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MEDICINE FIELD

The evaluation of epidemiological, bacteriological and clinical-evolutionary particularities of systemic and local infections occurred in severe burn patients in Romania

PhD THESIS SUMMARY

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Thesis summary

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The evaluation of epidemiological, bacteriological and clinical-evolutionary particularities of systemic and local infections occurred in severe burn patients in Romania

The doctoral thesis "Evaluation of the epidemiological, bacteriological and clinical-evolutionary particularities of systemic and local infections in patients with severe burns in Romania" is structured in two parts: a general part, which has 2 chapters, in which I presented a synthesis of the stage of current knowledge regarding burns and infections in patients with severe burns hospitalized in the Intensive Care Unit; a part of personal research structured in 4 chapters, in which the motivation, objectives, methodology and results of the research carried out throughout the doctoral studies are presented.

GENERAL PART

CHAPTER 1. Burns - General aspects

In chapter 1 of the doctoral thesis, I addressed notions related to burns, which represent with certainty one of the most severe pathologies in the field of acute traumatology and one of the most complex conditions from a physiopathological point of view. In addition to the trauma produced, burns present numerous complications in the medium and long term, starting from renal, cardiovascular and infectious damage and ending with post-burn sequelae, psychological sequelae as well as psycho-social implications.

CHAPTER 2. Infections in patients with severe burns

In chapter 2 of the doctoral thesis, notions related to burn wound infections were addressed, which occur mainly in patients with large total burn surface area (TBSA - over 20%), in elderly, immunosuppressed patients, with associated significant comorbidities, or in those who associate upper respiratory tract burns. Despite surgical and supportive treatment, a burn patient, who has lost the skin barrier and consequently the main antimicrobial defense mechanism, becomes susceptible to local or systemic infections and subsequent sepsis.

PERSONAL CONTRIBUTIONS
CHAPTER 3. Working hypothesis and research objectives

At the time of the start of this doctoral research, there were not many data related to the pathology associated with burns in general and about the infections associated with burns, in particular, neither in our country nor in Eastern Europe. Thus, in carrying out this doctoral research, I started from the hypothesis that the themes addressed and the studies that will be carried out will make a contribution to the knowledge of the pathology associated with severe burns in Romania, from a demographic, epidemiological, clinical and paraclinical perspective, emphasizing on the etiology of infections and on the antibiotic resistance of the identified bacteria.

Also, given the difficulty of evaluating local and systemic infections in patients with severe burns, I wanted to evaluate a group of patients from the point of view of several biological bio-markers (presepsin, C-reactive protein, etc.) that could help doctors in early diagnosis and evaluation of prognosis of this patients.

Another working hypothesis started from the desire to deepen the knowledge of the factors that influence the appearance of antibiotic resistance in the bacterial strains most often isolated from the wounds generated by burns. In this sense, we considered it useful and necessary to initiate a pilot research on a subset of patients in which molecular biology methods were used to identify the antibiotic resistance genes of some bacterial species.

The main objectives of the doctoral research were represented by:

- Evaluation of the incidence of skin and soft tissue infections and systemic infections in patients with severe burns in Romania

- Identification of the etiological profile of skin infections and systemic infections in patients with severe burns in Romania

- Evaluation of the antibiotic sensitivity profile of bacteria isolated from infections in patients with severe burns in Romania

- Evaluation of epidemiological, clinical-evolutionary characteristics and mortality in patients with severe burns in Romania

- Identifying and establishing the opportunity to use some biological markers in establishing the diagnosis and prognosis of sepsis and establishing the predictive values of these bio-markers for the occurrence of hemodynamic instability in patients with severe burns

- Identification of antibiotic resistance genes for bacterial etiological agents involved in infected wounds in burn patients

In order to achieve the proposed objectives, we carried out three individual studies.

- In the first study, presented in chapter 4, we sought to identify the main etiological microbiological agents involved in infections in patients with severe burns, their antibiotic resistance profile, survival rate, costs/patient on a group of 202 patients in 3 and a half years period.

- The second study of the doctorate, presented in chapter 5, was carried out on a group of 121 patients, in which we tried to identify the cut-off values for a series of bio-markers involved in the early diagnosis of sepsis in patients with burns and in the association of the prognosis of these patients. We specifically looked at C-reactive protein (CRP) and presepsin (PSP).

