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The bacterial strains isolated from trophic ulcers and their persistence factors

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Abstract

Background: Recently, a particular attention has been drawn to the study of the microbial persistence properties and their correlation with the rate of elimination from the source of infection, as well as the prognosis of the disease progression.
Material and methods: There were examined 44 samples taken from patients with trophic ulcers. The bacteriological examination, as well as tests on determining both the persistence factors and the antibiotic susceptibility of the isolated strains were carried out according to the current method.
Results: There were determined 80 isolated bacterial strains. Two and more strains were isolated in over half of these cases (52.3%). The most commonly involved strains were the genus *Staphylococcus*, followed by *Enterobacter spp*., *Pseudomonas spp., Candida spp.,* and enterococci. Both gram-negative and gram-positive species exhibited a high-level antimicrobial resistance. The study of the persistence factors revealed that the strains isolated in mixed culture showed a higher rate of virulence (1.0-1.5 times higher) compared to isolates in pure culture.
Conclusions: The main bacterial strains isolated from trophic ulcers are the genus *Staphylococcus* and the *Enterobacteriaceae* family. Isolated strains showed higher level of antimicrobial resistance and multiple persistence factors. The study results proved that treatment of trophic ulcers is still a major problem, requiring rational monitoring and management strategies.
Key words: trophic ulcers, microbial spectrum, antibiotic resistance, persistence factors.

Introduction

In recent years there has been a qualitative change of some microbial strains involved in the infectious disease pathology, which tend to increase the incidence of mixed infections caused by potentially virulent gram-negative and gram-positive bacteria and characterized by a marked clinical polymorphism due to a simultaneous exposure of several etiological agents, each of which exhibiting a range of pathogenicity factors [1].

Microorganisms, involved in mixed infections, commonly present antibiotic resistance and a number of pathogenicity factors, such as lecithinase, haemolytic, antilizozyme, DNA-staining, and adherent activity, etc. [2].

Long-term persistence of bacteria within the host organism is due to multiple factors that inactivate the antimicrobial mechanisms of the immune system. Thus, it is highly recommended to study the persistence properties of the microorganisms in purulent infections, since these are responsible for the elimination rate from the site of inflammation, as well as for the prognosis of the disease. It is well known that the bacterial persistent potential is dependent upon the length of pathogenic harboring within the macro-organism, whereas its suppression via drugs may weaken this infectious potential [3-7].

The studies, conducted across different countries, have revealed a range of species isolated from trophic ulcers and their antimicrobial resistance, as well as the incidence of multidrug resistance (MDR) cases, strains of methicillin-resistant *Staphylococcus* spp. (MRS) and extended-spectrum beta-lactamases (ESBL), thus, suggesting that administration of empirical antimicrobial therapy might increase the rate of a treatment failure [8-10].

Treatment of trophic ulcer is a challenging task for clinicians and remains a current and relevant issue [11].

As regarding to the aforementioned, the purpose of the study was to determine the spectrum of bacteria isolated from trophic ulcers, to study the antibiotic susceptibility of the bacterial strains and to determine their hemolytic, lecithinase, anti-lysozyme, and anti-complementary properties, as well as to prove their diagnostic importance in detection of the bacterial targets in order to select the appropriate drug therapy.

Material and methods

Studies were conducted on 80 microbial strains isolated from trophic ulcers. The microbiological investigations, as well as the persistence factors and antibiotic sensitivity assessment of the isolated strains were performed within the microbiological laboratory of the National Agency for Public Health. The bacterial strains were isolated and detected according to the methodology described in "Basic laboratory procedures in clinical bacteriology". Antibiotic susceptibility was tested by Vitek 2 compact and Kirby-Bauer disk diffusion method. Antibiotic susceptibility testing and data interpretation have been standardized, in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for current antibiotic assessment [12]. Antibiotic disk diffusion testing included: cefoxitin (30 mg), erythromycin (15 mg), clindamycin (2 mg), gentamicin (10 mg), cefepime (30 mg), ceftazidime (10 mg) , me-

ropenem (10 mg), aztreonam (30 mg), ciprofloxacin (5 mg), linezolid (10 mg), tetracycline (30 mg), amikacin (30 mg), chloramphenicol (30 mg), rifampicin (5 mg), ampicillin (10 mg). Strains that showed resistance to three or more antibiotic groups were considered polyresistant [13].

The persistence factors were determined in the most common isolates from trophic ulcers. The lecithinase activity was assayed on the egg yolk salt agar, the hemolytic activity on a blood agar plate and the anti-lysozyme and anti-complementary activity we determined according to the method described by Bukharin O. et al. [14-16].

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) reference strains were used for quality control. Statistical data analysis was carried out via EpiInfo 2000.

