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Prophylactic antibiotic therapy leads to the reduction of postoperative complications in colonized patients subjected to abdominal hysterectomy with/without appendages for gynaecological indications

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Summary

Introduction: Surgical gynaecological procedures, such as: total, abdominal hysterectomy or vaginal hysterectomy are a group of surgeries associated with a high risk of postoperative infectious complications (PICs). The aim of this investigation was to identify microorganisms responsible for evolution of infections' complications in the postoperative period of these patients and to assess a correlation between the appearance of certain microorganisms on the risk of inflammation development in postoperative period. *Materials and Methods:* The medical research recruited 115 patients of Gynecologic-Obstetrician Hospital of Medical University in Poznań (Poland) undergoing total abdominal hysterectomy, with or without adnexa by gynecologic indications. To participate in this investigation, patients under 55 years of age were considered and 111 patients qualified to undergo medical investigation once pharyngeal and vaginal swabs and twice skin swabs (at the first day of hospitalization from defined area of the surgical field and at the third day after surgery from the same area) were taken. Antibiotic prophylaxis was used, including ampicilin, netromicin, and metronidazole. *Results:* The abnormal results of vaginal biocoenosis diagnosed in patients before total abdominal hysterectomy with or without adnexa by gynecologic indications increase probability of postoperative antibiotic therapy, if antibiotic prophylaxis was not given to the patient before the surgery started. There was no defined positive correlation between the number microorganism colony cultured in pharyngeal, vaginal, and skin swabs taken from the patients at highest risk of infection occurrence during the postoperative period.

Key words: Vaginal biocoenosis; Antibiotic prophylaxis; Total abdominal hysterectomy.

Introduction

Surgical gynaecological procedures, such as: total, abdominal hysterectomy or vaginal hysterectomy are a group of surgeries associated with a high risk of postoperative infectious complications (PICs) [1]. The conditions present in the female urogenital system: closeness of the anus, chronic inflammatory processes, and vesicoureteral reflux or closeness of the reproductive system, predispose them to the development of bacterial infections. The studies by Houang *et al.* [2] confirmed that the occurrence of bacterial infection or disturbance in the balance between anaerobic and aerobic bacteria in the vagina of a woman during the preoperative period significantly increases the risk of development of postoperative complications in patients who had undergone hysterectomy by laparotomy. According to the data from literature, the majority of these complications are caused by Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis [3, 4].

The objective of the study was to identify microorganisms responsible for the development of complications during the postoperative period in patients subjected to abdominal hysterectomy with/without appendages for gynaecological indications.

Materials and Methods

The study recruited 111 patients of the Obstetrics and Gynecology Clinic at the University Hospital of Medical Sciences in Poznań, hospitalized in the Clinic of Surgical Gynaecology, in the Diagnostics and Observation Ward, and the 2nd Obstetrics and Gynaecology Ward, scheduled to undergo hysterectomy with/without appendages for gynaecological indications. Into the study patients aged under 55 years were considered. After gynaecological examination and ultrasound scan using the transvaginal linear probe with a frequency 5.5 mHz, with negative skin tests for penicillin allergy or negative medical history for allergy to drugs applied in the study.

The exclusion criteria for participation in the study were: the BMI < 18.5 and > 40, an active inflammatory process (including bacterial vaginosis), antibiotic therapy within the last 14 days, cirrhosis, renal failure, chronic diseases – diabetics, systemic diseases, haematologic proliferative processes, inflammatory dermal changes or skin necrosis, malignant cancerous processes, radio- or chemotherapy, use of adrenal cortex hormones, ASA III, ASA IV,

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ASA V, a prolonged duration of surgical procedure (> 2 hours), and intraoperative blood loss of over 1.5 L.

