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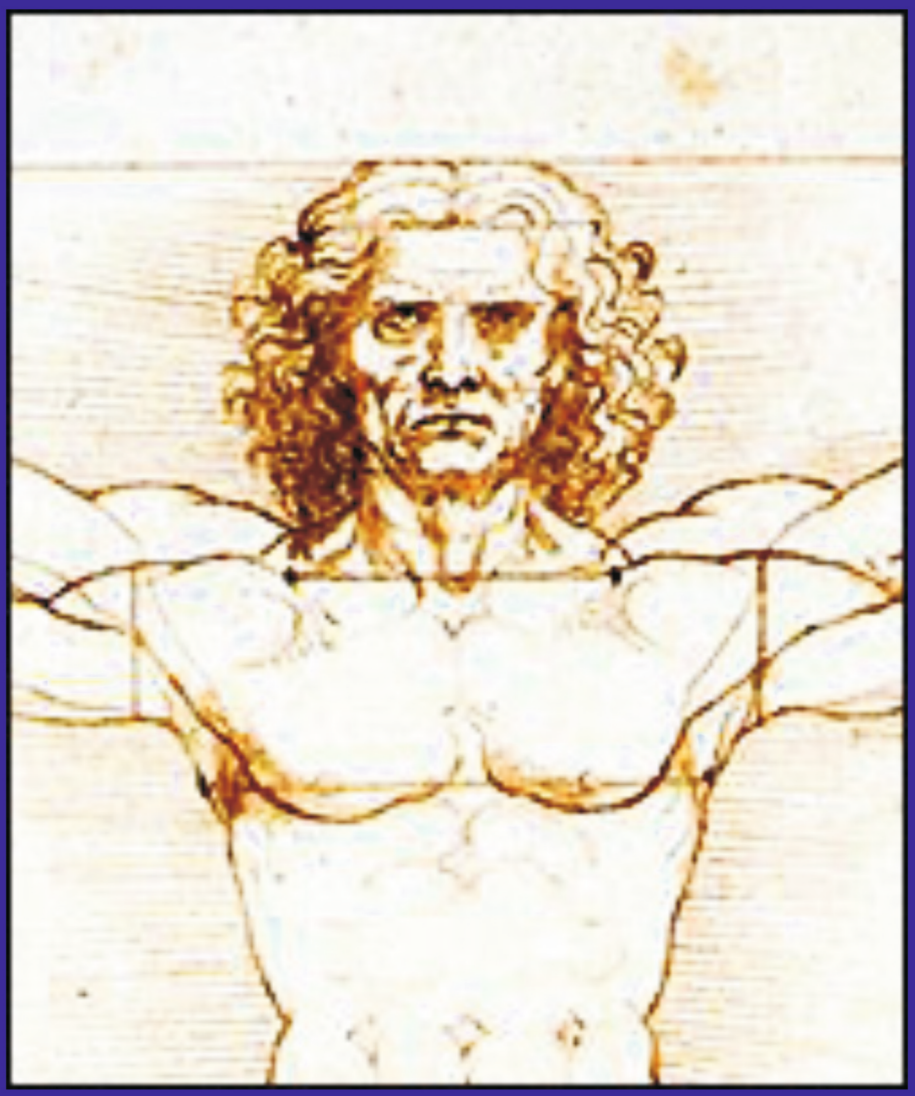

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A WORD FROM THE GUEST EDITOR

Dear Readers,

A very warm welcome to you all. It is my great privilege to present this unique issue of Sanamed to you as guest editor and I would also like to thank the Journal for this unprecedented initiative.

The current issue of the Journal focuses mainly on critical care medicine, including five papers from five countries from this feld of medicine. As you may all know critical has come a long way since the polio-epidemic in the 1950's when dr. Björn Ibsen and colleagues commenced mechanical ventilation with the help of medical students on an allocated ward, Poul Astrup provided the blood gas analyzer, then hundreds of lives had been saved and intensive therapy was born.