Ethical Issues

The strains used in this study were obtained from the routine analysis of clinical specimens. Sample collection did not involve direct contact with the patient, thus no consent was required. The study was conducted and approved by the Ethics Committee no. 65 / 12.04.2017 of Nicolae Testemitanu State University of Medicine and Pharmacy from the Republic of Moldova.

Results

The bacteriological study was conducted on 44 samples collected from patients with trophic ulcers. A single bacterial strain was isolated in 36.3% of cases, two and more species – in 52.3% and no strains – in 11.4% of cases.

A total of 80 bacterial species were isolated and identified. The most common strains, isolated from trophic ulcers, were the *Staphylococcus* (predominantly *S. aureus*), then enterobacteria (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Serratia* spp., *Escherichia* spp.), non-fermenting bacilli *Pseudomonas spp.,* levuriform fungi of *Candida* type and enterococci. (fig. 1).

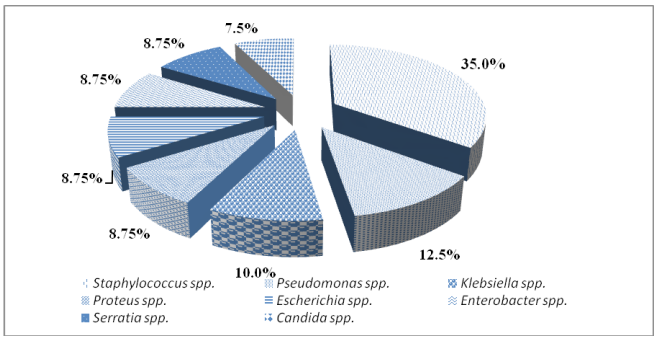

Fig. 1. The etiological spectrum of microorganisms isolated from trophic ulcers.

Among the infections caused by a single strain, the most common was found *Staphylococcus aureus* (43.75%), along with the other isolates such as *Pseudomonas aeruginosa* (18.75%), *Staphylococcus haemolyticus* (12.5%), *Proteus mirabilis* (12.5%), *Klebsiella pneumoniae* and *Enterobacter gergoviae* (6.25%).

S. aureus was determined in 82.6% of mixed infections, whereas 30.4% of cases were associated with *Klebsiella* and *Pseudomonas* species. Two-strain associations were recorded in 52.2% of cases, three-strain in 13.1%, 4 and 5 species were found in 13.1% and 4.3% of cases, respectively.

Staphylococcus spp. strains showed a marked sensitivity to vancomycin (96.4%) and only 1 strain showed intermediate resistance to vancomycin, tetracycline (89.3%) and linezolid (82.1%). Of the 28 tested staphylococcus strains, 13 (46.4%) were methicillin-resistant (MRS). MRS strains were more sensitive to vancomycin (100%), tetracycline (84.6%) and linezolid (76.9%), followed by chloramphenicol (79.2%), whereas a reduced sensitivity was recorded to erythromycin (27.5%) and ciprofloxacin (17.3%). Moreover, the obtained data highlighted a number of strains with multiple antibiotic resistance and only 3 (10.7%) of the 28 strains were sensitive to all the tested antibiotics.

Carbapenems were found to be the most effective antibacterial drugs (86.1%) in treatment of enterobacterial infections; however, the bacteria exhibited a marked resistance to aminoglycosides (> 70%), fluorquinolones and cephalosporins (> 80%).

Furthermore, this study detected 15 extended-spectrum beta-lactamase strains (BLSE), which showed susceptibility to meropenem (86.6%), followed by amikacin (60.0%), gentamicin (53.3%), ceftazidime (26.6%) and ciprofloxacin (13.3%).

The bacterial strains of *Pseudomonas* genus presented susceptibility rates to aminoglycosides (100%) and monobactam drugs (90%) and resistance to fluoroquinolones (100%), carbapenems (90%) and cephalosporins (80%).

Levuriform fungi of the genus *Candida* isolated from trophic ulcers made up 7.5%. In the present study, 66.7% of *Candida* spp. were susceptible to fluconazole, 100% to amphotericin B, 83.3% and 50.0% to voriconazole and itraconazole, respectively.

In the next step of our study, we determined the levels of expression for some persistence factors found in the most common bacterial strains, isolated from trophic ulcers.

The study of the persistence factors of bacteria isolated from trophic ulcers showed a higher- level expression in strains isolated from mixed infections (1.0-1.5 times) compared to those isolated in pure culture.

Lecithinase was among the studied persistence factors. This enzyme destroys lecithin and releases the receptors with which microorganisms interact [16]. Of the total 26 *S. aureus* strains, 24 (92.3%) showed lecithinase activity and 2 (7.6%) strains were inactive.