Control microbiological smears were collected each time using a new pair of disposable gloves, prior to prophylaxis, maintaining the principles of asepsis, from 1) posterior vaginal vault, 2) throat, and 3) skin integuments. Prior to the performance of gynaecological bimanual examination, a gynaecological speculum was inserted into the vagina, and a smear was collected from the posterior vaginal vault with sterile swaps. Subsequently, the collected material, in an agar transport sealed sterile container was transferred within 30 minutes from collection, to the microbiological laboratory in the Obstetrics and Gynecology Clinic at University Hospital of Medical Sciences (GPSK) to be cultured on growth media for aerobic and anaerobic bacteria and fungi. During the period of 24 hours before the collection of vaginal smear, the examined patients had no intercourse with their partner, did not apply any drugs intravaginally, nor did they use any intimate hygiene means. The material for culture was collected using two sterile swabs, while the material designed exclusively for microscopic evaluation of biocenosis with one swab. Simultaneously with the collection of the smears, the acidity of the fresh vaginal discharge was determined directly from the patient, and 10% KOH amine test (potassium bicarbonate) performed in order to confirm or exclude bacterial vaginosis (BV).

The degree of cleanness (biocenosis) of the vagina was determined according to the 4-stage Manu af Heurlin's Scale, where: Stage 1 is determined in the situation of the presence in the microscopic specimen of material from the vagina of exclusively lactic acid bacilli. Stage 2 is diagnosed in the case of detection of the additional presence in the specimen of vibrios, or fine curved Gram-negative bacteria, which are most often rods of the genus Mobiluncus, Stage 3 is recognized when in the specimen examined there are non-uniform groups of pathogens of various shapes and genera (e.g. Gram-positive rods, Gram-positive cocci), and Stage 4 is diagnosed in case of the presence in the specimen of fungi and/or trichomonas.

Throat smear was collected with a sterile swab moistened with a sterile solution of sodium chloride, after immobilization of the tongue with a sterile spatula. The material for microbiological testing was collected from the palatal arches, the site of palatine tonsils, and from the back wall of the throat. While collecting the material, other surfaces of the nasopharynx were not touched, nor was saliva collected. The material was placed in the transport medium and transferred to the microbiological laboratory at the GPSK, not later than up to two hours from the moment of collection.

Integuments smear was taken twice, on the day of surgery from the surgical site, and on the third day after the surgical procedure from the post-surgical site. Prior to the first collection, the skin at the collection site was disinfected with 70% ethyl alcohol, and after the alcohol dried, a smear was taken with a sterile swab moisturized with isotonic sodium chlorine solution, and subsequently placed on transport agar gel. On the third postoperative day, the material for microbiological examinations was collected from the edges of the wound, at the previous collection site, after previous disinfection of the wound site with 70% ethyl alcohol. Subsequently, within 30 minutes, the material was transferred to the microbiological laboratory at the GPSK. While collecting the material for microbiological tests, the 'Instruction for collection, storage and transport of materials for microbiological examinations' was observed, which has been in effect at the GPSK from May 15, 2006. In the microbiological laboratory, the bacteriological material collected twice from the skin was inoculated on culture media for aerobic, anaerobic bacteria and fungi, cultured, and the strains grown were then evaluated and microbiologically classified.

Patients were divided into three groups: Group 1 (G1) – (n=43) did not receive perioperative prophylaxis. According to the postoperative management method. The G1 group was divided into: subgroup G1a - in which there occurred the necessity for the application of antibiotic therapy during the postoperative period, and subgroup G1ba where the use of antibiotic therapy was unnecessary. Group 2 (G2) – (n=30) received antibiotic prophylaxis 30-60 minutes prior to the surgery, where it was necessary (subgroup G2a)$^{1+2}$ / and unnecessary (subgroup G2ba)^{1} to apply antibiotic therapy during the postoperative period. [^{1}30 minutes before surgery: ampicillin 2 g i.v., netromycin 2 mg/kg b.m. i.v., meronidazole 500 mg i.v., and ^{2}during the postoperative period: claforan 2 x 1g i. v. (for indications)]. Group 3 (G3) – (n=38) received antibiotic prophylaxis 30-60 minutes before the surgical procedure, and 12 hours after the first administration, where it was necessary (subgroup G3a)$^{1+2}$ / or unnecessary (subgroup G3ba)^{1} to use antibiotic therapy during the postoperative period. [^{1}30 minutes before surgery and 12 hours after first administration: ampicyllin 2g i.v., netromycin 2 mg/kg b.m. i.v., meronidazole 500 mg i.v., and ^{2} during the postoperative period: claforan 2 x 1g i.v. (for indications)].

