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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

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ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.2018.269

Baby-Led-Weaning (BLW) from maternal perspective: Polish experience

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ABSTRACT

Introduction. Baby-Led-Weaning (BLW) is increasingly popular as a complementary feeding practice although its safety, limitations and advantages have not been widely studied as yet.
Material and Methods. The present survey employed an anonymous online questionnaire to learn from experience (their concerns, perceived advantages, disadvantages, and overall satisfaction) of Polish mothers (n = 373) that adopted BLW.
Results. Most of surveyed mothers adopting BLW had tertiary education and good economic status, and inhabited urban areas. Non-scientific online resources were the most important source of knowledge on BLW; none of surveyed mentioned healthcare professionals as having played any role in this respect. The risk of choking was the greatest concern expressed while considering the BLW prospect. At least one choking event during B:W adoption was reported by 55.6%, mostly involving an apple, occurring at beginning of introduction, and perceived as non-serious. The BLW advantages included: (i) promotion of infant self-reliance, motor skills, biting and chewing of food and speech), sensory learning of food, and (ii) motivation to eat family meals and make more healthier dietary choices. The greatest disadvantage was an in-house mess. Nearly all mothers recommended the BLW adoption to other caregivers.
Conclusions. In view of the scarcity of data on this feeding practice, the maternal experience demonstrated in the present study may offer valuable information for health professionals as well as future caregivers who consider the adoption of BLW to be a complementary feeding practice.

Keywords: Baby-Led-Weaning, complementary feeding practice, maternal experience, online survey.

Introduction

Infant feeding practices raise discussions among parents and paediatricians as their guidelines have changed several times over the last 60 years [1]. Currently, WHO and UNICEF unanimously recommend exclusive breastfeeding for the first 6 months of life followed by nutritionally-adequate and safe complementary (solid) foods together with continued breastfeeding up to 2 years of age or beyond [2, 3]. It has, however, been shown that in certain infant groups introduction of complementary feeding prior to 6 months may be beneficial [4]. Nevertheless, it is generally advised to gradually increase food variety and consistency to decrease the risk of choking, allergic reactions and other unwanted effects [1, 5].

Traditional complementary feeding practice requires parents or caregivers to decide entirely on amount, type and consistency of food given to infants, and actively assist at spoon feeding, particularly during the first months of its introduction. Contrary to this approach, an alternative strategy, established in 2003 by a British midwife Gill Rapley, as "Baby-Led Weaning" (BLW; also referred to as "self-feeding"), allows an infant to have a greater control over its feeding from the onset of

complementary feeding by being offered a range of whole pieces of solid foods, preferably from the family meal, eaten by hand and of its own choice [6]. Over the last decade BLW has been anecdotally reported to increase in popularity. According to Google Trends data (available online at trends.google.com), the number of online hits related to this feeding practice in the Google search engine is continuously growing, and over 700,000 total records by September, 2017 it appears that BLW is gaining attention. However, a systematic search for English language articles published up to early 2017 in the MEDLINE/PubMed database with key term "baby led weaning" yielded less than 30 items of which some were review papers [7–9], one was a description of a randomized controlled trial yet to be conducted [10], one reported the results of a clinical trial on choking risk [11], and two were commentaries [12, 13]. Other have described the maternal experience with BLW, attitudes of healthcare professionals or assessment of energy and nutrient intakes although the studied groups varied in size, and were mostly small; the need for further investigations on BLW was repeatedly indicated [14–18].

Learning from the maternal perspective may be valuable in understanding the main risks, disadvantages and advantages of BLW, and further to develop efficient support from health professionals in mothers' decision-making on infant feeding. In this context, BLW has been studied rarely and mostly on small sample sizes [16, 17, 19]. This study surveyed a group of Polish mothers (n = 373) adopting BLW, characterized it, and assessed its overall experience and accompanying feelings, fears and satisfaction. BLW has been promoted in Poland since 2011 following the translation Gill Rapley book. The experience of Polish mothers with BLW has not yet been the subject of any previous study.

Material and Methods

The study assessed the overall maternal experience with BLW using a self-report, anonymous questionnaire. The inclusion criteria included: Polish citizenship, adoption of BLW regardless of whether it was adopted consequently or with temporary exceptions. The questionnaire examined:

- the mother's intention(s) behind the BLW adoption,
- main sources from which mothers learned about BLW,
- the mother's fears (if any) that accompanied the BLW adoption of BLW,
- the attitude of relatives (family and friends) towards the BLW adoption,
- the experience of the mothers with BLW as regards consistency in its adoption,
- the occurrence of adverse events,
- main advantages and disadvantages of BLW as identified by mothers.

The questionnaire also assessed the demographic characteristics of each mother (age, education, economic status, place of living). The online survey was undertaken during a period of one year (June 2015 – July 2016). The invitation to complete the questionnaire were frequently posted on Polish parental online message boards and websites.

Results

Demographic characteristics

The demographic characteristics of the polled group (n = 373) are presented in **Table 1**. The vast majority had completed their education at a tertiary level, inhabited urban areas, and had good economic status.

Table 1. Demographical characteristics of Polish mothers enrolled in the study

Characteristic	n = 373
Age	
Mean (years ± SD)	29.8 ± 3.71
Median (range) years	30 (20-43)
Place of living *n* (%)	
Urban > 100,000 residents	221 (59.2)
Urban 50,000 – 100,000 residents	27 (7.2)
Urban 10,000 – 50,000 residents	52 (13.9)
Urban < 10,000 residents	22 (5.9)
Rural	51 (13.7)
Education *n* (%)	
Primary	0 (0)
Secondary	55 (14.7)
Tertiary	316 (84.7)
Vocational	2 (0.5)
Income status *n* (%)	
Very good	71 (19.1)
Good	176 (47.2)
Satisfactory	118 (31.7)
Poor	8 (2.2)
Very poor	0 (0)

Motivations to adopt BLW and knowledge sources

The main motivation to adopt BLW by studied group included plain curiosity (51.5%), an infant showing a lively interest in solid foods eaten by adults (43.5%), and the conviction that this feeding practice is associated with health benefits for child development (38.3%; **Figure 1A**).

The vast majority of women (98.3%) indicated non-scientific online resources to be the source of information on BLW (**Figure 1B**). A significant number (72.8%) also indicated books, and less often – family and friends. Only a small percentage had read scientific sources (e.g. publications from peer-reviewed journals) while none of them indicated health professional (e.g. paediatrician, dietician) as a source of information on this feeding approach (**Figure 1B**).

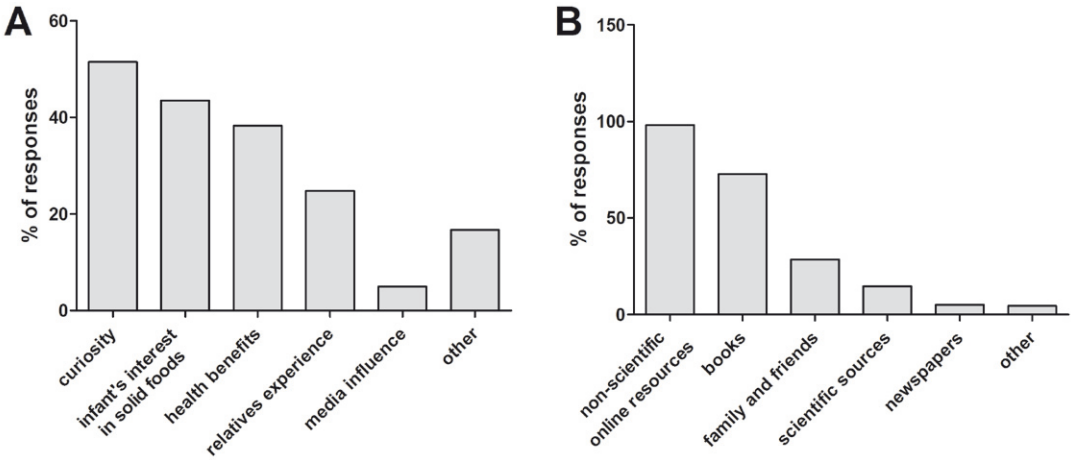

Figure 1. Motivation to adopt BLW (A) and sources of information on this feeding practice (B) among the studied mothers (n = 373)

Experiences with BLW

The vast majority of mothers (85.3%) introduced BLW between 6 and 9 months of their infants' lives. BLW was introduced before 6 months and after 9 months by 9.4 and 5.4%, respectively. As reported, most children (93.8%) were highly interested in solid foods when BLW was introduced. Nearly one-third (29.5%) of mothers declared that they had adopted BLW consistently while the rest (70.5%) adopted it with slight and temporary exceptions, namely spoon-feeding soup and yogurt at home, and spoon-feeding in restaurants and other public places.

The possibility of choking was a major fear (68.4%) that was present during BLW adoption (**Figure 2A**). In fact, 55.6% of mothers reported that their children experienced at least one event of choking with solid food, particularly during the first weeks of BLW adoption. The food involved in such events included fruits (46.0%), mostly an apple (35.4%), cooked or raw vegetables (31.7%; usually a carrot – 43.1% or a piece of broccoli – 23.5%) and less often, bread (13.0%). Choking was more frequently reported by mothers adopting BLW before 6 months than afterwards (62.9 *vs* 42.4%; $p < 0.05$; Pearson chi-square test). None of these events was reported to be serious and the surveyed mothers viewed them as a normal step in the child's education in self-feeding. No other adverse events related to BLW adoption were reported by the polled group. Nearly one-third of mothers (29.2%) feared that BLW could lead to undernourishment (**Figure 2A**) although problems in weight gain were reported only by 2.7%.

As many as 71.6% of mothers faced some form of criticism and negative attitude towards BLW from their relatives, family and friends. Again, the concern that was most often expressed was the risk of choking (59.6%). The other BLW disadvantage seen by relatives included the generation of in-house mess (30.0%), and risk of undernourishment (38.0%). Some relatives also indicated that BLW is unsuitable for such young children (18.4%) and that this approach generates large food waste (12.9%) (**Figure 2B**).

The majority of the surveyed mothers (87.1%) declared that their children were very interested in new food during BLW adoption. Nearly all of them (97.3%) reported that their children willingly ate meals together with the rest of the family. A number of advantages related to the BLW approach

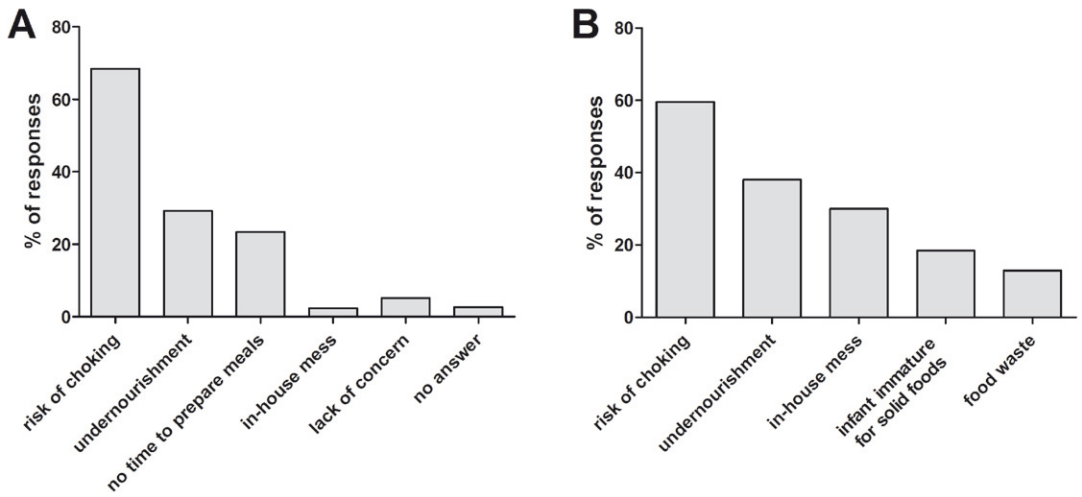

Figure 2. Fears and concerns associated with adoption of BLW expressed by mothers (A) and their relatives – family and friends (B) (n = 373)

Table 2. Advantages of BLW as reported by the mothers surveyed in the present study (n = 373)

Reported advantages of BLW	% (n)
Supporting child self-reliance	176 (47.2)
Supporting child development	117 (31.4)
Eating family meals together	103 (27.6)
Supporting child decision making	94 (25.2)
Supporting sensory learning of food	85 (22.8)
Supporting healthier and more diversified diet	79 (21.9)
Supporting interest in food and new flavors	57 (15.3)
The joy which child has during eating	49 (13.1)
Child eats the same food as adults	42 (11.3)
Giving child a sense of trust	24 (6.4)
Possibility to feed child in non-stressful way	24 (6.4)
No answer	40 (10.7)

were identified (**Table 2**). The most often indicated included promotion of self-reliance during a meal and independence in food choices. Over 30% indicated that BLW supports child development including manual and motor skills, biting and chewing of food as well as speech. In view of mothers, BLW promoted sensory learning of food by involving not only taste but also smell and touch. A relatively high share of mothers indicated that BLW is a way to experience shared family meals which motivate adults to make more balanced, more diversified, well-thought-out and healthier dietary choices (e.g. by including fresh, unprocessed, seasonal foods; decreased salt and sugar free consumption). It is worth noting that nearly all mothers surveyed in the present study (97.6%) would recommend BLW to other parents as a complementary feeding practice.

