's linear correlation coefficient, a single correlation between area 7 – *Comprehensibility* (p = 0.009) and duration of dialysis. Taking into account the **functional status** a statistically significant correlation between the efficiency in IADL and the answer to questions: 1– (p = 0.000), 3 – (p = 0.020), 4– (p = 0.000), 5– (p = 0.017), and 6 – (p = 0.020) was found. Worse perception of illness was observed with increasing limitations and inefficiency (IADL).

Two-factor variance analysis (ANOVA AB)

Demographic data, apart from age and education, were not variables significantly influencing the perception of illness in the study group. Hence, in further analyses an attempt was made to explain whether age in combination with other variables had an impact on the perception of illness. Differences in the perception of illness were found only when both age and marital status were taken into account (F 4.256, p = 0.017). Older and unmarried people perceived the disease worse. Education was another significant variable. The perception of illness differed with sex and education taken into account in the following areas: 5 – *Identity* (p = 0.028), 6 – *Illness concern* (p = 0.009), and 8 – *Emotions* (p = 0.012). In the above-mentioned areas women with higher education level perceived the illness less negatively. Among men, education did not affect the perception of illness. In area 7 – *Comprehensibility* (p = 0.000) both women and men with higher education perceived their state of knowledge about the disease as subjectively better.

There was no significant change in the perception of illness taking into account age and sex together (F 0.368, p = 0.546); age and education (F 1.210, p = 0.274); age and time of haemodialysis (F 0.516, p = 0.474); and sex and haemodialysis time (F 0.008, p = 0.928).

Brief IPQ *vs.* ESAS

Of the 10 symptoms analysed, the most severe (7–10 points) were fatigue and somnolence in 18.37%. The least severe (0–4 points) were nausea (92.86%), depression (84.69%), dyspnoea (83.67%), and other problems such as constipation (76.53%).

The relationship between some areas of Brief IPQ scale and particular ailments included in ESAS scale has been demonstrated (Tab. 4). Most relationships were found between area 4 and 2 and individual symptoms. Assessment of area 4 – *Treatment control* (p < 0.05) depended on the persistence of symptoms: pain, fatigue, anxiety, loss of appetite, mood, and dyspnoea. On the other hand, assessment of area 2 – *Timeline* was dependant (p < 0.05) on severity of symptoms: pain, fatigue, anxiety, mood, and dyspnoea.

One symptom that showed correlation with all areas of the Brief IPQ scale (p < 0.05) was dyspnoea. Another symptom with which correlation was found relatively often was fatigue (five areas). Other symptoms had different effects on the perception of illness.

Table 4. Correlation between sub-scales of the Health Questionnaire Specific for End-Stage Renal Disease and functional status and time on haemodialysis

Scale/ Variable	PS	AS	IOLS	IGA	IW	SQOLS	OQOLS	KPSS	BI	IADL
KPSS	0.72	0.65	0.76	0.76	0.79	0.85	0.81	–	0.81	0.83
IB	0.65	0.55	0.60	0.60	0.62	0.72	0.70	0.81	–	0.92
IADL	0.62	0.57	0.63	0.65	0.66	0.74	0.73	0.83	0.92	–
THD	0.04	0.12	0.07	–0.06	–0.01	–0.03	–0.01	–0.05	0.03	0.02

*Statistically important; PS — The physical symptom scale; AS — The affect scale; IOLS — Index of overall life satisfaction; IGA — Index of general affect; IW — Index of well-being; SQOLS — Subjective QOL Scale; OQOLS — Objective QOL Scale; KPSS — Karnofsky Performance Status Scale; BI — Barthel Index; IADL — IADL Lawton-Brody Scale; THD — Time on Haemodialysis

Discussion

The main area of interest of the study was to assess perception of illness with the use of the Brief IPQ tool among patients with ESRD treated with haemodialysis. The evaluation included: severity of symptoms, functional efficiency, and selected sociodemographic factors.

According to the literature, perception of illness and the development of coping strategies is conditioned by sociodemographic, medical, psychological, and behavioural factors [3, 16–18]. In this study it was shown that perception of illness in the study group was most affected by the level of severity of symptoms, especially those of dyspnoea and fatigue. Individual areas of perception of illness were influenced by functional efficiency, age, and education.

Considering the results related to the perception of illness based on the Brief IPQ tool, it was shown that the perception of illness is at a moderate level.

Other researchers report [19–21] that ESRD patients undergoing renal replacement therapy experience many physical and emotional symptoms, which were evaluated in the present study with the use of the ESAS scale. It was noticed that physical symptoms of the disease, with the exception of chronic fatigue and dyspnoea, were not significantly severe and in terms of frequency they were similar to those of other researchers [19, 21]. Low severity of symptoms was probably due to the still short period of dialysis therapy and had an impact also on better functional capacity of the subjects.

The influence of the frequency and severity of symptoms on the perception of illness was also observed by other researchers [20, 22]. The degree of this influence and attitudes towards the illness, in turn, are quite diverse. In this research physical symptoms had a greater impact on the perception of illness than in other authors' reports [23–26].

The authors of the works cited above concluded that identity and mental health are of decisive importance for the perception of illness. The quoted researchers found that a negative emotional background accompanying chronic disease, and even depressive or anxiety disorders, are more significant for the perception of illness than are physical symptoms. Our study also looked at the impact of depression, mood, and anxiety on the perception of illness, but this did not prove to be significant. It is possible that differences in the results obtained stem from the type of a study tool used by authors and/or the heterogeneity of the group. Respondents in this study differed from other groups in terms of their socio-demographic situation, the duration of the disease, and the duration of dialysis.

Following the relationship between functional capacity exponents and socio-demographic variables with the way the disease is perceived, worse perception of illness was found in those who scored as more dependent on the IADL questionnaire as well as younger and less educated respondents. Similar results were also obtained by other researchers [1, 7, 27–31]. These researchers emphasise that more functional patients with ESRD show better health, less severe symptoms, and usually have a better understanding of their illness. The manner of experiencing and subjective reception of the illness change over time and can be strongly dependent on the stage of the chronic illness [32].

To summarise the results obtained in this study, it should be emphasised that a moderate, borderline negative way of perceiving illness may result from the awareness of the chronic nature of the illness and the necessity of undergoing frequent and exhausting dialysis therapy. The positive assessment of the situation may be influenced by a shorter duration of dialysis treatment and a slight increase in the symptoms associated with it. Studies carried out by other authors have shown that in patients with end-stage renal disease undergoing haemodialysis, the perception of illness gradually worsens as the duration of the disease progresses [6, 33–35].

This research had some limitations. The results regarding functional capacity were based on information

obtained directly from patients, i.e. they are declarative in nature. Therefore, they should be interpreted with caution.

Conclusions

1. The perception of illness in the study group was at a moderate level.
2. The perception of illness in the study group was most influenced by the severity of symptoms, especially dyspnoea and fatigue.
3. Functional efficiency, age, and education significantly affected the perception of illness.

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VIA MEDICA

ORIGINAL ARTICLE

Marta Zalewska-Zacharek, Jolanta Zegarska, Lena Nowak-Łoś, Magdalena Kuligowska-Prusińska, Grażyna Odrowąż-Sypniewska, Marek Grabiec, Karina Chmielarz

Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

The assessment of usefulness of HE4 and CA125 quantification for the diagnostics of endometrial cancer

Corresponding author:

Karina Chmielarz, Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland,
e-mail: karina.chmielarz@gmail.com

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ABSTRACT

Endometrial cancer is one of the most prevalent uterine malignancies. This disease occurs mostly in older women, frequently affected with other comorbidities. Hence, it is important to search for novel, less burdensome diagnostic modalities, enabling the objective assessment of the patient's status and facilitating qualification to relevant risk groups prior to surgical treatment. The aim of this study was to verify the usefulness of CA125 and HE4 in the evaluation of endometrial cancer.

The study included 308 women treated at University Hospital No. 2 in Bydgoszcz. The study group included 180 patients operated due to endometrial cancer. The control group included 128 women operated due to perineal statics disorders. The concentrations of tumour markers were measured with ELISA-based ready-to-use diagnostic kits.

Patients with endometrial cancer and healthy women differed significantly in terms of HE4 concentrations (P = 0.001). The serum concentration of HE4 in stage I endometrial cancer patients was significantly higher (Me = 88.37 pM) than in healthy women (Me = 46.14) (P = 0.007). The analysis of ROC curves with the determination of the area under curve showed 66.7% sensitivity and 78.1% specificity of HE4. AUC for HE4 amounted to 0.721 and was the highest of all markers. Our analysis revealed that HE4 is useful in the detection of endometrial cancer, while Human Epididymis Protein 4 can potentially be used for screening purposes. CA125 antigen, previously used in the diagnostic process, is useless or may possess limited usefulness. There is a need for further studies on larger populations of female patients.

Key words: endometrial cancer; endometrial carcinoma; HE4; CA125; biomarkers; tumour; diagnostics

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Introduction

Endometrial cancer is one of the most common uterine malignancies. It is the sixth most common cancer in women in the world [1]. The incidence of endometrial cancer is higher in countries with high economic status, as compared with poorer countries. In the United States, the incidence rate is 42/100 k/year, while in Western Europe the incidence rate is 34/100 k/year. The occurrences in people above 45 years of age represent more than 90% of the cases and are cumulated mainly in two peaks: at 54–59 and 65–70 years of age [2, 3]. It has been observed that the disease develops in an increasing number of young women of reproductive age (5–8% of patients). Recent studies suggest an increase in the number of deaths due to endometrial cancer, despite a significant decline in mortality rates observed to date [4].

Up to 90% of cases of endometrial cancer are characterised by early onset of symptoms, which allows an early diagnosis of the disease [5]. The diagnostics allowing the identification of the disease are based on histopathological analysis of the material obtained via fractional curettage of the uterine cavity and the cervical canal or via endoscopic methods. Due to the presence of the tumour mainly in elderly women, often burdened with additional diseases, it is important to seek less invasive diagnostic techniques. Determining new, highly sensitive, and specific tumour markers appears to be one such method.

The markers known to date cannot be routinely used in the diagnostics of uterine cancer due to their

relatively low sensitivity and specificity, and the predictive value of the tests that allow the quantification of these substances.

The proportion of cases of operable stage 1 endometrial cancer is 75%. However, 20–30% of patients classified to FIGO (International Federation of Gynaecology and Obstetrics) stage 1 are at high or medium risk of relapse related with infiltration of the uterine muscle, invasion of lymphatic vessels, and a high degree of differentiation [8, 9]. This group of patients may benefit most from markers that permit detection of early relapse and monitoring the response to the treatment used. At the same time, a sufficiently sensitive and specific marker can be used as a prognostic indicator before the planned surgical intervention in order to ensure the proper therapeutic decisions.

It is important to search for a tumour marker to be used for screening in women at high risk of endometrial cancer, including those with Lynch II syndrome or the PTEN gene defect, as well as patients treated with tamoxifen or obese patients with diabetes. A sensitive marker is essential in the monitoring of patients with a high risk of relapse or a systemic form of endometrial cancer. Currently, the monitoring of patients after and during treatment is based mainly on the clinical assessment of symptoms and diagnostic imaging, which results in a late diagnosis of an already advanced disease [10].

Studies published to date suggest an increased expression of HE4 in endometrial cancer [11–13]. Moore, R.G et al. reported that the HE4 concentration is increased at all stages of endometrial cancer and is characterised by higher sensitivity in the detection of the tumour compared to CA125 (78.7% vs. 67.1%) [12–13]. Therefore, the aim of this study was to evaluate the suitability of the quantification of the tumour markers HE4 and CA125 for the diagnostics of endometrial cancer.

Material and methods

Permission to conduct this study was obtained from the Bioethics Committee of the Ludwik Rydygier Collegium Medicum of Nicolaus Copernicus University in Toruń (no. KB/248/2010).