Hemolysin was determined as another persistent factor, leading to development of chronic infectious disease. Hemolytic activity was detected in 38 (47.5%) bacterial strains isolated from trophic ulcers. A higher level of expression was also recorded in strains isolated from the mixed infections, compared to those isolated in pure culture.

Lysozyme is a carbohydrase that selectively breaks down the glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine and is a component of the peptidoglycan cell wall. If the peptidoglycan network is destroyed, the osmotic pressure between the inside and the outside of the cell is no longer balanced and thus is being destroyed [17]. Therefore, an important strategy of the bacteria is to protect themselves against this enzyme, aiming to survive longer

in the host organism. Of 26 strains of *S. aureus*, 14 (53.8%) strains showed antilysozyme activity and 12 (46.2%) were inactive. The antilysozyme activity was assessed by lysozyme titres in the medium, which revealed that out of 14 Staphylococcus aureus strains, 5 (35.7%) strains showed a lysozyme concentration greater than 10 μg/ml, 6 (42.9%) – a concentration of 5-10 μg/ml and 3 (21.4%) – 5 μg/ml.

The antilysozyme activity of *Enterobacteriaceae* strains was also assessed, showing that of 36 strains, 24 (66.7%) strains exhibited an antilysozyme activity, of which 6 (25.0%) in concentration greater than 10 μg/ml, 8 (33.3%) – in concentration from 5-10 μg / ml, 10 (41.7%) – in concentration of 5 μg/ml and 12 (33.3%) strains did not present any activity ($p < 0.05$).

Another important factor that provides persistence for the microorganisms within the infection site is the ability of the bacterial cells to inactivate the complement system of the macroorganism [16] . The study of the anti-complement activity of the strains isolated in pure culture showed that 62.5% of the strains inactivate the complement and 37.5% of the strains did not present anti-complement activity.

Of the 26 Staphylococcus aureus strains, 24 strains (92.3%) exhibited complementary activity, of which 7 (29.2%) strains inactivated the complement at a concentration of 5 CH50/ml, 3 (12.5%) – at concentration from 5 – 15 CH50/ml and 35 (58.3%) in a concentration greater than 15 CH50/ml. Only two strains did not exhibit anti-complement activity (7.7%).

Anticomplementary activity is a common feature among the bacteria of the *Enterobacteriaceae* family. 34 (94.4%) strains of enterobacteria out of 36 isolates from trophic ulcers showed anti-complementary activity. 1 (2.9%) strain inactivated the complement at a concentration of 5 CH50/ml, 6 (17.6%) at a concentration from 5-15 CH50/ml and 27 (79.4%) strains at a concentration greater than 15 CH50/ml.

The data study of the anti-complementary activities in monocultures compared to isolated cultures in associations (co-culture isolates) showed that the latter are often related to medium and high anti-complementary activity ($p<0.05$).

Conclusions

1. The study of the spectrum of microorganisms isolated from trophic ulcers has revealed the significant role of strains belonging to the genus *Staphylococcus*, followed by enterobacteria, *Pseudomonas* spp., levuriform fungi of the genus *Candida* and streptococci. *Staphylococcus aureus* strain was predominantly isolated in both pure and mixed cultures.

2. Both gram-positive and gram-negative strains isolated from trophic ulcers showed a marked resistance to the antimicrobial drugs tested.

3. The study of the persistence factors confirmed that the strains isolated from trophic ulcers exhibit a range of properties to inactivate the natural resistance factors of the macroorganism.

4. It is essential to understand the pathogenic persistence factors, since it might provide effective targeted therapies for controlling the microbial growth in trophic ulcers.

5. The study results have proved that treatment of trophic ulcers is still a major medical concern, requiring current management strategies.

Conflict of Interest Declaration

There are no known conflicts of interest and financial or non-financial support associated with this publication.

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Survival predictive models in severe trauma patients' transportation within Moldovan medical system

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Abstract

Background: Trauma remains an unresolved medical problem and its severity often requires the transfer of patients to specialized trauma institutions (centers). The elaboration of the predictive models represents an effective tool for improving the prognosis of the transported patients by optimizing the management of the trauma and/or improving the national interhospital transfer system. The survival probability predictive models in severe trauma were proposed in this pilot research.
Material and methods: Data were collected from 39 patients with severe trauma (NISS > 15) transported to the Emergency Medicine Institute (EMI), Chisinau, the Republic of Moldova, from district hospitals. These data were statistically processed using multivariate logistic regression where NISS, MPMoIII, age and biological gender were considered as covariates.
Results: There were developed three predictive models: based on the estimation of anatomical lesions (NISS), based on physiologic parameters estimation and conditions during/immediately after hospital admission (MPMoIII) and their combination (NISS + MPMoIII). The last of these showed significance only after the resampling, the characteristics of the model being superior (the coefficient of determination over 0.8, the sensitivity and the specificity over 80%) compared to the first two taken separately. Age and biological gender were insignificant and were not included in the equations.
Conclusions: Developed models are perspective (especially a combined one) in predicting survival rate of severe trauma patients transported to EMI from district hospitals. At the same time, taking into account the particularities and limitations related to the pilot study, the models can be recommended for use in clinical practice after validation procedure only.
Key words: severe trauma, predictive models, interhospital transportation.

