HEALTH SCIENCES
RECEIVED 06.07.2014 ACCEPTED 10.09.2014 PUBLISHED 15.09.2014
DOI: 10.15550/ASJ.2014.10.050

THE BACTERIAL CHARACTERISTIC OF THE PURULONECROTIC FOCUS IN COMPLICATED DIABETIC FOOT SYNDROME DEPENDING ON ITS CLINICAL FORM

S. Shapoval, I. Savon, D. Ryazanov, M. Sofilkanych

**Zaporizhzhya Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine,
20, Vintera Blvr., Zaporizhzhya 69096 Ukraine
shapoval.s.d@gmail.com**

Abstract. A study of the microflora of 1037 patients with complicated diabetic foot syndrome (DFS) was conducted over the period of 2010 through 2014. The quantity of gram-positive flora among aerobic germs increased from 52.7% (2010) to 61.4% (2014) with Staphylococcus aureus (62.5%) and Enterococcus faecalis (23.5%) prevailing. Enterobacter spp. and Escherichia coli prevailed among aerobic gram-negative flora. The plating of Pseudomonas aeruginosa grew from 17.2% (2010) to 22.4% (2014). The number of microbe associations extracted from the primary suppurative focus increased from 46.9% to 53.1%. Both common features and differences were discovered during the study of the microbial landscape of patients with different forms of complicated DFS. The increase in the dynamics of gram-positive flora study and the number of microbe associations, as well as the growth of MRSA, was a common pattern. The results of the antibiogram study are indicative of the high resistance of the detected microflora to I-II generation antibiotics.

Key words: complicated diabetic foot syndrome, infectious agent qualitative characteristic, antibiotic sensitivity, resistance.

Introduction

Bacterial infection is one of the key pathogenic factors outlined in the report of the International Working Group on the Diabetic Foot (IWDGF, 2013). The development of infection on the feet of patients with diabetes mellitus (DM) is responsible for amputation in over 50% cases, therefore, it is of paramount importance to detect and identify the causative agent in the early stages of the condition (Dedov, 2011; Bakker, 2012).

The data concerning the microflora of the suppurative focus in the lower limbs of patients with DM are highly contradictory (Amit Kumar, 2013; Beloborodov, 2013). Most studies testify that the association of aerobic and anaerobic microorganisms contributes to the progression of the purulonecrotic process during diabetic foot syndrome (DFS) in the vast majority of cases, and the specific gravity of non-clostridial flora in infection nidi tends to increase steadily (Galstjan, 2013; Gorobejko, 2013; Milne, 2013).

Nonpathogenic bacteria may cause infection development under normal conditions in patients with DM (Podprjatov, 2012). The consequences of the emergence of infection are more severe due to the anatomy structure of the foot and the presence of tendons and tissue spaces over which infection spreads proximally (Kalachev, 2013; Zarivchackij, 2013).

The differences in the interpretation of microbiological studies may be connected with the fact that the microbial landscape is characterized on the whole, regardless of the various clinical forms of complicated DFS (Strbova, 2011). Thus, the identification of the bacterial flora of the purulonecrotic focus provides the basis for the formation of rational antibacterial therapy and assessment of wound process dynamics (Fincke, 2010; Faroqi, 2013).

The objective of the study is to improve the results of treatment of patients with complicated DFS through the study of the bacteriological condition of the primary suppurative focus and the sensitivity to antimicrobials.

Method

1037 patients with complicated DFS receiving medical treatment in the "City Clinical Hospital No.3 PUS" in-patient suppurative-septic diabetic foot center, Zaporizhzhya, were examined over the period of 2010 through 2014.

All patients had DM type 2 with an average duration amounting to 12.4±2.6 years. The average age of the patients amounted to 62.4±2.3 years. There were 498 (48%) men and 539 (52%) women.

According to the classification of DFS by clinical manifestations (International Working Group on the Diabetic Foot, the Netherlands, 1999), 295 (28.4%) patients had its neuropathic form, 168 (16.2%) had the ischemic one and 574 (55.4%) had the mixed one.

In view of the depth and prevalence of the infectious inflammatory process, 282 (27.2%) of the patients were diagnosed with 2nd damage degree, y 376 (36.3%) of patients – with 3rd degree, 246 (23.7%) – with 4th degree, 133 (12.8%) patients – with 5th damage degree according to the classification of P.M. Wagner, 1979.