Study design and subjects

The study included 308 women who were patients in the Clinic of Obstetrics, Gynaecological Diseases, and Oncological Gynaecology of the J. Biziel University Hospital no. 2 in Bydgoszcz in 2010–2011. Included were only postmenopausal women with the last menstrual period at least one year prior to the study.

The patients for each group were recruited separately. The patients were divided into two groups. The study group consisted of 180 patients operated due to endometrial cancer diagnosed preoperatively in a histopathological examination. The control group consisted of 128 women operated due to perineal statics disorders. The diagnosis was based on a histopathological examination performed at the Institute of Pathomorphology of the J. Biziel University Hospital no. 2 in Bydgoszcz. The mean age of the patients diagnosed with cancer was 61 years (SD = 10.8), while the mean age of the patients from the control group was 61 years (SD = 9.5).

All women included in the study underwent a medical history, gynaecological examination with rectal examination, and ultrasound examination.

Medical history concerned the age, the date of the first and the last menstruation, the number of pregnancies and deliveries, the occurrence of chronic diseases (diabetes, hypertension, cancer), and drugs (tamoxifen). The gynaecological examination included cytology, speculum examination, and bimanual pelvic examination. Ultrasonography was performed using standard transvaginal probes with a frequency range of 5-9 MHz.

Body mass index (BMI) was calculated based on the formula: BMI = weight [kg] / height [m^2]

Hypertension was diagnosed when the value of systolic blood pressure was above 140 mmHg and the value of diastolic blood pressure was above 90 mmHg (as defined by the World Health Organisation).

Clinical diagnosis was based on histopathological examination of uterine cavities that were performed at the Department of Pathomorphology of the J. Biziel University Hospital no. 2 in Bydgoszcz.

Statistical analyses did not reveal any statistically significant differences between the compared groups.

Blood sampling

The examined material was blood serum obtained from fasting patients preoperatively by basilic vein puncture. A volume of 10 ml was collected into sterile glass tubes without anticoagulant. In order to obtain blood serum, blood samples were centrifuged for 10 minutes at about 1500 ´ g. The obtained sera were stored at -80°C until the determination of markers was performed.

Methods and statistical analysis

The concentration of HE4 and CA125 in the blood serum of the women were determined using ready-to-use ELISA test kits based on a sandwich immunoenzymatic system.

To determine the serum concentration of HE4, we used the HE4 EIA test from FUJIREBIO Diagnostics, Inc. (Gothenburg, Sweden), with a range of detection

of 15–900 pM. CA125 was quantified using ELISA tests from Demeditec Diagnostics GmbH (Kiel, Germany) with a range of detection of 0–500 U/ml.

Statistical analysis

Statistical analysis was performed using the statistical software STATISTICA 9.1 from StatSoft® and employed Shapiro-Wilk normality test and Student's t-test for independent quantitative variables with a distribution similar to normal. Other variables were assessed using the Mann-Whitney U test. The analysis of the qualitative variables was performed using the nonparametric chi-square test, and the results were presented in a contingency table with percentages calculated by columns. Differences at a significance concentration $P < 0.05$ were considered as statistically significant and marked. Multidimensional dependencies were shown in logistic regression models using independent variables of a dichotomous nature that were determined based on the cut-off values estimated in ROC analysis. The assessment of the diagnostic accuracy of the tumour markers was conducted on the basis of the producer's reference values and our own data, including the 95% confidence interval of the arithmetic mean (95% CI). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the method were established. The presented ROC charts including the area under the curve (AUC) also proved helpful in the assessment of the diagnostic suitability of the markers.

Results

The patients were divided into two groups. The characteristics of the investigated groups are given in Table 1.

Table 1. Characteristics of the study groups

	Cancer N = 180 [M ± SD]	Control Group N = 128 [M ± SD]
Age (years)	61.1 ± 10.8	61.4 ± 9.5
FM (age)	13.8 ± 1.7	14.1 ± 1.8
LM (age)	49.8 ± 3.1	50.0 ± 3.1
BMI (kg/m^2)	30.0 ± 6.1	28.6 ± 4.8
Number of pregnancies	2.5 ± 1.3	2.8 ± 1.4
Number of births	2.2 ± 1.2	2.6 ± 1.4

M — mean; SD — standard deviation; FM — first menstruation; LM — last menstruation

The mean age of the patients diagnosed with cancer was 61 years (SD = 10.8), while the mean age of the patients from the control group was 61 years (SD = 9.5). The difference between the two compared means was not statistically significant ($P = 0.897$). Similarly, the investigated groups did not differ in terms of the time since the last menstrual period ($P = 0.514$ and $P = 0.735$, respectively), BMI ($P = 0.285$), and the number of pregnancies and deliveries in the patients' lifetimes ($P = 0.260$ and $P = 0.244$, respectively).

Statistically significant differences between the investigated groups were observed in the concentration of HE4 ($P = 0.001$), with medians of 96.7 pM and 46.1 pM in the group of women with endometrial cancer and in the healthy women, respectively. The obtained results are shown in Figure 1.

An analysis of the concentration of the tumour markers CA125 and HE4 in the blood serum of women with endometrial cancer and women from the control group was conducted. The median CA125 concentration was 5.8 U/ml in the group of women with endometrial cancer and 5.2 U/ml in the group of women without cancer. The difference between the compared values was not statistically significant ($P = 0.661$) (Fig. 2).

Among the 180 patients operated due to endometrial cancer, 140 (77.8%) were classified to stage 1 of clinical advancement according to FIGO (2009), 24 (13.3%) were classified to stage 2, and 16 were classified to stages 3 and 4 (eight per stage, 4.4%). No statistically significant differences in the concentration of the tumour markers were observed between the group

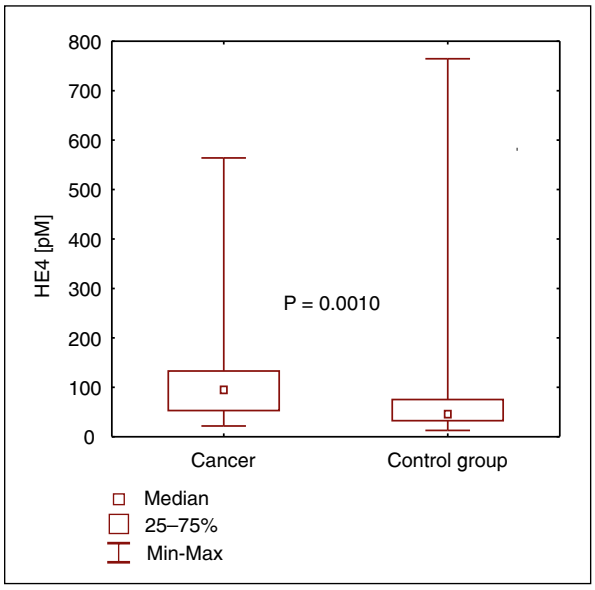

Figure 1. Serum concentrations of HE4 in endometrial cancer patients and in controls

of women with stage 1 endometrial cancer and the groups with further stages of the disease according to FIGO (Tab. 2).

It was found that the blood serum concentration of HE4 was significantly higher in the patients with stage 1 endometrial cancer compared to the group of healthy women (P = 0.007), with medians of 88.37 pM and 46.14 pM, respectively (Tab. 2).

The statistical significance of CA125 markers (P = 0.795) and HE4 (P = 0.873) was not found depending on the incidence of type I and type II endometrial carcinoma in groups N = 164, N = 16, respectively

Tumour marker concentration stratified according to the histopathological grade of endometrial cancer to two groups G1 (N = 36) and G2/G3 (N = 136). In statistical analysis of CA125 marker, the P-value was near-borderline significance (P = 0.101). Analysis of HE4 showed no statistical significance (P = 0.363).

An analysis of the effectiveness of endometrial cancer detection depending on the concentration of the tested marker was conducted. The adopted cut-off values were based on the reference values of the diagnostic tests from Demeditec Diagnostics GmbH (for CA125) and FUJIREBIO Diagnostics, Inc. (for HE4). No statistical significance was found in the results of the assessment of cancer detection effectiveness relative to the reference values for CA125 and HE4. The results are shown in Table 3 and 4.

In the presented study, no correlation was observed between the concentration of CA125/HE4 at 95% CI and the effectiveness of endometrial cancer detection (P = 0.652 and P = 0.276, respectively).

High specificity of tumour markers with clearly unsatisfactory sensitivity and low predictive value, positive and negative, causes the accuracy to be estimated at a concentration no greater than 60% (Tab. 5).

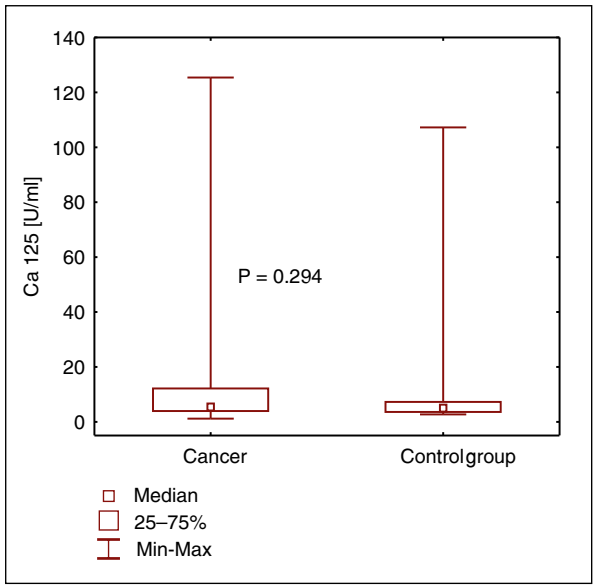

Figure 2. Serum concentrations of CA 125 in endometrial cancer patients and in controls

Table 2. Tumour marker concentration in patients with endometrial cancers representing FIGO stage I, higher stages of the FIGO classification, and in the controls

Markers	Statistical parameter			FIGO	
		Control group N = 128	Endometrial cancer group N = 180	I N = 140	II-IV N = 40
CA125 [U/ml]	Me	5.2	5.8	4.8	8.1
	Q1	3.4	3.7	2.7	5.6
	Q3	7.5	12.4	12.4	13.6
HE4 [pM]	Me	46.1	96.7	88.4	122.5
	Q1	31.2	51.8	44.6	87.9
	Q3	76.3	134.2	125.8	211.6

FIGO — The International Federation of Gynaecology and Obstetrics; Me — median; Q1 Q3 — quartile

Table 3. Endometrial cancer detection rates depending on a cut-off value for the CA125 test

Serum concentration CA125	The occurrence of endometrial cancer				Total
	present		absent		
	N	%	N	%	N
>= 29.6 U/ml	16	9	12	9	28
< 29.6 U/ml	164	91	116	91	280
Total	180	100	128	100	308
p = 0.742					

Table 4. Endometrial cancer detection rates depending on a cut-off value for the HE4 test

Serum concentration HE4	The occurrence of endometrial cancer				Total
	present		absent		
	N	%	N	%	N
>= 150 pM	40	22	16	13	56
< 150 pM	140	78	112	88	252
Total	180	100	128	100	308
p = 0.276					

Table 5. Summary of diagnostic accuracy of the analysed tumour markers

Markers	Criterion	Sensitivity	Specificity	PPV	NPV	Accuracy
CA125	reference value (29.6 U/ml)	8.89%	90.63%	57.14%	41.43%	42.86%
	95% CI (18.26 U/ml)	15.56%	90.63%	70.00%	43.28%	46.75%
HE4	reference value (150 pM)	22.22%	87.50%	71.43%	44.44%	49.35%
	95% CI (141.62 pM)	22.22%	87.50%	71.43%	44.44%	49.35%

NPV — negative predictive value; PPV — positive predictive value

CA125 offers the best diagnostic usefulness within the 95% CI, reaching 90.63% specificity and 70% positive predictive value, with 15.56% sensitivity and a negative predictive value of 43.28%. The diagnostic accuracy for this cut-off value was 46.75%.

The reference value for the HE4 test within the previously established 95% CI allows the detection of endometrial cancer with 22.22% sensitivity and 87.5% specificity, with a satisfactory positive predictive value of 71.43%. The diagnostic accuracy of this tumour marker is up to 49.35%.