- The third study within the research, presented in chapter 6, was carried out in collaboration with the "Cantacuzino" National Military Medical Institute for Researach and Development, Bucharest, Romania, for the identification of antibiotic resistance genes of some strains of *P. aeruginosa* isolated from burns patients admitted to the intensive care unit of C.E.H.P.R.S.B. (Clinical Emergency Hospital of Plastic, Reparatory Surgery and Burns, Bucharest) , using phenotypic and molecular methods. The antibiotic susceptibility profile of these strains was also identified.

CHAPTER 4. Epidemiological, demographic, clinical-evolutionary, bacteriological and therapeutic aspects in patients with severe burns in Romania (2018-2022)

This chapter presents the first study in which we aimed to evaluate and identify the etiological profile and the resistance profile of bacteria to antibiotics, this issue representing the main cause of treatment failure in infections in patients with burns over 20% total body surface area.

We also aimed to evaluate and identify demographic and epidemiological aspects related to the pathology of the severe burn.

The main objectives were to identify the etiological profile of infections/ colonizations in patients with severe burns in Romania and then to identify the susceptibility of the identified bacteria to antibiotics, which decisively influences empiric antibiotic therapy and patient prognosis. We also followed the relationship between the severity of the burns and patient prognosis, epidemiological factors related to the transfer of patients from

other medical centers not specialized in the treatment of burns to our unit (Clinical Emergency Hospital of Plastic, Reparatory Surgery and Burns, Bucharest - C.E.H.P.R.S.B.) , demographic factors related to gender, age and patient comorbidities.

Materials and method

We performed a longitudinal observational prospective study, in which 202 adult patients admitted to the Intensive Care Unit of C.E.H.P.R.S.B. were included.

The criteria for inclusion in the study coincide with the criteria for admission to the Intensive Care Unit (ICU) of C.E.H.P.R.S.B with few exceptions. Patients are admitted to the ICU if they have at least IIA degree burns (burn involving the papillary dermis), affecting at least 20% total body surface area and/or associating upper respiratory tract burns. Another admission criterion for ICU is represented by the presence of burns of the upper respiratory tract alone, regardless of the total body surface area or the severity of the burn.

The following parameters were observed for the patients included in the statistical analysis: sex, age, presence or absence of comorbidities, total affected body surface area, transfer status (yes/no) from another medical unit to C.E.H.P.R.S.B., presence or absence of upper respiratory tract burns , the total costs associated with patient care, the mortality rate, the bacteria identified, the method of collecting samples for bacteriological determinations, the year in which they were enrolled, the ABSI (Abbreviated Burn Severity Index) score.

Data were collected from patients' clinical observation sheets and C.E.H.P.R.S.B.'s informatic system (Hipocrate) , then stored in a database using Microsoft Office Excel Worksheet. Statistical analysis was performed using: SPSS Statistics 15.0.0 (SPSS Inc – 2006 - for ANOVA analysis, Crosstabulation - Chi square, Kaplan Meier and part of the graphs, Bar Chart) and MedCalc 14.8.1 (MedCalc software – 2014 - for the ROC analysis and the estimated calculation of the OR risk). A p value < 0.05 was considered statistically significant.

Informed consent was obtained from patients or their relatives for treatment and data use. The study was observational, without directly interfering with the treatment of the patients. In order to carry out this doctoral research, the approval of the Ethics Commitee of C.E.H.P.R.S.B. was obtained, through document no. 3/ 26.04.2021.

Results

Description of patients group

- out of the 202 patients, (132) 65% of them were Male

- (90) 45% of patients were aged between 51-70 years.

- 69 (34%) were survivors and 133 (66%) non-survivors.

- 110 (55%) patients in the study had associated comorbidities at the time of hospitalization, while 92 (45%) did not.

- (122) 60% of patients hospitalized at C.E.H.P.R.S.B. were transferred from other medical units, while 80 (40%) were presented directly to our medical unit.