Since then critical care has become a multidisciplinary specialty requiring the team work of physicians, surgeons, neurologists, radiologists etc., and not the least highly trained devoted intensive care specialist. As technology advances and the population is ageing, and since we've become more sophisticated on the intensive care units (ICU), the demand for ICU admissions, elective and acute alike, are growing year by year stretching the limits on both the workload on the ICU staff and on the hospitals' financial burden as well. Therefore, understanding the way we work, what we do, and who we can help on, is fundamental for a successful teamwork, which is mandatory for our patients' benefit.

In this issue of the Journal we present five papers from the field of intensive care medicine to aid the above mentioned mission. A mission we started a few years back together with a very enthusiastic group of intensivists here in Central EUROPE, which manifested itself in an international conference what we called SepsEast (www.sepseast.eu). The aim of this initiative was to help Eastern and Central European talented physicians to present on stage together with our Western European counterparts. Let's face it, in general Central and Eastern European intensive care is still way behind the West as far as research and impact on clinical practice is concerned.

But there is light at the end of the tunnel. In addition to SepsEast now we have this initiative from Sanamed. International experts and opinion leaders from five countries gave five reviews on interdisciplinary topics of critical care. Professor Paolo Pelosi from Genoa, Italy, the former president of the European Society of Anaesthesiology, who is also one of the most influential researcher in the world in the field of mechanical ventilation, presents with his colleagues a thorough review paper on the subject and if you read it you will understand why we need well trained experienced doctors on every ICU. Professor Frank Brunkhorst from Jena, Germany, who is a key representative the German Sepsis Society, and he and his team is one of the most influential research group in the world in the field of sepsis, highlights state-of-the-art issues on biomarkers and pathogen detection in sepsis. Professor Evangelos J.Giamarellos-Bourboulis from Athens, Greece, who has been collaborating the abovementioned research group in Jena for years, and he is also a great promoter of the idea of the Global Sepsis Alliance and the World Sepsis Day (www.world-sepsis-day.org), writes a review and their results of more than 10 years of research on immunomodulation and the additional effects of clarythromycin in septic shock. Professor János Fazakas from Budapest, Hungary, our key transplant and liver expert in Hungary has chosen the topic on the role of immunoglobulins in the transplanted patient but their review also gives an insight on how the immune status changes and adapts within the first

year after transplantation. And last, but not least, Dr. Radmilo Jankovic, Nis, Serbia, my great "comrade" in this mission of getting Eastern Europe closer to the West and us to each other, presents state-of-the-art issues about safe extubation of the trachea after mechanical ventilation.

There are also four other papers in this issue of the Journal, from four different fields of medicine. Drs. Kostov Hristijan and Kostova Elena, Skopje, Macedonia, report the results of prospective observational study on patients undergoing knee arthroscopy, and evaluate the diagnostic accuracy of two clinical tests. Dr. Evlijana Hasanovic and coworkers from Tuzla, Bosnia and Herzegovina, performed a retrospective analysis on the value of ultrasound in the diagnostics of kidney parenchymal lesions in type-I diabetic children. Drs. Stojanović Zlatan and Vukadinović Stojanović Sanja, Banja Luka, Bosnia and Herzegovina, investigated the relationship between the anatomical localisation of stroke and after stroke depression. Dr. Poštić D. Srđan and colleagues, Belgrade, Serbia, present the results of real team work, in which they found that systemic osteoporosis leads to decreased bone density of the mandible and causes reduction of edentulous ridges.

Very interesting papers, well worth reading indeed!

Finally, I would like to thank all authors for their contribution, Sanamed and especially Dr. Dzemail Detanac for this great initiative, which should be an example to be followed by other national journals in our region, and I wish you, kind Readers, very pleasant and fruitful time while reading these exceptional articles.

Prof. Dr. Zsolt Molnar
Guest Editor Sanamed
Chair of the Scientific Committee of the Hungarian Society of Anaesthesiology and Intensive Therapy
Head of department, Anaesthesiology and Intensive Therapy, University of Szeged, Hungary
Chairman of SepsEast

REČ GOSTUJUĆEG UREDNIKA

Poštovani Čitaoci,

Želim Vam svima toplu dobrodošlicu. Velika mi je privilegija da Vam, kao gost urednik, predstavim ovo jedinstveno izdanje Sanamed-a i da se zahvalim uredništvu časopisa na ovoj jedinstvenoj inicijativi.