A ROC curve analysis was performed in order to assess the diagnostic value of the tumour markers. Assessing the blood serum HE4 concentration allowed determination of the presence of endometrial cancer with 66.7% sensitivity and 78.1% specificity, with extinction values above 80.487 pM. The area under the ROC curve for HE4 was 0.721, which indicated a good diagnostic value (Fig. 3).

The area under the ROC curve for CA125 indicated a lack of discriminative value of this parameter as a prognostic factor in the diagnostics of endometrial cancer (Fig. 4).

In a summary of the diagnostic value of the investigated markers based on the AUC of the ROC curves, HE4 obtained the highest sensitivity at 66.7% with 78.1% specificity and a satisfactory positive predictive value (PPV).

When comparing the logistic regression models in terms of the ability to detect endometrial cancer by the investigated tumour markers, the optimal model in the diagnostics of endometrial cancer from a statistical point of view is model 2 (P = 0.0001) (Tab. 6).

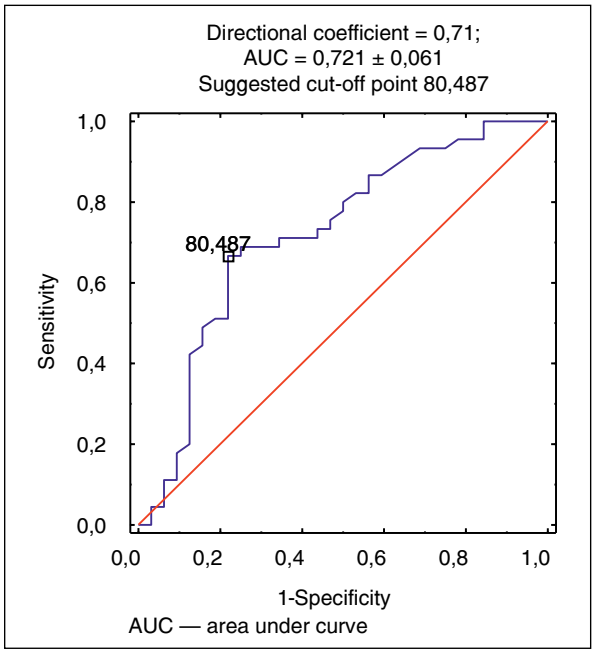

Figure 3. Area under the receiver operating characteristic (ROC-AUC) curve for HE-4 as a diagnostic marker of endometrial cancer

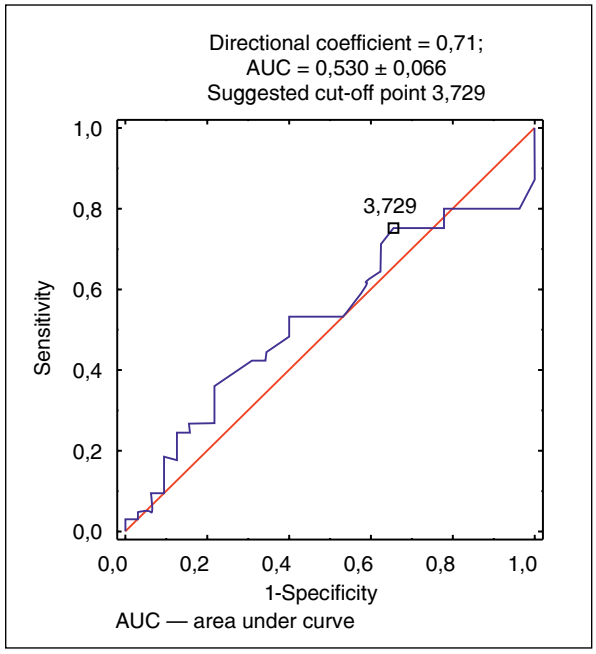

Figure 4. Area under the receiver operating characteristic (ROC-AUC) curve for CA 125 as a diagnostic marker of endometrial cancer

Table 6. P-value from the analysis of logistic regression models that describe the ability to detect cancer by the analysed markers. Dichotomous independent variables are associated with the cut-off points determined during analysis of the ROC curves

	Chi² P	Marker 1 P	Marker 2 P
Model 1	0.3437	CA125 0.3466	
Model 2	0.0001*	HE4 0.0004*	
Model 3	0.0003*	CA125 0.5545	HE4 0.0005*

*P < 0.05

Discussion

Endometrial cancer produces symptoms at the early stages of clinical advancement, which improves the prognosis. However, due to the lack of a safe, noninvasive screening method for detecting this cancer, it is reasonable to search for new substances, particularly useful for patients with increased risk, including people with severe obesity and diabetes, Lynch syndrome, PTEN gene defects, or women treated with tamoxifen. Quantification of tumour markers in the blood serum of women seems to be acceptable as a potential diagnostic method for detecting endometrial cancer due to the low cost of the method, the possibility of repeating the test at any interval, as well as the safety and diagnostic usefulness of the method. The quantitative nature of the obtained result, although not conclusive in the diagnostics of the disease, allows the clinical assessment to be more objective.

In this study, an analysis of the concentration of tumour markers was conducted using the blood serum of female patients with endometrial cancer in comparison with the control group. The median HE4 concentration was higher in the cancer patients to a statistically significant degree (P = 0.001), while CA125 did not demonstrate such a correlation (P = 0.661). The HE4 concentration in the cancer group and in the healthy women were 96.7 and 46.1 pM, respectively. Similar values were reported by Moore, R.G. et al. (71.5 and 35.4 pM, respectively), as well as by Znang, A.M. et al. [13–14]. The authors confirmed the presence of higher concentrations of HE4 in cancer patients as well, using another diagnostic method [11–13, 15–16].

There are also many reports on the increased concentration of CA125 in the blood serum of women with endometrial cancer. According to some authors, only 10–30% of women with endometrial cancer have increased concentration of this glycoprotein in the blood, which is confirmed by our results [17–18]. However, many publications report significant differences in the concentration of CA125 in the blood serum of women with endometrial cancer compared to healthy individuals [19–22]. In this study, the median CA125 concentration in the blood serum of cancer patients was only 5.8 U/ml and was virtually comparable to the median concentration of CA125 in the blood serum of healthy individuals (5.2 U/ml). The cause of such low values of the CA125 concentration in the blood serum of the investigated group may be, e.g., the prevalence of women at an early stage of disease advancement. This was confirmed by Powell, J.L. et al., who obtained the correct values of the blood CA125 concentration in approx. 87.7% of the cases of FIGO stage 1 and 2 disease [22]. On the other hand, Nicklin, J. et al. obtained medians of approx. 14 U/ml and observed statistically significant differences in the CA125 concentration in the blood serum of women with endometrial cancer and healthy individuals [23]. The findings of Powell, J.L. and the authors of this study are confirmed in a study conducted by Bignotti, E., who did not observe any statistically significant differences in the group of patients with stage 1 and 2 of clinical advancement and the G1 degree of histopathological differentiation in comparison with healthy individuals [15]. The results demonstrate the limited usefulness of the antigen CA125 in detecting endometrial cancer.

An interesting issue considered in this study is the evaluation of the concentration of the investigated mark-

ers in relation to the stage of clinical advancement according to FIGO. The obtained statistical differences in the concentration of HE4 in the blood serum of healthy women and women with FIGO stage 1 endometrial cancer suggest possible usefulness of this marker in screening tests (P = 0.007). Human Epididymis Protein 4, as the only one of the investigated markers, demonstrated a different concentration in the control group and in patients with FIGO stage 1 cancer. This correlation has also been described by Moore, R.G. et al. and Bignotti, E. [13, 15]. However, these results were not confirmed by Kalogera, E. et al. (P = 0.49) [12]. Differences in the published studies suggest the need for further research on this topic.

Some authors have reported a statistically significant difference between the concentration of HE4 in the blood serum of patients with FIGO stage 1 endometrial cancer and those with further stages of the disease [12, 15]. In this study, the analysis of the concentration of HE4 was performed either at FIGO stage 1 or at further stages [2–4] of clinical advancement. Due to the small size of the groups with further stages of clinical advancement, patients with FIGO stages 2–4 were assessed together. As opposed to other authors, we observed no statistically significant difference in the marker concentration (P = 0.120); however, the median HE4 concentration at further stages of clinical advancement was higher (122.46 pM) compared to stage 1 according to FIGO (median = 88.37 pM).

The differences in the blood serum concentration of CA125 in women with FIGO stage 1 and further stages of clinical advancement were close to statistical significance (P = 0.062). The presented results are consistent with reports by other researchers, which confirm the correlation of the CA125 concentration with increasing degree of clinical advancement [15, 18, 22, 24].

In the analysis of the concentrations of the investigated markers in relation to the aetiopathogenetic type of endometrial cancer, the median marker concentration values established in the blood serum of women with either type I or type II cancer were higher than those found in healthy individuals. No statistically significant differences with respect to the blood serum concentration of CA125 and HE4 were observed between women with type I and type II endometrial cancer (P = 0.795; P = 0.873). However, the median CA125 concentration was higher in patients with type II disease.

The concentration of HE4 shows an opposite relationship, in which the median concentration of this marker in oestrogen-dependent cancer is higher than in the hormone-independent type. However, it should be pointed out that the group of patients with type II cancer in this study was small, which justifies the need for continuing the research in a larger group of patients. Our results of the HE4 quantification in patients with type I and II endometrial cancer are confirmed in the study published by Kalogera et al. [12]. As for the other markers, the correlation of higher marker concentrations with type II cancer allowed the identification of patients with poor prognosis, requiring constant monitoring for a possible relapse of the disease and a plan for more aggressive treatment.

The comparison of the marker concentration in relation to the degree of histopathological differentiation of endometrial cancer revealed no statistically significant differences. A tendency of the CA125 and HE4 concentration to reach higher values in the blood serum of patients with G2 and G3 cancer was demonstrated, with CA125 being nearest to statistical significance.

A similar correlation was shown in the studies by Powell, J.L. et al. and Dvalishvili, I. et al. [22, 25]. Differences in the concentration of HE4 depending on the degree of histopathological differentiation were observed by Bignotti, E. et al. [15].

For a reliable assessment of the diagnostic usefulness of the investigated tumour markers, the diagnostic sensitivity and specificity were established in relation to the different cut-off values for a given marker.

The first assessment regarded the predictive value and utilised the suggested upper limit of the reference values (> 95% CI) used in the study of diagnostic tests based on the ELISA method. On the basis of the authors' own observations, the 95% CI value was established in the investigated group of women, and the effectiveness of the investigated tumour markers in endometrial cancer detection was assessed in relation to the results obtained. The study estimated the predictive capacity of CA125 with a sensitivity as low as 8.89% and a specificity of 90.63%, with the cut-off value based on the reference value upper limit from the CA19-9 ELISA test by Demeditec Diagnostics GmbH, which was ≥ 29.6 U/ml. No correlation with cancer occurrence was observed above this value (P = 0.742). The low positive and negative predictive value resulted in an accuracy estimate of only 42.86%. A slightly higher accuracy was obtained for the ≥95% CI established in the authors' own study. For concentrations ≥ 18.26 U/ml, the accuracy was 46.75%. The low sensitivity and NPV values cause this marker to be ineffective in the diagnostics of endometrial cancer. Despite the unsatisfactory results obtained in the assessment of diagnostic usefulness, CA125 is used in clinical practice for monitoring patients with a high risk of relapse of the disease. It is also used for planning the extent of surgery. Values ≥65 U/ml strongly correlate with the spread of the disease outside the uterus [26].

Very disappointing results were obtained in the assessment of the effectiveness of endometrial cancer detection with HE4. The marker considered as the best marker in the diagnostics and monitoring of the treat-

ment of endometrial cancer obtained only 49.35% accuracy. The obtained sensitivity of 22.22% is clearly unsatisfactory in the assessment of the test marker, while the specificity of 87.5% is lower compared to CA125.