- 91 patients (45%) presented upper respiratory tract burns

- the total body surface area affected by burns was on average 35.45%, with a maximum of 95% and a minimum of 5%

The non-microbiological characteristics of the patients in the studied group

- cost/ patient in the subset of patients transferred to C.E.H.P.R.S.B. versus the subgroup of non-transferred patients, shows that in the transferred patient the cost was significantly higher (RON 58,515) than in the non-transferred patient (RON 44,088) (P = 0.020)

- patients with comorbidities have a higher average age than patients without comorbidities (64 years old vs. 52 years old)

- the average age of patients who survived was 51y.o., while the age of patients who died was significantly higher, 62 y.o

- the average affected area in the group of patients who died was 38.2%, while in the group of patients who survived was 30.1%

- in the surviving patients subset, the average ABSI severity score was 8 (50-70% chance of survival), while in the deceased patients subset, the average severity score was 9.6 (20-40% chance of survival)

The microbiological particularities of the samples from the patients with severe burns from the studied group

- from the total of 737 isolated bacterial strains, the most frequently isolated microorganism was *Pseudomonas aeruginosa* (298/40%), followed by *Staphylococcus aureus* (88/12%) and *Klebisella spp.* (80/11%)

- among the pathological collected specimens, the most frequently sampling site was the wound swab (51%), followed by endotracheal aspirate (29%), urine culture (14%) and blood culture (6%)

- from wound swabs *Pseudomonas aeruginosa* was the most frequently isolated (43%) followed by *Staphylococcus aureus* (14%) and *Klebisella spp.* (10%)

Antibiotic resistance profile of bacterial strains isolated from patients with severe burns

- out of the total of 298 strains of *Pseudomonas aeruginosa* identified, 278 (93%) were MDR. *Acinetobacter baumannii* followed with 91% MDR strains, and in third place was *Klebsiella spp.* with 31% MDR strains.

- among the 88 strains of *Staphylococcus aureus*, 27 (31%) were methicillin-resistant (MRSA)

- Colistin resistance of *Acinetobacter baumannii* (27.14%) was much higher than that of *Pseudomonas aeruginosa* (6.71%).

- for *S. aureus*, 29 of the 88 strains (33%) isolated were resistant to the combination Amoxicillin + Clavulanic Acid, 27 (31%) resistant to Oxacillin, while 32 of 88 (36%) were sensitive only to antibiotics from the glycopeptide class (Teicoplanin and Vancomycin) and to Linezolid [1].

Discussions and Conclusions

The microorganisms most often identified from the severe burn patients hospitalized in the ICU ward of C.E.H.P.R.S.B. were, in order, *Pseudomonas aeruginosa*, *Candida spp.*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Acinetobacter baumannii*. Different studies, starting from those carried out by Herndon in the USA, have identified a similar hierarchy of the microorganisms isolated from the patient with burns. Other studies have reported a different hierarchy and quantity of the infectious agents involved, either because of the subtropical climate or because the studies were conducted in developing countries. Overall, the etiology remained similar [2,3,4].

A review article highlights a 5-year study at a burn center in Seoul, South Korea that identified MDR strains of *A.baumanni* and *P. aeruginosa* in proportions of 97.5% and 92.2%, respectively sampled from blood cultures. Another study conducted in the USA identified MDR rates of 90.8% for *A. baumanni* in an intensive care unit specifically dedicated to burn patients and 82% for *S. aureus*. Also, an article included in this literature review, conducted in North Carolina, USA in 2012, revealed that strains isolated from patients with severe burns admitted in ICU from their respiratory tract have a rate three times higher of multi - drug resistant strains than patients who are not in intensive care units. [5]

A study recently published by Prof. Lascăr's team from the Clinical Emergency Hospital of Bucharest, carried out in 2019 on a group of 68 patients admitted to the intensive care unit, revealed the presence of a large number of strains from the ESKAPE group

(*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter species*). Thus, a bacterium from the ESKAPE group was isolated from 75% of patients during hospitalization, the most frequently reported being *Staphyloccocus aureus*, ranked 2nd in our study. *Acinetobacter spp.* was also observed to have the highest rate of MDR, this pathogen also being the second most frequently multi-drug resistant bacteria in our study. [6]

The results obtained in the present doctoral research are comparable to those in the international literature and to the few studies carried out in our country. This is important to the burn treatment in our unit because it is a reflection of the sustained activity and successful efforts in the treatment of severe burns.

Chapter 5. Identifying the predictive values of inflammatory biomarkers in the diagnosis of sepsis and in improving the prognosis in patients with severe burns

Introduction

After an analysis of the main diagnostic tools in sepsis from the specialized literature and after evaluating the logistical resources available at the C.E.H.P.R.S.B.. laboratory, in chapter 5 we identified a series of bio-markers that can be used in our PhD research. Thus, we tried to identify the cut-off values at which presepsin (PSP) and C-reactive protein (CRP) could be used as prognostic markers for systemic infection in patients with severe burns. Other factors of severity that we followed in order to evaluate the prognosis in severe burns, were the values of sideremia, platelets and albumin. The ABSI score, also used in the previous study (chapter 4), was also usefull in this group of patients as an indicator of severity.