Ovo izdanje časopisa se fokusira uglavnom na intenzivnu medicinsku negu, koju obrađuju autori iz pet zemalja. Kao što svi znate, razvoj intenzivne nege pacijenata je prošao dug put, od polio epidemije u '50-im, kada su dr Bjorn Ibsen i kolege započeli mehaničku ventilaciju uz pomoć studenata medicine na izdvojenom odeljenju, a Pol Astrup obezbedio analize gasova u krvi, nakon čega su stotine života spašeni, rođena je ova grana medicine.

Od tada intenzivna nega je postala multidisciplinarna i zahteva timski rad lekara, hirurga, neurologa, radiologa i drugih, kao i visokoobrazovanih specijalista intenzivne nege. Kako tehnologija napreduje i populacija stara, i kako smo postali sofisticiraniji u jedinicama intenzivne nege, prijem pacijenata u intenzivnoj nezi, elektivnoj i akutnoj, raste iz godine u godinu, sve više opterećujući osoblje jedinica intenzivne nege, kao i finansijski budžet bolnica. Zato, razumevanje načina na koji mi radimo, šta mi radimo i kome možemo pružiti pomoć je od fundamentalnog značaja za uspešan timski rad, koji je obavezan za dobrobit naših pacijenata.

Ovo izdanje časopisa predstavlja pet radova iz oblasti intenzivne nege radi sprovođenja već pomenute misije, koju smo započeli pre nekoliko godina, zajedno sa entuzijastičnom grupom intenzivista u Centralnoj Evropi, internacionalnom konferencijom koju smo nazvali SepsEast (www.sepseast.eu). Cilj ove inicijative jeste da se pomogne doktorima istočne i centralne Evrope da se predstave na sceni zajedno sa kolegama iz zapadne Evrope. Da se razumemo, u centralnoj i istočnoj Evropi intenzivna nega je još uvek iza zapadne kada se radi o istraživanjima i uticaju na kliničku praksu.

Ali, ipak ima svetla na kraju tunela. Kao dodatak SepsEast-u, sada imamo ovu inicijativu iz Sanamed-a. Internacionalni eksperti i lideri u ovoj oblasti, iz pet zemalja, prezentovali su aktuelne teme iz oblasti intenzivne nege. Profesor Paolo Pelosi iz Đenove, Italija, prethodni predsednik Evropskog Saveta za Anesteziologiju, koji je takođe jedan od najuticajnijih istraživača u svetu što se tiče polja mehaničke ventilacije, prezentuje sa svojim kolegama detaljan rad na ovu temu i ako ga pročitate shvatićete zašto nam na intenzivnoj nezi trebaju doktori sa iskustvom, dobro obučeni.

Profesor Frank Brankhorst iz Jene, Nemačka, koji je glavni predstavnik Nemačkog Udruženja za Sepsu, zajedno sa svojim timom predstavlja jednu od najuticajnijih istraživačkih grupa na polju sepse, ističe najbitnije probleme vezane za biomarkere i detekciju patogena u sepsi. Profesor Evangelos J. Giamarelos-Burbolis iz Atine, Grčka, koji je godinama sarađivao sa već pomenutom grupom iz Jene, i takođe je promoter ideje o Globalnoj Alijansi za Sepsu i Svetskog Dana Sepse (www.world-sepsis-day.org), u svom radu iznosi rezultate desetogodišnjeg istraživanja na temu imuno-modulacije i dodatnim efektima klaritromicina u septičkom šoku. Profesor Janos Fazakas iz Budimpešte, Mađarska, ključni ekspert za transplantaciju jetre i jetre uopšte, je odabrao temu o ulozi imunoglobulina kod pacijenata koji su imali transplantaciju. Ali, njegov rad nam takođe daje informacije kako se imunološki status menja i prilagođava u prvoj godini nakon transplantacije.