The lack of established standards for HE4 prevented the assessment of the marker's usefulness in detecting endometrial cancer in relation to the cut-off value adopted for the population of healthy women. The attempts to establish the reference values performed to date have produced divergent results and have not taken into account the influence of many factors. A recent study by Bolstad, N. et al. involving material from 1591 women and men presents the correlation between the HE4 concentration and smoking status and age. The cut-off values cited by the authors are significantly lower than the reference value from the HE4 EIA test by FUJIREBIO Diagnostics, Inc. (~150 pM), as well as the values proposed by Molina, R. et al. (> 130 pM) and Lenhard M. et al. [27–29].

Due to the unsatisfactory results obtained in the assessment of the diagnostic usefulness of the investigated tumour markers, and in order to further compare them, ROC curves were established on the basis of the extinction value distribution for each parameter. Cut-off values characterised by the highest diagnostic value were identified, and the assessment of the area under the curve (ROC-AUC) was performed in order to estimate the discriminative value of each parameter.

The cut-off value for HE4 was established at 80.487 pM, which allowed the detection of endometrial cancer with 66.7% sensitivity and 78.1% specificity above the established extinction value. The positive predictive value of 81% allowed the detection of the disease based on a positive test result above the established cut-off value. When calculating the area under the ROC curve for HE4, a good discriminative value of this parameter was observed (ROC–AUC = 0.721). Moor, R.G. et al. and Kalogera, E. et al. obtained similar results of 0.79 and 0.67, respectively [12–13]. The differences between the individual studies may be due to the differences in the distribution of stages of clinical advancement in the investigated groups of patients.

The values obtained with CA125 allowed confirmation of the presence of a cancerous disease with 75.6% sensitivity and a very low specificity of 34.4%. The cut-off value (3.729 U/ml) established in this study differed significantly from the results obtained by Kim, B.W. et al., who reported a value of 18.7 U/ml. Moreover, the authors obtained a fairly good discriminative capacity for this parameter because the area under the ROC curve was 0.689 with 49.3% sensitivity and 83.1% specificity [30]. The observed differences may arise from the different distribution of the clinical status of the patients included in the study.

In the present study, an analysis of the correlation between the concentration of tumour markers and the occurrence of endometrial cancer was also performed. For this purpose, three logistic regression models with different combinations of the tested substances were established and assessed. Model 3, which took into account the influence of CA125 and HE4 on the detection of cancer, was characterised by a lower predictive capacity than the single-component model 1 (P = 0.0003 vs. 0.0001), which confirms the lack of any advantage of combined CA125 and HE4 determination in the diagnostics of endometrial cancer. Other researchers have also attempted similar analyses and obtained data suggesting an advantage of combined CA125 and HE4 determination over the assessment of single markers [13].

To conclude, the obtained results confirm that HE4 is the most sensitive and specific marker for the early detection of patients with endometrial cancer, as compared with CA125, which is commonly used in the monitoring of patients with endometrial cancer. However, the numerous discrepancies that are still observed in the assessment of the usefulness of the tumour markers indicate the need for further research involving larger groups and careful conclusions with reference to the established cut-off values for the healthy population.

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VIA MEDICA

ORIGINAL ARTICLE

Ali Aldujeli[1], Kasparas Briedis[2], Montazar Aldujeili[3], Auguste Stalmokaite[4], Ramunas Unikas[1]

[1]Department of Cardiology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Liverpool Heart and Chest Hospital, NHS foundation trust, Liverpool, United Kingdom
[3]Medical Academy, University of Brescia, Brescia, Italy
[4]Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania

Cardiac Biomarker Levels After a Football Match in Professional Versus Amateur Lithuanian Football Players

Corresponding author:

Ali Aldujeli, Department of Cardiology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, e-mail: Ali.Aldujeli@kaunoklinikos.lt

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ABSTRACT

Background: There are very limited scientific data available on cardiac troponin I release after intermittent exercise. To know the different factors that mediate cTnI release after exercise is of concern for scientists. In this regard, our study is based on two major goals: 1) to evaluate the cTnI release in individuals during a sports match; and 2) to understand the impact of the status of the athlete (biological) on the release of individual cTnI.

Methods: A total of 44 players, including "22 adult professional [PFP]: 24.2±4.5 years, 22 adult amateur [AFP]: 26.5±3.6 years" were involved in a match simulated as real. Successive observations of cTnI release were obtained at different settings such as at rest, pre-exercise, and instant-post-exercise at regular intervals of 3, 6, and then 24 h post-exercise.

Results: From the obtained results it was observed that the individual highest values were vastly varied, with higher levels of cTnI release baseline and post-exercise for PFP players as compared to those of AFP (all $p < 0.05$). Moreover, the cTnI levels were increased (peak post: 0.024 [0.004-0.244] μg/L; $p < 0.05$). Additionally, the cTnI peak values surpassed the upper limit of reference in 77.3% of PFP (17 PFP).

Conclusions: Our study data results affirm that the cTnI release is highly affected by the status of athletes. The cTnI release is enhanced by intermittent exercise.

Key words: football, troponin I, cardiac biomarkers, exercise

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Introduction

There is huge interest among scientists in studying the biomarkers released by cardiomyocytes at different levels of physical workout. Cardiac troponins (cTn) have gained great importance among researchers because they are a key biomarker [1, 2]. It is an established fact that many athletes after long-duration exercise have more than the upper reference limit (URL) release of cTn [3]. Some studies have also shown high values for cTn release among sportspersons, even for short-duration exercise [4], moderate duration [5], and continuous exercise [6]. There has also been an effort to study the different factors that affect the cTn release after physical workouts. Several research works have associated the increase in cardiac troponin I (cTnI) with exercise style [7], individual age [8], high throughput exercises, and long duration [5] and level of physical adaptation to exercise [9]. However, the results obtained in different studies do not cohere with each other.

Different studies were performed on the release of cTn after a physical workout is done on a regular basis, such as weightlifting [10], floorball [11], basketball [12], or marathon running [13]. These research works have inadequate data to be utilised for a significant evaluation. For instance, some studies have improper sampling time for post-exercise [10–13]; some have a poorly controlled condition for evaluation [11, 12]. Moreover, the athletes monitored in the studies are amateur rather than professional. Since it is an established fact that amateur athletes usually show cTn levels that are detectable after exercise more than the values exhibited by experienced and professional athletes [9, 14–18]. Similarly, the data is usually obtained from activities that are field based, and post-activity sampling intervals are fewer, which shows data with inaccurate values for cTn release [19]. Simi-

larly, in some studies, higher values of cTn values have been observed from pre- and post-exercise conditions [8] due to controlled interventions during endurance training. From the literature, it is evident that in adolescent athletes, their immature cardiac muscles result in increased cTn detection after exercise [8, 17, 18, 20]. Therefore, these data obtained are not significant for a better understanding of the cTn release mechanism.

Despite all the mentioned studies in literature, more studies are required to understand the impact of training level on cTn release from a training or athletic status point of view. Full understanding of the cTn release in professional athletes is essential, although some data are available with sample frequency deficiencies [21, 22]. Therefore, it is very important to deal with these issues; hence, a study was designed by hypothesising that among the amateur players and adolescent ones the cTnI release is increased during recovery. The study was designed by employing multiple sampling points within 24 h after exercise. The release of cTnI monitoring was articulated to observe the effect of a football match among players in a simulated manner similar to a real one. To have comprehensive data, the players of different training levels were selected from among both professionals and amateurs.

Material and methods

Subjects and design

A cohort of players of football (n = 44) was selected. The players comprised 22 adults, professional football players (PFP) from the Lithuanian A League and 22 adults, amateur football players (AFP) from a local university football team. All the players gave written informed consent for the participation in an imitative football game, and during the first 24 h after exercise, continuous evaluation of cTnI release was performed. Pertinent information about the study purpose, testing procedures, and possible risks was given to the players and their parents. Furthermore, they were given the liberty to terminate participation at will. This study was given approval by the Research Ethics Committee of the republic of Lithuania. The study complies with the principles laid down in the Declaration of Helsinki, adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and recently amended at the 59th World Medical Assembly, Seoul, Korea, October 2008.

Table 1 shows the major features of the study cohort. Both AFP had training history lower than PFP and lower current training volume as well (all $p < 0.05$). The PFP and AFP players' ages were not very different. All the players were scheduled to attend the laboratory for three sessions. However, before attending the first session, all the players were instructed not to do any vigorous athletic activity for at least 48 h before attending the laboratory session. Instructions were given for maintaining their routine life with a normal diet and their sleeping routine etc. Furthermore, subjects were asked to not take any meal at least 3 hr before the test and avoid any energetic products.

In the first session, a general physical check-up was done, which included height and body weight, measured using Health O Meter 500KL Series Digital Adult Scales. In order to get personal information, a history of players' training and any cardiac problems, a questionnaire was filled in by them in with pre-organised questions. In the first session, to exclude any player having significant cardiac history or pathological electrocardiograph, a 12-lead ECG was done. For determining the VO2max, all the athletes were asked to perform a shuttle run test of 20 m [23]. The maximal heart rate (HR) was measured by an Acentas team monitoring system (Acentas GmbH, Hörgertshausen, Germany).

In the second round of laboratory visits, two teams were made to play a match. All players were asked to play their game in the position where they usually perform. The players were asked to treat the game as a regular competitive match and perform a similar warm-up as they usually would. FIFA regulations were applied to the played match. To make it possible for all players to participate in the game, every team was allowed to make changes after an interval of 4 min of the actual game time. By using the Acentas team monitoring system, routine and continuous monitoring was performed for HR during the match. To assess serum cTnI venous blood samples were taken at several stages of the game, i.e. before the game, immediately after (usually 5 min), and post-exercise at 3, 6, and 24 h.

The blood samples taken were immediately put in a centrifuge to separate out plasma and serum and then stored at –80°C for later research work. A URL

Table 1. Characteristics of the football players

	Age (years)	BMI (kg/m^2)	VO2max (ml/kg/min)	Football training history (years)	Football training magnitude (hours/week)
Professional football players (n = 22)	24.2 ± 4.5	23.5 ± 3.4	60 ± 4.3	10 ± 3.3	20 ± 2.5
Amateur Football players (n = 22)	26.5 ± 3.6	19.9 ± 6.1	57 ± 5.0	6 ± 4.0	7 ± 3.2
P value	P = 0.1	P = 0.025	P = 0.001	P = 0.02	P = 0.03

value of 0.04 μg/L [26] for cTnI was considered as the 99th percentile of healthy players.

Statistical analysis

The data obtained was statistically treated by using the IBM Statistical Package of Social Sciences (IBM SPSS Statistics, v. 20.0 for WINDOWS). All the data are usually stated as the mean ± SD values, except where stated in particular. For normal distribution Kolmogorov-Smirnov tests were used for analysis. A mixed model two-way ANOVA was performed with post-hoc Bonferroni tests (employed when appropriate) for the measurement of the effect of sampling time (pre, 5 min, 3, 6, and 24 h post-exercise). In the whole research work, bivariate Pearson's product moment correlation coefficients were used to assess the different variables. These parameters included the association between baseline cTnI, increases in cTnI, peak post-exercise value, and mean and max exercise HR during simulated gameplay. The values were taken to be significant when p >0.05.

Results

The results obtained (Table 2) from the shuttle run test of 20 m showed no difference between the groups for HRmax. However, the PFPs had lower mean HR than values for AFPs.

Table 2. Heart rate during the football match

	Mean HR (BPM)
Professional football players (n = 22)	139 ± 12.9
Amateur Football players (n = 22)	168 ± 17.8
P value	P < 0.05

Figure 1 revealed, in all the subjects, an increased value of post-match cTnI in comparison to the base level of cTnI. From Figure 1, it is evident that 14 PFPs had high values for URL of cTnI. For post-match maximum cTnI value was observed at three hours in 34 subjects, the other 10 individuals reached a maximum at six hours. The study showed that the peak value of troponin I in plasma post-match was not related to the role of the player in the game. However, base level values were correlated with mean HR.