The use of CRP and PSP in the present study is based on the many studies that place CRP and PSP, along with PCT (procalcitonin) and IL-6 in the top biomarkers to monitor and study in relation to sepsis. [7,8,9]

The main objective we pursued in the present study was to identify the cut-off values for which PSP and PCR could predict a systemic infection (sepsis) or mortality in patients with severe burns. We also followed the correlation between the values of these bio-markers

and the need to use vasopressors, since hemodynamic instability is a negative prognostic factor that contributes to the increase in mortality rates.

Materials and method

We conducted a longitudinal prospective observational study that included 121 adult patients admitted to the Intensive Care Unit (ICU) of the C.E.H.P.R.S.B., Bucharest. Patients were included in the study between May 1, 2018 and December 31, 2021. Data were collected from the hospital's informatic computer system (Hipocrate) retrospectively and prospectively. Only the data of patients who had at least 2 determinations of bio-markers were taken into account.

The inclusion criteria in the statistical analysis of this study were similar to those of the previous study, presented in chapter 4 (2 of the abstract).

The following information was collected and entered into the database: demographic data (sex, age), PSP values, CRP, sideremia, serum albumin, platelet count; the ABSI score calculated at admission; the use or not of vasopressor medication.

The informed consent for the treatment and the use of the data was signed by the patients or their relatives, while the approval for the research was obtained from the Ethics Committee of C.E.H.P.R.S.B., similarly to the previous study, through document no. 3/ 26.04.2021.

Results

Description of the characteristics of the patients in the studied group

- the statistics group included 121 patients hospitalized for severe burns in the ICU ward of C.E.H.P.R.S.B.

- 75 (61%) patients were male, the ratio of male/female being 1.5:1.

- the average age of the studied group was 58 y.o.

- 67 (55%) patients were diagnosed with sepsis during the admission to the ICU, while the remaining 54 (45%) did not meet the diagnostic criteria for sepsis.

- 44 (36%) of the total of 121 patients admitted to the Intensive Care Unit survived, while 77 (64%) did not survive.

- 50 (41%) patients did not require vasopressor support, while 71 (59%) did.

- of the 71 patients who did need vasopressor support, 58 (81%) did not survive.

Results regarding the dynamics and statistical significance of the inflammatory biomarkers analyzed

The study group consisted of 121 patients. They were divided into several subgroups (sepsis/non-sepsis, survivors/deceased, vasopressor/non-vasopressor) to be able to visualize more easily the median values of the bio-markers used.

- the median value of PSP in the Sepsis subgroup was 1702 pg/mL, significantly higher than the value of the Non-sepsis subgroup, in which it was only 470.5 pg/mL

- in patients with vasopressor use, the median PSP value was statistically significantly higher - 1452 pg/mL compared to that of patients without vasopressor use, which was only 191 pg/mL.

- CRP had a median value of 203 mg/L in patients with sepsis and 173.5 mg/L in patients without sepsis

- a lower number of platelets is observed in patients who did not survive (191000/µL) compared to those in the group of survivors (282000/µL)

- univariate analysis was also performed for the biomarkers used, including the ABSI score at admission and the need for vasopressor treatment as predictive factors for mortality. Except for sideremia, all other parameters included in the analysis had statistical significance ($p<0.001$)

- we also performed a multivariate analysis that included PSP, the number of platelets and the ABSI score, which demonstrated that these parameters have a greater statistical significance than the other parameters included in the study, ABSI having a value of p =0.005

- the cut-off value for albumin in the prediction of sepsis was ≤ 30.3 (g/l) with a sensitivity of 75.7%,

- the cut-off value for CRP in the prediction of sepsis was > 194 mg/L with a sensitivity of 64.6%

- the cut-off value for predicting hemodynamic instability of PSP is >868 pg/mL, with a sensitivity of 71.5%, while the threshold value for predicting hemodynamic instability of CRP is >180 mg/L, with a sensitivity of 69.3%. (both statistically significant $p<0.001$) [10]

Discussions and Conclusions

Cut-off values for the prediction of hemodynamic instability of PSP and CRP were identified, having moderate specificity and sensitivity, but reaching the statistical significance value. The values of the AUC-ROC curves for PSP and CRP were below 0.8, which allows the use of the 2 biomarkers as predictive prognostic factors, but we must recommend the use of these biomarkers in combination with other prognostic factors.