I poslednji, ali ne i manje bitan, dr Radmilo Janković iz Niša, Srbija, moj veliki „saborac" u ovoj misiji približavanja istočne Evrope zapadnoj, kao i približavanja nas međusobno, predstavlja najuticajnije probleme vezane za sigurnu ekstubaciju nakon mehaničke ventilacije.

Tu su takođe, četiri dodatna rada, iz četiri različita polja medicine. Dr Hristijan Kostov i Elena Kostova iz Skoplja, Makedonija, pišu o rezultatima prospektivne studije posmatranja pacijenata podvrgnutih artroskopiji kolena, gde ocenjuju dijagnostičku tačnost dva klinička testa. Dr Evlijana Hasanović i saradnici iz Tuzle, Bosna i Hercegovina, dali su retrospektivnu analizu vrednosti ultrazvuka u dijagnozi parenhimalnih lezija bubrega kod dece sa dijabetesom tipa I. Dr Zlatan Stojanović i dr Sanja Vukadinović Stojanović iz Banja Luke, Bosna i Hercegovina, istraživali su vezu između anatomske lokalizacije moždanog udara i depresije nakon njega. Dr Srđan D. Poštić i kolege iz Beograda, Srbija, predstavili su rezultate pravog timskog rada, u kojima su pronašli da sistematska osteoporoza vodi do redukcije koštane gustine mandibule i redukcije bezubih grebenova vilice.

Jako interesantni radovi, svakako vredni čitanja!

Na kraju, želeo bih da se zahvalim svim autorima na njihovom doprinosu, zatim uredništvu Sanamed-a i posebno Dr Džemailu Detancu za ovu predivnu inicijativu, koja bi trebala biti primer ostalim nacionalnim časopisima u našem regionu, i želim Vama, dragi Čitaoci, ugodno i produktivno vreme u čitanju ovih izvanrednih radova.

Prof. Dr Zsolt Molnar
Gost urednik u Sanamedu
Predsednik Mađarskog Naučnog Komiteta
za Anesteziologiju i Intenzivnu terapiju
Šef katedre za Anesteziologiju i Intenzivnu terapiju,
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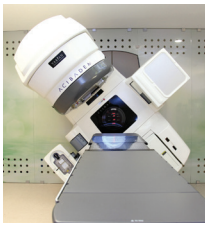

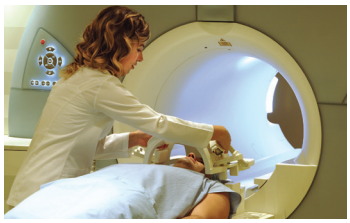

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SYSTEMIC NON-MALIGNANT OSTEOPOROSIS AND REDUCTION OF EDENTULOUS ALVEOLAR RIDGES

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Abstract: **Introduction.** Systemic osteoporosis damages skeletal bones to different degrees.

The aim of this study was to determine the intensity and correlation of the osteoporotic changes in the bone density of the skeleton and body mass index (BMI) with a reduction in edentulous mandibles, and to assess possibility of reparation of layers of mandibles with increase of mineral content in jaws of patients affected by osteoporosis.

Material and Methods. In this study, 99 edentulous patients with decreased bone density comprised the experimental group, and 48 edentulous patients with normal bone densities formed the control. The age of the examined patients was 69.02 ± 7,9, range 53–74 of females and 69.11 ± 7.1, range 59–76 years. Radiographs of the hands and panoramic radiographs were done for all the patients. The values of BMI, metacarpal index , density of lumbar spine (L2–L4), in the phalanx and in segments of the mandibles as well as the edentulous alveolar ridges heights were measured, assessed and calculated.

Results. The lowest value of the total skeletal density was established in the osteoporotic patients on the basis of the average T-score of – 2.5 in men, and – 2.6 in women. Minimum values of the edentulous ridges heights (right/left, in mm) were measured in both osteoporotic females (21.84/22.39) and males 24.90/24.96) patients. By comparison of the densities of the metacarpal bones, proximal phalanx, segments of the edentulous mandibles and based on the numerical values of the edentulous ridges heights, $\chi^2 = 3.81$ was found in men and $\chi^2 = 4.03$ was found in women with normal bone densities; $\chi^2 = 5.92$ was found in men and $\chi^2 = 6.25$ was found in women with osteopenia; $\chi^2 = 2.63$ was found in men and $\chi^2 = 3.85$ was found in women with osteoporosis, on the level of probability of 0.05. After application of calcium and calcitonin in solutions, moderate increment of density ($p < 0.05$; $p < 0.01$) was verified, compensating up to 4% of total loss of mass, minerals and solidity of denture bearing areas of osteoporotic mandibles.