Nevertheless, the surveyed group also pointed to some disadvantages of BLW, the in-house mess that an infant usually generates while eating being a major one. This was often accompanied by dirty clothes and a need for their more frequent washing, and often baby bathing. Nearly 10% of mothers expressed the view that there were no disadvantages to this feeding practice (**Table 3**).

Table 3. Disadvantages of BLW as reported by the mothers surveyed in the present study (n = 373)

Reported disadvantages of BLW	% (n)
Generation of in-house mess	186 (49.9)
Possibility of chocking	46 (12.3)
Time-consuming	23 (6.2)
Lack of public acceptance of BLW	21 (5.6)
No answer	49 (13.1)
No disadvantages	35 (9.4)

Discussion

This study has provided an insight into the world of Polish mothers practicing BLW, identified their basic demographic characteristics, described their experience with BLW and indicated its benefits, fears and disadvantages from their own perspective. In view of the scarcity of data on BLW, the maternal experience may offer valuable information for health professionals and future caregivers who consider the BLW adoption

Considering the growing interest in BLW [19], it is important to deliver accurate information to parents interested in alternative practices of

infant feeding. Unsurprisingly, the non-scientific Internet websites were found to be the most important source of information on BLW. Their influential role in diet promotion has already been documented [20, 21]. The surveyed individuals also anticipated information from scientific literature – over 10% had already used them to learn about BLW. This advocates the need to disseminate the research results, e.g. by Open Access publishing mode. Through valuable free, full-text, online resources, Open Access greatly enhances the possibility to accurately communicate science to the general public and health professionals.

As demonstrated previously, healthcare professionals can and should play a significant role in decision-making on breastfeeding and introducing solid foods [22, 23]. Strikingly, not a single mother surveyed in our study indicated that a medical specialist or dietician had played any role in this respect. This further highlights the urgent need to increase awareness of BLW among different paediatricians and dieticians in Poland, for the benefit of future mothers and their children. As found in a mini-survey conducted in New Zealand, some healthcare professionals had concerns about the risk of iron deficiency, inadequate energy intake and choking, and as a result most felt reluctant to recommend it [16]. A recent investigation revealed that infants following BLW (n = 25) had a lower intake of iron, zinc and vitamin B12 than those on traditional spoon-feeding (n = 26) although energy intake was similar and there was a higher fat intake [18]. These observations still require assessment through further studies, preferentially randomized clinical trials or cross-sectional investigations on a larger sample size.

It appears that the risk of choking is one of the greatest concerns related to the BLW adoption. In our study this was expressed by both mothers and their relatives. Although more than half of mothers actually found their children to choke at least once, none of those events were reported to be serious. As recently shown using randomized controlled trials, infants that follow BLW do not appear more likely to choke than infants that follow traditional feeding practice [11]. Moreover, the mothers surveyed in our study admitted to treating these choking events as a natural step by which a child learns how to eat solid foods.

Undernourishment was the other important risk that mothers feared while adopting BLW and which also formed part of negative attitude expressed by their relatives. It should be noted that the surveyed mothers did not report this issue as a BLW disadvantage, indirectly indicating that in their opinion, the children were fed appropriately. As shown in other study, weaning style may have an important impact on childrens' food preferences and growth. BLW has been shown to promote regulation of food intake better when compared to spoon-feeding, leading to lower BMI, and preference for carbohydrates rather than sweets [24]. Other study demonstrated that infants weaned by BLW were significantly more satiety-responsive [25]. Considering that childhood obesity related partially to excessive free sugar consumption is becoming a worldwide health issue [26], BLW may potentially represent a strategy to lessen its devastating consequences. As declared by some mothers surveyed in our, BLW adoption supported a healthier diet for the whole family by decreasing salt, free sugars and processed food consumption. This aspect would be worth further investigations on a more objective level. A previous study found only a low percentage of individuals underweight (< 5%) in children fed by BLW [24]. This indicates that fears that BLW may lead to insufficient nutrient supply expressed by healthcare professionals [16], mothers and their relatives may be unsupported. As suggested , BLW allows for a gradual transition to solid foods, in the childrens' own time and at their own pace. In fact, nearly half of the respondents in our study noted that BLW promotes childrens' self-reliance [9].

Nearly one-third of respondents declared that BLW promoted eating family meals together and vast majority of mothers also noted that their children ate basically the same food as adults. This is an entirely different pattern in comparison to the traditional approach in which parents are forced to spoon-feed and often cannot eat at the same time. BLW promotes eating meals together not only because of the fear of an infant choking and the necessity to carefully supervise but also because of the chance afforded to the infant to observe its parents may be supportive in acquiring new motor skills [16]. A number of benefits of eating family meals together have been evidenced including improvement of psychological well-be-

ing and healthier diet patterns [28, 29]. The frequency of family meals, due to various reasons, is likely to decrease in some regions [30, 31]. Eating a meal together with an infant may be difficult to coordinate on a daily basis but BLW adoption may somewhat enforce this and provide potential benefits for the whole family.

The surveyed mothers indicated at high frequency that BLW promotes child development, specifically manual and motor skills, biting and chewing, and speech. It is unknown whether BLW may actually be more efficient in this aspect compared to traditional practices. It is worth further investigation, specifically using a clinical trial approach with a considerable number of recruited and compared infants due to the large number of factors implicated in reaching developmental "milestones" [31].

Although the study provides some valuable information regarding maternal experience with BLW, it also have some limitations, therefore its data should be interpreted cautiously. The study did not assess the frequency at which BLW is being used by Polish parents; this would require cross-sectional investigations on a population level. Although a relative high number of mothers was surveyed, the study was limited only to one country. Moreover, benefits of BLW were self-reported and while some provide important observations, those particularly related to child development would require further confirmation on a clinical level.

Conclusions

In the opinion of the surveyed mothers the pros of BLW outweighs the cons, and nearly all of them would recommend its adoption to other parents/caregivers. There is a need for further investigations, preferably randomized trials, that would focus on the involvement of BLW in promotion of infant development, and supporting family to eat meals together with its potential social and health beneficial outcomes. Considering the increase in BLW popularity, there is a need for healthcare professionals to serve as a reliable source of information on this feeding practice

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Histological evaluation of kidney development in young rats after a surgery and management of wound with fibrin glue

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ABSTRACT

Aim. The subject of this study is evaluation of histological hallmarks of kidney growth in rats after a surgery and subsequent management of the wound with fibrin glue.
Material and Methods. Sixty-five Wistar rats underwent a surgical procedure resulting in parenchymal injury by incision followed by an application of fibrin glue. Kidneys have been examined and evaluated histologically after 4 weeks (group I) and 26 weeks (group II) after the surgery
Results. When fibrin glue was used, in both groups, regular anastomoses of the wound edges of the kidney after surgery were noted. Histological features of kidney growth (renal glomeruli, renal tubules and blood vessels) were observed in the parenchyma and in the postoperative scar. In the second group kidneys grew on average by 3.5mm in the longitudinal section and by 1.89 in the cross section. In this group, the number of renal glomeruli nearly doubled. Glomeruli and tubules were also found in the postoperative scar.

Keywords: kidney, histology, surgery.

Introduction

Because good adhesive and haemostatic properties [1–4], fibrin based glues are used in surgical management of parenchymal organs in elective surgeries and post-traumatic wounds. Tissue adhesion occurs via activation of physiological haemostatic pathways. As a result, the inflammatory reaction around the wound is minimal, which shortens the healing period. In the end deposited fibrin is completely resorbed [5–7]. In this study Beriplast glue by Behring Company was used. It is a two-component adhesive containing fibrinogen, thrombin, Ca^{++} ions, factor XIII and fibrinolysis inhibitor. It causes fibrinogen to convert into fibrin with clot formation. Thrombin activates factor XIII, which catalyses the process of bonding fibrin polymers and clot stabilisation [8, 9].

We chose to study wound healing properties of fibrin glue in kidneys surgeries because they are technically difficult and carry a high risk of complications [10–12].

Additionally, interesting question was how histological structure of kidney is affected by

surgery and application of fibrin glue in developing organs. We have found that fibrin glue not only shortens the healing time of the wound, but also enables a regular development of the organ.

Aim

The aim of this work was to evaluate histological changes in developing rat kidney in rats that underwent a surgery and had the wound treated with fibrin based glue.

Material and Methods

A total of 65 Wistar rats, both male and female, aged 5–7 weeks, weighing 43–57g were chosen for the study. The research was approved by Bioethics Committee.

Surgery

All animals underwent a surgery of bipolar incision of the renal parenchyma. The resulted wound was treated with Beriplast FS fibrin glue (CSL Behring, King of Prussia, PA). Rats were anaesthetised with inhalatory ether. The limbs were secured with tape. Anaesthesia was maintained by administering drops of ether onto a mask of cotton wool. The shaved skin of the abdomen was disinfected with water solution of Hibitan. The abdominal cavity was opened with a left flank cross incision below the coastal margin. In order to secure skin flaps, two stitches were placed on the edges of the skin. This provided free access to the kidney. Left kidney has been visualized by moving away the intestinal loop. Subsequently, kidney was rolled up to the level of the lining of the stomach and put on a wet gauze pad. A two millimetre deep longitudinal incision of parenchyma along the side edge from upper towards lower pole was performed with a surgical knife.

After brief separation of the incised kidney, fibrin glue was applied to the bleeding surface of the parenchyma. The edges of the cut were brought together and lightly pressed with a gauze pad for about 30 seconds.

The animals were sacrificed and kidneys were harvested for histological examination 4 weeks after the surgery (group I) and 26 weeks (group II).

Histological examination

Examination of rat kidneys fixed in 10% buffered neutral formalin was carried out according to the routine histological technique. The specimens were embedded in paraffin, cut at 3–5 μm thickness using a Leica microtome and mounted on microscope slides. The sections were stained with haematoxylin and eosin.

Photographic documentation of the whole kidney, 40-fold magnified, was made using an Olympus BX 43 microscope and a XC 30 digital camera. Using computer software cellSens (Olympus), calibrated with the microscope, renal glomeruli were counted, and the postoperative scar was measured at the base in the cortex of the kidney.

Statistical analysis

Following parameters were calculated, for each of the testing group: average values, standard variance, minimal and maximal values.

All experimental results were analysed with Shapiro-Wilk method for assessing normal distribution. When the variant did not show normal distribution, a nonparametric test of Mann-Whitney test was used.

Significance of the statistical analysis results was set as $p < 0.05$.

The above analyses were performed using *Statistica 12* (Statsoft, Inc.) software.

Results

Group I

In all animals the wound healed. Average width of the scar, measured at the base of the cortex of the kidney was 1167.155 μm. In the bonding area lymphocytic infiltration was observed, with hemosiderin deposits. In 8 animals apart with lymphocytic infiltration, foci of parenchymal necrosis were visible. Presence of blood vessels in the scar was observed (**Table 1**).

Group II

In all animals the wound healed. Average width of the scar, measured at the base of the cortex of the kidney was 764.77 μm. In the area of the scar glomeruli and tubules were found. Proliferation of blood vessels was also visible in this area. Small foci of lymphocytes were observed in the scar or its proximity (**Table 2**).

Table 1. Descriptive statistics of results in Group I

	Group I				
	N	Average value	Minimum	Maximum	Std variance
Number of glomeruli	33	118.545	98.000	151.000	11.832
Longitudinal section (cm)	33	6.485	6.000	7.000	0.507
Cross section (cm)	33	4.455	4.000	5.000	0.506
Scar width (μm)	33	1167.155	688.580	1454.870	217.348

Table 2. Descriptive statistics of results in Group II

	Group II				
	N	Average value	Minimum	Maximum	Std variance
Number of glomeruli	32	212.344	159.000	247.000	22.075
Longitudinal section (cm)	32	10.000	8.000	11.000	0.622
Cross section (cm)	32	6.344	6.000	7.000	0.4823
Scar width (μm)	32	764.779	539.510	1100.640	150.265

Discussion

Fibrin glue is commonly used in laparoscopic procedures and in partial nephrectomy surgeries [13, 14].

Results of our research showed that when fibrin glue was applied to surgical wounds in the experimental model of parenchymal injury the organ development continued normally and histological structure has been maintained.

Based on the calculations of macroscopic length of longitudinal and cross sections of the kidney, the difference in both groups studied is statistically significant.

In animals examined 26 weeks after the incision of renal parenchyma, the scar was significantly thinner than in the group 4 weeks after the surgery. The kidney in those animals was also bigger. A larger number of glomeruli and tubules was also observed. The difference in the number of glomeruli between both groups was statistically significant ($p < 0.05$). The surgery did not result in disruption of kidney development.

It should also be noted that in both groups the markers of inflammation in the scar were visible. However, in the group examined 26 weeks after the surgery the histological findings were indicating less intensity of the inflammatory process.

In group II in the area of the scar, regular glomeruli, tubules and blood vessels appeared. No inflammation of the scar or renal interstitial fibrosis were observed. Similar results regarding the presence of renal tubules were presented by Wang PF and co-authors. The authors noted the presence of additional tubules on the 21st day after the surgery in rats [15].