Given the cut-off values identified for CRP and PSP (>180 mg/dL respectively >868 pg/mL) using ROC-AUC curves for identifying the occurrence of hemodynamic instability and considering the negative predictive value (75%), we can state that if CRP and PSP values are below the cut-off values identified in the study, it becomes extremely unlikely that the patient will develop hemodynamic instability.

The values obtained in our study for the AUC curve for CRP fall within the predictive power of values obtained by the same method and by other studies in septic patients with burn injuries (0.463 - 0.749) [11,12].

The predictive values identified for PSP in the assessment of the risk of mortality (AUC-ROC = 0.68) are considered to have moderate statistical power, but are similar to a cut-off value in the prediction of mortality in intensive care unit patients without severe burns observed in an international study (AUC-ROC = 0.703) [13].

PSP, platelet count and ABSI score were also observed to be the most usefull clinical and paraclinical parameters in predicting mortality by univariate and multivariate analyses.

We achieved the primary objective of identifying cut-off values for CRP and PSP in predicting the use of vasopressor medication in patients with hemodynamic instability and identifying a cut-off value for PSP in predicting the mortality rate. We have also achieved a series of secondary objectives, such as identifying the cut-off values for the other bio-markers analyzed, which may prove important in defining a future prognostic score.

CHAPTER 6. Research on the identification of antibiotic resistance genes of *Pseudomonas aeruginosa* strains isolated from patients with severe burns

Introduction

The results obtained in study 1, presented in the paper in chapter 4, identified *Pseudomonas aeruginosa* as the main etiological agent in wound swabs. It was also observed that *P. aeruginosa* is the microorganism most resistant to antibiotics (over 90%) among those identified, but still maintaining a very good susceptibility to Colistin. Thus, starting from these results of the first doctoral study, the need for a more in-depth evaluation of antibiotic resistance of *P. aeruginosa* isolated from patients with severe burns emerged and the need to identify resistance genes found in an intensive care unit for severe burns was imperative.

Objectives

The main objective we pursued in this study was to identify the susceptibility to antibiotics by phenotypic methods and the screening of resistance genes of *P.aeruginosa* strains isolated from wound swabs from the patient with severe burns.

Materials and method

The study was carried out by C.E.H.P.R.S.B.. in collaboration with the "Cantacuzino" National Military Medical Institute for Researach and Development, Bucharest, Romania within a research project funded by the Ministry of National Defense, based on a collaboration agreement between the 2 institutions. The guidance and supervision of the good conduct of the study was carried out in collaboration with microbiologists, epidemiologists and infectious disease doctors from the National Institute of Infectious Diseases "Prof. Dr. Matei Balș" Bucharest.

16 strains of *P. aeruginosa*, isolated from wound swabs from burns from 13 adult patients admitted to the intensive care unit of C.E.H.P.R.S.B.., were analyzed prospectively. between August 2021 and October 2022.

Given that this study also dealt with the processing of some parameters from usual practice and did not intervene on the treatment of patients, the approval of the Ethics Committee of the C.E.H.P.R.S.B. was obtained. (document no. 3/ 26.04.2021) and the patients' consent was obtained for processing data according to the observation sheets.

In this study, the following were practiced: proteomic analysis by MALDI-ToF mass spectrometry for bacterial species identification, antimicrobial susceptibility testing by

phenotypic methods, detection of carbapenemase production by phenotypic methods and screening of resistance genes by molecular methods

Results

The restricted group analyzed included 13 patients with severe burns hospitalized in the intensive care unit of C.E.H.PR.S.B.., most of them being female (7/13), aged between 28 - 90 years, with total burn surface aread affected between 20 and 55%

Antimicrobial Susceptibility Testing (AST)

Resistance to antibiotics from the beta-lactam class was high, only 3 and 4 isolates (19-25%) being sensitive to Ticarcillin + clavulanic acid, Piperacillin + tazobactam, Ceftazidime, Cefepime, Meropenem, Imipenem and Aztreonam.

The results are also similar for aminoglycosides (amikacin, tobramycin, with n=4 susceptible strains) and quinolones (ciprofloxacin and levofloxacin, with n =3 susceptible strains), suggesting an increased resistance of *P. aeruginosa* to these antibiotic classes as well.