The continuation or implementation of postoperative antibiotic therapy (claforan 2 x 1g) depended on the exponents of the ongoing inflammatory process, i.e. body temperature ≥ 38°C measured twice within 24 hours after surgery, and/or exponents of postoperative wound infection (pain, tenderness, reddening, edema, wound exudate), evaluated by the same person, based on physical examination. The infiltration of tissues > 5 mm from the edge of the wound was adopted in the study as edema at the surgical site.

The obtained results, expressed in a nominal scale, i.e. species of bacteria grown on cultures of the material collected from throat, vagina, and the skin at post-surgical site, were described using the numbers of patients and the equivalent percentages. The relationships between the characteristics were investigated using the Fisher-Freeman-Halton test. In order to compare two percentages, the Gauss' U test was applied. The calculations were performed using statistical software (2005), STATISTICA (data analysis software system), v. 7.1, Cytel Studio v. 7.0.0 (2005), and Analyse-it v. 1.62 (2001).

All participants voluntarily agreed to participate in the study. Data confidentiality and survey procedures were reviewed with each participant before the questionnaire. Researchers assured participants that the contents of the questionnaire would be used solely for research purposes. The patients gave written obtained consent for this study. Ethics committees approved this consent procedure. This study was specifically approved only for this study by the Bioethics Commission at the Poznan University of Medical Sciences No 574/2011.

Results

The groups in the study were characterized by high homogeneity from the aspect of the characteristics analyzed: age, height, body weight, and the BMI (Table 1). In the first

Table 1. — *Distribution of characteristics: age, body weight, height, and BMI in particular groups in the study.*

Group	Subgroup	Age (years) mean		Body weight (kg) mean		Height (cm) mean		BMI mean	
G1	G1ba	47.76	47.48	69.68	67.15	164.18	165.13	25.82	24.61
	G1a		48.10		72.60		163.10		27.21
G2	G2ba	48.83	50.00	69.6	69.65	163.8	164.65	26.04	25.88
	G2a		46.50		69.50		162.10		26.38
G3	G3ba	48.28	48.56	68.91	68.72	164.52	164.70	25.28	25.13
	G3a		46.00		70.50		163.00		26.55
***p**	#p	**0.54**	0.878	**0.94**	0.519	**0.87**	0.296	**0.70**	0.692
	#p		0.675		0.134		0.750		**0.048**

**The level of statistical significance was assumed at $p < 0.05$ (Kruskal-Wallis One Way Analysis of Variance on Ranks).*
 # The level of statistical significance was assumed at $p < 0.05$ (Levene's test).

Table 2. — *Biocenosis in individual subgroups examined.*

Subgroup	No.	Biocenosis Stage 1	Biocenosis Stage 2	Biocenosis Stage 3	Biocenosis Stage 4
G1ba	23	0	17	6	0
G1a	20	0	8	7	5
G2ba	20	1	11	4	4
G2a	10	0	6	3	1
G3ba	34	0	25	3	6
G3a	4	0	1	0	3
Total	111	1	68	23	19