The therapeutic program included mandatory use of antimicrobials. In order to define the sensitivity of microorganisms to antimicrobials, the standard disk diffusion method and the rapid test method (Shapoval, 2005) were used. The studies were conducted during hospital stay and over time.

The statistical analysis was conducted using the «Statgraphics Plus for Windows 7.0» software package.

Table 1

The quantitative and qualitative characteristic of causative agents of patients with complicated DFS over time

Infection type	2010 n=217		2011 n=198		2012 n=233		2013 n=176		2014 n=233	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Aerobic monoinfection	**103**	**47.7**	**92**	**46.5**	**100**	**43.4**	**75**	**42.6**	**96**	**41.2**
gram-positive	54	52.7*	51	55.2*	60	60.0*	46	61.3*	59	61.4*
gram-negative	49	47.3*	41	44.8*	48	40.0	29	38.7*	37	38.6*
Anaerobic monoinfection	**12**	**5.4**	**9**	**4.5**	**12**	**5.2**	**10**	**5.7**	**12**	**5.2**
clostridial	0	0**	0	0**	0	0**	0	0**	0	0**
non-clostridial	12	5.4**	9	4.5**	12	5.2**	10	5.7**	12	5.2**
Mixed infection	**102**	**46.9**	**97**	**49.0**	**121**	**51.4**	**91**	**51.7**	**125**	**53.6**
Aerobic associations	95	93.1***	93	95.9***	118	97.5***	91	100***	118	94.4***
Anaerobic associations	0	0***	0	0***	0	0***	0	0***	0	0***
Aerobic-anaerobic associations	7	6.9***	4	4.1***	3	2.5***	0	0***	7	5.6***

Notes:

* - percentage in aerobic monoinfection;

** - percentage in anaerobic monoinfection;

***- percentage in mixed infection.

Results

The study of the plating of microflora in patients with complicated DFS over the period of 2010 through 2014 showed that the nature of the wound infection underwent both quantitative and qualitative changes.

The planting of aerobic monoinfection from primary suppurative foci in patients with complicated DFS decreased from 47.6% in 2010 to 41.2% in 2014 (Table 1). However, the balance among the causative agents of the aerobic flora changed to the advantage of gram-positive flora from 52.7% in 2010 to 61.4% in 2014.

The bulk of the gram-positive aerobes is accounted for by Staphylococcus aureus (62.5%) with Enterococcus faecalis plating second in quantity (23.5%).

The dynamics of the microbiological studies are indicative of the growth of the percentage of MRSA in the pure culture among coagulase-positive staphylococci: 20.7% in 2010; 23.3% in 2011; 29.4% in 2012; 34.7% in 2013; 42.8% in 2014.

The aerobic gram-negative flora of initial suppurative foci in patients with complicated DFS decreased from 47.3% (2010) to 38.6% (2014) over the period under study, which amounts as a percentage of the total planting rate to 21.2% and 16.4%.

The Enterobacter spp. and Escherichia coli groups were plated with the same frequency (21% (19%) in 2010 – 22% (18%) in 2014). Consequently, the planting of Pseudomonas aeruginosa in the pure culture among gram-negative flora grew from 17.2% (2010) to 22.4% (2014).

The recovery of anaerobic causative agents of surgical infection remains topical, as the pathological course of disease has its peculiarities which have to be taken into account in the process of treatment.

Anaerobic infection has been diagnosed in 5.4% in 2010 and in 5.2% in 2014. The anaerobic process is characteristic of closed wounds, abscesses, hollow trophic ulcers and myonecrosis; thus, it is not always possible to detect anaerobic infection in the wound while examining the traumatic discharge. The appearance of skin and the wound, pus, presence of smell and bacteria in impression smear microscopy are specific for anaerobic infection; thus, the corresponding bacterial therapy would be prescribed for the cases of presence of the above symptoms and the absence of bacterial growth in the standard medium.

The quantity of microbial associations recovered from the initial suppurative foci of patients with complicated DFS increased from 46.9% to 53.1%. The increase occurred due to aerobic associations. Aerobic-anaerobic associations were plated in isolated cases, and no growth of anaerobic associations in patients with complicated DFS was detected over the period under study.

The major representative of mixed aerobic infection was Staphylococcus aureus. Staphylococcus aureus was usually plated together with gram-positive rods: Enterococcus faecalis, Corynebacterium xerosis and Staphilococcus epidermidis.