Cefiderocol is a new generation cephalosporin with a siderophore-like mechanism, to which 15 of the 16 analyzed strains (93.75%) were susceptible. All strains were found to be susceptible to Colistin.

Phenotypic tests for the identification of carbapenemases

Among the 16 strains of P. aeruginosa, the mCIM test was positive in 7 of them, while the Blue Carba test was positive in 10 strains. The imipenem-cloxacillin disc-diffusion method was positive in 9 of the strains. Immunochromatographic assays revealed VIM carbapenemase production in 9 strains

Molecular detection of antimicrobial resistance

The results of the PCR analysis revealed the presence of the blaVIM gene in 9 strains and the presence of resistance genes conferring the ESBL phenotype in 6 strains, which were positive for the blaCTX-M gene.

Some strains expressed a single antibiotic resistance gene (7/16) either blaVIM or blaCTX-M, while others co-expressed 2 resistance genes (4/16) and some strains expressed no resistance genes at all (5/16).

Discussions and Conclusions

In the present study we identified the antibiotic susceptibility and demonstrated the existence of antibiotic resistance genes of some *P. aeruginosa* strains isolated from wound

secretions of patients with severe burns. We also evaluated the expression of carbapenemases in these strains by phenotypic methods.

Antibiotic resistance levels of bacterial strains isolated from patients with severe burns were high, especially to beta-lactams, quinolones and aminoglycosides (25% susceptible). Thus, the results obtained in chapter 4 are confirmed.

Cefiderocol is a promising therapeutic option as 15/16 strains of *P.aeruginosa* (93.75%) were susceptible. In almost half of the patients, cefiderocol was the only remaining therapeutic option besides Colistin. The strain that was resistant to Cefiderocol was resistant to all other antibiotics tested except Colistin.

Imipenem-relebactam was noted to be a good option for patients with carbapenem-resistant *Pseudomonas aeruginosa* without Ambler class B carbapenemases, in our study, 5/16 strains were susceptible to Imipenem-Relebactam.

Resistance genes of the studied strains were identified, the blaVIM gene being identified in 9/16 strains, and the blaCTX-M gene in 6/16 strains, some of the patients simultaneously presenting both resistance genes. [14]

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Conclusions

Burns have been and will remain one of the most difficult pathologies to treat worldwide, both from a surgical and a medical perspective. In the desire to limit as much as possible the severe effects of post-burn shock and the effects of systemic burn-triggered inflammatory response syndrome, we can achieve some milestones that can lead to improved prognosis in thesevere burn patient. Also, in certain situations, wound infection control can be achieved in this particular type of patient, through the use of complex algorithms, for which this doctoral work could represent a starting point.

This doctoral thesis represents one of the most extensive researches carried out on patients with severe burns in Romania over time. Through the 3 studies conducted (chapters 4, 5, 6) we wanted to obtain a series of evidence that would contribute to improving survival rates, prognosis and optimizing the care management of burn patients in Romania.

1. Within the framework of the doctoral research, I reached the proposed objectives one by one. In study 1 (chapter 4), we evaluated the incidence and etiological profile of skin infections and systemic infections in patients with severe burns, in a large cohort of patients (202) and over a period of 3.5 years. Thus, we identified as the main etiological factors for

skin infections/colonization in patients with severe burns *Pseudomonas aeruginosa*, which was the most frequently isolated bacterium (40%), followed by *Staphylococcus aureus* (14%) *and Klebisella spp.* (10%) . From blood cultures, *Pseudomonas aeruginosa* was the most frequently isolated bacterium (45%), followed by *Staphylococcus aureus* (26%) and *Klebisella spp.* (10%).

2. Another objective achieved in study 1 (chapter 4) was to evaluate the antibiotic susceptibility profile of the main bacteria isolated from infections in patients with severe burns. It was thus demonstrated that, of the total of 298 strains of *Pseudomonas aeruginosa* identified, 278 (93%) were MDR. *Acinetobacter baumannii* followed with 91% MDR strains, and in third place was *Klebsiella spp* with 31% MDR strains. The lowest percentage of MDR strains was observed in *E. coli* (6%). It was also observed that Colistin remains the best therapeutic option in the treatment of *P. aeruginosa* and *A. baumanni* infections, their susceptibility to Colistin being very good.