The results obtained indicate that the surgery did not have an adverse effect on the development of the kidney in general and its gross microscopic structure. Despite of concern for haemorrhage in a well-vascularised organ as kidney no macroscopic or microscopic events were observed. This may be secondary to beneficial haemostatic properties of fibrinogen glue.

Conclusions

1. In all subject animals, when fibrin glue was used for bonding of experimental surgical parenchymal wound, the observed scar edges were regular.
2. The development of the kidney was not disturbed, which was confirmed by macroscopic and microscopic studies.

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The ability to perform a post-traumatic examination as an indicator of the effects of teaching emergency medicine at the successive stages of the educational process: medical students, trainee physicians

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ABSTRACT

Introduction. Emergency management at the accident site is often of key importance for the later fate of patients who have sustained severe injuries. The scheme for post-traumatic examination has been developed to improve dealing with trauma patients.

Aim. The aim of this study was to analyse the effects of teaching emergency medicine to students and graduates of the Faculty of Medicine in Szczecin, on the basis of their ability to carry out post-traumatic examination. The skills at recognizing a direct threat to life and performing basic life-saving procedures were appraised.

Material and Methods. The study involved 81 individuals, who were evaluated three times at different stages of their educational process. Groups I and II comprised of fifth year students before and after an emergency medicine course, and group III consisted of trainee physicians. The Laerdal MegaCode Kelly manikin was employed in the project. Each of the simulated patients had the same external injury symptoms and parameters of vital functions.

Results. Evaluation of vital functions was correctly done by 14.8% of group I, 59.3% of group II, and no one in group III. A quick post-traumatic examination was performed properly by 11.2% of group I, 55.5% of group II, and no one in group III.

Conclusions. Group I lacked the ability to perform post-traumatic examination and first aid procedures. Participation in emergency medicine courses had positive effects on the participants' skills (group II). The ability of trainee physicians (group III) to perform the majority of the tested elements of post-traumatic examination, including first aid procedures, noticeably declined and reached the initial level.

Keywords: emergency medicine, post-traumatic examination, medical students, postgraduate trainee physicians, teaching effects.

Introduction

Implementation of the appropriate procedures for the management of trauma patients is an important element of healthcare workers' services. A shortening of the time spent by the emergency team on the spot of an accident is critical for survival of a patient, and necessary for the 'golden

hour' rule to be applied. In prehospital care, we can even talk about 'platinum ten minutes', during which the emergency team should identify patients, make diagnostic and therapeutic decisions, and start transport to hospital. Elements that reduce the time on the accident site include a properly performed examination of a patient, identification of all life-threatening injuries, and execution of procedures that save life and sustain vital functions. To improve the management of trauma patients, the scheme for post-traumatic examination has been developed, showing step by step how it should proceed and what emergency procedures should be involved. A fundamental part of post-traumatic examination is the monitoring of basic vital functions (consciousness, airway patency, breath, blood circulation). Therefore, its methodology not only makes it possible to verify the way of carrying out the post-traumatic examination, but also to test the ability to recognize a direct threat to life and perform basic life-saving procedures [1]. Medical skills in first aid and first aid from a qualified practitioner are crucial for giving aid in each situation of a direct threat to life, irrespective of whether it is caused by the consequences of an injury or a sudden cardiac arrest produced by other factors. All decisions must be then made very quickly, and so the skills needed must be well-learned and repeatedly practiced. A lack of them results in delayed recognition of respiratory and/or cardiac arrest, and the late onset of cardiopulmonary resuscitation. Each educational process should be thoroughly checked and evaluated, both during the process and after it, and the results of such evaluation should serve as a basis for drawing conclusions about the potential changes (for example, a teaching manner). Appraisal of the effects of teaching emergency medicine to medical students is a difficult task. It seems that the first instrument to determine the level of knowledge gained during studies and postgraduate residency training is the Physician Final Exam.

Aim

The aim of this study was to assess the effects of teaching emergency medicine to students and graduates of the Faculty of Medicine, the Pomeranian Medical University in Szczecin on the basis of their ability to carry out post-traumatic examination as an emergency procedure of choice. Versatility of this skill is the reason why it was used to verify the effects of the educational process. Since the monitoring of basic vital functions (consciousness, airway patency, breath, blood circulation) is a fundamental part of post-traumatic examination, we checked if the fifth year medical students and trainee physicians had the skills needed to identify an imminent threat to life, and whether they were able to perform basic life-saving procedures, just before the completion of their postgraduate residency training.

Material and Methods

The project involved 81 individuals, who were evaluated thrice at different stages of the educational process. The participants were denoted as group I, group II, and group III, depending on which stage they were evaluated. Group I consisted of fifth year students before starting the course in emergency medicine, group II included the same fifth year students after the completion of the course in emergency medicine, and group III comprised of trainee physicians — graduates of the Pomeranian Medical University in Szczecin who 2–2.5 years earlier were the members of groups I and II — being just before the completion of their postgraduate residency training. The study was to verify the ability of the participants to perform preliminary emergency management of a patient who has sustained numerous life-threatening injuries as a result of an accident. All participants were randomly assigned to one of four scenarios of the event. The simulated patients were: (1) a 25-year-old woman knocked down by a car, (2) a 30-year-old man battered with a baseball bat, (3) a 40-year-old man after a fall from the second floor, and (4) a 50-year-old woman after a fall from a height of six meters. The Laerdal MegaCode Kelly manikin was employed in the project. Despite different injury patterns, each of the simulated patients sustained the same injuries, resulting in identical external symptoms and the same parameters of vital functions in post-traumatic examination, namely unconsciousness and no reaction to stimuli (AVPU = U — *unresponsive*), the number of breaths per 10 seconds: 1 breath (6 breaths per 1 minute), pulse: 23 beats per 10 seconds

(140 beats per 1 minute), pulse wave perceptible on both the carotid and ulnar arteries, overfilled carotid veins, no respiratory murmur at the right side of the chest, a tympanic percussion note at the right side of the chest, pelvis unstable on palpation, the right shin fracture, and a laceration on the back. The participants' task was to perform a full post-traumatic examination according to the scheme, starting with the assessment of the accident site in terms of the threats posed to the emergency team, and evaluation of the mechanism of an injury. The next element was a preliminary examination of the patient's state, which included approaching an injured person and stabilizing his/her head with hands; assessing the level of consciousness according to the AVPU scale; restoring airway patency; measuring breath, and feeling the pulse. What was also taken into account was whether all components of post-traumatic examination were executed according to the following scheme: examination of the head, neck, chest, abdomen, pelvis, lower and upper limbs, and back. Furthermore, the participants were expected to identify injuries critical for the injured person's life. These included right-sided pneumothorax, unstable pelvic ring fracture, fractures of both right shin bones, and a laceration on the back. Another element gauged in this study was the provision of the patients with emergency medical treatment essential for their safety. Due to the type of injuries, it involved putting on a cervical collar to stabilize the cervical spine, the right oxygen therapy (active oxygen therapy with the use of a self-reinflating bag with a reservoir of oxygen) and maintenance of airway patency by means of mandibular protrusion and/or pharyngeal intubation, decompression of pneumothorax, and lifting patients on a separable-type scoop stretcher, putting patients on a spinal board, fastening patients to a spinal board with stabilizing belts, and putting on head stabilizers. These emergency procedures should be performed in the above-mentioned order. Attention was also paid to the execution of medical procedures regarded as unnecessary in the initial stage of giving aid, such as measuring blood pressure with a manometer, neurological examination beyond assessing the level of consciousness, preparation of the vascular access or intratracheal intubation. These procedures should be done at the successive stages of medical assistance. The results were statistically analyzed by means of Statistica PL version 10.0 (StatSoft Inc.), USA (2011) (www.statsoft.com., AXAP502C295820AR-K license). In this study we analyzed dichotomus qualitative (or discrete) variables. Differences between the groups expressed as a difference of fraction were analyzed using Pearson's chi-square test for independence, McNemar's test for paired data, and the fraction comparison test. The level of statistical significance was set at $p < 0.05$.

Results

The accident site was correctly assessed in terms of the emergency team's safety, the number of the injured individuals, and the need for additional means and forces by 15% of the participants in group I, 82% of those in group II, and 14% of those in group III ($P_{I–II;\ II–III} < 0.0001$; $P_{I–III}$ = n.s.). The procedure of approaching an injured person and stabilizing his/her head with hands was correctly performed by 11.2% of group I, 66.7% of group II and 7.2% of group III ($P_{I–II;\ II–III} < 0.0001$; $P_{I–III}$ = n.s.). The level of consciousness according to the AVPU scale was assessed by 92.5% of group I, 62.9% of group II, and 28.6% of group III ($P_{I–II;\ I–III;\ II–III} < 0.0001$). Restoration of airway patency was performed by 22.3% of group I, 96.3% of group II, and merely 14.2% of group III ($P_{I–II;\ II–III} < 0.0001$; $P_{I–III}$ = n.s.). Breath was correctly assessed by the majority of group I (92.6%), and all participants in groups II and III ($P_{I–II;\ I–III} < 0.05$; $P_{II–III}$ = n.s.). Feeling the pulse — which was the last stage of quick evaluation of basic vital functions — was correctly done by 59.3% of group I, 92.5% of group II, and 10.8% of group III ($P_{I–II;}\ P_{II–III;\ I–III} < 0.0001$). The assessment of all four elements — level of consciousness, airway patency, breath, and pulse — performed in the right order enables the monitoring of vital functions. In group I, all four elements were correctly assessed by 14.8% of the participants, three — by 44.4%, two — by 33.4%, and one — by 7.4%. Group II obtained better results: four elements were assessed by 59.3%, three — by 33.3%, and two — by 7.4%. None of the participants in this group assessed only one element. No one in group III assessed correctly all four elements (0%). Three elements were evaluated by 7.14% of the participants, two — by 39.29%, and one — by 53.57% ($P^{\chi 2}{}_{I–II;\ I–III;\ II–III} < 0.0001$).

Table 1 shows the proportions of the participants in all groups who performed particular elements of quick post-traumatic examination correctly. What all groups (especially I and III) found the most difficult was the examination of the neck and the back. Considering that it consists of eight elements, we can say that quick post-traumatic examination was correctly performed by 11.2% of group I, 55.5% of group II, and no one in group III ($P_{I-II;\ II-III} < 0.0001$; $P_{I-III} < 0.05$).

Table 2 shows the results of the execution of necessary emergency procedures performed due to sustained injuries. The parameters of vital functions and clinical symptoms in each of four scenarios were matched in such a way that the patients required the following necessary emergency medical procedures: (-) putting on a collar to stabilize the cervical spine; (-) oxygen therapy; (-) decompression of a right-sided pneumothorax; (-) carrying patients on a separable-type scoop stretcher; (-) putting patients on a spinal board, fastening them with belts, and immobilizing their heads with side stabilizers.

Discussion

The task of the participants at the successive stages of their educational process was to carry out a full scheme-based post-traumatic examination on a randomly chosen simulated patients, to recognize life-threatening states, and to perform necessary emergency procedures. Despite different injury patterns, each of the simulated patients had the same injuries, which resulted in identical parameters of vital functions and external symptoms in post-traumatic examination. Each of the simulated patients required the same emergency medical treatment. Age and sex of the patients had no influence on the course of the examination and interpretation of the results. In our study, 25 points of the scheme for post-traumatic examination were assessed. What was striking in the majority of the tested situations, was a statistically significant difference between the skills demonstrated by fifth year students before (group I) and after (group II) the emergency medicine course. Participation in

Table 1. The results of the execution of the successive elements of quick post-traumatic examination

No.	Examination	Group I	Group II	Group III	Statistical result $P^{\chi^2}_{I-II;\ I-III;\ II-III}$
1	Head	40.8%	88.9%	46.5%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
2	Neck	22.3%	81.5%	14.3%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
3	Chest	70.8%	100.0%	17.9%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
4	Abdomen	44.4%	100.0%	50.0%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
5	Pelvis	41.0%	100.0%	60.7%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
6	Lower limbs	51.9%	100.0%	55.6%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
7	Upper limbs	22.6%	100.0%	37.1%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
8	Back	11.2%	55.5%	0.0%	$P_{I-II;\ II-III} < 0.0001$; $P_{I-III} < 0.05$
The mean proportion of the physicians who performed all elements of post-traumatic examination		39.9%	90.7%	35.7%	$P_{I-II;\ II-II;\ I-III} < 0.0001$

Table 2. Emergency medical treatment essential for the patient's safety

No.	Emergency medical treatment	Group I	Group II	Group III	Statistical result $P^{\chi^2}_{I-II;\ I-III;\ II-III}$
1	A collar stabilizing the cervical spine	81.4%	88.8%	89.0%	$P_{I-II;\ II-III;\ I-III}$ = n.s.
2	Active oxygen therapy and maintenance of the upper airway patency	48.0%	100.0%	71.5%	$P_{I-II;\ II-III} < 0.0001$; $P_{I-III} < 0.05$
3	Decompression of a right-sided pneumothorax	33.4%	92.6%	39.3%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
4	Using a separable-type scoop stretcher	41.0%	100.0%	60.7%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
5	Putting patients on a spinal board, fastening them with stabilizing belts, and immobilizing their heads with side stabilizers	59.2%	100.0%	75.0%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.
6	Procedures regarded as unnecessary in the initial stage of giving aid	59.2%	22.2%	75.0%	$P_{I-II;\ II-III} < 0.0001$; P_{I-III} = n.s.

this course increased the proportion of students who performed post-traumatic examination and assessed basic vital functions correctly. The data concerning group II are similar to those obtained by Skitek et al. in Poznań [2]. The comparison of groups I and II with group III (trainee physicians) revealed that after two years, the ability to perform the majority of the tested elements of post-traumatic examination substantially and statistically significantly declined, reaching again the level noted in group I. The same observation was also made about the ability to perform first aid procedures, consecutive elements of post-traumatic examination, and emergency medical treatment essential for the patient's safety. The results presented and discussed in our study confirm the effectiveness of teaching practical skills in the field of post-traumatic examination. The scheme for post-traumatic examination and practical skills were taught during the emergency medicine course, therefore good results achieved by group II are not surprising for us. An especially worrying aspect is trainee physicians' performance on quick post-traumatic examination just before the completion of a postgraduate residency training program (group III). In this group, many participants made critical mistakes from the point of view of emergency management efficiency. If underlying elements of post-traumatic examination are neglected or performed incorrectly, the results can include improper examination of a patient, false conclusions drawn from the data, and not undertaking essential medical life-saving procedures.