Introduction

Trauma remains an unresolved medical problem. According to data from the literature, traumatic lesions occupy the third place in the overall structure of the lethality and are the first cause of death in the category of patients between the ages of 1 and 44 years [1]. The mentioned trends are also characteristic for the Republic of Moldova. According to the data of the National Management Center of the National Agency of Public Health for the period 2008-2017, traumas are placed on the fourth place, constituting 8.1% (36889 cases) of all the death cases registered after the diseases of the circulatory system (61%, 226195 of cases), tumors (15.8%, 58518 cases) and diseases of the digestive system (10%, 36889 cases). The analysis of the lethality structure by age shows that in the first year of life the traumas are placed second (30.3%) after the diseases of the respiratory system (57.9%). Subsequently, as the age progresses, the rate of deaths caused by trauma increases and reaches maximum values at 18 years (81.3%), after which it is decreasing, predominating until the age of 45 years (27.2%) compared to other causes of death, continuing to decrease to zero at old age [2].

Often, patients with trauma are admitted to a medical institution and subsequently, for different reasons, require transfer to the trauma center, sometimes being in a serious or critical condition during transfer. On the one hand, transporting patients from one institution to another represents an increased risk for complications and even death. On the other hand, the transfer of patients to specialized institutions has benefic effects for patients. But, unfortunately, currently for patients with severe trauma are not unanimously accepted criteria for the need, the right time, and the mode of transport between two medical institutions [3, 4, 5].

One of the criteria to determine the tactics for transferring to a specialized institution is to determine the severity of the injuries and the prognosis of the patient's condition. These are crucial for trauma management. Currently, two approaches need to be considered in order to mark patients at high risk of complications, including death. The first is the use of terms such as "severe trauma", "major trauma" and "polytrauma". The analysis of the number of records / documents in the Web of Science database in 2016 highlighted 24441, 19471 and 2813 records for these notions, respectively. The terms "severe trauma" and "major trauma" are very close, interchangeable, but the criteria are not well established, the critical value of ISS (Injury Severity Score) or NISS (New Injury Severity Score) varies in different studies at the level of 16-17 points [6, 7, 8]. Polytrauma is one of the most complicated and unexplored categories of trauma, being a restricted notion compared to severe trauma or major trauma. According to the Berlin definition, polytrauma is

defined as lesion of at least two regions of the body, assessed by AIS (Abbreviated Injury Scale) with score ≥ 3 and presence of at least one of the 5 physiological parameters (systolic pressure ≤ 90 mmHg, GCS ≤ 8, acidosis, coagulopathy and age ≥70 years [9]. This approach has as a disadvantage – the lack of the possibility of individualizing the management of a patient with traumatic lesions arising from the particularities of their evolution, the circumstances of the trauma, etc. As a result, the most severe patients within each group cannot be identified and there are no indications of the probability of survival/death, of developing complications, which of the parameters/variables are effective in determining the treatment results, which of the examined factors would have the greatest influence, which of the patients requires admission in Intensive Care Unit or how rational it is to benefit from a procedure, etc.

Another approach – the use of traumatic scores (NISS, ISS, MPMoIII, ASCOT, TRISS etc.) as well as the development of predictive models, which represent effective tools for solving the mentioned disadvantages. Thus, the predictive models have a potential for improving the prognosis of the transported patients by optimizing the management and/or by improving the interhospital transfer system in the Republic of Moldova [3].

In the pilot research, three predictive models have been proposed and analyzed for estimating the survival probability of patients with severe trauma, transferred from the district hospitals to the EMI through the AVIASAN service.

Material and methods

Analyzing the observation data of the patients admitted to the EMI for 2012, a retrospective pilot study was performed. The study included 39 severely traumatized patients transferred through the AVIASAN service from district hospitals to EMI by the reanimatologic team. The criteria used for severe trauma was the NISS score greater than 15 points [10].

The research project was approved by the ethics committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy.

The elaboration of the predictive models was carried out by the logistic regression analysis, taking into consideration the recommendations for the multivariate analysis. The minimum number of respondents was estimated by the ratio 1:10 (for each covariate included in the model at least 10 respondents) [11].