3. Identifying all these aspects, we can make available to the specialized committees from the Ministry of Health and professional societies this database resulting from the doctoral research in order to be used as a working tool for the development, financing and implementation of unique management protocols (diagnosis and treatment) and for infections in patients with severe burns, to be applied and respected by all emergency departments and intensive care units of regional hospitals. Thus, hospitals can properly supply themselves with the necessary medication for the treatment of patients with severe burns. As a result, it is important that an infectious disease specialist and an epidemiologist are employed in each hospital in order to prescribe the correct antibiotic therapy and to supervise the (healthcare associated infections) HAIs.

4. Altough the aim of this work was not to identify the incidence of healthcare-associated infections, recommendations can be made that isolation, compliance with universal precautions, hygiene and epidemiological surveillance of the patient with severe burns should be treated more carefully than of patients with other pathologies.

5. Epidemiological, clinical-evolutionary particularities and mortality rate in patients with severe burns in Romania were also evaluated and some aspects were identified from which we can outline the overall picture of the severely burned patient. It was observed that most of the patients included in the study came by transfer from other health facilities (60%) and that most of them had comorbidities at the time of admission (54%). These aspects are very important from the point of view of patient management, and could help public health authorities to develop policies regarding human resources – hiring plastic surgeons

specialized in the treatment of burns in regional centers without burn units in order to help with the timing and optimizing the treatment of the severely burned patient, an infectious disease doctor, a microbiologist and an epidemiologist. Also, the inclusion in the residency curriculum of internships in hospitals that care for severe burns may represent an opportunity for future human resources involved in receiving patients with severe burns to be familiar with the complexity of this pathology.

6. The second study, included in chapter 5, on a significant subset of 121 patients, followed for 3 and a half years, achieved the main objective of identifying and establishing the opportunity of using some biological markers in establishing early diagnosis and prognosis in patients with severe burns. It was shown that cut-off values identified for CRP and PSP (>180 mg/dL and >868 pg/mL respectively) using ROC-AUC curves for identifying the occurrence of hemodynamic instability had a negative predictive value of 75%. Thus, we can say that if the CRP and PSP values are below the cut-off values identified in the study, it becomes extremely unlikely that the patient will develop hemodynamic instability, a very important element for the ICU physician and for the patient's management. This information is extremely important for the intensive care physician who can take all therapeutic measures when patients are at risk of developing hemodynamic instability.

7. The third study, from chapter 6, had its main purpose the identification of antibiotic resistance genes of *P. aeruginosa* strains, the bacterium most often involved in skin infections of patients with severe burns. Resistance genes were thus identified and it was demonstrated that Cefiderocol is an effective antibiotic against *P. aeruginosa* and can be used as an alternative to Colistin (to which all analyzed strains were susceptible). In the future, it can be proposed to include this antibiotic in the current practice of the ICU departments of hospitals that care for patients with severe burns, but with the observance of clear prescription protocols and consulting the infectious disease doctor to avoid compromising its efficiency through an inappropriate and unjudgmental use.

8. The secondary objectives of the study, related to the costs of care for patients with severe burns, were also achieved, and it was observed that patients who came directly to specialized medical facilities in the treatment of burns had 20% lower costs than those who came by transfer. This information can be important for the relevant ministry to calculate and identify options to reduce the financial pressure in the health system, given the high costs related to this type of patients.

9. Other clinical and biological parameters with role in the therapeutics and prognosis of patients, severity scores and the number of days of hospitalization were followed in

chapter 4. These aspects related to the evolution and epidemiology of severe burned patients can represent valuable information for health facilities in the attempt to optimize the management of these patients.

10. The doctoral research had a number of limitations, related to the collection of data from a single center and the multidisciplinary nature of the research which required the collaboration between the plastic surgeon, the infectious diseases doctor, the epidemiologist, the laboratory doctor and the intensive care doctor . The limitations of the C.E.H.P.R.S.B. laboratory in carrying out certain bacteriological and fungal identifications and in the evaluation of some bio-markers do not currently allow us to make more in-depth evaluations of the analyzed data. Limitations related to the construction of statistically significant groups in phenotypic and molecular research could be compensated in the future by a post-doctoral study. Also, multicenter research would certainly provide better insight into the pathology of major burns and its treatment.

11. The results obtained from this paper are comparable to those at international level, with similar conclusions and discussions, which leads to the validation of research and medical management of the large burn patient in Romania.