These findings are supported by the study of Dąbrowski et al., who analyzed a population of 72 fourth year medical students of Poznań Medical University and 82 trainee physicians — graduates of this university. Although the study only tested theoretical knowledge (single choice questionnaire), the results obtained were highly unsatisfactory [3].

When starting our research, we could expect that in all three groups, comprised of the same individuals at various stages of the educational process, the knowledge and skills needed to carry out post-traumatic examination will only improve. This assumption seemed even more certain, as the participants continued their education in the sixth year of their studies, and then completed one-year of postgraduate residency training over the whole time of our research. What is more, participation in our project was not a new experience for the individuals in group III, because they had already performed post-traumatic examination as the members of groups I and II. And yet, our expectations have been thwarted. The fact that simulated events took place in a classroom, and an injured person was represented by a manikin may have influenced the participants' attitude to the task. On the other hand, the worldwide teaching of emergency medicine is based on training on technically advanced manikins in simulation laboratories. Medical simulations, which reconstruct a specific physiological process complicated by a disease or an injury, are modern teaching methods. The simulation scenario is decided by the type of complication. The role of a patient is played by a manikin on which all procedures can be performed that are normally executed on a living person. Simulation is thus not only a method of learning, but most of all the way of checking on the ability to provide emergency treatment with no harm to the patient if the procedure is performed incorrectly [4]. Common application of simulators in training programs was preceded by the period of using various types of manikins in the objective system of clinical exams (OSCE) [5]. According to Ali et al. high-quality simulators can substitute for human beings as models of injured people [6]. Taking into account a wide variety of medical procedures — resulting from preliminary diagnosis and post-traumatic examination — that can be trained on simulators, it is difficult to overestimate their role [7–10]. It is probable that those who have not performed medical procedures on manikins during their education, will not perform them in their professional duties either.

Based on our research, we found that neither the number of didactic hours of emergency medicine for medical students, nor the teaching manner are sufficient to achieve educational goals. Postgraduate residency training does not give a guarantee of success either. Both the organization of a didactic process during medical studies, and the form of a postgraduate residency training program require new solutions. To improve the situation, emergency medicine classes for medical students should be divided into competency levels, and conducted at various stages of studies. Students and trainee physicians, during their postgraduate residency training, should

take part in refresher courses in the field of basic and advanced resuscitation techniques used on adults and children [3]. The sense of organizing the training in the field of post-traumatic examination in that way in order to achieve good educational effects is confirmed by the study of Li and Jawaid. An extension of the training program with an additional course, refreshing the previously-taught contents, noticeably improves both theoretical and practical exam results [11, 12]. Nevertheless, according to Grześkowiak et al. even physicians specializing in anesthesiology and intensive therapy have inadequate resuscitation skills, despite the fact that resident physicians take part in refresher courses. This shows that we are still far from normality [13].

Conclusions

1. Fifth year students starting the emergency medicine course (group I) were unable to perform post-traumatic examination and so called first aid procedures correctly.
2. Participation in the emergency medicine course improved the proportion of the procedures performed correctly (group II).
3. The ability of trainee physicians (group III) to perform the majority of the tested elements of a post-traumatic examination, including so called first aid procedures, noticeably declined and returned to the initial level.

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Women and men diagnosed with acute coronary syndrome – sex-related differences

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ABSTRACT

Introduction. Coronary artery disease (CAD) is one of the most important causes of death in both men and women. There are many gender differences among patients with CAD, including risk factors and acute coronary syndrome (ACS) outcomes. The latest reports showed that mortality due to CAD is higher in women than in men. Cardiac biomarkers play an important role in risk stratification and choice of treatment strategy in patients with ACS, however some of cardiac biomarkers show worse sensitivity and specificity in women.

Aim. The aim was to investigate the sex-related differences in patients with acute coronary syndrome and to compare their sex-related risk of in-hospital mortality.

Material and Methods. Single-center study of patients diagnosed with ACS who underwent precutaneous coronary intervention (PCI). Patients were diagnosed and treated according to ESC Guidelines. Statistical analysis was performed using StatSoft Statistica.

Results. Of the 297 patients included in this study, 32% were women and 68% were men. There were 26.94% STEMI patients, 22.22% NSTEMI patients and 50.84% unstable angina patients. Compared with males, females were significant older (68.2 ± 10.6 vs. 64.8 ± 11.0; p = 0.0175). Levels of biomarkers of myocardial injury were significantly lower in women: CK-MB (p = 0.0241), troponin I (p = 0.0417) and CK (p = 0.0035) than in men. Women were less frequently treated with PCI or CABG (p = 0.0016) but the in-hospital outcomes (cardiogenic shock, sudden cardiac arrest or cardiac death) were similar in both groups (p = 0.8557).

Conclusions. Women with ACS were older and have higher incidence of non-ST-elevation ACS than men. In-hospital mortality show no significant difference between genders. Women were less likely to receive invasive treatment.

Keywords: acute myocardial infarction, coronary artery disease, in-hospital mortality.

Introduction

Coronary artery disease (CAD) is one of the most important causes of death in Europe in both men and women [1]. According to the European Society of Cardiology (ESC) guidelines, the definition of acute coronary syndrome (ACS) includes unstable angina (UA), ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) [2, 3]. There are many gender differences among patients with CAD, including risk factors, clinical presentation and ACS outcomes [4]. Statistically women develop CAD ca. 10 years later than men [5], therefore this disease was believed to be 'a men's domain'. One of the reasons for this is the atheroprotective effect of estrogen [6]. However, some latest reports have concluded that mortality due to CAD is higher in women than in men [7], and that women with ACS have higher rates of in-hospital complications and risk of death than men [4]. Less specific symptoms of CAD in women [8] cause

significant diagnostic difficulties. Cardiac biomarkers, such as cardiac troponins and MB fraction of creatine kinase (CK-MB), being important indicators of myocardial injury [9], play an important role in risk stratification and choice of treatment strategy in patients with ACS. Blood tests for these biomarkers in women show worse sensitivity and specificity than in men [10]. Because diagnosis and risk stratification of ACS is more difficult in women, a multimarker approach may be appropriate in female population. Sex-related differences in patients with ACS require searching for a strategy providing better risk stratification and aiding choice of treatment.

Aim

The aim of this study was to investigate sex-related differences in clinical status, biomarker profile, echocardiographic parameters and treatment strategy in patients with ACS and to compare their sex-related risk of in-hospital mortality.

Material and Methods

This study analyzed data from a prospective single-center registry of patients with ACS. 297 consecutive patients were included; they were admitted to hospital between the 1st January and 31st December 2014 and diagnosed with ACS. All the patients were diagnosed and treated according to the ESC Guidelines [2, 3]. The exclusion criteria were end-stage renal disease, severe liver disease and pregnancy. On admission, the patients underwent physical examination, ECG and had blood collected for laboratory tests, and the investigators collected demographic data and medical history. All patients underwent coronary angiography – immediately, if they presented with a persistent chest pain, a significantly elevated troponin concentration and/or ECG changes; or within 24 hours after admission, if not. Pharmacological therapy in accordance to ESC guidelines [2, 3] was initiated in all the patients. The primary endpoint was defined as occurrence of cardiogenic shock, sudden cardiac arrest or cardiac death during hospitalization. This study was conducted according to the Declaration of Helsinki, and it was approved by the Ethics Committee of Poznan University of Medical Sciences.

Continuous data is presented as mean and standard deviation (SD). The patients were divided into three groups according to diagnosis. Both sexes were compared within each of the groups. Statistical analysis was performed using Statistica 10, StatSoft. Probability distribution of continuous variables was tested with Lillefors and Shapiro-Wilk tests, and it was found non-normal for all the variables. Mann-Whitney U test was used for continuous variables with non-normal distribution. Chi square tests were used for categorical variables. The data is expressed as mean values with standard deviation for continuous variables and percentages for categorical variables. A p value of < 0.05 was considered statistically significant for all the tests.

Results

Of the 297 patients included in this study, 96 (32%) were women and 201 (68%) were men. There were 80 (27%) patients with STEMI, 66 (22%) with NSTEMI and 151 (51%) with UA. Men presented significantly more frequently with STEMI, and women with UA. Female sex was associated with a higher incidence of non-ST-elevation acute coronary syndrome (NSTE-ACS) (UA and NSTEMI) (82% vs. 69%, p = 0.0378). Incidence of NSTEMI was similar in both groups **(Table 1)**. Women were older and had significantly higher systolic blood pressure on admission. They were more likely to have lower red blood cells count and hemoglobin level, and higher count of blood platelets. There were no significant differences in body mass index (BMI), heart rate on admission, and comorbidities such as arterial hypertension

Table 1. Incidence of types of acute coronary syndrome (ACS)

Diagnosis	All patients (n = 297)	Men (n = 201) (68%)	Women (n = 96) (32%)	p
STEMI (%)	80	31 (n = 63)	18 (n = 17)	0.0378
NSTEMI (%)	66	22 (n = 44)	23 (n = 22)	
UA (%)	151	47 (n = 94)	59 (n = 57)	

STEMI – ST-segment elevation myocardial infarction; NSTEMI – non-ST-segment elevation myocardial infarction; UA – unstable angina

and diabetes mellitus between genders (**Table 2**). Higher left ventricular ejection fraction (LVEF) (p = 0.0001), smaller end-diastolic diameter of left ventricle (p < 0.0001), smaller diameter of left atrium (p = 0.0001), thinner intraventricular septum (p = 0.0009) and posterior wall of left ventricle (p = 0.0042) were observed in female gender (**Table 3**). Women had significantly lower blood concentrations of troponin I, creatine kinase-myocardial band (CK-MB) and lower creatine kinase activity (CK); but there was no significant difference in concentrations of CK-MB mass between both genders **(Table 4).** In detailed analysis of ischaemic cardiac enyzmes in separate groups of ACS patients, there were no significant differences observed in levels of troponin, CK-MB mass, CK-MB, CK between men and women (**Table 5**). All patients underwent coronary angiography. Of the patients, 66% were qualified for percutaneous coronary intervention (PCI), 4% for coronary artery bypass grafting (CABG) and 30% for conservative treatment. Percutaneous coronary intervention was performed in 142 (70.65%) men and 54 (56.25%) women (p = 0.0016), coronary bypass grafting was conducted more often in men (11 (5.47%) vs. 1 (1.04%); p = 0.0015). Conservative treatment was carried out in 48 (23.88%) men and 41 (42.71%) women (p = 0.0015). Female gender was less likely to receive invasive treatment **(Figure 1).** Women and men did not differ significantly as to endpoint occurrence (6.25% vs. 6.47% respectively, p = 0.86).

Table 2. Baseline characteristics of the investigated patients

	All patients (n = 297)	Men (n = 201) (68%)	Women (n = 96) (32%)	p
Characteristics				
Age (years)*	65.9	65.0 ± 11	68.0 ± 11	0.0174
BMI (kg/m^2)*	27.9	28.0 ± 4.52	27.6 ± 4.21	0.7294
Medical history				
Diabetes mellitus (%)	30.07	29	32	0.7994
Heart failure (%)	28.62	33	20	0.0286
Arterial hypertension (%)	77.7	75	83	0.1613
Clinical presentation				
SBP (mm Hg)*	145 ± 27	142 ± 27	149 ± 27	0.0340
HR adm (bpm)*	74 ± 18	73 ± 18	76 ± 17	0.0689
RBC (10^{12}/L)*	4.66 ± 0.56	4.74 ± 0.58	4.51 ± 0.49	0.0004
HGB (mmol/L)*	8.70 ± 1.09	8.93 ± 1.06	8.23 ± 0.88	< 0.0001
PLT (10^9/L)*	230 ± 73	222 ± 71	249 ± 74	0.0002
WBC (10^9/L)*	9.23 ± 6.13	9.05 ± 3.02	9.61 ± 9.89	0.0906
ESR (mm/h)*	17 ± 18	14 ± 18	23 ± 18	< 0.0001
Glc adm (mmol/L)*	7.43 ± 2.97	7.34 ± 2.70	7.62 ± 3.50	0.4564
Anemia (%)	7.77	8	7.29	0.9850

* mean values and SD
BMI – body mass index; SBP – systolic blood pressure; HR adm – heart rate at admission;; RBC – red blood cells count; HGB – hemoglobin; PLT – platelet count; WBC – white blood cells count; ESR – erythrocyte sedimentation rate, Glc adm – glucose at admission.