Additionally, for AFPs, a significant effect of groups was observed in the context of recovery cTnI as well as the baseline, which was lower than that of PFPs (p = 0.001). Furthermore, there was also dissimilarity in the data of the player groups for the highest after-exercise values of cTnI (AFP: median [range]; 0.029 [0.024–0.04]; PFP: 0.11 [0.02–0.3]).

Discussion

From the reviewed literature it can be confirmed that our research work is the first such work for investigating the cTnI "kinetics" post-football match, after the exercise is performed, in meticulous conditions, with separate groups of players distinguished by the status of the athlete and their age. The major findings from our studies were the variation in cTn, magnitude differences between baseline and post-football match cTn, and similarity of post-football match cTn response found in the professional players and amateur players.

Due to the application of fewer cTnI sampling intervals in the previous studies, several contradictory results have been reported for cTn release. However, in our study, a post-exercise cTnI increase was observed in every participant, which in general is contradictory

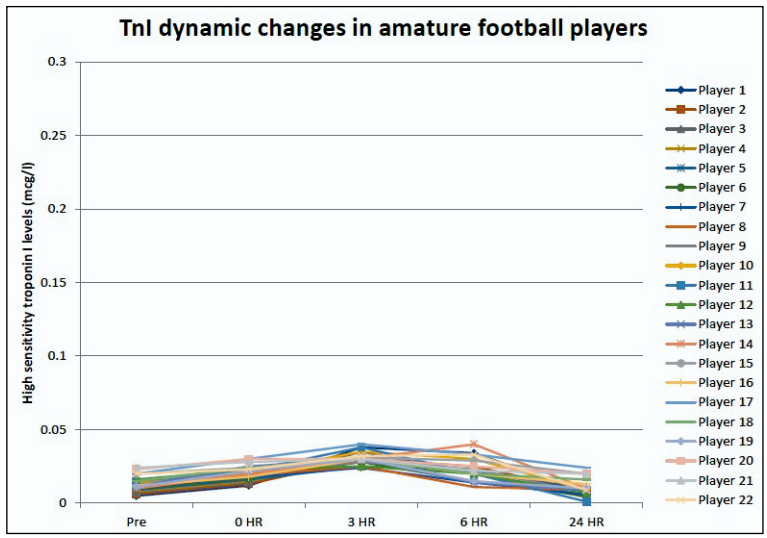

Figure 1. TnI dynamic changes in amateur football players

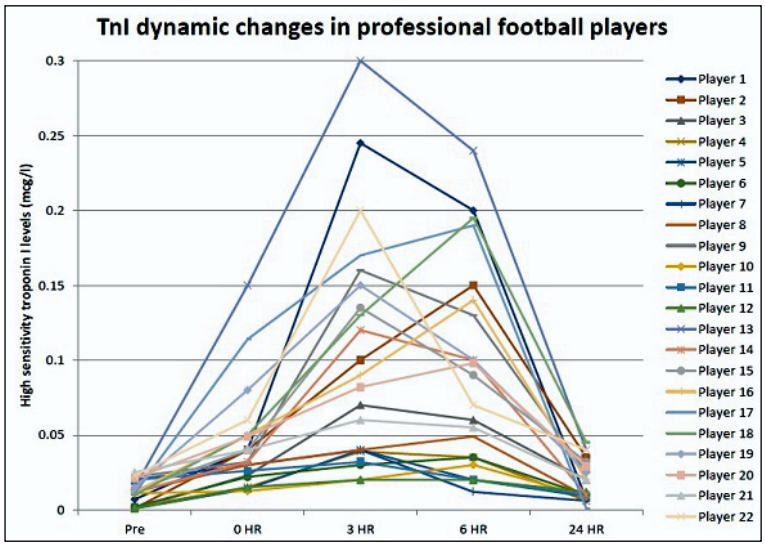

Figure 2. TnI dynamic changes in professional football players

to the results in which no cTnI increase was observed, as reported by George et al. [25] and Rahnama et al. [24]. This difference can be better explained due to the use of a simple sampling plan. Our data is in accordance with results reported by Nie et al. [12], who also observed a cTn increase in the several hours after simulated gameplay. Therefore, this research work shows the importance of taking several samples during recovery to monitor the maximum post-exercise release values of cTn.

We report the individual variation of peak cTnI in 26% of subjects with only one individual having a cTnI value greater than the URL. However, this value is much lower than the reported percentages for other game exercises, such as for the long run marathon [28] or cycling [29]. Our findings confirm the diversity of cTnI release in peak time, and at that specific time the analysis suggests some degree of variance in "kinetics" of the cTnI appearance. It has been reported by previous researchers [5, 27] that when the magnitude of exercise is controlled, the exercise duration usually mediates the increase in cTnI or cTnT.

The results of many previous studies [17, 22] reported that post-exercise cTn peak is obtained within three to four hours post-exercise. However, our results are different in that most of the cTnI peak was observed six hours post-exercise, which affirms that many factors affect the peak post-exercise value, such as the intensity of exercise, duration, and mode of exercise. However, further studies are in demand to clear any such ambiguous data.

In cases of coronary syndromes, generally, the mode of cTn release and then clearance of cTn in post-exercise are at odds with the changes in the observed cTn. This could be due to the fact that after the exercise stimulus, post-exercise cTn levels may be related to a physiological response rather than a pathological response. It has been hypothesised that high-tension exercise usually results in an increase in the permeability of the membrane and causes membrane damage of the cell. This damage induces transient cytosolic leakage [30].

Among some reports of recent papers [14–18] it can be found that post-physical stress cTn release is more prominent in amateur players as compared to more physically fit players. This is because of the lower myocardial stress efficiency, which is due to the non-adaptability of the myocardial cells in amateur players, similar to the same adaptability mode of skeletal muscles [14, 15]. However, our results are in contradiction to this theory because higher post-physical stress values were expressed by PFPs than those of AFPs. During the simulated game, the lower HR and %HRmax are in accordance with recent field-based studies with marathon runners [31] training under controlled mediation [21].

The PFPs had higher post-exercise cTnI values as compared to AFP values, which could be associated with changes in baseline cTnI. In previous studies [5] it was reported that there is a strong relation between the values of baseline and post-exercise cTnI release. Usually, no attention is given to the variation in baseline cTn values found in healthy people. It has been postulated that men and skilled athletes usually have larger hearts than women and lower trained players, respectively. This postulation has also been put forth by Mingels et al. [16], who observed very high hs-cTnT values. In the same way, we can rationalise the elevated concentration for both base-level and post-match cTnI in PFP; however, it cannot be clearly stated that the hypertrophied hearts of PFPs are larger than those of AFPs. Therefore,

further research is needed to make clear the associations between the differences of exercise-related cTn values at baseline and the factors involved.

It is noteworthy for clinicians that cTnI release is not only related to highly intense exercises, because URL higher than cTnI can be observed even after intermittent exercises such as a sports game. Therefore, for clinicians it is important to consider any recent exercise activities in addition to any clinical signs or even in the absence of any symptoms but with positive cTn concentrations.

In this research work, we observed a diversified response of cTnI release among all players. Our results contradict past reports, which indicate a relation between professional players and cTnI plasma concentration after physical stress. Additionally, in our results, we postulate the relationship between physiological response after the exercise stimulus and changes in cTn release.

Conclusions

In our study, it is clear that intermittent exercise, such as a football game, causes an upsurge in cTnI plasma concentration. However, all the players had different cTnI values for peak and time-to-peak cTnI. Another major conclusion of the study is that the elite athletes showed higher values of cTn appearance as compared to amateur athletes, confirming the association of athlete status and cTn appearance.

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REVIEW ARTICLE

Wojciech Rogóż[1], Karolina Kulig[1], Magdalena Knopik-Kocleęga[2], Agnieszka Szkudlarek[1], Małgorzata Maciążek-Jurczyk[1]

[1]Department of Physical Pharmacy, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland
[2]Apteka Zdrowit, Piekary Śląskie, Poland

Analysis of antibiotic resistance genetic conditioning of *Enterobacteriaceae* NDM-1 family members and the related epidemiological threat in Poland

Corresponding author:

Wojciech Rogóż, Department of Physical Pharmacy, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland, e-mail: wrogoz@sum.edu.pl

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ABSTRACT

Antibiotic resistance is an extremely serious threat to the modern world. Since 2008, Gram-negative rods from the *Enterobacteriaceae* family gained the possibility of β-lactam degradation using NDM-1 carbapenemase, encoded by the *blaNDM* gene. It often occurs in the genome of *Klebsiella pneumoniae* and can occur on both bacterial chromosome and plasmids. This creates a very high risk due to the widespread occurrence of bacteria from this family both in the environment and in human microflora. Lack of sensitivity to popular β-lactam antibiotics is especially dangerous for patients hospitalised for a long time with reduced immunity. In Poland, since 2011, the number of registered NDM+ isolates and related infections are constantly increasing, reaching 1780 cases in 2016. Bacilli showing the presence of the *blaNDM* gene are registered very often in the Mazowieckie and Podlaskie regions, while the number of such cases is the lowest in the Opolskie region. Inhibiting the growing number of infections caused by *Enterobacteriaceae* NDM+ is extremely difficult, and one of the methods to reduce this phenomenon is strict compliance with hygiene rules.

Key words: NDM-1, antibiotic resistance, Enterobacteriaceae

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Introduction

The phenomenon of antibiotic resistance is one of the biggest threats for public health in the modern world. It is a problem not only for the inhabitants of countries where hygiene rules are not respected, or for people with reduced immunity who have been hospitalised for a long time, but also for all those who may potentially have contact with bacteria that is insensitive to antibiotics. A particularly dangerous example of such a microorganism is *Klebsiella pneumoniae* "New Delhi", which produces metallo-β-lactamase rod from the *Enterobacteriaceae* family, the frequency of occurrence of which is constantly increasing in Poland and throughout the world.

Enterobacteriaceae family

The family of Gram-negative *Enterobacteriaceae* is an extremely diverse and very broad group of bacteria. They belong to the most common microorganisms in nature such as: *Enterobacter, Klebsiella, Escherichia, Shigella, Salmonella, Yersinia, Proteus*, or *Serratia*. They include hundreds of species that live in water, soil, plants, and animals and are an extremely important element of human microflora. Most of them are not dangerous to people. Individual species, such as *Yersinia pestis*, are always pathogenic to humans, while others may cause opportunistic infections (e.g. *Proteus mirabilis*). The majority of bacilli belonging to this family, especially opportunistic, can move by cilia and are relative anaerobes. All of the *Enterobacteriaceae* members have the following common features: the ability to ferment glucose and reduce nitrates, and a lack of cytochrome oxidase and catalase production. An extremely important species of the rod that belongs to the mentioned family was discovered in the 19th century: *Klebsiella pneumoniae*. It is an element of the natural human microflora [1, 2]. It occurs in the oropharynx and rectum of people who are its carriers

[3, 4]. *Klebsiella pneumoniae* exists under anaerobic conditions, has the ability to ferment glucose and fructose, and on the MacConkey medium it grows in the form of a highly characteristic colonies, slightly pink, sometimes milky pink, *reminiscent* of morning *dew drops,* often with round, smooth shapes. Due to the very thick, mucous envelope, *K. pneumoniae* colonies have a slippery, moist surface [1, 2]. This envelope is also an important feature of virulence [1]. Bacteria from this species produce urease, enabling the breakdown of urea into ammonia, which facilitates the colonisation of the infected organism by limiting the function of the immune system. *K. pneumoniae* is an extremely important aetiological factor in hospital infections. People treated with long-term antibiotic therapy are particularly vulnerable to colonisation with this pathogen. Unfortunately, *K. pneumoniae* is acquiring resistance to new, commonly used groups of antibiotics and chemotherapeutics, which is a growing problem [3].