Conclusion. Systemic osteoporosis leads to decrease of densities of bones of mandibles and causes reduction of edentulous ridges.

Key words: osteoporosis; mandible; stomatognathic system; density; mineral content; bones.

INTRODUCTION

Osteoporosis decreases bone mass (1, 2, 3). Also, osteoporosis increases bone fragility (1, 2, 3).

Systemic osteoporosis has a greater influence to quality and quantity of jaw bones than expected when compared to the influence of local factors among which loss of a tooth is the prevailing one (4–9). However, there is still controversy concerning whether osteoporosis significantly reduces edentulous alveolar ridges (8, 10, 11, 12), or whether its impact on the jaw reduction is insignificant (13, 14, 15). Also, the results based on the previous studies showed the significant role of the metabolic factors and osteoporosis in the initiation of the reduction of the edentulous residual alveolar ridges (10).

Reduced blood flow to the jaw — particularly in the lower jaw and difficulty in circulation associated

with systemic osteoporosis can cause loss of bone substance in the edentulous residual alveolar ridges (16, 17). In addition, if there are signs of alveolar bone reduction under a denture that fits comfortably in the intimate underlying tissue, the causes, in the first place, should be sought in action and influence of systemic osteoporosis (18, 19).

Metabolic disturbances and systemic osteoporosis are directly responsible for the appearance of manifested reduction in the residual alveolar ridges of the maxilla and mandible (20).

In many of these studies the panoramic radiographs were used, which is confirmed in the literature as the correct approach in the diagnosis and therapy for many diseases of the stomatognathic region of edentulous patients as well as in dentate patients (21).

A low BMI has also been described as one of the risk factors that may indicate the beginning or the presence of atrophy and reduction of the alveolar ridges, with the fact that patients with low bone mineral density (BMD) may not always have reduced edentulous alveolar ridge, and vice versa. It is also acceptable that patients without supple bone structure and anatomically minor dimensions of the bones usually have significantly marked signs of atrophic jawbone when compared with a subject with normal BMD (22, 23, 24, 25).

The aim of this study was to determine the intensity and correlation of the osteoporotic changes in the bone density of the skeleton and body mass index (BMI) with a reduction in edentulous mandibles, and to assess possibility of reparation of layers of mandibles with increase of mineral content in jaws of patients affected by osteoporosis.

MATERIAL AND METHODS

In this research 147 edentulous subjects, aged from 53 to 73 years, were examined. A study group consisted of 99 edentulous patients (39 men and 60 women) with low bone density, based on data obtained by densitometry of lumbar vertebral bodies, and control group consisted of 48 edentulous patients (25 men and 23 women) with normal bone density (Table 1).

In order to collect data, the parameters of weight (in kg) and height (in cm) were measured, to provide information on body mass index (BMI) of every patient. Antero-posterior radiographs of hands (APCR) (Siemens Rtg apparatus, tube Dofoks RX 150/30–50, focus 2 mm), and standardized panoramic radiographs (Kodak T-MAT G) of all patients were taken in accordance with predetermined distance on a scale on the plastic chin-holder of the panoramic X-ray unit (Orthopantomograph 10, Serial No. 01492, Siemens, Germany) under the determined conditions (68 KV and 5 mA). After collecting data on BMI, the most important analyses on skeletal densities at systemic level were provided on the basis of scanning of lumbar vertebrae (Lunar DPX-L scanner, USA). Analyses of changes of qualities and quantities of bones of interest were done taking into consideration four parameters: body mass index (BMI), metacarpal index (MI), heights (in mm) of edentulous mandibular ridges (VBG) respecting areas where molars were previously extracted and densities of spongy parts of metacarpal bones, proximal phalanx, as well as edentulous ridges of mandibles.