In addition to NISS, for the determination of patient status, the MPMoIII (Mortality Probability Admission Model) score was used [12]. The age and gender of the respondents were also taken into account. Specifically, these four skills were considered as effective maintenance variables in the predictive models of survival rate for transferred severe trauma patients. Considering the relatively small number of respondents for the mixed model, the resampling was performed by bootstrapping.

Results

Age of the studied group varied from 20 to 74 years (Median 45, interquartile range 32), from which 30 were males evaluated with NISS 16-66 (Median 48, interquartile range10), MPMoIII variated between 17.1 and 91.2 (Median 73.8, interquartile range19) upon admission to IMSP IMU.

Totally, three models were developed: a model based on anatomical lesions (NISS), another model based on the physiological parameters and some patient parameters during/immediately after admission to the hospital (MPMoIII) and the third, mixed one (NISS + MPMoIII), results being adjusted to the age and gender only in the case of the NISS score. Age and biological gender were insignificant and were not included in the final equations.

Model based on the estimation of anatomical lesions (NISS)

For the NISS-based model, the following hypotheses were formulated: *The null hypothesis* - the covariates included in the model cannot predict the probability of survival in severely traumatized transported patients better than a model that is based only on constant. *Alternative hypothesis* – at least a variable can predict the probability of survival in patients with severe trauma better than a model that is based only on constant.

The model presented the following characteristics. *Omnibus Test of Model Coefficients* ($\chi 2$ (df=1) = 23.05 p<0.001). The test was a significant one, which allowed us to reject the null hypothesis and to analyze further, which of the studied covariates is relevant for predicting survival rate in severe trauma. The coefficient of determination, *Nagelkerke R Square*, was estimated at 0.641 (64.1%), which tells us that the variables included in the model (NISS) determine about 2/3 of the dispersion of the examined variable (probability of occurrence of an event). *The Hosmer-Lemeshow test*, ana-

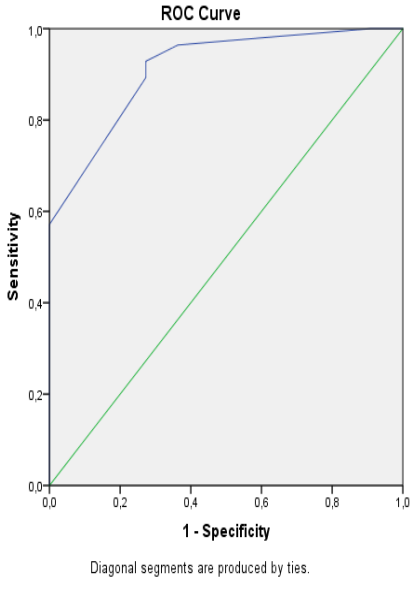

Fig. 1. The ROC curve of the predictive model for the probability of survival in patients transported with severe trauma. SPSS 22 Output.

Table 1

Variables in the equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
NISS	-.369	.128	8.255	1	.004	.692	.538	.889
Constant	18.983	6.405	8.783	1	.003			

Note: Constant – the value of the equation constant; B – the coefficients B; S.E. – standard errors; Wald – Wald statistic; df – degrees of freedom; Sig. – significance threshold; Exp (B) – values for odds ratio; 95% C.I. for EXP (B) – confidence interval for odds ratio.

lyzing the model in terms of the ability to predict positive and negative results, presents the result as insignificant ($\chi 2$ (df = 6) = .332, p = 0.999), which tells us about the increased fidelity of the obtained results. The classification table highlighted a *sensitivity* of 96.4% (27 cases out of 28), the *specificity* being 63.6% (7 out of 11 cases), the average validation appreciated at the level of 87.2%.

The surface under the ROC curve, for the proposed model, was 0.912, with 95% confidence interval (0.819, 1.000) and with a significant difference from the value 0.5 (p <0.001) (fig. 1). Thus, the logistic regression classified the model as significantly better model than the random model.

The model includes the constant (B = 18.983) and the NISS values (B = - 369) (tab. 1). NISS is a predictor for survival of patients with severe trauma, OR (odds ratio) = .692 (95% CI, .538, .889). If the NISS value increases by one point, the probability of survival will decrease by approximately 30% (tab. 1). Age and biological gender showed no significance. The analysis of the classification graph (fig. 2) did not reveal possibilities for improving the specificity.

The model based on the estimation of physiological parameters and indicators during/immediately after hospital admission (MPMoIII)

The following hypotheses were formulated. *The null hypothesis* – the covariates included in the model cannot predict the probability of survival in transported severely traumatized patients better than a model that is based only on constant. *Alternative hypothesis* – at least a variable can predict the probability of survival in patients with severe trauma better than a model that is based only on constant.