The phenomenon of antibiotic resistance

There are many definitions of antibiotic resistance as well as forms, causes, and consequences of this phenomenon. The simplest of them says that it is a condition in which bacteria are not sensitive to the effects of an antibiotic, and it is caused by certain specific features of bacterium [2, 4, 5]. Incorrect behaviour of patients, doctors, farmers, cattlemen, and food industry employees, resulting from ignorance or disregard of current recommendations, have contributed to the rapid increase in the importance of bacteria defence mechanisms against antibiotics. Antibiotic resistance of bacteria can be natural when insensitivity to a given antibiotic or a whole group is a constant characteristic for a species, e.g. due to the lack of a structure on which the antibiotic can act, or acquired nature, when the gene structures determining drug resistance are introduced into bacterial cells together with DNA by conjugation, transduction, or transformation [2, 4, 5]. The reservoir of genes determining antibiotic resistance are not only pathogenic bacteria, but also those harmless to humans, commonly found in the environment [5]. Bacteria showing resistance to antibiotics realise this feature using various mechanisms that neutralise the drug, e.g. prevention of drug penetration into the cell due to a thick cell wall, enzymatic degradation of the antibiotic, or its modification by adding new functional groups to its molecule, overproduction of the factor being an antibiotic placeholder, active removal of the drug with other toxins using efflux pumps, or such a change in metabolic pathways in the cell to replace that one on which the antibiotic acts [4, 5].

β-lactam antibiotics

β-lactam antibiotics are the most important and commonly used group of antimicrobial drugs. The key element of their structure, characteristic for all representatives of this group of drugs, is the presence in their molecule of a four-element β-lactam ring, which determines their biological function. They show bactericidal activity by the inhibition of the synthesis of peptidoglycan, which is an element that builds the cell wall in bacterial cells. This is possible due to the interaction with penicillin-binding protein (PBP) involved in the final stage of cross-linking murein subunits in a process called transpeptidation. They are bactericidal against many Gram-positive bacteria, as well as some of the Gram-negative bacteria, including those belonging to the *Enterobacteriaceae* family. This is possible due to the activation of autolysis, disruption of the ion balance of Gram-negative bacteria, as well as stimulation of holin formation. There are five main groups of β-lactam antibiotics: penicillins, cephalosporins, cephamycins, carbapenems, and monobactams. Unfortunately, many mechanisms of resistance to β-lactams are known. These include the production by bacteria of enzymes that hydrolyse the binding in the β-lactam ring and thereby inactivation an antibiotic molecule, mutations within the PBP protein sequence – leading to changes in its structure, the formation of a completely new form of the PBP protein, not showing affinity for β-lactam antibiotics, and disruption of the transport (specific for Gram-negative bacteria) to the cell of the antibiotic by changing the amount and structure of porin proteins and the active removal of the drug from the bacterial cell [1, 6, 7].

Enzymatic degradation of antibiotics

Enzymatic degradation of the antibiotic, especially β-lactams (the basis of their construction is a β-lactam ring), is a very common defence mechanism for bacteria. They have a characteristic enzyme that is necessary to exhibit biological activity, involving the inhibition of the synthesis of Gram-positive bacteria cell wall. A very large group of enzymes that inactivate β-lactam antibiotics are β-lactamases [8, 9]. β-lactamases that show activity towards only one type of antibiotics from this group are named penicillinases, cephalosporinases, or carbapenemases (the names indicate their most important substrates) [7]. Their simplest split by the mode of action includes: serine β-lactamases (SBL) and metallo-β-lactamases (MBL). The first of these groups is very large, and belonging enzymes causes the acylation and hydrolysis of the antibiotic using the active centre serine. On the other hand, metallo-β-lac-

tamases in the active centre have a zinc cation, which enables β-lactam ring damage by the interaction with the antibiotic [8, 9, 10]. There are also other β-lactamase classification systems, e.g. the one proposed by Amber et al. This classification relies on analysis of the evolutionary relationship of enzymes and distinguishing classes A, B, C, and D. Metallo β-lactamases, such as NDM (New Delhi *metallo-β-lactamase)*, belong to class B [11, 12]. Class A includes such carbapenemases as KPC, GES, or MSP. Representatives of classes C and D are CMY and OXA carbapenemases, respectively [13]. Bush and Jacoby are the authors of a classification that assumes the split due to the speed of antibiotic degradation reactions and the potential sensitivity of enzymes to their inhibitors. This split includes groups I, II, III, and IV – metallo-β-lactamases belong to group III. Their most important common feature is the lack of any sensitivity to inhibitors, as well as high sensitivity to the presence of EDTA that binds metal cations [9, 14, 15]. With the application of β-lactams that are effective against Gram-negative bacteria, an increase in bacterial infections showing the presence of acquired forms of β-lactamases has been observed. They are usually encoded on plasmids, and in cells they show constitutive expression [8]. Within class B of β-lactamases, several key groups can be distinguished, which are acquired by bacteria. These are: β-lactamases with extended substrate spectrum, called ESBL, AmpC cephalosporinases, and carbapenemases. The first group provides the bacteria with a resistance to penicillins, cephalosporins, and monobactams, while retaining sensitivity to cephamycins, carbapenems, and β-lactamase inhibitors. AmpC provides resistance to penicillins, cephalosporins of I-III generation, and monobactams. Carbapenems, considered to be examples of 'last resort' antibiotics, may be degraded by carbapenemases. Class B is their most important class. Its representatives are metallo-β-lactamases (MBL), which effectively hydrolyse all β-lactam antibiotics, including carbapenems, with the exception of monobactams [9]. Class B β-lactamases contain two zinc cations in the active site – the first cation is associated with three histidines, and the second one with the system of three amino acids: Cys-His-Asp [16]. They are the most active and therefore the most dangerous group of carbapenemases. They were first observed in representatives of the *Pseudomonas aeruginosa* species in the 1980s, and then in the 1990s in bacteria belonging to the *Enterobacteriaceae* family. The most important enzymes from the MBL group that a bacterial cell can acquire include IMP, VIM, and NDM [15]. Many groups of researchers are constantly conducting research aimed at obtaining effective NDM-1 metallo-β-lactamase inhibitors [17, 18]. Until recently, only inhibitors of serine β-lactamases, such as clavulanic acid, sulbactam, or tazobactam, which unfortunately showed no activity against MBL, were known. According to the research of Cahill et al., there are compounds that can inhibit both SBL and MBL activity. They are cyclic boroorganic compounds having in their structure a bond between carbon and boron [10]. Another NDM-1 carbapenemase inhibitor is the natural compound isolated from mould, Aspergillomarasmine A (AMA). According to the results from the studies of King et al., it restored bactericidal activity to antibiotics such as meropenem against members of the *Enterobacteriaceae* family, containing in their genome the bla_{NDM-1} gene [19]. There is also a group of compounds that are analogues of penicillins with a free thiol group, called bistiazolidines. They show a high ability to block NDM-1 β-lactamase activity, leading to inhibition of imipenem hydrolysis by enzymes produced by *Escherichia coli* NDM+ [20]. Attempts to overcome NDM+ strains using a combination of several currently known and used antibiotics are ongoing. Ojdana et al. showed that the combination of third generation of cephalosporin (ceftazidime), class A, B, and D β-lactamase inhibitor (avibactam) and an organophosphate compound (fosfomycin) shows high efficiency against *K. pneumoniae* NDM + [21]. Figure 1 shows a simplified classification scheme of acquired β-lactamases [9].

K. pneumoniae can show the presence of many mechanisms of resistance to antibiotics. Data published in 2015 by KORLD from the European network EARS-Net, the aim of which was to control antibiotic resistance among bacteria in Europe, comprising several hundred tested isolates from about 60 hospitals, show that 100% of *K. pneumoniae* in Poland are resistant to aminopenicillins. From the reported data, it follows that more than 60% of the bacilli from this species are insensitive to fluoroquinolones and third-generation cephalosporins, and more than half to aminoglycosides. A very large proportion of the isolates turned out to be resistant to all three groups of drugs. Only 0.3% of all isolates showed resistance to carbapenemases [22].

Genesis *K. pneumoniae* NDM-1

In 2008 the first person in the world to be diagnosed with infection of a bacteria strain (now called *K. pneumoniae* NDM-1) was a Swede of Indian origin. He was diagnosed in a hospital in New Delhi with a urinary tract infection. Bacteria isolated from his urine proved to be extremely resistant to ampicillin, cefoxitin, cefuroxime, ceftazidime, and piperacillin. They were sensitive only to colistin and tigecycline [23]. One of the reasons for the appearance of this form of antibiotic resistant bacteria was insufficient compliance in South Asia with the rules of hygiene and environmental pollution. Bacteria showing the presence of NDM-1 carbapenemase also very

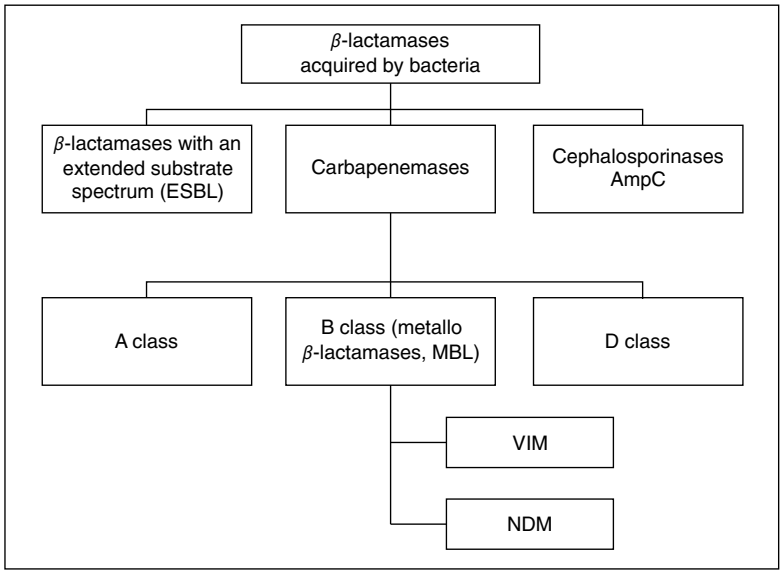

Figure 1. A simplified classification scheme of acquired β-lactamases [9]

often belong to the species *E. coli* and *Enterobacter cloacae* [24, 25]. A high risk of infection with bacteria showing the ability to produce NDM-1 occurs in India and other regions of southern Asia [26]. The list of countries where the risk of infection is high, apart from such Asian countries as China or Japan, includes also many European countries, such as: France, Belgium, the Netherlands, Germany, Austria, Sweden, and Norway [27]. A difficult, and sometimes impossible to eradicate, infection with *K. pneumoniae* NDM+ poses a number of threats with available medical devices. One of them is the possibility of infecting kidney transplant recipients, which has been documented by Karczewski et al. [28].

Genetic conditioning of the NDM resistance mechanism

The bla_{NDM} gene is responsible for coding the NDM-1 enzyme [29]. It can be located both on the bacterial chromosome and on plasmids [27]. The second of these locations promotes horizontal gene transfer and the spread between bacteria of the gene encoding the carbapenemase [27, 29]. The bla_{NDM} gene has been located on plasmid types such as IncF [30, 31], IncA/C [30, 32], or IncL/M [33]. Particularly dangerous are cases are those in which, apart from the bla_{NDM} gene, other genes that determine antibiotic resistance are also found on the plasmid. The results of this type of analysis were presented by Gamal et al., who from Egyptian patients isolates of *K. pneumoniae* isolated IncR plasmids containing both bla_{NDM} and [34]. rmtF gene codes methyltransferase, which is responsible for modifying the aminoglycoside attachment site in 16S rRNA bacterial ribosome. This leads to bacterial resistance to this group of antibiotics [35]. Data from Yan et al. show that in isolates obtained in China multidrug resistant *K. pneumoniae* were present, which apart from the bla_{NDM} gene also had bla_{KPC-2} gene encoding carbapenemase belonging to class A according to Ambler and enzyme group 2 according to Bush and Jacoby [14, 36, 37]. Ho et al. from Hong Kong isolated and sequenced the pNDM-HK plasmid. It belongs to the group of IncL/M plasmids. It was observed that beyond to the gene responsible for the mechanism of resistance associated with NDM carbapenemase (bla_{NDM-1}) it had other genes determining resistance to: β-lactams such as bla_{TEM-1} and bla_{DHA-1}, aminoglycosides such as *armA* and *aaaC2*, macrolides such as *mph2* and *mel*, and sulphonamides such as *sul1* [38, 39].