The parameter MI was calculated based on the measurement of the differences between the width of trabecular-spongy part of the bone and the width of the cortical bone in the middle segment of the second metacarpal bone according to the formula: MI equals width of the spongy portion minus cortical thickness divided with the numerical value of the overall diameter of the middle shaft of the second metacarpal (2, 50).

The region of the lower jaw was determined in this study for measurements of both of bone density and edentulous ridge height. Transparent films with mesh (squares of dimension 2 mm x 2 mm) were then positioned onto each of the panoramic radiograph of each patient. Numerical values of the digital light-optical densities were recorded using a densitometer (DT 11 05, England) by transmission of a light beam through each square of the region of interest. In the assessment of the quality of bone of each of the subject examined BMI, densities of a skeletons (g/cm^3) (Lunar DPX-L GE Healthcare, Piscataway, NJ,USA), and densities of mandibles on the basis of measurements on panoramic radiographs and digital densitometer were considered.

The procedure of measurement and calculation was performed as follows:

The absolute density of the extracted segment of the jaw bone, as measured by the pycnometer was in the range of values of $0 < \rho < 0.95$ g/cm^3.

Bone density was measured using a digital densitometer. It was measured in the range $-3 < \mu < 3$, because the value of a digital densitometer depicted in the range of –3 to +3 U/mm^2.

In calculating the values of bone density in arbitrary units corresponding to a determined and specified values of absolute density g/cm^3 (ie mg/cm^2) general formulas were applied with a coefficient equations (μ) and two unknowns ($a + b$), $F = a + \mu b$. To determine values measured using the pycnometer the formula was $\rho = \rho o + c\mu$. Value c is calculated from the quotient value = 0.95 6c, where c = 0.95:6. calculating the procedure is then applied to the calculation of the value of formulas 3 – 1. 05 + $c\mu = \rho o$ and ρo = (3 x 0.95) 6, so that the established basic value was $0 = \rho o - 3C$.

Calcitonin (Miacalcic, Novartis, Switzerland), and calcium (Calcium-gluconate Sterop, Belgium; Calci-

Figure 1. *Segments of interest in mandible*

um-Sandoz 10% calcium glubionas amp., Switzerland) were used locally to improve solidity of oral bones in fifty three osteoporotic edentulous patients — 11 men, aged 64 to75 yrs. (mean age 71 yrs.) and 42 women, aged 55 to 75 yrs. (mean age 56 yrs.) of the experimental group. Thirty seven edentulous patients — 17 men, aged 55 to 75 yrs. (mean age 59 yrs.), and 20 women, aged 45 to 56 yrs. (mean age 55 yrs.), with well pronounced edentulous ridges after extractions of teeth and with normal bone density, were controls. Patients were selected concerning dental history, questionnaire on previous medical treatments of osteoporosis, skeletal density, history of fractures of any of bones, menopausal periods in women, calcium and microelements of blood-plasma, as well as oral status on bone consistency or reduction. Patients had not manifested destructions in the mouth because of malignancy. After local application of anaesthetic, submucosal injection of up to 1.5 ml of solution containing calcitonin and calcium (2:1 ratio in one dose) has been administered (26, 27, 28). This solution was injected towards mandibular bone surfaces (Figure 1). Total of six doses to the right side of osteoporotic mandible and six doses to the left side of bone were administered in each patient of the experimental group. Regions of interest were determined on the basis of assigning of reference line under the base of mandible, as well as according to determining of vertical lines. Region Pr was determined regarding area of bone where roots of the second premolars were missing. Region M1r was determined for areas in which roots of the first molars were missing after extractions, and region M2r was determined on the basis of areas without roots of the second molars. Using the same parameters and reference lines, regions Pl, M1l and M2l were determined. Measurements of density of mandibles have been done after 4 months of administration of solution of calcitonin and calcium and after 6 months. T scores were applied for analysis of density of bone of experimental group patients, at systemic level.