The model presented the following features. After performing the *Omnibus Test of Model Coefficients* ($\chi 2$ (df = 1) = 17.094 p <0.001) The null hypothesis was rejected. The coefficient of determination, *Nagelkerke R Square* = 0.51 (51%) was reduced from the model based on anatomical lesions. The fidelity of the results was confirmed by performing the *Hosmer-Lemeshow test*, ($\chi 2$ (df = 7) = 3.338, p = 0.847). The classification table shows a *sensitivity* of 89.3% (25 cases out of 28), the *specificity* being 72% (8 out of 11 cases), the average validation appreciated at the level of 84.6%.

The surface under the ROC curve, for the proposed model, was 0.878, with 95% confidence interval (0.773, 0.983) and with a significant difference from the value 0.5 (p <0.001) (fig. 3). Thus, the logistic regression classified the model developed as significantly better model than the random model.

The model includes the constant (B = 14.385) and the values of MPMoIII (B= -.178) (tab. 2). MPMoIII is a predictor with OR = .837 (CI95% .735, .954), that means that if the value of MPMoIII increases by one point, the probability of survival decreases to almost 16% (tab. 2). Age and biological gender are the components of the score and were included in the model.

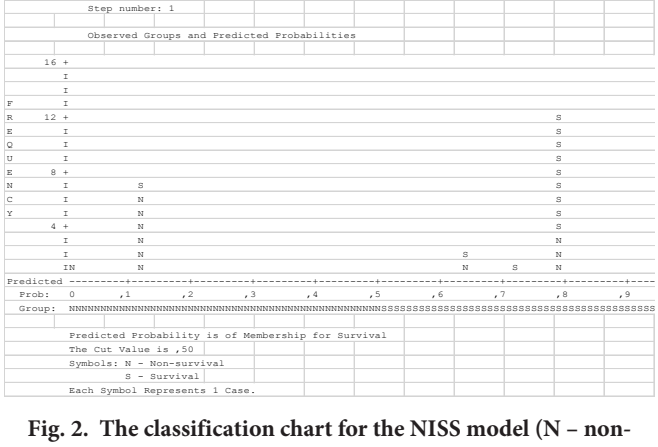

Fig. 2. The classification chart for the NISS model (N – non-survived, S – survived).

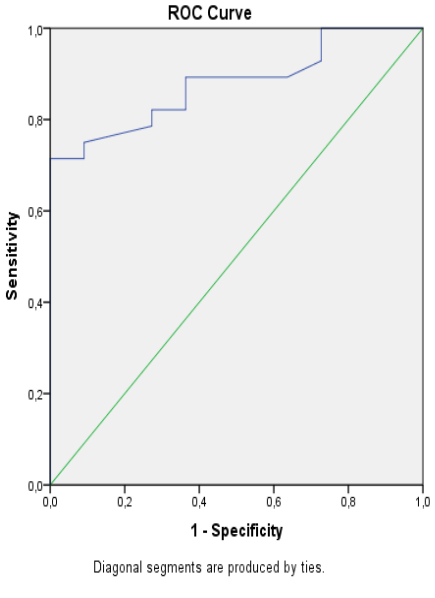

Fig. 3. The ROC curve of the predictive model for the probability of survival in transported patients with severe trauma. SPSS 22 Output.

Table 2

Variables in the equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
MPMoIII	-.178	.067	7.144	1	.008	.837	.735	.954
Constant	14.385	5.272	7.445	1	.006			

Note: Constant – the value of the equation constant; B – the coefficients B; S.E. – standard errors, Wald – Wald statistic; df – degrees of freedom; Sig. – significance threshold; Exp (B) – values for odds ratio; 95% C.I. for EXP (B) – confidence interval for odds ratio.

The analysis of the classification graph (fig. 4) highlights the possibility of improving the specificity (increasing of the cut-off to .62 instead 0.5), but due to the fact that the results are not stable (standard error of MPMoIII coefficient had more than 30% of B), there is a chance of not highlighting the survivors (*overfitting*).