group (G1), the necessity for continuing administration of antibiotic during the postoperative period occurred in 20 (46.5%) patients (subgroup G1a), in the second group (G2) postoperative antibiotic therapy was implemented in ten (33.3%) patients (subgroup G2a), whereas in the third group (G3), it was necessary to apply antibiotic therapy in only four (subgroup G3a) of 38 patients analyzed, which is 10.5% of the group in the study. The surgery was performed in 36 (83.72%) patients from group G1, 26 (86.67%) patients from group G2, and in 33 (86.84%) patients from group G3. Abdominal hysterectomy saving appendages (HTASA) was definitely more rarely performed: seven (6.28%) patients in G1, four (13.33%) patients in G2, and five (13.16%) patients in G3. Transverse suprapubic incision was performed in 27 (62.8%) patients in G3, 13 in G2, and ten (26.3%) in G3. In the remaining patients, the abdominal integuments were opened by vertical incision.

The most frequently observed stages of cleanness of the vagina were Stages 2 and 3. Stage 2 was found in 68 patients (61.3%), while Stage 3 - in 23 patients qualified for the study (20.7%) (Table 2). In the group of G1 patients, where antibiotic prophylaxis was not applied, Stages 3 and 4 of vaginal cleanness were observed in 26.09% patients who did not require postoperative antibiotic therapy, and in as many as 60% of those who required the use of antibiotics ($p > 0.05$). Respectively, for G2 patients receiving antibiotic therapy in the form of one dose of the series of antibiotics, this ratio was 40%: 40% ($p > 0.05$). For group G3 receiving a double series of antibiotics in prophylaxis, this relationship was as follows: 26.47%: 75% ($p > 0.05$).

The bacteria most frequently found in the nose in patients that qualified for the study were: the species *S. viridi*ans, family of Gram-negative diplococci, to which belong the genera Neisseria, Kingella and Branhamella (Moraxella), and anaerobic bacteria. In the group of patients that did not qualify for antibiotic therapy, 95 colonies of bacteria and fungi were found. In the group that received a single series of antibiotics in prophylaxis - 73 colonies, and in the group administered a double dose of antibiotics in prophylaxis - 94 colonies. A total number of 262 colonies were found among 111 patients: 2.36 colony per patient, on average. A considerably larger number of colonies were found in the subgroups of patients who did not require postoperative antibiotic therapy: 53 in 'G1ba' (2.3 colony/patient on average), 51 in 'G2ba' (2.55 colony/patient on average), and 86 in 'G3ba' (2.5 colony/patient, on average); for subgroups receiving postoperative antibiotic therapy – 42 colonies, on average, in the subgroup 'G1a', 22 – in 'G2a', and eight in 'G3a'.

The bacteria which were most frequently seen in the examined patients were coagulaso-negative staphylococci (species S. epidermidis), and anaerobic bacteria. Gram positive rods of the species Corynebacterium and enterococci grew on culture media more rarely. In the material collected