Three questionnaires were designed for the patients in the experimental group in this study. The first questionnaire aimed to learn what the patients thought about their old dentures before therapy by calcitonin and calcium. The second questionnaire aimed to document what the patients thought about their dentures after therapy by calcitonin and calcium. The patients were asked to rate their dentures as good, fair, or poor in the categories of retention, ability to chew, and comfort. Each item was scored on the basis of good = 3, fair = 2, and poor = 1. Total score was termed the "Patient satisfaction score".

The third questionnaire was provided for the dentist to evaluate the technical quality of the complete dentures that were fabricated for the patients immediately after therapy. The scoring of denture quality was on the same scale as for questionnaires 1 and 2.

Statistics

For statistical calculations of numerical values of densities of bones, mean values and standard deviations were initially calculated. In statistical processing of numerical values of heights of edentulous ridges, mean values, standard deviations and coefficients of variations were used. Correlations of radiologic representation of height of edentulous ridge Y1 on panoramic radiographs and digital optical densities of determined segments of mandibles were estimated by Pearson's index of correlation.

Calculation of measured numerical values of densities of metacarpal bones, proximal phalanx, segments of edentulous ridges and numerical data of heights of segments of edentulous ridges of mandibles was done applying χ^2 test with comparison of 4 independent samples by McNemar's test.

Student's T test, one-way ANOVA and coefficients of correlations were used for calculation of numerical values of densities of mandibles in P, M1 and M2 regions.

The correlation of functional assessment and patient satisfaction with complete dentures with increased density in mandibular segments after therapy was calculated by Pearson's correlation coefficients. The correlation of retention and quality of the bases of new complete dentures with increased density was calculated by Spearman's correlation coefficients.

The Institutional Review Board of the University approved this study and each participant agreed with an informed consent form before the study began.

RESULTS

Total skeletal density, established on the basis of T scores was measured on densitometer, and minimum values were calculated for osteoporotic patients: T = –2.5 g/cm^3 in men and T = –2.6 g/cm^3 in women (Table 1).

Values of BMI were the lowest in osteoporotic patients (Table 2).

The measured values of metacarpal indexes were in upper levels for patients of the control group, but in very low levels for osteoporotic patients (Table 2).

The measured density values of metacarpal bones were as a minimum in women (0.68 and 0.65 U/mm^2) and men (0.77 and 0.82 U/mm^2) of patients with osteoporosis (Table 3).

The numerical values of densities of proximal phalanx were no less than 0.51 and 0.52 U/mm^2 in osteoporotic women, and no less than 0.53 and 0.56 U/mm^2 in osteoporotic men (Table 4).

The measured values of densities in the segments of the first missing molars in mandibles were as a minimum in women (–1.80 and –1.81 U/mm^2) and in men (–1.19 and –1.27 U/mm^2) suffering of osteoporosis (Table 5).

Minimum heights of edentulous alveolar ridges were measured in osteoporotic women (21,84 mm and 22,39 mm) and in osteoporotic men (24,90 mm i 24,96 mm) (Tables 6 and 7).

Positive correlations of the radiographically revealed and measured height loss of the edentulous ridge Y1 and the digital light density in the determined segments of the mandibles (Table 8) as well as of measured height loss of the edentulous ridge Y1 and the BMI (Table 9) were established.

Based on comparison of numerical values that were observed in the analysis of densities of metacarpal bones, proximal phalanges, edentulous ridge segments and numerical data on the edentulous ridge using the χ^2 test and McNemar's test results are established: $\chi^2 = 3.8182$ (degrees of freedom $\upsilon = 11$) in men with normal bone density, $\chi^2 = 4.0384$ (degrees of freedom $\upsilon = 12$) in women with normal bone density, $\chi^2 = 5.9238$ (degrees of freedom $\upsilon = 9$) in men with osteoporosis, bone, $\chi^2 = 6.2571$ (degrees of freedom $\upsilon = 11$) in women with osteopenia, bone, $\chi^2 = 2.6359$ (degrees of freedom $\upsilon = 5$) in men with osteoporosis, bone, $\chi^2 = 3.8519$ (degrees of freedom $\upsilon = 6$) in women with osteoporosis, at the level of probability 0.05 (ie 5%).