Table 3. Echocardiography parameters

Parameter	All patients (n = 297)	Male (n = 201) (68%)	Female (n = 96) (32%)	p
LVEF (%)*	49.85 ± 10.73	48.27 ± 10.62	53.13 ± 10.25	0.0001
LVEDd (mm)*	50.73 ± 6.85	52.2 ± 6.9	47.66 ± 5.63	< 0.0001
LAD (mm)*	40.32 ± 5.93	41.33 ± 6.01	38.20 ± 5.15	0.0001
RVD (mm)*	28.32 ± 4.53	28.89 ± 4.55	27.15 ± 4.28	0.0027
Ao (mm)*	32.35 ± 5.05	33.12 ± 5.17	30.78 ± 4.46	< 0.0001
PW (mm)*	10.6 ± 1.56	10.78 ± 1.70	10.23 ± 1.13	0.0042
IVS (mm)*	10.93 ± 1.58	11.13 ± 1.54	10.52 ± 1.58	0.0009

* mean values and SD
LVEF – left ventricular ejection fraction; LVEDd – left ventricular end-diastolic diameter; LAD – left atrium diameter; RVD – right ventricular diameter; Ao – aortic bulb diameter; PW – posterior wall of left ventricle; IVS – intraventricular septum diameter.

Table 4. Cardiac biomarkers

Cardiac biomarkers	All patients (n = 297)	Male (n = 201) (68%)	Female (n = 96) (32%)	p
TnI (ng/mL)	14.447	18.615	5.611	0.0417
CKMB mass (ng/mL)	22.59	26.06	15.58	0.0779
CKMB (ng/mL)	49.22	56.83	31.29	0.0241
CK (U/L)	326	370	234	0.0035

TnI – Cardiac Troponin I; CK-MBmass – mass concentration of creatine kinase-MB, CK-MB – creatine kinase-myocardial band; CK – creatine kinase.

Table 5. Cardiac biomarkers characteristic among types of ACS and gender

Biomarker	API		p	NSTEMI		p	STEMI		p
	Male	Female		Male	Female		Male	Female	
TnI (ng/mL)	0.081	0.162	0.8433	2.667	7.267	0.3967	56.622	22.165	0.1369
CK-MBmass (ng/mL)	2.57	2.16	0.5707	31.53	27.36	0.8789	71.95	50.72	0.1966
CK-MB (ng/mL)	18.87	18.64	0.7704	29.00	36.11	1.0000	110.31	65.38	0.1394
CK (U/L)	135	116	0.1036	177	368	0.4924	854	522	0.0739

TnI – Cardiac Troponin I; CK-MBmass – mass concentration of creatine kinase-MB, CK-MB – creatine kinase-myocardial band; CK – creatine kinase.

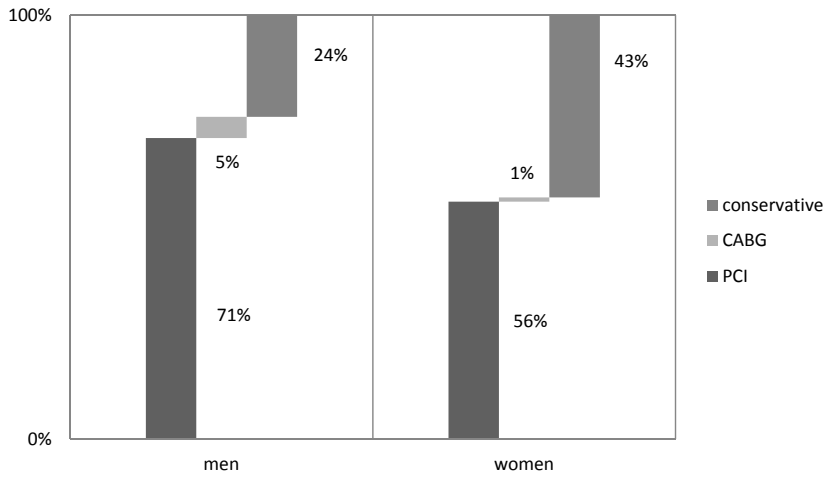

Figure 1. Treatment qualification (p = 0,002). CABG – coronary artery bypass grafting, PCI – percutaneous coronary intervention

Discussion

The hereby-presented analysis shows a number of interesting information referring to effect of gender on course and treatment of ACS. The main finding is that female sex was associated with a higher incidence of non-ST-elevation ACS and lower of ST-segment elevation acute coronary syndrome (STE ACS) than male gender. In-hospital mortality was not different between men and women despite lower levels of injury markers, lower prevalence of heart failure and higher ejection fraction in women. But women were less likely receiving invasive treatment.

Higher incidence of NSTE-ACS in women was observed also in a study [of] a huge population of 199.690 patients [11]. Kragholm et al. showed that 33.3% of NSTE-ACS patients were women, and it has changed minimally over the 17-years observation [12]. We have observed a similar percentage of female patients in ACS patients (32.3%), however this included also STEMI. Our results are consistent with the previous findings showing that ACS women are older than men [12]. Average age at first myocardial infarction (MI) is 64.5 years for men and 70.3 years for women [1]. The incidence of hypertension and diabetes mellitus was similar in men and women, although some previous studies show that women present more frequently with these comorbidities [12, 13], however, cited studies investigated only NSTE-ACS. Other sur-

veys showed the prevalence of hypertension and diabetes mellitus with the same frequency in men and women [14]. Incidence of heart failure was lower in women, which is in opposite with the previous studies [12]. Comorbidities occur with similar frequency in both women and men, but other authors show that women have a greater number of comorbidities [15]. Usually there are modifiable risk factors for heart diseases, such as smoking, obesity, hypertension or hyperlipidemia. Differences between studies may mainly be due to different inclusion criteria, whether patients with confirmed or suspected ACS were included.

Referring to a recent study, heart rate may be an important prognostic factor [16] and may help in stratification of risk [17] connected with incidence of ACS. We showed no significant difference in heart rate on admission between men and women. It should be noted that heart rate on admission is considered one of the ACS prognostic factors.

There was no significant difference between genders in BMI, whereas in other studies higher BMI correlated with higher occurrence of ACS and male gender [18]. Previous studies show the impact of obesity on the development of CAD appears to be greater in women than in men [19]. The Framingham Heart Study showed that obesity increased relative risk of CAD by 64% in women, as opposed to 46% in men [20]. Moreover other surveys showed that patients who have higher BMI have higher risk of STEMI than any other type of ACS, but also they have the same risk for in-hospital outcomes [21]. It was also shown that obese patients, especially men, have better prognosis after ACS [22].

We found no difference in mortality between genders. Numerous investigators observed higher early mortality after STEMI in women than in men [21]. In comparison, another analysis showed no differences in early (30 days) mortality in STEMI in women versus in men [23]; and a similar incidence of in-hospital MACE was demonstrated also in NSTE-ACS [13]. Long-term mortality was not taken under consideration in our study. What is more, according to other studies, in-hospital outcomes are very rare in NSTEMI patients. Several studies showed that mortality after NSTE-ACS is similar in both men and women [13]. Sex-related differences in early mortality after MI are the topics of numerous studies. After MI, younger women, but not older women, have higher mortality during hospitalization than men of the same age [5]. The younger patients were, the higher the risk of death of female patients was compared to males [5].

We noticed also that women underwent coronary revascularization relatively rarely compare to men, as it was observed in other studies [13]. This observation may be consistent with lower biomarkers level and lower risk-baseline characteristics in women. The majority of women from investigated population were treated conservatively. Other trials explain, that different manifestations of CAD in women are associated with their smaller coronary arteries, higher coronary blood flow, and higher endothelial shear stress, which have major effects on endothelial function and resistance to coronary atherosclerosis [24]. In women complex interactions of focal stenosis, diffuse epicardial coronary narrowing, related endothelial shear stress, and microvascular dysfunction often make diagnostic process difficult by use of standard noninvasive or invasive technologies [24].

Several studies examined relationship between troponin concentration and gender, and showed women to have lower concentrations of troponins [14]; these results are consistent with our findings. However in detailed analysis of every ACS group there were no differences between men and women in level of cardiac biomarkers. According to another survey [25], predictive value of troponin concentration is greater in women than in men, thus knowing the differences and establishing cut-off points separating women and men might be useful. The high sensitivity troponin assay with sex specific diagnostic thresholds may double the diagnosis of myocardial infarction in women and identify those at high risk of reinfarction or death [14]. The risk of under-diagnosing of myocardial infarction in women can contribute to inequalities in the management and treatment of myocardial infarction. In clinical practice it is important, to take into consideration, differences among genders to improve diagnosis and treatment strategy in both men and women.

Limitations of the study

This was prospective single-center registry of patients with ACS, so the number of patients is not very high.

Conclusions

Female sex was associated with a higher incidence of non-ST-elevation ACS and lower of ST-elevation ACS than male gender. In-hospital mortality was not different between men and women despite lower levels of heart injury markers, lower prevalence of heart failure, and higher ejection fraction in women. Women were less likely to receive invasive treatment.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Reactions of bone, liver and kidney tissues to orthopaedic implants with silver nanoparticle doped hydroxyapatite coatings: microscopic examination in a rabbit model

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ABSTRACT

Introduction. Periprosthetic joint infections are severe complications of arthroplasty, difficult to manage due to biofilm formation on the components. Recently, the use of silver nanoparticles (SNs) has emerged as a method of preventing biofilm formation on orthopaedic implants, however little is known about the systematic toxicity of SNs.
Aim. This study used a rabbit model to examine the tissue response of bone, liver and kidney to prototype components with SN doped hydroxyapatite (HA) coatings.
Material and Methods. Twelve prototype implants (six with HA, six with SN doped HA coatings) were implanted into the femora of twelve New Zealand Rabbits. After 6 weeks, the animals were euthanised, their femora and samples of livers and kidneys harvested to prepare microscopic slides. The slides were examined for the presence inflammatory or toxic reactions to SNs, implants were examined using scanning electron microscopy (SEM) to determine structural changes related to implantation and verify retention of SNs *in vivo*.
Results. SEM demonstrated that SNs formed submicron conglomerates, which were retained after 6 weeks *in vivo* and did not interfere with osseointegration. Histologic studies of bone fragments demonstrated no signs of acute toxicity and inflammation. No inflammatory reaction was observed in kidneys, although in some samples signs of acute renal failure related to euthanasia were found. No severe toxic reaction was found in liver samples, however fatty degeneration of liver was found in some animals.
Conclusions. This study documented good osteointegration of implants with SN doped HA coatings with low systematic toxicity of SNs.

Keywords: osseointegration, silver nanoparticles, hydroxyapatite coatings.

Introduction

Total joint arthroplasty is perhaps the most successful orthopaedic procedure, performed in cases of end-stage osteoarthritis of the hip, knee and other joints [1, 2]. The number of such surgeries performed in developed countries is increasing due to ageing populations and it is estimated that approximately 50 thousand arthroplasties are performed annually in Poland. Arthroplasty is often performed in young, more active patients due to encouraging long-term clinical outcomes. In such cases, long-term function of the implants

relies on biologic fixation with host bone by means of osseointegration, ingrowth of bony tissue into various coatings of the components, predominantly hydroxyapatite (HA) [1, 2].

Unfortunately, as the number of joint replacements performed annually is increasing worldwide, the financial burden related to complications of these procedures is becoming more severe [1]. Perhaps the most devastating complication of arthroplasty is periprosthetic joint infection (PJI). This is because infections involving orthopaedic implants are associated with formation of biofilms, complex polymeric conglomerates of proteins, polysaccharides and DNA [3]. Biofilms encapsulate bacterial cells present on the surface of implants and protect them from antibiotics or the host immune response [3]. To date, no biofilm-disrupting drugs are available, consequently surgical exchange of components and prolonged antibiotic therapy are a necessity in the management of most patients with PJIs [1]. Unfortunately, this type of treatment is associated with unacceptably low success rates and suboptimal clinical results.

For this reason, development of methods allowing prevention of PJIs has become a research priority. In recent years, application of silver nanoparticles (SNs) on the surface of orthopaedic components has been suggested [2–4] based on data from laboratory studies, which indicated that SNs have antimicrobial properties and can prevent biofilm formation. This is also an attractive alternative for the industry, as such modification could be easily used in existing implant designs with long-term clinical record. However, there is a lack of data regarding the safe application of SNs on orthopaedic coatings and their possible local and systematic toxicity, especially in kidneys and liver.

Aim

This study used a rabbit model to examine tissue reactions of bone, liver and kidney to implantation of prototype components with SN doped hydroxyapatite coatings.