from the body integuments on the day of admission, a total number of 180 colonies of bacteria were found, which is 1.64 colonies per patient, on average, whereas in the material collected on the third day after surgery, there were 120 bacterial colonies, i.e., 1.08 colonies per patient. A decrease was observed in the number of colonies found during hospitalization. In group G1 of patients without antibiotic prophylaxis, on the day of admission, the presence of 60 bacterial colonies were found, on average, i.e. 1.4 per patient, on average. Respectively, in group G2, with a single dose of antibiotics in prophylaxis, 51 bacterial colonies were isolated, i.e. 1.7 per patient. In the group of patients who received a double series of antibiotics in prophylaxis, 70 colonies were found, i.e. 1.84 per patient. In cultures from the third day (SC2), the presence of 58 bacterial colonies was discovered, in patients from G1 (1.35 per patient, on average), in G2 - 34 colonies (1.13 per patient, on average), while in G3 - 38 colonies (1.0 colony per patient). In the group receiving antibiotic prophylaxis the number of colonies grown in the skin smear collected on the third day (SC2) was lower, compared to the first day (SC1). The more antibiotic doses administered in prophylaxis, the lower the mean number of colonies per patient in these groups. While analyzing the number of bacterial colonies in patients from subgroups without postoperative antibiotic therapy, it was found that among these patients the mean number of colonies per patient was 1.73 in SC1, and 1.14 in SC2. A similar relationship was observed in the subgroups requiring postoperative antibiotic therapy: 1.53 in SC1, and 0.94 in SC2. The implementation of prophylactic antibiotic therapy led to the reduction of the number of bacteria on the skin on the third postoperative day, especially in the group SC administered antibiotic after surgery. The bacteria most often found in these patients belonged to the species Candida, Lactobacillus, and coagulaso-negative staphylococci. More rarely than the aforementioned, however, relatively often found on culture media were anaerobic bacteria, Gram-negative rods E. coli, of the species Mycoplasma hominis, Ureaplasma urealyticum, and the Gram-negative species S. agalactiae. In cultures inoculated on the third day, bacteria of the species Agrobacterium were found, which were not present in the first culture of material collected from the skin integuments, whereas the number of colonies of coagulaso-negative staphylococci (S. epidermidis) considerably decreased, the Lactobacillus bacilli disappeared, Gram-negative rods of the genus Acinetobacter, which are components of bacterial flora of the skin of healthy individuals, colonize gastrointestinal tract, nasopharynx, and vagina, as well as Streptococcus agalactiae of group B pyogenic staphylococci. Among patients that qualified for the study, from the cultures of material collected from the vagina, 273 colonies of bacteria and fungi were obtained (101-G1, 75-G2, 97-G3), where fungi constituted 3.66% of all colonies. The mean number of bacteria and fungi per patient participating in the study was 2.46, respectively, in the group without prophylaxis there were 2.35 colonies per patient, in the group with a single dose of antibiotics – 2.5 colonies, and in the group receiving a double preventive dose of antibiotics – 2.55 colonies. In the subgroups of patients without postoperative administration of antibiotic, a total number of 191 colonies were grown; 3.42 per patient on average, while in groups with postoperative antibiotic a total number of 82 colonies; i.e. 2.41 colonies per patient, on average. Respectively, in subgroups 'G1ba' and 'G1a', 'G2ba' and 'G2a', 'G3ba', and 'G3a', the mean number of colonies grown per patient was: 2.14 and 2.6, 2.7 and 2.1, 2.59 and 2.25. The aforementioned presented data show that the number of colonies of bacteria and fungi found in the vagina of patients qualifying for laparotomy for gynaecological indications does not exert any effect on the increase in the risk of postoperative antibiotic therapy, and therefore does not increase the probability of occurrence of an inflammatory state during peri- and postoperative periods.

Discussion

The species of microorganisms infecting the wound are closely related with the site of surgery, because these are microorganisms from the closest neighbourhood [5]. Surgical infections may be of an endogenous type, with own bacterial flora, in the situation where the line of surgical incision goes along abundantly colonized mucous membranes, and the wound becomes infected with these microorganisms, or of an exogenous character – when bacteria initiating the process come from the external environment or the patient's nearest surroundings [5, 6]. Staphylococcus epidermidis, Staphylococcus aureus, aerobic and anaerobic rods G (-), and enterococci, most frequently lead to the infection of postoperative wounds [6, 7]. According to Sopper *et al.* [8], the bacteria most often isolated from infected postoperative wounds are: the genus Staphylococcus, including mainly S. epidermidis, S. aureus and S. agalactiae, β-hemolytic staphylococci, the genus Enterococcus, Acinetobacter, and the species Proteus mirabilis. Analysis of the frequency of wound infections in Poland, according to the degree of cleanness of the surgical site, showed a relationship between the frequency of infections and the degree of contamination of the wound with microorganisms from 1.02 % for surgeries at a clean site, 1.68% for clean infected surgeries, to 5.9% for surgeries at an infected site [9]. The removal of the uterus with appendages by laparoscopy is considered as a surgery at a clean-contaminated site, and its most frequent complications are inflammations of soft tissues, haematomas, and ulcers of the lower pelvis, and infections of postoperative wound (14-57%) caused by mixed aerobic and anaerobic bacterial flora [10]. According to the results of the prospective study conducted by Sopper *et al.*, the risk of infection of a postoperative wound in patients subjected to abdomi-