Despite the fact that the Enterobacteriaceae family is one of the basic components of the human gut microflora, they were plated as part of aerobic associations together with gram-positive flora in over half the instances: 46.9% (2010) and 53.1% (2014).

Pseudomonas aeruginosa bears the greatest clinical relevance among the group of non-fermentative microorganisms belonging to aerobic associations. The blue pus bacillus holds a special place among suppurative microflora due to its peculiar channels and existence conditions, as well as its ability to suppress the growth of other microflora. The planting of Pseudomonas aeruginosa as part of microbial associations amounted to 10.6% (2010) and 13.4% (2014). The frequency of their planting increased with the extension of the patients' hospital stay period.

Acinetobacter spp. holds the second place after the blue pus bacillus among the non-fermentative microorganisms in the composition of microbial associations: 8.2% (2010) and 8.4% (2014). Acinetobacter spp. are characterized by a high level of natural sensibility to the majority of antibiotics; however, its characteristic feature is the quick formation of resistance to numerous groups of antimicrobials. The hospital strains of Acinetobacter spp., according to our research, were mostly multiresistant.

Both common features and differences were discovered during the study of the microbial landscape of patients with different forms of complicated DFS.

The increase in the dynamics of gram-positive flora study and the number of microbe associations, as well as the growth of MRSA strains of staphylococci in the pure culture, was a common pattern (Table 2).

The difference consisted in the following: aerobic monoinfection with a prevalence of staphylococci was plated in patients with the neuropathic form. The frequency of staphylococci fluctuated from 82.3% (2010) to 84.6% (2014). The group of enterobacteria was plated with the same frequency during the study over time, and the content of blue pus bacillus in the pure culture never exceeded 20%.

In 18-22% of the cases of the ischemic form of complicated DFS, the growth of microflora was absent or skin saprophytes and pathogenic fungi of the Candida genus were detected.

Microbial associations were plated most often (57.4%) in the case of the mixed form of complicated DFS in comparison with the above forms of the condition. Enterococcus spp. was plated most often in the associations.

The data of the nature of the causative agent and its sensitivity to antimicrobials are necessary for rational antimicrobial therapy. The data of the sensitivity of microorganisms to antibiotics for the patients with complicated DFS of our center in 2013 are presented in Table 3.

Table 2

The quantitative and qualitative characteristic of microorganisms in various clinical forms of DFS

The generic and specific assignment of the microorganisms	The clinical form of the diabetic foot syndrome					
	Ischemic form, n = 168		Neuropathic form, n = 295		Mixed form, n = 574	
	Abs. number	%	Abs. number	%	Abs. number	%
Staphilococcus aureus	89	53.2	145	49.2	293	51.1
Staphilococcus epidermidis	15	8.9	16	5.4	18	3.2
Staphilococcus haemoliticus	11	6.4	4	1.4	8	1.4
Streptococcus pyogenes	1	0.7	1	0.3	12	2.1
Enterococcus faecalis	13	7.7	44	14.9	76	13.3
Enterococcus faecium	2	1.2	7	2.4	4	0.7
Corynebacterium xerosis	9	5.5	12	4.1	15	2.6
Esherihia coli	9	5.5	8	2.8	9	1.6
Proteus spp.	3	1.8	1	0.3	5	0.8
Pseudomonas aeruginosa	8	4.8	54	18.4	82	14.3
Acinetobacter spp.	2	1.2	0	0	3	0.6
Citrobacter spp.	2	1.2	0	0	2	0.4
Enterobacter spp.	3	1.8	2	0.7	9	1.6
Klebsiella spp.	0	0	0	0	2	0.4
Peptostreptococccus spp	0	0	1	0.3	0	0
Bacteroides fragilis	0	0	1	0.3	1	0.2
Candida spp.	1	0.6	4	1.4	9	1.6

Table 3

The sensitivity of S. aureus (MSSA) and Ps. aeruginosa to antibiotics in patients with complicated DFS for 2013 (M+m)