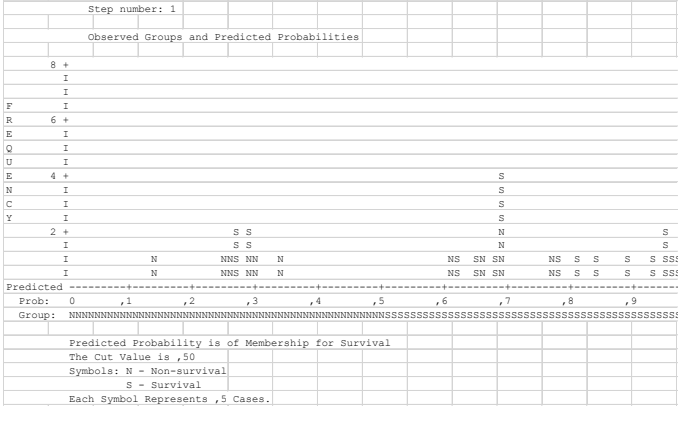

Fig. 4. The classification chart for the model MPMoIII (N – non-survived, S – survived).

The mixed model (NISS + MPMoIII)

For the mixed model, the following hypotheses were formulated. *The null hypothesis* – the covariates included in the model cannot predict the probability of survival in transported severely traumatized patients better than a model that is based only on a single constant. *Alternative hypothesis* – at least a variable can predict the probability of survival in patients with severe trauma better than a model that is based only on a single constant.

The Omnibus Test of Model Coefficients ($\chi 2$ (df = 1) = 32.023 p <0.001), being a significant one, allowed us to reject the null hypothesis and to analyze further, which of the studied covariates is relevant. The coefficient of determination, *Nagelkerke R Square* = 0.805 (80.5%), explaining 4/5 of the dispersion of the examined variable (probability of occurrence for an event), reached the optimal level for the prognostic models. The *Hosmer-Lemeshow test*, analyzing the model in terms of the ability to predict positive and negative results, presents the result as insignificant $\chi 2$ (df = 8) = 2.037, p = 0.980 and increased fidelity to the obtained results. The classification table shows a sensitivity of 96.4% (27 out of 28 cases), the specificity being 81.8% (9 out of 11 cases), the average validation appreciated at 94.5%. As with the coefficients of determination, the optimum values were reached.

The surface under the ROC curve, for the proposed model, constituted 0.977, with 95% confidence interval

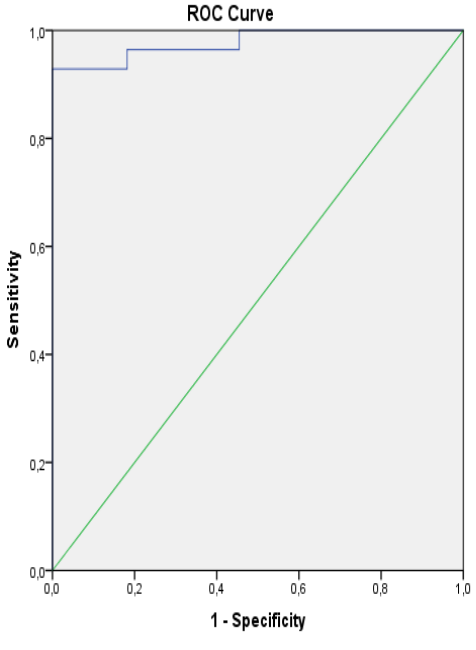

Fig. 5. The ROC curve of the predictive model for the probability of survival in transported patients with severe trauma. SPSS 22 Output.

Table 3

Variables in the equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
MPMoIII	-.331	.202	2.686	1	.101	.718	.484	1.067
NISS	-.400	.175	5.247	1	.022	.670	.476	.944
Constant	46.233	22.835	4.099	1	.043			

Note: Constant – the value of the equation constant; B – the coefficients B; S.E. – standard errors, Wald – Wald statistic; df – degrees of freedom; Sig. – significance threshold; Exp (B) – values for odds ratio; 95% C.I. for EXP (B) – confidence interval for odds ratio.