Material and Methods

This experimental study based on a New Zealand White Rabbit model compared the response of bone, liver and kidneys to implantations or prototype orthopaedic implants with conventional HA and SN doped HA coatings. First, prototype implants (cylinders with a diameter of 4 mm and length of 22 mm) were manufactured from TiAl6V4 alloy, plasma sprayed with a 50 µm thick HA coating (2PS, Montbazens, France). Coatings of randomly selected implants were then doped with SNs with a mean diameter of 66 nm (Particular GmBH, Hannover, Germany). Briefly, implants were immersed in a suspension of SNs with a silver concentration of 106 mg/l and stirred for 14 hours at room temperature. The components were then rinsed with physiological saline, dried and sterilised. Next, characterisation of the coating from both types of implants was performed using scanning electron microscopy (SEM): implants were rinsed in acetone to remove any fatty residues, vacuum dried, glued to aluminium stubs using a conductive carbon tape and coated with a 10-nm layer of gold using a JEOL JFC 1200 sputter coater. Samples were then examined using SEM (JEOL JSM-6400) with an accelerating voltage of 20–30 KV. The chemical composition of osseointegrative layers was verified using Energy-Dispersive X-ray Spectroscopy (EDS, Oxford Instruments Inca); analyses were performed in randomly selected, square regions (10 x 10 µm) at an accelerating voltage of 25 KV, with a bandwidth of 10 KeV and noise peak cut-off at 0.3 KeV. Analyses of larger regions were performed to minimise differences in silver content related to random distribution of SNs on the surface of the implants.

Six components with SNs (study group) and six implants with conventional HA coatings (controls) were implanted into the femora of New Zealand White rabbits (a total of twelve animals), according to approval granted by the local bioethical committee. Briefly, animals were fist anaesthetised (ketamine + medetomidine) and distal epiphyses of their left femora were exposed using a lateral approach. A 4-mm hole was then drilled through the bone, following by implantation of a prototype component and wound closure. All rabbits survived the procedures with no major complications, and were kept in cages receiving food and water *ad libitum* for 6 weeks. At that time, animals were euthanised (intracardiac phenobarbital injection) and their femora containing the implants, livers and kidneys were imme-

diately harvested. Fragments of bone adjacent to the implants were removed using a 10-mm chisel and used for further studies. All obtained tissue samples were fixed in 10% buffered formalin for 72 hours.

Liver and kidney samples were used to prepare routine pathological slides (H+E staining). Since toxic effects of silver may result in fatty degeneration of liver, these samples were evaluated according to the NAS scoring system developed by Kleiner et al. [5]. The scale comprised 14 histological features, 4 of which are evaluated semi-quantitatively: steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–4). In this system scores higher than five are required to diagnose steatohepatitis.

Bony fragments underwent electrochemical decalcification in Romeis fluid (solution of hydrochloric and formic acid) using a current of 100 mA and were then stained using conventional H+E and McManus PAS techniques. The healing response of bone around implants was then evaluated using a modified Solchaga score [6]. The system comprised the presence of new bone (0–3), vascularisation of the bone (0–1), presence of osteoblasts/osteocytes/osteoclasts (0–3 each), presence of immature bone (0–3), trabecular bone (0–3) and Haversian channels (0–3), inflammation (0–2) and granulation tissue (0–2). The scale yields 0–26 points and was used to quantify bone healing within a mantle of 4 mm around the implant.

Finally, all retrieved prototype implants were vacuum dried, prepared for electron microscopy using the same protocol as for characterisation of newly manufactured coatings and examined using SEM to verify bone ingrowth into the surface of components and retention of SNs using EDS.

Results

Coating characterisation

A uniform HA layer was obtained on all implants fabricated for this study. SEM analysis demonstrated that the coatings were composed of small irregular splats and grains of hydroxyapatite, which formed a complex three-dimensional structure (**Figure 1a**). The deposition of nanoparticles had no effect on the morphology of the HA coating, which was identical to the control samples (**Figure 1b**). SEM examination under high magnifications showed a tendency of SNs to form larger conglomerates (approx. 100 nm – 1 µm in diameter; **Figure 1c**). EDS analysis of the coatings confirmed the presence of calcium and phosphors in all samples; a well-defined peak

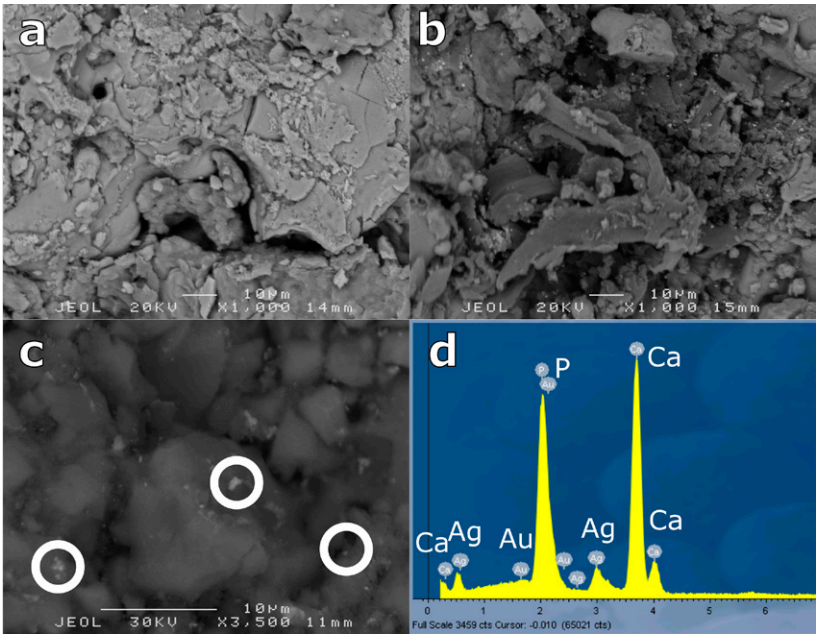

Figure 1. SEM studies of HA coatings used in the study: a) microstructure of the HA coating in control group; b) microstructure of the HA coating doped with SNs; c) high magnification image of SN doped HA coating demonstrating conglomerates formed by the nanoparticles (in circles); d) EDS analysis of SN doped HA coating confirming presence of silver

confirming presence of silver was observed in all SN doped implants (**Figure 1d**). Dependent on the region, the quantitative EDS analysis indicated a mean silver content of 3.3% (range 2.7–4.2%).

Characterisation of bone tissue reactions

There were no qualitative differences between bone samples around the implants in both groups. In all cases, the periosteum was properly structured from the connective tissue, and osteoblasts were only observed in regions adjacent to the implant border, where the periosteum was disrupted during implantation (**Figure 2**). The cortical bone was formed from significantly mineralised tissue with typical Haversian canals in all samples, while the trabecular bone exhibited a typical structure, with an increasing gradient of trabeculae towards the epiphyses. Areas around the implants had a mixed pattern of various features including formation of fibrous tissue (fibroplasia), small regions of cartilaginous tissue (chondroplasia) and new bone elements (osteoplasia) with features of lamellar and trabecular bone (**Figure 2**). There were no cases of cellular infiltration around the implants, indicating a lack of inflammation. The presence of implants did not influence the structure of bone marrow; intertrabecular sinuses contained yellow and red bone marrow with a typical morphology and occurrence of myelogenesis. A good healing response was observed in all cases, with comparable mean healing scores for HA (mean 18.3; range 15–23) and HA+SN (mean 17.8; range 16–22) samples.

Characterisation of kidney and liver tissues

Kidney samples from all animals retained a typical microscopic structure of nephrons (**Figure 3a**); the border between the cortical and medullar parts were clearly visible. Hyperaemia was observed in some cases within the glomerular (3 HA and 2 SN animals) and borderline (2 HA and 2 SN rabbits) renal layers, indicating acute renal failure, and are often described as "shock kidney".

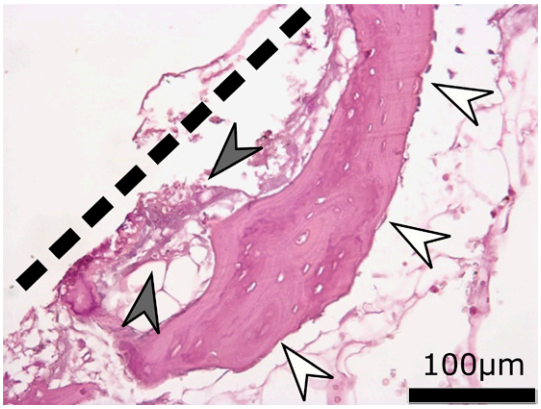

Figure 2. Bone formation around the implant. Dashed line indicates the location of implant, white arrows indicate trabecular bone, and grey arrows indicate localised chondroplasia

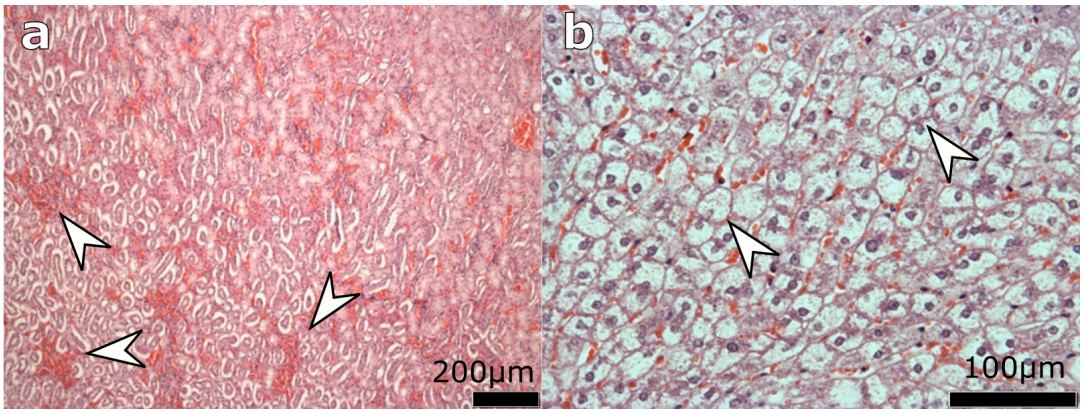

Figure 3. Microscopic images of kidney and liver samples: a) fragment of kidney tissue with localised hyperaemia indicated by arrows; b) liver sample from one animal with severe fatty degeneration (white arrows)

A normal morphology of liver with typical hepatic lobules was observed in most rabbits. However, a varying extent of hyperaemia in hepatic vessels (3 HA and 3 SN rabbits), localised lymphocytic infiltrations (1 HA and 3 SN rabbits) as well as fatty and vacuolar degeneration of hepatocytes (2 HA and 4 SN rabbits) were observed in some samples from both groups. In five cases where signs of fatty degeneration were present, the NAS score was 4 (borderline steatohepatosis), while pronounced damage with a score 6 was observed in one SN rabbit (**Figure 3b**).

Retrieval analysis

SEM examination of explanted components demonstrated ingrowth of trabecular bone into implants with conventional HA coatings and components with SNs (**Figure 4a**, **b**). In all implants the coating remained intact, except for localised damage caused by surgical tools used to extract bony fragments for histologic analysis. There were no qualitative differences between the newly formed bone on the surface of the components; in all cases formation of well attached trabeculae was confirmed. In all implants with SN doped coatings, conglomerates of silver nanoparticles were visible after six weeks *in vivo*, and EDS analysis confirmed their atomic composition (**Figure 4c**).

Discussion

In current clinical practice, the use of implants anchored by means of biologic fixation is becoming the gold standard in joint arthroplasty, including hip, knee, shoulder and ankle replacements [4, 7]. Such implants are being used in a growing number of patients, including young, active individuals. Many arthroplasties performed in developed countries are associated with an increasing number of complications following these procedures, and PJIs are perhaps the most severe [1]. Since the development of PJIs heavily depends on formation of biofilms on implants, it has been suggested that application of SNs to their surface could minimise the risk of infection [2–4]. Although antimicrobial properties of SNs are widely recognised, it is unclear whether their application in orthopaedic components is associated with localised or systematic toxicity [2, 3, 8, 9]. This study demonstrated that hydroxyapatite coatings doped with SNs are not associated with a localised toxic response of host bone, kidneys and liver.

There are several limitations to this study, predominantly due to the fact that it includes a small number of samples and animals. This was due to ethical reasons, which required the lowest possible number of animals to be used, and this applies

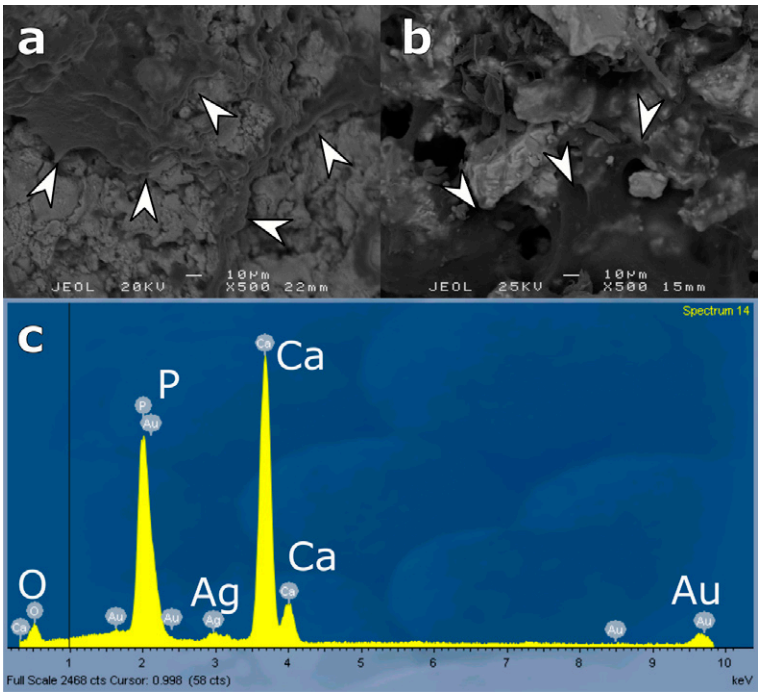

Figure 4. SEM analysis of retrieved samples: a) bony ingrowth (arrows) into conventional HA coating; b) ingrowth of bone (arrows) into SN doped HA coating; c) EDS analysis of fragment of SN doped coating not covered by bone confirming presence of silver nanoparticles

to other similar studies [3, 9]. Another limitation is that conventional, decalcified bone samples were included [10, 11]. It is possible that the decalcification procedure could potentially alter the morphology of bone tissue, however performing this procedure allowed the SEM examination of the retrievals. Lastly, the examination of the toxic effects related to implantation of components with SNs has limited precision since it is based on histologic examination [5, 8, 11].