12. From this doctoral research, 3 articles were published in journals indexed in international databases, with a total impact factor over 5.5, which were in turn cited in other works. Also, from the results of this work, I made a presentation at the UMPCD Bucharest Congress, and the summary of this presentation was published in a BDI rated magazine. The validation of the results by these prestigious journals only strengthens the viability of the research.

PERSONAL CONTRIBUTIONS

1. Through the study carried out in chapter 4, we were able to outline the profile of the patient with severe burns from Romania, namely: male patient, approximately 60 years old, with comorbidities, with 35% total burn surface area, with severe burn degrees (IIB-III), transferred from another health unit.

2. Also, by identifying the bacteriological profile and antibiotic susceptibility of bacteria isolated from patients with severe burns, we can make the entire database available to the Ministry of Health, in order to correctly inform all departments and hospitals in Romania where burn patients are cared for. Future research based on this paper could outline in depth microbiological, epidemiological and infectious diseases aspects that would make the mechanisms identified in this paper more efficient.

3. In the future, more studies starting from the research presented in chapter 5, on larger groups and with more collected samples could establish better cut-off values for PSP in the prognosis of patients with severe burns. The results obtained for PSP and CRP revealed the superiority of presepsin over C-reactive protein as a prognostic factor. Starting from this observation, future studies could observe the correlation of PSP with other more specific and sensitive factors than CRP . Also, starting from the values obtained for all the markers involved, a new prognostic score could be developed in the specialized literature and its predictive capacity could be followed in a future study in patients with severe burns.

The correlation of the results of the bacterial cultures together with the ABA criteria and the values of PSP and CRP could be the foundation for a protocol that could lead in the future to the optimization of the therapeutic conduct and to a better understanding of the septic phenomenon in patients with severe burns.

4. The results obtained in the third study (chapter 6) demonstrated that there are alternatives to colistin treatment. Given its nephro- and neurotoxic potentials, alternative treatments need to be implemented in the treatment of major burns. Identification of Cefiderocol and Imipenem-Relebactam as effective antibiotics against *P. aeruginosa* could represent a starting point. I suggest that in the future the achievement of molecular studies on larger groups of patients to confirm the present results. After this, the implementation in medical practice of some therapeutic guidelines based on these new antibiotics should be achieved. This could make the treatment more efficient and improve the prognosis of patients with severe burns from intensive care units, leading to a decrease in mortality.

The present contributions can certainly represent a solid background for future studies related to the pathology of severe burns, already providing valuable information in directions related to epidemiology, etiology, antibiotic treatment, inflammatory biomarkers and antibiotic resistance genes of the bacteria involved in this type of trauma. The depth of future studies and their multidisciplinary and multicentric character could lead to maximizing the efficiency of the care of major burns.

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Publications list

1.   **Nițescu, B**.; Pițigoi, D.; Tălăpan, D.; Nițescu, M.; Aramă, S.Ș.; Pavel, B.; Streinu-Cercel, A.; Rafila, A.; Aramă, V. Etiology and Multi-Drug Resistant Profile of Bacterial Infections in Severe Burn Patients, Romania 2018–2022. *Medicina* **2023**, *59*,1143. https://doi.org/10.3390/medicina59061143 (F.I. 2.6, articol original, prim autor) - capitolul 4

2.   **Nițescu B**; Pavel B; Tache A.I.; Pop I.A.; Balan I.C.; Aramă S.Ș.; Isac S.; Streinu-Cercel A.; Talapan D.; Pitigoi D.; Aramă V. Predictive values of presepsin and C-reactive protein on prognosis in severe burns. *Farmacia*, 2023, Vol. 71, 3 . https://doi.org/10.31925/farmacia.2023.3.8 (F.I. 1.55, articol original, prim autor) – capitolul 5

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Comunications in Scientific Congress

1.   **Nițescu Bogdan**, Aramă Victoria, Rafila Alexandru, Streinu-Cercel Adrian, Aramă Sorin, Pițigoi Daniela, Nițescu Maria, Pavel Bogdan, Tălăpan Daniela - Bacterial infections in severe burn patients -A prospective study in a burn-dedicated intensive care unit, Bucharest 2018-2022 – presented at Congresului Universității de Medicină și Farmacie "Carol Davila" – București, 10-12 NOV, 2022 – with summary published in Maedica - A Journal of Clinical Medicine, Volume 17, Supplement, 2022, pg 83