Antimicrobial	Sensitivity of the culture, %	
	S. aureus (MSSA)	Ps. aeruginosa
Azithromycin	37.4±3.1	0
Amikacin	82.7±2.4	74.3±1.7
Amoxicillin/clavulanate	76.3±1.9	0
Ampicillin/sulbactam	78.6±1.4	0
Vancomycin	100.0	0
Gentamicin	66.3±2.1	23.4±1.1
Imipenem/cilastatin	96.4±1.7	74.8±3.3
Clindamycin	81.4±1.6	0
Linezolid	100.0	0
Levofloxacin	68.3±1.5	68.6±1.7
Lincomycin	64.7±2.7	0
Meropenem	95.4±2.1	75.6±2.9
Metronidazole	0	0
Moxifloxacin	67.1±2.4	32.3±1.7
Norfloxacin	42.3±1.4	0
Oxacillin	72.4±2.3	0
Ofloxacin	47.4±2.1	34.6±1.2
Pefloxacin	47.6±1.8	0
Piperacillin/tazobactam	83.2±2.7	69.4±2.2
Rifampicin	68.3±3.3	0
Tetracycline	42.7±1.4	0
Cefazolin	74.6±1.8	0

continue Table 3

Antimicrobial	Sensitivity of the culture, %	
	S. aureus (MSSA)	Ps. aeruginosa
Cefepime	86.4±1.6	81.6±3.4
Cefoperazone	37.4±2.1	68.4±3.1
Cefoperazone /sulbactam	43.6±1.5	72.9±1.3
Cefotaxime	67.3±2.4	0
Ceftazidime	0	81.2±2.3
Ceftaroline	69.3±1.7	71.4±2.1
Ceftriaxone	66.8±1.6	0
Cefuroxime	63.7±2.4	0
Ciprofloxacin	51.4±2.2	71.4±2.6
Erythromycin	28.2±1.8	0
Ertapenem	83.4±1.3	0

The sensitivity of the microflora to chemotherapeutic compounds was defined using the rapid test method, which was done simultaneously with the "classical method". However, the latter produced the results of the research after two days. With the help of the rapid test method the results for the sensitivity of the microflora to antimicrobials were received as early as four hours later; thus, targeted antibiotic therapy of the patient was conducted on the day of hospital admission. The verification of the causative agent was obtained in 48 to 72 hours. The bacterioscopy of the wound smears allowing to define the group of the microbes was obtained in 1 to 1.5 hours.

Discussion

Relying on the results of the antimicrobial sensitivity of the microflora, we were guided by the following principles: in case of sensitivity to multiple antibiotics we would prescribe them increasingly, from generation 1 and up. Based on their grouping, we would begin with semisynthetic penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems. We would apply the "reserve" antibiotics when the indications of systemic inflammation response syndrome were indisputable.

For instance, the microorganism is sensitive to cephalosporins of all generations and fluoroquinolones of generations 1-3. In such cases I generation cephalosporin or I generation fluoroquinolone was prescribed.

The results of the study are indicative of the high resistance of the detected microflora to I-II generation antibiotics. The quantity of methicillin (oxacillin) resistant Staphylococcus aureus is on the increase.

Monoinfection sensitive to most antimicrobials was, as a rule, present in the wound contents seeding of those patients who had underwent no antibiotic therapy at the pre-hospital stage.

In the cases of transfer of patients from other surgery facilities or after unsuccessful domestic treatment (as a rule, these are patients in critical condition with disseminated purulent infection), high frequency of aerobic association plating was observed. In all likelihood, the infection contamination of the wounds resulted from hospital infection acquired in the previous surgery facility and uncontrolled use of antimicrobials at the pre-hospital stage.

These circumstances need to be taken into account in the complex therapy system for patients with complicated DFS.

We suggest that antimicrobials should be used in accordance with the developed schedules and patterns of complex therapy of complicated DFS with due regard to the clinical form of the condition, the anatomical location of the suppurative focus and the etiological agent (Optimizatsija antibakterialnoj terapii u bolnyh s oslozhnjonnym sindromom diabeticheskoj stopy. Kiev, 2010).

Unfortunately, there is no single policy of rational antibiotic use in state medical institutions in Ukraine. It may be due to this fact that the excessive adherence of surgeons to the prescription of new, original medication is observed.

In order to hinder the development of resistance, the most widely used antimicrobials are changed once every three months in our center.

Conclusion

The plating quantity of the monoinfection from the primary suppurative foci in patients with complicated DFS decreased from 47.6% in 2010 to 41.3% in 2014.