nal hysterectomy is 11.3%, on average, and increases when the thickness of subcutaneous fatty tissue is above 3 centimetres, the patient's body weight exceeds 92.6 kilograms, and the BMI is above 35.6 [8]. For comparison, investigations confirmed that vaginal hysterectomy is related with a higher risk of infections (14-57%), compared to abdominal hysterectomy (15-24%), and the use of antibiotic prophylaxis may decrease this percentage down to 6.3-32% and 9-9.8%, respectively [11, 12].

While performing surgeries at a 'clean' surgical site, the spectrum of the antibiotic applied should cover with its action streptococci, whereas in procedures performed at a 'clean-contaminated surgical site', to which belongs abdominal hysterectomy with/without appendages performed in patients participating in this study – a mixed aerobic-anaerobic bacterial flora. Therefore, the antibiotics used in prophylaxis should be characterized by a wide spectrum of action which would cover both aerobic and anaerobic bacteria, typical of the urogenital system, the skin, and oral cavity. The results of meta-analyses published in 1993 demonstrated that in as many as 21.1% of patients not subjected to antibiotic prophylaxis by any preparation during the perioperative period, prior to the performance of abdominal hysterectomy, there may develop serious infectious complications, whereas in patients who received this prophylaxis, the risk of development of these complications is only 9-10% [13-15]. Houang *et al.* [2], based on the analyses performed, drew the conclusion that this is the number and not the pathological potential of the strains isolated from patients from the cervix and vagina that exerts a significant effect on the risk of development of postoperative infection after abdominal hysterectomy. The mean number of bacterial and fungal colonies from the vagina per one patient participating in the study was 2.46. In the group not receiving prophylaxis, the mean number of colonies per patient found on media was 2.35, in the group receiving one prophylactic antibiotic therapy dose 2.5, and in the group subjected to two prophylactic doses 2.55. Apparently, these values do not differ significantly. The situation is slightly different in the case of comparing subgroups which require postoperative antibiotic therapy or not, where only in the group of patients without antibiotic prophylaxis the number of colonies of bacteria and fungi cultured on material collected from the vagina was higher in the subgroup requiring postoperative antibiotic therapy (2.14 *vs.* 2.6), which might confirm the relationships described by Hounag *et al.* [2]. However, a comprehensive analysis of the subgroups showed that in the subgroups of patients who did not require postoperative antibiotic therapy, 3.42 colonies were cultured per patient, while in subgroups administered an antibiotic postoperatively only 2.41. Obviously, the thesis by Hounag *et al.* [2] was not confirmed in the presented study. These discrepancies may be explained by the fact that analyses performed by Hounag *et al.* [2] also concerned cultures of material from the cervix, not covered by this study. In addition, the species of bacteria and fungi obtained in cultures of material collected from the vagina in the examined patients did not differ from the data mentioned in literature [3, 16]. Considering the synergistic action of netromycin, ampicyllin, and metronidazole, the spectrum of action which covers the flora of the reproductive system, good tolerance, and low percentage of adverse effects, the use of these drugs in antibiotic prophylaxis in patients subjected to laparotomy for gyneacological indications is fully justified.

Conclusions

Abnormal biocenosis of the vagina, the lumen of which is opened during abdominal hysterectomy, increases the probability of postoperative antibiotic therapy in patients who had not previously received antibiotic prophylaxis. No correlation was found between the presence of microorganisms in the cultures from the throat, skin, and vagina, and the risk of development of infection during the postoperative period.

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