Good osseointegration of SN doped HA coatings and retention of nanoparticles on the surface of the coatings is perhaps the most important finding from this study. Although results of EDS analyses suggest that a large portion of silver was retained after six weeks *in vivo*, it is not possible to precisely determine the extent of silver release from such coatings [9]. The HA layer has a complex porous structure, and EDS analysis allows the silver content to be determined only in areas directly exposed to the electron beam of the SEM. Unfortunately, the small size and weight of particles make it practically impossible to determine the amount of SNs retained in areas overgrown by trabecular bone. Theoretically focused ion beam milling could be used to gradually remove fragments of bone and expose the coating and SNs. However, even the use of such advanced techniques would yield very imprecise results due to irregularities in particle distribution and the complex three-dimensional coating structure [3]. Nonetheless, histological studies demonstrated that the presence of SNs does not affect bone ingrowth, with no inflammatory reactions associated with the presence of SNs.

The examination of kidney and liver tissues performed in this study aimed to determine the systematic cytotoxicity of SNs. Although some of samples examined in this study exhibited abnormal findings, they are most likely not associated with SNs. Kidney tissue samples exhibited findings typical of acute kidney failure, which are considered characteristic for hypovolemic shock rather than chronic damage, in agreement with data from other authors [11]. We believe that they were most likely formed when the animals were euthanised. However, some pathologies observed in the liver samples, predominantly fatty degeneration, developed over a relatively long time. It is essential to consider other etiological factors in these cases which may cause the hepatic lesions, such as the type of culture, nutrition, infections and stress-inducing conditions [2, 7–9]. Taking the above into consideration, it is not possible to confirm that lesions noted in experimental groups of rabbits are associated with the type of implant used.

Conclusions

This study demonstrated good osseointegrative properties of SN doped HA coatings, comparable to that of conventional hydroxyapatite layers. The presence of pathologic lesions in livers and kidneys of some animals can be attributed to the harvesting procedure (shock kidney) or have multifactorial origin (fatty degeneration in some livers). The findings suggest the antimicrobial potential of SN doped HA coatings to prevent biofilm formation *in vivo,* but this requires confirmation in further studies.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

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Current principles underlying clinician and pathologist cooperation in pathological and genetic diagnostics in breast cancer patients in the times of personalised medicine

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ABSTRACT

Positive long-term treatment outcome in cancer patients depends mainly on the disease stage. It also depends on selection of an optimum therapeutic management. In breast cancer patients, the final treatment arrangements result, to a large extent, from a quality of cooperation of medical personnel providing cancer diagnostics and therapy. This requires knowledge of mutual expectations of doctors of different specialisations. The most common problems in interdisciplinary communication are relatively easy to notice in relations between a clinician (surgeon) and a pathologist. This paper discusses the most important aspects of that relationship.

Keywords: breast cancer, pathological diagnostics, genetic diagnostics, interdisciplinary communication.

Introduction

In breast cancer patients, a possibility to select an optimum treatment results, to a large extent, from a quality of cooperation of medical personnel providing diagnostics and therapy of neoplastic lesions in the breast gland. This also concerns full understanding of mutual expectations of doctors of different specialisations.

In patients, positive long-term treatment outcome significantly depends on a disease stage. It is also a result of a correct cancer treatment applied. Its final form is decisively influenced by a status of prognostic factors determined during a multidirectional pathological assessment (histopathological examination, immunohistochemical techniques, and molecular and genetic tests). According to classification of prognostic factors determined to this date and used in breast cancer patients as proposed by The College of American Pathologists, they belong to one of three separate groups. The first group consists of factors of proven clinical value used in a standard way to establish a necessary treatment method for patients (including a size of a primary lesion, an axillary lymph node status, a cancer histological type and histological malignancy, and a status of oestrogen (ER) and progesterone (PgR) receptors). The second group includes factors which final prognostic value is a subject of current controlled clinical studies. The third group consists of markers currently not meeting criteria established for groups I and II [1].

As it was shown in conclusions to conducted studies, the initial classification of prognos-

tic factors became partly obsolete [2, 3]. The factors of the highest prognostic value (group I) also include a status of the human epidermal growth factor receptor 2 (HER2), a value of proliferation markers (mitotic activity index, Ki-67), and a biological type of breast cancer. Establishing the biological type of breast cancer requires determination and joint assessment of the status of ER, PgR and HER2 receptors together with the mitotic activity index, Ki-67. This way, the cancer type (luminal A, luminal B1-HER2 negative, luminal B2-HER2 positive, HER2-positive, and triple negative – basal-like) can be established unambiguously and decisions concerning planning of a required treatment scope and correct course are implied [4–12].

The tasks for pathological diagnostics for malignant breast cancer were precisely specified. Diagnostic categories for individual types of biological material were determined and introduced, to facilitate communication between different groups of specialists. The developed management standards were based on guidelines specified by scientific associations (a need to implement these standards also resulted from recommendations included in the European Commission guidelines). They concern both cytological tests, as well as pathological evaluation of tissue material (from a core needle biopsy of cancer lesions and from intraoperative samples).

The problems established in the title of this paper, concerning interdisciplinary communication, are relatively easy to notice in relations between a clinician (surgeon) and a pathologist. They may involve several interfaces of mutual communication:

- clinician (surgeon) expectations at the disease diagnostic stage;
- expectations associated with the treatment process (including the surgery);
- expectations at a stage of establishing indications for more radical surgical treatment (also applies to qualification for auxiliary treatment procedures – local and systemic).

Cytological tests

It should be remembered that besides many clear advantages (low invasiveness, low cost, low difficulty of the test, and a short time of waiting for its results), the fine-needle aspiration is a diagnostic method with numerous limitations [13, 14]. The most important of them are:

- final disease diagnosis is not possible (concerns distinguishing between atypical ductal hyperplasias and ductal carcinomas, ductal carcinomas and invasive cancer, sarcoma and metaplastic cancer);
- too high rate of incorrect diagnoses (an increased risk of a false negative result – when evaluating highly differentiated breast cancer, or a false positive result – when evaluating post-radiation lesions and cases of local recurrence).

The collected cell aspirate allows determination of the cancer lesion only on a basis of morphological parameters of individual cells (or their groups). To facilitate the multidisciplinary communication, diagnostic categories were introduced for obtained cytological material (from C1 – inadequate aspirate smear to C5 – malignant cell parameters) [14].

The cytological test allows final determination of the oestrogen and progesterone receptor status (a nuclear reaction using immunohistochemical techniques). However, this does not apply to reliable determination of the HER2 receptor status (a membrane reaction), particularly, when a negative test result is obtained (during fixing of the obtained biopsy specimen cell membranes can be damaged resulting in an incorrect reading). For correct evaluation of this prognostic factor, immunohistochemical determinations on histological material are recommended [14].

Histopathological examinations (core needle biopsy material)

The main source of problems in relations of a clinician and a pathologist is a possibility that material was collected from a fragment of the studied mass that has not been not fully representative. In the event of inconsistencies between the obtained results and a radiological image of the mass (particularly, when radiological diagnostic of malignant neoplasm is possible), the biopsy must be repeated.

In accordance with the clinician's expectations, a histopathological evaluation of specimens allows determination of the mass nature (primary lesion, mass of a metastatic origin),

forms (invasive, in situ), and a histopathological type of the cancer. This also applies to determination of the cancer histological malignancy grade and receptor tests (to evaluate prognostic and predictive factors). However, this way the size of the lesion and its surgical margins cannot be determined [15–17].

Analogically to the system of diagnostic categories specified above, and used to describe the cytological tests, similar rules apply to verification of material obtained in the core needle biopsy. Introduced categories of lesions include diagnoses coded with symbols starting with B1 (denominating presence of the normal tissue) to B5 (diagnosed malignant lesion, with its type specified, marked with a–d) [15].

Histopathological examination (post-surgery specimens)

Of main issues concerning interdisciplinary communication in this area, limitations associated with the intraoperative examination must be mentioned. Furthermore, a need for ordering histopathological evaluation of tissue specimens in this mode is also an issue. For detailed discussion, these issues need to be analysed, taking into account specific characteristics of each histopathological presentation of malignant lesions in the breast gland:

1. Invasive breast cancer forms:
   - an ad hoc test allows an evaluation of margins for mass resection; however, for multiple lesions (particularly, in a presence of additional cancer microfoci), it may be of limited value;
   - in a selected group of patients with macrometastatic lesions present in the sentinel node (in accordance with the inclusion criteria for the study ACOSOG Z0011) [18], conservative treatment can be selected (auxiliary axillary lymph node dissection is not necessary); therefore, in such cases, it is justified not to perform the routine ad hoc evaluation of lymph nodes sampled during the surgery [19, 20];
   - it should be remembered that the ad hoc examination of the sentinel node reduces the amount of tissue material to be used for routine tests (this applies, of course, to all cases when intraoperative tests are used);
   - data that the clinician expects to be provided in the pathological examination report from evaluation of material collected during biopsy of the sentinel node includes information on the total number of nodes resected, number of nodes with metastases and sizes of these lesions (macrometastasis, micrometastasis, isolated tumour cells, with dimensions of the largest mestasis specified); the presence and type of the node capsule infiltration must also be specified (focal or massive infiltration) together with information on test methods used (routine tests, serial sectioning, use of immunohistochemical reactions or molecular methods) [14];
   - cancer treatment failure (in form of a local recurrence or metastatic lesions) may concur with a conversion in a status of initially determined receptors; thus in the event of the above-mentioned recurrence, reassessment of ER, PgR and HER2 is required, it is also recommended following neoadjuvant therapy [21, 22].
2. Pre-invasive cancer – ductal carcinoma in situ (DCIS):
   - concerning the common DCIS presentation in form of multiple lesions, lack of palpable lesions, as well as a frequently found characteristic mammographic picture (groups of suspected microcalcinations without accompanying "mass" symptoms), intraoperative pathological verification of the specimen is not recommended; for reasons described above, the result of ad hoc histopathological evaluation is frequently unreliable, this concerns, in particular, difficulties in assessment of the resection margins, as well as significant problems with diagnosing microinvasion foci [23, 24];
   - as it was noted, DCIS lesions are characterised by a possibility to develop in one of two separate differentiation directions (low-risk DCIS – indolent disease vs. high-risk DCIS – "*extensive pure ductal carcinoma in situ*"); thus, a very important expectation of a clinician is a precise determination of the mass type by a pathologist (diagnosis of cancer of the second type requires surgical procedures used for treatment of invasive breast cancer) [25];

- a detailed pathological report allows making a decision whether a patient with DCIS should be qualified for a surgical verification of the regional lymph node drainage (a biopsy of the sentinel node); the so-called "poor prognostic factors" concerning a risk of coexistence of in situ and invasive forms of cancer, on a basis of data included in the description of the histopathological result, the high histological malignancy grade, multifocal nature, significant size, presence of necrosis (*comedonecrosis*), and steroid resistance can be confirmed (or excluded) [23, 24].

3. Pre-invasive cancer – lobular carcinoma in situ (LCIS):
   - similarly as for DCIS-type lesions, the pathologist must specify a histopathological type of LCIS (analogically to DCIS masses, they are characterised by a different course of the disease, thus they require planning and implementation of a different treatment type); the histopathological evaluation should determine a presence of the classic LCIS type (with a minimum risk for co-existence of other breast cancer forms) or of any other type of this cancer (a *comedo* type with necrosis, *florid* LCIS, or a pleomorphic type) requiring radical resection of the diagnosed lesion [26, 27];
   - the histopathological LCIS type diagnosed also determines further management in the event of non-radical resection of the mass that underwent a surgical biopsy (the classic form does not require a radical operation) [28, 29];
   - contrary to breast cancer types described above, a diagnosis of isolated LCIS lesions does not require patients to undergo surgical procedures involving the axillary lymph nodes [26–29].

Molecular tests, genetic diagnostics

Regardless of the valid determination of the "classic" prognostic and predictive factors, increasingly often patients with breast tumours undergo tests of gene expression patterns. The breast cancer molecular signatures obtained this way allow to determine a likelihood of the disease recurrence and benefits of chemotherapy in specific clinical cases [30–39]. This concerns, in particular, patients in whom a need for this form of the systemic treatment was excluded following the "standard" assessment of the cancer type. In accordance with reported data, use of the Oncotype DX test may change qualification for chemotherapy or hormone therapy in about 30 % of patients evaluated with this validator [30, 31, 34, 35]. Use of the gene expression test (a consensus of the expert panel at the St. Gallen conference, Vienna 2017) is not justified solely in breast cancer of a low clinical risk. This concerns, in particular, patients with a tumour of pT1a/b size, of a low histological malignancy grade (G1), with a simultaneous high expression of ER receptors and no metastatic lesions in lymph nodes (pN0) [4].