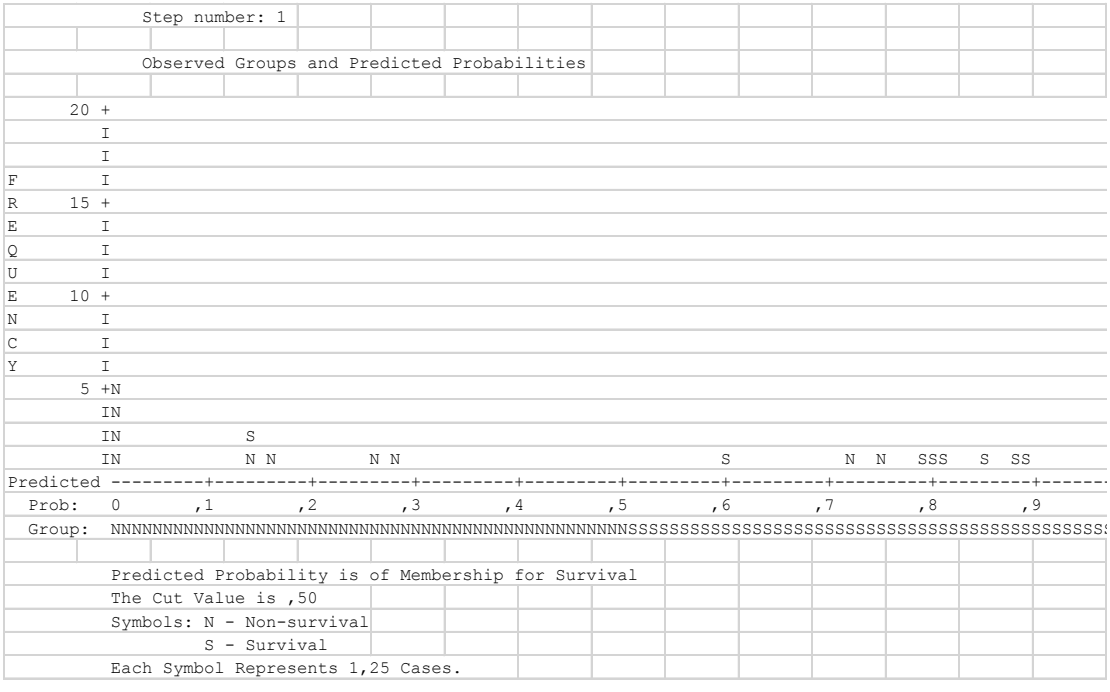

Fig. 6. Chart of classification for the model MMMIII and NISS (N – non-survived, S – survived).

(0.938, 1.000) and with a significant difference from the value 0.5 ($p < 0.001$) (fig. 5). Thus, the logistic regression classified the model as significantly better model than the random model.

The model includes the constant (B = 46.233), the values of MPMoIII (B = -.331) and NISS (B = -.400). The adjustment of NISS to MPMoIII improved the characteristics of the model (the coefficient of determination, sensitivity and specificity). At the same time, NISS represents an efficient covariate, OR = 0.670 (CI95% .476, .944), MPMoIII being insignificant. OR = .718 (CI95% .484, 1.067) (tab. 3). The resampling by bootstrapping showed the significance of parameter MPMoIII (tab. 4). The analysis of the classification graph (fig. 6) does not reveal possibilities of improving the specificity/sensitivity.

Thus, this model is one of perspective, combining an anatomical score with a physiological one, in order to predict the survival in patients with severe trauma transported to the specialized institution.

Table 4

Resampling by bootstrap (997 samples)

	B	Bias	Std. Error	Sig.	95% C.I. for B	
					Lower	Upper
MPMoIII	-.331	.202	2.686	.004	-40.230	-.166
NISS	-.400	.175	5.247	.005	-47.976	-.202
Constant	46.233	22.835	4.099	.002	29.144	5836.065

Note: Constant – the value of the equation constant; B – the coefficients B; Std. Error – standard errors;
Sig. – significance threshold; 95% C.I. B – confidence interval for the coefficients.

Discussion and conclusions

In our research, three predictive models have been developed for the survival rate of severely traumatized patients transferred from district hospitals to the EMI. The model that includes NISS, an anatomical score, has been proven as relevant, the effect of anatomical lesions for this category of patients being estimated quantitatively by the coefficient of determination, the OR and the coefficient for the logistic regression equation. The coefficient of determination constituted .641 (64.1%), compared to .41 (41%) estimated for traumatized hospitalized directly in the EMI. Thus, it can confirm that interhospital transfer of patients increases the effect of the anatomical lesions on the treatment results (outcomes).

The MPMoIII model also predicted the survival rate of a patient with severe trauma. The coefficient of determination constituted .51 (51%), being close to the NISS effect and confirms the idea that the physiological parameters, as well as some indicators of the patient's condition at the admission to the hospital have a prospective predictive potential. The combination of NISS and MPMoIII in the mixed model increased the coefficient of determination over .80 (80%), a practically ideal value, the sensitivity and specificity also being over 80%. But, the significance for MPMoIII was obtained only by resampling.

Thus, all these models are perspective models (especially the combined model) for predicting survival in severely traumatized patients transported to the EMI from district hospitals.

On the one hand, the proposed models can't be recommended for use in daily practice due to limitations related to

the particularities of the pilot study. The most important of them – a small number of respondents was analyzed, which cannot ensure a high level of accuracy of the coefficients in the logistic regression equation (for example in the NISS covariate mixed model it had a coefficient B = -0.400 and a standard error = .175). On the other hand, the models have a potential to be improved by supplementing with efficient variables.

The implementation procedure can't be initiated without obtaining an accuracy of the coefficients (narrow confidence intervals) and validation of the elaborated models, both obtained in studies with higher level of evidence.

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