Types of NDM metallo-β-lactamases

There are many different types of NDM metallo-β-lactamases, depending on their structure. The differences between them in the primary structure of the protein are minimal, and they are caused by point mutations resulting in the exchange of one amino acid for another. The main type of NDM enzyme is NDM-1. This form of carbapenemase was discovered in 2008 in a Swedish patient in New Delhi, India [23]. It has a mass of about 28 kDa and is made of 269 amino acids [13]. Subsequent variants of this enzyme, marked sequentially from 2 to 17, are very rare. NDM-3, -4, -5, -6, -7, -8, -11, -12, -13, -15, and -17 were detected in *E. coli*, while NDM-9, -10, and -16 were detected in *K. pneumoniae* [29, 40]. Importantly, NDM metallo-β-lactamases (especially

NDM-1) are sometimes present in individual representatives of *Vibrionaceae* and other non-*Enterobacteriaceae* species [41].

Carbapenemase-producing *Enterobacteriaceae* (CPE)

The *Enterobacteriaceae* family, which have the ability to produce carbapenemases, are called CPE (Carbapenemase-Producing *Enterobacteriaceae*). According to the KORLD definition, in order to confirm their presence in a patient, it is necessary to either obtain a positive Carba NP test (Nordmann-Poirel), or to determine the production of carbapenemase in a KORLD facility, or to obtain the confirmation of carbapenemase presence using genetic tests. The study may include, for example, the patient's blood, an intraoperative segment taken from an infected wound, or bronchoscopy from a person diagnosed with pneumonia [42]. The mentioned Carba NP test is an extremely simple, cheap, and easy detection method, including bacilli from the *Enterobacteriaceae* family that produce carbapenemases [43]. For this group of bacteria, it has almost complete sensitivity and specificity compared to molecular methods [44]. This test involves the hydrolysis of imipenem by carbapenemases derived from the lysate of a suspension of bacteria derived from a biological sample. The pH of the solution decreases due to the acidity of antibiotic breakdown products, which causes the red colour in the alkaline phenol solution to change to yellow [43, 44].

CPE in Poland in the years 2011 to 2016

There is a very large reservoir of bacteria in Poland that can obtain the ability to produce carbapenemases. This is due to the widespread occurrence of both *E. coli* and *K. pneumoniae* representatives. In the years 2011 to 2016, as much as 98.8% of all bacteria in which the NDM-1 enzyme was detected belonged to the species *K. pneumoniae* (Fig. 2). Approximately 1% of cases are representatives of the *E. coli* species. This means that every case of NDM+ strain in Poland is associated with the *Enterobacteriaceae* family [45]. In Poland, the first case of infection caused by a representative of the *Enterobacteriaceae* family producing New Delhi metallo-β-lactamase 1 (NDM) appeared in 2011 in the Mazowieckie region [46]. He was a middle-aged man transported by air to Warsaw from Congo, where he was admitted to hospital due to sudden cardiac arrest. In his urine, an *E. coli* bacterium was identified showing the presence of New Delhi metallo-β-lactamase 1. In spite of almost two weeks of colistin treatment, the patient

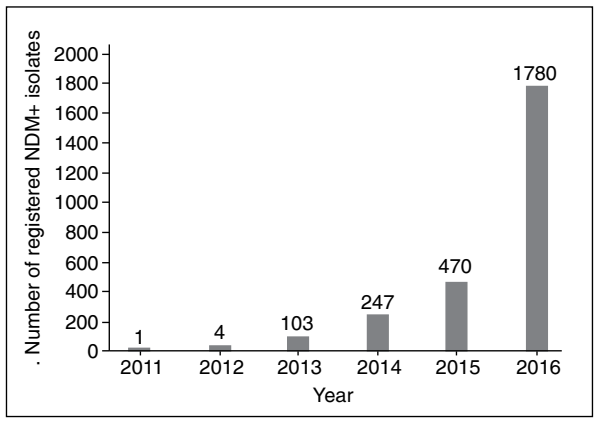

Figure 2. Number of registered cases of bacteria from the *Enterobacteriaceae* NDM-1 family from 2011 to 2016 [46, 49]

died due to multi-organ failure [47]. In the following year, four cases of infection with bacteria showing the presence of NDM enzymes were registered in the Wielkopolska region. A huge increase in the number of observed infection cases, equal to 103, took place in 2013. The number of infections in 2014 was more than twice as high as in the previous year, and since 2016 it has exceeded 1000 cases [46]. The KORLD data shows that, in the years between 2011 and 2016, 2605 cases of NDM+ isolates were recorded [45, 46]. According to data published by Baraniak et al., many cases of NDM+ from the *Enterobacteriaceae* family occurring in Poland, especially in the early years of their occurrence (2012–2014), were acquired abroad. For example, three cases of infection from 2013 were reported in patients from Montenegro, Afghanistan, and India. Due to the fact that the species showing the presence of the bla_{NDM-1} gene was *P. mirabilis*, the case of infection of a person returning from Afghanistan was particularly interesting [48].

The vast majority of cases, about 93.3%, were identified in hospitals, hospices, and in the course of admitting patients to hospitals. Analysing the age of infected patients, it can be stated that about 65% of them were people over 65 years of age, and under the age of 19 years the cases of isolation of NDM+ strains practically did not occur. Perhaps due to insufficient compliance with hygiene rules, observed more often in men than women, in the first mentioned group the number of infected was slightly higher [45]. From the data collected at the surgery department of the Medical University of Warsaw in 2012–2014 and published by Milner et al., it appears that from 236 *K. pneumoniae* strains isolated from patients' biological materials 14 isolates (6%) showed a mechanism of resistance to antibiotics associated with the NDM-1 enzyme. None of these isolates was

obtained in 2012, while as many as 11 of them were from 2013. All NDM+ was sensitive to gentamicin, but at the same time none of the isolates was sensitive to ertapenem. As many as 14% of isolates were not sensitive to colistin [3]. Nawfal Dagher et al. proved that registered in Lebanon, insensitive to colistin the *K. pneumoniae* isolate showed the presence of NDM-5 carbapenemase [50].

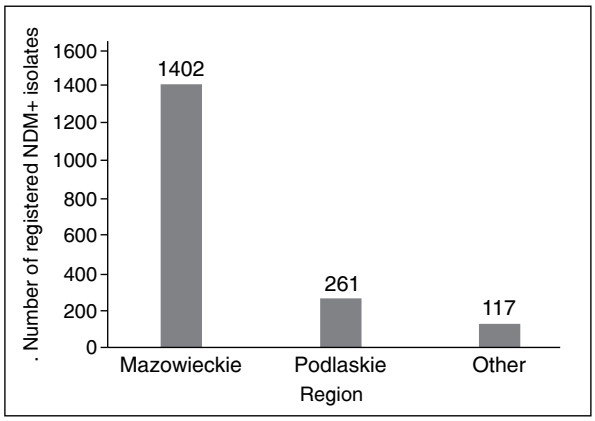

Figure 3. Number of registered cases of bacteria from the *Enterobacteriaceae* NDM-1 family in 2016 from the Mazowieckie and Podlaskie regions, relative to others [46]

1780 cases of *Enterobacteriaceae* NDM-1 bacteria were registered in 2016. About 78% of these cases took place in the Mazowieckie region (Fig. 3). In 14 regions (Fig. 4), the number of cases of bacteria containing the NDM-1 enzyme did not exceed 120, and it was more than two-fold lower than in the Podlaskie region. In the Podkarpackie, Opolskie, and Lubuskie regions no cases of NDM carbapenemase occurred in microorganisms [46].

CPE in Poland in 2017

Figures 5 and 6 show the numbers of recorded cases of bacteria from the *Enterobacteriaceae* NDM-1 family in the first three quarters registered in Mazowieckie, Podlaskie, and others (Fig. 5) and in regions outside Mazowieckie and Podlaskie (Fig. 6).

In 2017 about 2405 cases of NDM+ isolates were recorded – more than half were registered in the Mazowieckie region (Fig. 5) [46]. The Opolskie, Podkarpackie, and Lubuskie regions, similarly as in 2016, were the places of registration with the smallest number of NDM+ strains. Both in the first and second quarter of 2017, the number of registered cases of infection was higher than in the third quarter (Fig. 6) [51]. In the first quarter of 2017 the information about infection was

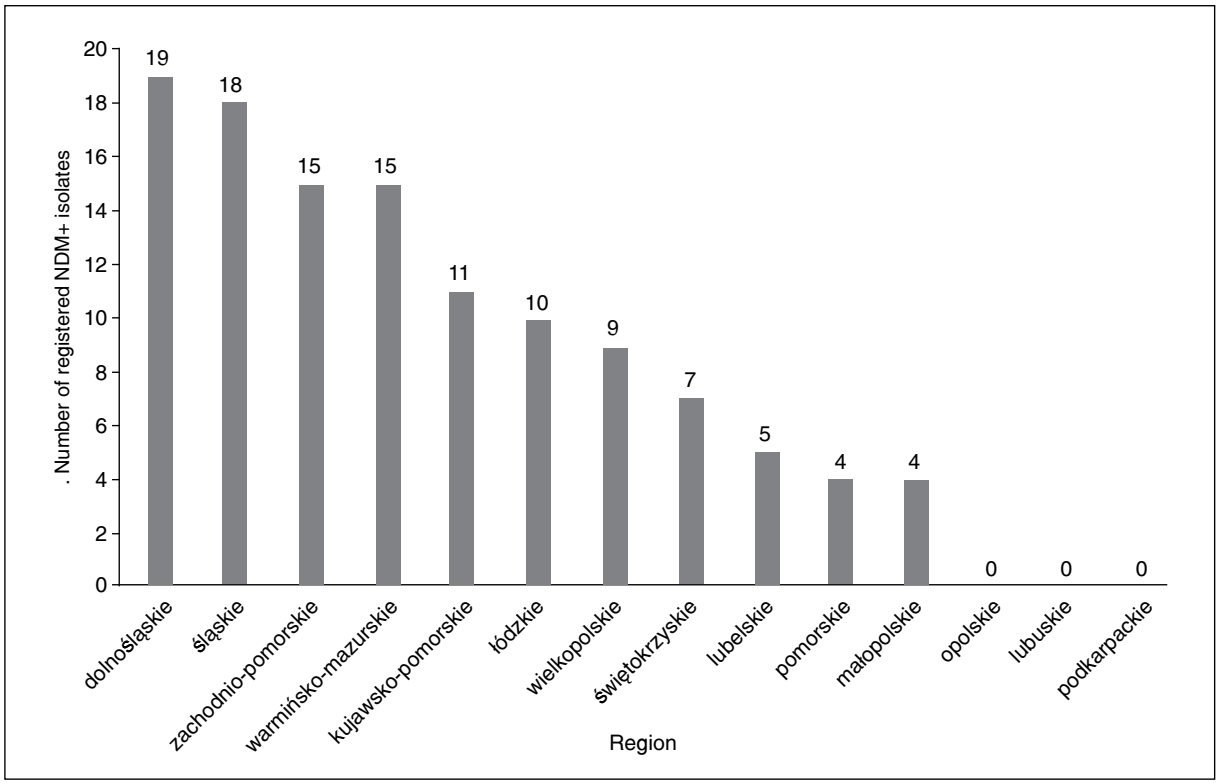

Figure 4. The number of recorded cases of bacteria from the *Enterobacteriaceae* NDM-1 family in 2016 [46]

obtained as a result of screening tests more often than infection diagnosis [52]. All the data available for the Mazowieckie region, due to the very large number of samples, from the period after the second quarter of 2017 can be considered undervalued, due to restrictions concerning the confirmation of the presence of carbapenemases in some *K. pneumoniae* strains producing metallo-β-lactamases [46, 51].