As it was proven in the results of randomised clinical studies (including MINDACT [40], NSABP B-14 [41], NSABP B-20 [41], TransATAC [42], SWOG 8814 [42], TAILORx, and RxPONDER [43]), analysing usefulness of most commonly used multi-gene tests (MammaPrint 70-gene test, Oncotype DX 21-gene test), their use provides additional information about the disease, they are a valuable supplement of data obtained by evaluation of "classic" clinical and histopathological factors. A main obstacle for a general use of tests determining the expression levels for selected gene panels in breast cancer is their high price (ca. EUR 3–4 thousand).

Of the tests determining a status of single genes, the test for mutation of BRCA1 and BRCA2 genes still remains an irreplaceable diagnostic standard, in particular, for members of families with an increased rate of breast cancer occurrence [4, 44, 45]. It should be remembered that when a person is found to be a carrier of a clinically significant mutation of BRCA1 gene (mainly founding mutations and recurring mutations), the risk of them having the breast cancer is 56–84%. Of all mutations in the BRCA1 gene found so far, the most commonly determined in the Polish population (of clinical significance) are genome changes of 85delAG (ex2), 300T > G (ex5), 3819del5 i 4153delA (ex11), and 5382insC (ex20) type [46]. Its diagnosing in the breast cancer patient is also more frequently associated with a presence of disease with poorer prognosis (triple negative cancer) [47].

Other genetic anomalies predisposing to breast cancer development are mutations involving genes TP53, PTEN, ATM, BRIP1, CHEK2, and

PALB2 [48–50]. A need to determine their presence has a clinical importance comparable to cases of other malignant neoplasms [51, 52].

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Conflict of interest statement
The authors declare no conflict of interest.

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HPV-related HNC – new challenge and hope for head and neck cancer subjects

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ABSTRACT

In recent years there are observed substantial changes in epidemiology of head and neck cancer. An incidence of laryngeal cancer is declining as a consequence of decreased tobacco smoking. Contrary, oropharyngeal cancer associated with HPV infection transmitted on sexual way is becoming much more frequent. The latter one is characterized with a better prognosis that most likely does not require intensive therapy. De-escalation of therapy in case of HPV-associated tumor is a matter of current studies.

Keywords: HPV-related cancer, head and neck, epidemiology, treatment.

A casual involvement of Human Papilloma Virus (HPV) infection in gynecological cancer is being known for a long time. Owing to the experimental and intellectual contribution of Harald zur Hausen (awarded by the Nobel prize at 2008) it has become clear that HPV preferentially penetrates mucosa that can be followed by tumor formation in genital, anal and head and neck regions of both sexes [1]. The latter remains in the frame of our research and clinical interest.

Head and neck cancer mostly affecting squamous cells (abbreviation: HNSCC) is the 6th leading cause of world cancer mortality. Tobacco smoking and abusing of strong alcoholic beverages have been the well recognized causing factors. The main group of tobacco smoke carcinogens include polycyclic aromatic hydrocarbons, aromatic amines, N-notsrosoamines and reactive oxygen species. An intensive anti-smoking campaign have produced a decline of tobacco smoking at least in the developed countries that was followed by changes in epidemiologic data concerning HNSCC [2]. A detailed analysis has shown a decrease of laryngeal cancer and increase of oropharyngeal cancer incidence that altogether indicated for a stagnation of an incidence of HNSCC [3]. The noticed trends were explained with by a growing number of HPV-associated HNSCC [3–5]. HPV infection affects oropharynx much more frequently than larynx. Within oropharynx tonsils [6] and the base of tongue were found to be the primary targets for HPV infection [7]. However, the data concerning a partition of HPV-associated HNSCC cancer are not very convergent for two reasons. First, a percentage of HPV(+) HNSCC is constantly growing. As an example the meta-analysis done by Termine et al. [8] established a prevalence of HPV infection in oropharyngeal cancer for 38.1% as compared to 24.1% in not site-specific HNC. Next, the established techniques for HPV determination are operating on protein, RNA and DNA level exploring a battery of techniques including immunohistochemistry, Southern bloting, PCR, and *in situ* hybridization [9]. The most commonly applied technique is derived from an observation concerning overexpression of p16 protein highly correlating with HPV integration. Though, p16 staining is serving

as surrogate but highly reliable marker of HPV infection [10, 11]. It is clear that the techniques vary with their sensitivity, accuracy, complexicity and costs [9, 10]. To summarize, a prevalence of HPV-associated oropharyngeal cancer could be estimated for roughly almost 50%, when a casual impact of HPV in laryngeal cancer is estimated for 5% only [12].

Contrary to inhaled tobacco smoke carcinogens HPV is sexually transmitted [13]. Further, it was found that sexual history is notable for by multiple sexual partners and focus on oral sex. For this and other reasons it affects mostly young adults [14, 15]. Altogether, at presentation it appears a new face of head and neck patient that is much younger than typical patients at their 6^{th}–7^{th} decade of life, lacking commonly recognized risk factors as drinking and tobacco smoking, having a reasonably style of life and enjoying rather high economic status [15]. It is to remind that in the typical HNC patients a social margin is having a good share.

The mechanism of HPV-associated carcinogenic transformation was established to be different than that following activity of tobacco smoke carcinogens. Human papilloma viruses form a group of over 150 members that could be divided according to their oncogenic potential for high- and low-risk HPVs. HPV16 and HPV18 represent high risk types and are found most frequently in human tumors. A fully recognized functional structure of HPV16 circular DNA encodes the long control region (LC) and genes coding early proteins (E1–E7) and two late proteins (L1 and L2) (**Figure 1**). HPVs infect basal epithelial cells characterized with a high proliferation capacity. Infected cells provide two options: viral latency that is a type of abortive infection or integration of viral DNA sequence into human genome. Integration is necessary to maintain expression of viral proteins [16–18]. At this point a cell could be driven onto oncogenic transformation (**Figure 2**). Integrated viral genome does not affect directly host genetic information that is the case for chemical carcinogenesis. Tobacco smoke carcinogens frequently mutate *TP53* gene bearing a function of tumor suppressor gene. The protein coded by mutated *TP53* gene is losing its function on control the cell cycle and does not provide time sufficient to remove DNA lesions. Contrary, HPV integration leaves *TP53* gene undamaged,

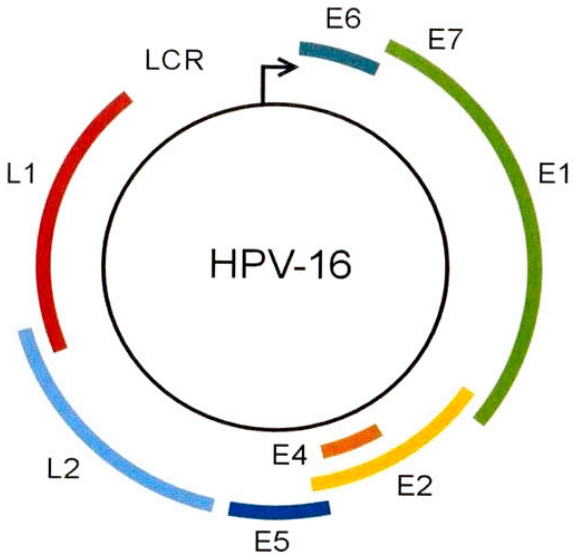

Figure 1. Genomic map of HPV-16 structure

producing fully functional TP53 protein. However, E6 viral protein inactivates TP53 protein via complex formation when E7 is responsible for inactivation of Rb. On this way the whole TP53/Rb anticancer pathway is blocked [19, 20]. Further, is was shown that the number a viral copy number and viral integration sites are having an impact on progression of cancer [20, 21]. Another consequence of HPV integration is an appearance of genomic instability manifested by centrosome dysregulation, division errors, chromosome rearrangement and replication stress that altogether promotes oncogenesis [16, 18, 22].

Later on, an attention was paid on HPV-positive HNSCC subjects who were also current or former tobacco smokers. Mirghani et al. [23] using next-generation sequencing examined mutation profile in 39 genes known to be most frequently mutated in HNSCC. It was established that smoking does not contribute to an increased level of mutations in HPV-positive HNC subjects. The finding is in agreement with the publication of Farsi et al. [24] who claim a lack of synergy between HPV infection and tobacco smoking that are independent etiological agents in HNSCC. Nevertheless, the research group of Thomas Carey [25] who were following up the group of already treated HPV-positive HNSCC subjects have found that current tobacco smokers as compared to never-tobacco users are at higher risk of disease recurrence.

Concerning characteristics of HPV-positive HNSCC subjects are lacking commonly recog-

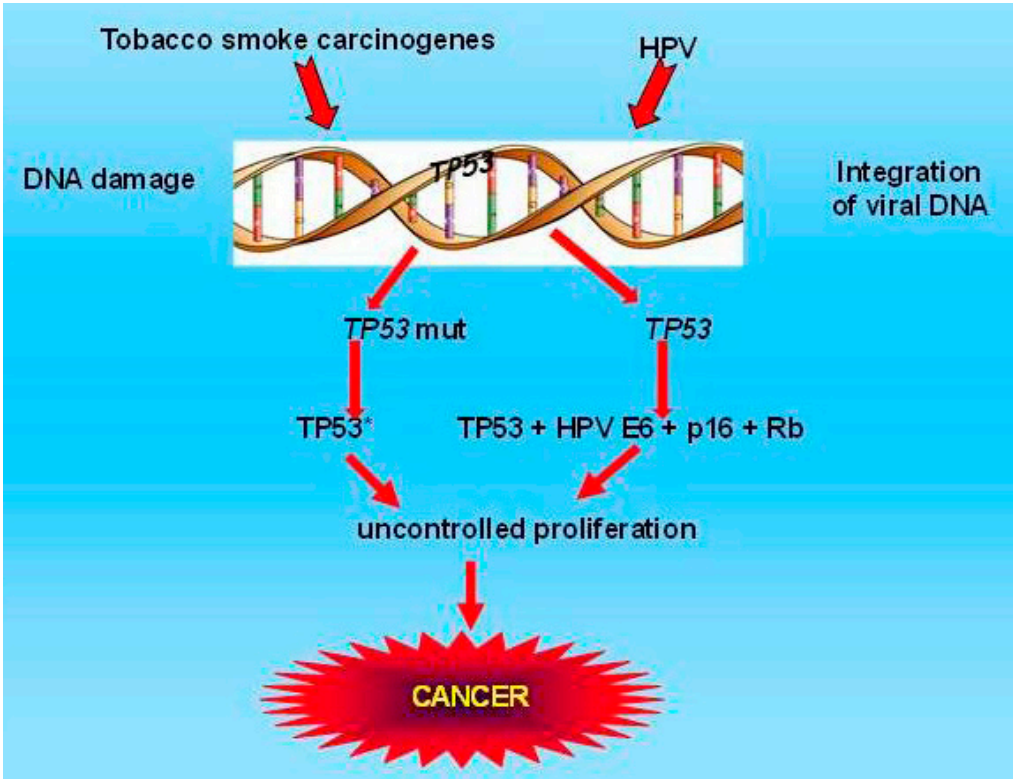

Figure 2. Molecular pathways of carcinogenic transformation initiated by tobacco smoke carcinogenes or HPV infection. The pathways indicate a difference in involvement of tumor suppressor gene TP53

nized risk factors as drinking and tobacco smoking, having a reasonable style of life and enjoying rather high economic status and are younger than typical patient [15]. Young age as well as sexual activity was confirmed in other studies as e.g. [26]. Moreover, high risk HPV was detected significantly more often than in aged patients [27].

A favorable prognosis for HPV-positive HNSCC compared to HPV-negative was recognized early. The contributing factors are smaller size tumors at presentation, good health conditions that offers a longer disease-free period and longer survival [28]. Such findings published in numerous papers have been confirmed in a large meta-analysis [29]. Altogether a less severe progression and a better prognosis in case of and HPV-positive HNSCC subject have implicated a question of therapy de-intensification. Nowadays this is one of the topic questions in oncological laryngology. Although there is no agreement about sensitivity to radio- and chemotherapy [compare: 29 v. 30] in HPV-positive HNSCC subjects a therapy avoiding acute toxicity is being suggested [30–32]. Nevertheless, so far as official recommendations do not differentiate between HPV-positive and -negative HNSCC, there is no safe way both for patients treatment as well for clinicians civil responsibility.

So, nowadays laryngologists are recognizing two types of HNSCC dependently on its origin. The first one is derived from an exposure onto chemical carcinogens most commonly present in tobacco smoke when the second results from HPV infection. Progression of the HPV-associated is connected with a better curability land longer survival but on optimal strategy of therapy requires still more studies. The other consequence is that highly heterogenous group of HNSCC is becoming even more differentiated and oropharyngeal and laryngeal cances should be definitively recognized as separate entities.

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Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

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The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References

All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

List all authors if there are six or fewer; if there are seven or more, list first six follower by "et al.". Journal names should be abbreviated according to Index Medicus.

Some examples

Standard journal articles

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

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SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process

All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

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