Aerobic gram-positive flora, 62.5% of which is accounted for by Staphylococcus aureus, dominates monoinfection. The growth of MRSA strains from 20.7% in 2010 to 42.8% in 2014 is observed. Среди аэробной грамотрицательной флоры доминируют Enterobacter spp., Escherichia coli and Pseudomonas aeruginosa dominate the aerobic gram-negative flora.

The common feature observed during the study of the microbial landscape in patients of various forms of complicated DFS was the increase in the dynamics of gram-positive flora and the number of microbial associations, as well as the growth of MRSA strains in the pure culture. The difference consisted in the following: aerobic monoinfection with a prevalence of staphylococci was plated in patients with the neuropathic form; in 18-22% of the cases of the ischemic form of complicated DFS, there was no microflora growth or skin saprophytes and pathogenic fungi of the Candida genus were detected; microbial associations were plated most often in the cases of the mixed form of complicated DFS.

The results of the study of the antibiotic sensitivity of microorganisms in patients with complicated DFS are indicative of the high resistance of the detected microflora to I-II generation antibiotics.

References

Amit Kumar, C. Jain et al. (2013) A decade of experience in managing diabetic foot at Amrita, India's largest diadetic limb Salvadge Centre. Journal Diabet Foot Complications, (2) pp. 15-17.

Bakker, K., Apelqvist, J., and Schaper, N. (2012) International Working Group on Diabetic Foot Editorial Board: Practical guidelines on the management and prevention of the diabetic foot 2011. Diabetes Metab Res Rev, pp. 225-231. Available at: http://dx.doi.org/10.1002/dmrr.2253

Beloborodov, V., Frolov, A. and Rudyh, K. (2013) Lechenie gnojno-nekroticheskih oslozhnenij sindroma diabeticheskoj stopy. Suchasni medichni tehnologii, (3) pp. 23-25.

Dedov, I. et al. (2011) Algoritmy specializirovannoj medicinskoj pomoshhi bolnym saharnym diabetom. Saharnyj diabet, (5) pp. 4-72.

Faroqi, L et al. (2013) Antibiotic Therapy and Culture Results For Diabetic versus Non-Diabetic Foot Wounds. Journal Diabet Foot Complications, (4) pp. 24-28.

Fincke, B., Christiansen, C., and Turpin, R. (2010) Variation in antibiotic treatment for diabetic patients with serious foot infections: a retrospective observational study. BMC Health Serv Res. (10) pp. 193. Available at: http://dx.doi.org/10.1186/1472-6963-10-193

Galstjan, G. et al. (2013) Kliniko-ekonomicheskoe obosnovanie stoimosti kvoty na lechenie pacientov s sindromom diabeticheskoj stopy. Saharnyj diabet, (3) pp. 71-83.

Gorobejko, M. (2013) Rezultaty lechenija sindroma diabeticheskoj stopy s pozicij dokazatelnoj mediciny. Suchasni medichni tehnologii, (3) pp. 48-49.

International Working Group on the Diabetic Foot (IWDGF), (2013). International consensus on the diabetic foot. Available at: http://iwgdf.org/consensus/epidemiology-of-the-diabetic-foot/ [Accessed 1 Jul 2014].

Kalachev, E., Butyrskij, A. and Vlasov, N. (2013) Osobennosti hirurgicheskoj infekcii pri gnojno-nekroticheskih oslozhnenijah sindroma diabeticheskoj stopy. Suchasni medichni tehnologii, (3) pp. 68-70.

Milne, T. et al. (2013) Healing Times of Diabetic Foot Ulcers: Investigating the Influence of Infection and Peripheral Arterial Disease. Journal Diabet Foot Complications, (1) pp. 29-38.

Podprjatov, S. Gychka, S. and Parshykov, O.(2012) Zbilshennja efektyvnosti dii antybakterialnyh preparativ pry likuvanni stopy diabetyka. Hirurgichna perspektyva, (1) pp. 30-31.

Shapoval, S. (2005) Modificirovannyj ekspress-metod opredelenija chuvstvitelnosti mikroorganizmov k antibakterialnym preparatam. Klinichna hirurgija, (11-12) pp. 62.

Strbova, L. (2011) Influence of infection on clinical picture of diabetic foot syndrome. Bratislava Medical Journal, (4) pp. 177-182.

Zarivchackij, M. et al. (2013) Kompleksnoe lechenie gnojno-nekroticheskih oslozhnenij sindroma diabeticheskoj stopy. Suchasni medichni tehnologii, pp. 66-67.