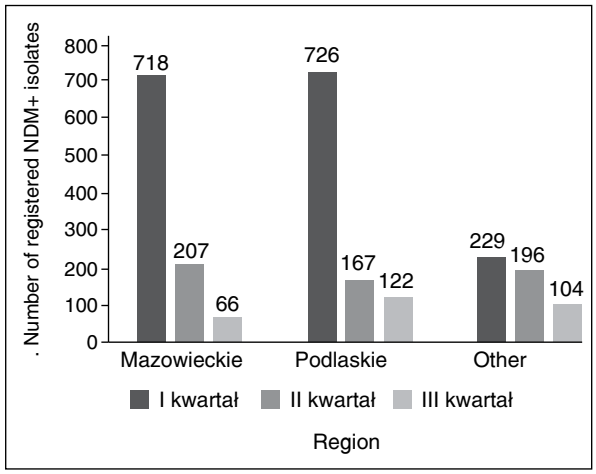

Figure 5. Number of recorded cases of bacteria from the *Enterobacteriaceae* NDM-1 family in the first three quarters of 2017 in the Mazowieckie and Podlaskie regions, relative to others [51]

Another interesting KORLD report allows for a more accurate analysis of data on the number of NDM+ isolates from 2016 and 2017. In the first quarter of 2017, more cases of patients with *Enterobacteriaceae*, mainly *K. pneumoniae*, NDM+ were recorded than in the first quarter of 2016 (about 150%), particularly in the Mazowieckie and Podlaskie regions. In the first of them, in both years, a carrying of bacteria than infection was observed in twice as many people. On the other hand, in the Podlaskie region in the first quarter of 2016 the number of patients infected and those who were carriers of bacteria was similar, and in the first quarter of 2017 the number of carriers became almost twice as high as those infected [52].

CPE in Poland after 2017

In 2018, probably due to the change in the procedures for obtaining and registering test results by

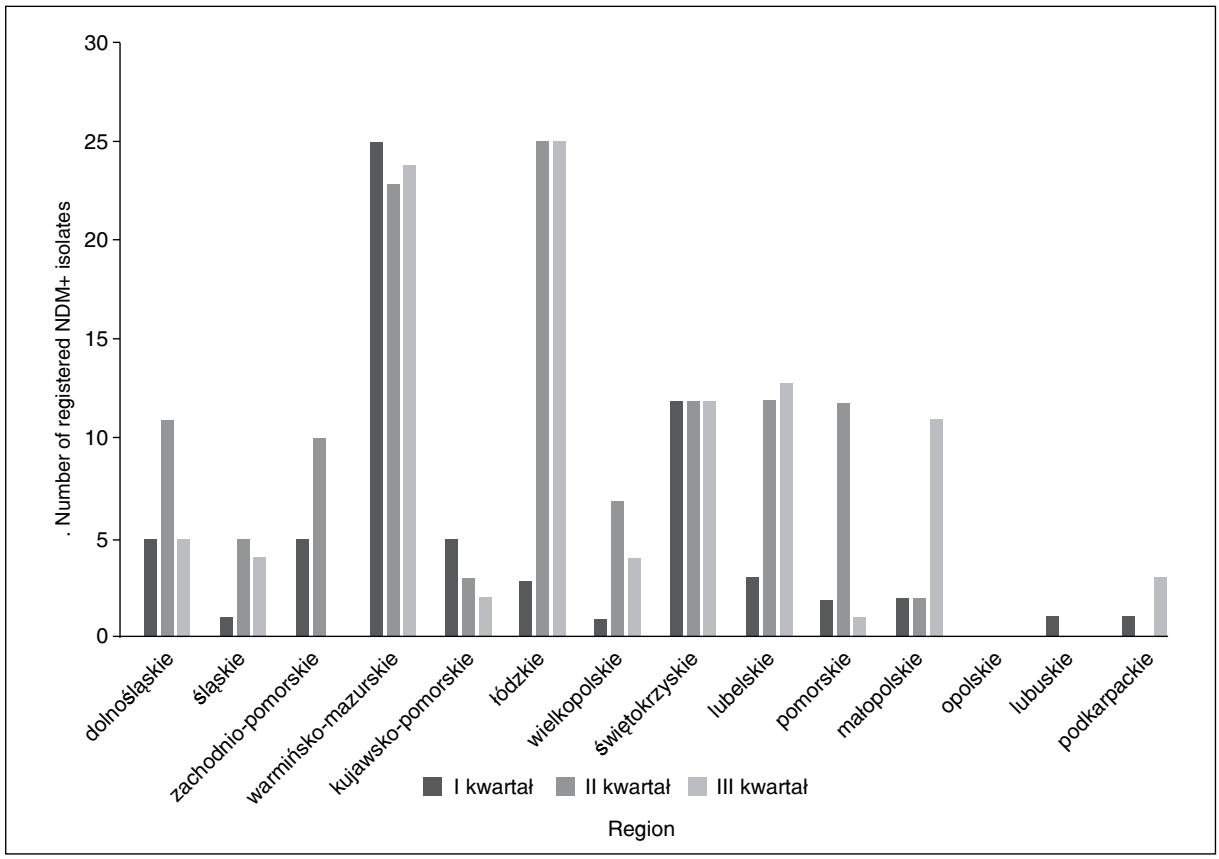

Figure 6. Number of recorded cases of bacteria from the *Enterobacteriaceae* NDM-1 family in the first three quarters registered in regions outside Mazowieckie and Podlaskie [51]

Table 1. Summary of the numbers of reported occurrences of NDM+ isolates in 2016–2018 from Podlaskie, Śląskie, Wielkopolskie, and Zachodniopomorskie regions

Region	2016	2017	2018
Podlaskie	261	675	786
Śląskie	18	12	53
Wielkopolskie	9	20	35
Zachodniopomorskie	15	16	31

KORLD in the Mazowieckie region, the number of registered NDM-1 isolates was lower than in 2017 and equalled 2355 [46]. However, this does not mean a decrease in the risk of bacteria from the *Enterobacteriaceae* family that produce NDM-1 carbapenemase. Not counting the Mazowieckie region, the number of registered NDM+ isolates in 2016–2018 increases in almost every region [46]. Table 1 presents a summary of the numbers of reported occurrences of NDM + isolates from the four selected Polish regions.

The data from the Region Sanitary and Epidemiological Station in Warsaw show that 23 outbreaks of nosocomial infections were reported in 2018, which were caused by *K. pneumoniae* with the ability to form metallo-β-lactamase. From these 23 outbreaks, seven recognized by KORLD were caused by bacteria-producing enzymes NDM-1. Apart from these cases, 51 transmissions of *K. pneumoniae* MBL-type colonisation took place [53].

Summary and conclusions

Bacteria belonging to the *Enterobacteriaceae* family, having the bla_{NDM} gene in their genome, are a growing problem not only in Poland but also throughout the world. The spread of multiresistant strains whose genes determining antibiotic resistance are located on plasmids is particularly worrying. Therefore, it is necessary to constantly try to discover new substances that inhibit or eliminate the effects of carbapenemases, such as their inhibitors, and new substances with bactericidal activity. In stopping the spread of antibiotic-resistant faecal sticks, hygiene, frequent hand washing, strict compliance with sanitary standards, and the responsibility of all who have contact with antibiotics are extremely important.

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Mikołaj Kamiński, Tomasz Głowacki, Daria Koczara, Zbigniew Fabiszewski

Department of General Surgery with subdivision of Urology, District Hospital in Oborniki, Poland

Angioedema of the small intestine in a 28-year-old woman

A 28-year-old obese women visited the emergency department with dyspnoea and umbilical abdominal pain intensifying for two weeks. The patient suffered from hereditary angioedema type (HAE) I. Three months earlier the woman delivered a child by caesarean section. The patient denied diarrhoea, constipation, or vomiting. A duty doctor administered the patient's plasma-derived C1-inhibitor intravenously. The dyspnoea withdrew, and abdominal pain was slightly relieved. The woman went home but returned after seven hours. The abdominal pain persisted. The patient was consulted by gynaecologists, who observed an excessive volume of fluid in the peritoneal cavity in vaginal and abdominal ultrasound. Besides that, no gynaecological abnormality was observed. White blood cell count was 17,000 per cubic millimetre and C-reactive protein (CRP) was 37 milligrams per cubic decimetre. Blood pressure, heart rate, and respiratory rate were within normal range. No physical signs of hydrothorax, obturation of respiratory tract, ascites, or bowel obstructions were presented. The patient received analgesics.

CT scan of the abdomen and pelvis revealed oedema of the small intestine and excessive fluid in the peritoneal cavity (Figure 1). The abdominal pain was diagnosed as angioedema of the small intestine. A second dose of C1-inhibitor was administered. After one hour the patient experienced relief of pain. The patient had never experienced abdominal attacks of angioedema before. The woman was observed for 48 hours and was discharged without complaints. On the day of discharge her white blood count was equal to 6.6 per cubic millimetre and CRP was 14 milligrams per cubic decimetre.

Discussion

Angioedema is a consequence of an episode of increased capillary permeability with extravasation of intravascular fluid and subsequent oedema. HAE of the small intestine appears as a mural and mucous thickening with fluid accumulation in the lumen but rarely with signs of ileus [1]. This case has several aspects worth detailed discussion. Firstly, abdominal attacks of hereditary angioedema tend to be self-limiting [2]

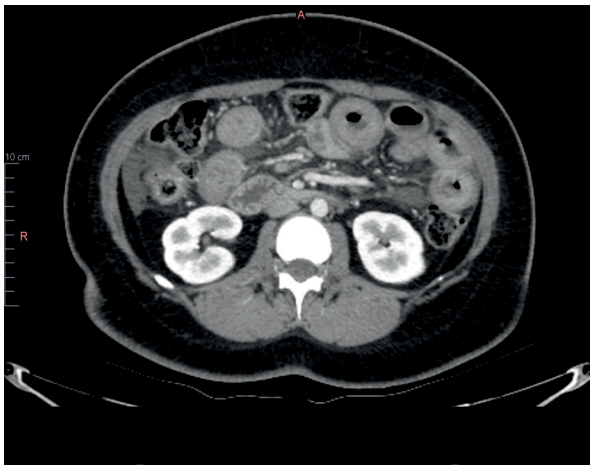

Figure 1. CT scan of the abdomen of a 28-year-old women with hereditary angioedema type 1. Arterial phase. **See Supplementary files:** Full CT scan of the abdomen of a 28-year-old women with hereditary angioedema type 1. Arterial phase

Corresponding author:

Mikołaj Kamiński, Department of General Surgery with subdivision of Urology, District Hospital in Oborniki, Poland, Szpitalna 2 Str., 64-600 Oborniki, Poland, e-mail: mikolaj.w.kaminski@gmail.com

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and sensitive to C1q inhibitor concentrate administration [3], while in the described case the attack lasted for two weeks and did not resolve 12 hours after the first dose. Secondly, the patient history did not reveal any potential triggers of the exacerbation. However, we hypothesise that the reconstitution of the female hormone levels after the postpartum period may lead to exacerbation of the disease [4]. The leukocytosis and elevated CRP serum level are uncommon in HAE attacks, but some patients present increased inflammatory markers regardless of different conditions [5, 6]. After 48 hours the white blood count of the patient dropped to 6.6 per cubic millimetre and CRP to 14 milligrams per cubic decimetre. Therefore, we concluded that HAE attack was the reason for elevated leucocytes and CRP in the case. The patient experienced significant relief of abdominal pain and did not disclose any ailment. Moreover, we observed that the subsequent decrease of white blood count and CRP and other parameters were in the normal range. Therefore, we decided not to perform a control CT scan. Importantly, patient awares of the malady equipped with the specific drug can be diagnosed and treated even in a district hospital. Nevertheless, the care of this patient required careful diagnosis and the cooperation of different specialists.

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