On the basis of χ^2 tests it was established that assessed data were relevant and consistent.

Increment of density of mandibular segments after the therapy was significant for Pr and Pl segments ($p < 0.01$). Following administration of calcium and calcitonin in solutions, moderate increment of jaw-bone density $p < 0.05$ and $p < 0.01$, was observed, compensating up to 4% of bone's mineral loss (Table 10). Increment of solidity of bone in the control group was present but not in significant levels.

The median score on the questionnaire for functional assessment of new complete dentures which patients used after the calcitonin/calcium therapy was 2, and scores ranged from 2 to 3. There were positive correlations of fit of lower dentures, ability to eat foods, and comfort with the therapy, with increased density in mandibular segments (Tables 11–14).

DISCUSSION

Osteoporosis is a systemic skeletal disease characterized by reduced bone mass and structural changes of the bone tissue, leading to bone fragility and an increased risk of fracture (1, 2, 3, 29, 30). Socio-economic factors and global trends have caused increased number of people suffering of osteoporosis (1, 2, 25).

According to the literature, the extraction of teeth in the jaw last activated osteoclasts during the first 6 to 24 months (10, 31, 32, 33) with later builds slower but progressive atrophy with reduction of the edentulous ridge. In addition, under certain allegations of professional studies systemic factors have a greater influence on the reduction of the edentulous jaw, with the systemic factors activated immediately and directly after the action of local factors (3, 5). In one study where the problematic of osteoporosis was focused 128 edentulous patients with osteoporosis were examined, with use of DXA to determine skeletal density and edentulous ridge resorption around the mental foramen and distally. Based on the results of this study statistically significant correlation between the reduction of the alveolar ridge of mandible and osteoporosis was showed (8). In a similar study conducted in 92 edentulous patients aged between 75 and 85 years, osteoporosis was defined based on the analysis of the panoramic radiographs of patients with the most intensified reduction in edentulous ridges, and statistically significant difference was found in the values of the minimum and maximum measured values of bone mineral density (34). Statistically significant differences in structure and bone density were tested with normal bone density and tested with osteoporosis, however, have not been established in a study performed on 12 edentulous postmenopausal women. Despite of diagnosed persistency of edentulous ridge resorption of the jaw, the presence of osteoporosis is determined solely on the basis of performed scan the hips and the existence of fractures of long bones (7).

In order to determine the total skeletal mineral density in this study only vertebral bone densities were assessed, although, two folded scanning of the lumbar vertebrae (L2–L4) and the scanning of a head of neck of the femoral bone is considered to be "the gold standard" in the diagnosis of osteoporosis because of precision, functionality and yet allowed the radiation dose (35).

In order to determine the absorption-reduction and the height of the edentulous alveolar ridges in this

Table 1. *Distribution of patients' age by different groups of bone mineral density and T values (scores)*

Group	Number of patients	Average age ± standard deviation of ages	T value (score)
Males with normal bone density	25	67.41 ± 5.27	– 0.2
Females with normal bone density	23	65.12 ± 6.11	– 0.5
Males with osteopenia	10	69.45 ± 7.93	– 1.9
Females with osteopenia	14	67.32 ± 8.47	– 2.1
Males with osteoporosis	29	70.27 ± 8.11	– 2.5
Females with osteoporosis	46	74.89 ± 9.05	– 2.6

Table 2. *Values of metacarpal indexes and BMIvalues in male and in female patients*

Group	Metacarpal index — right			Metacarpal index — left			BMI
	Minimum	Maximum	Average	Minimum	Maximum	Average	
Males with normal bone density	48.23%	56.65%	52.6%	48.23%	56.65%	52.6%	24,1
Females with normal bone density	46.93%	52.03%	49.7%	46.93%	52.03%	49.7%	22,2
Males with osteopenia	40.08%	43.37%	41.9%	41.03%	43.96%	42.4%	19,9
Females with osteopenia	38.52%	41.14%	40.1%	39.11%	42.12%	41.2%	18,1
Males with osteoporosis	31.89%	41.93%	36.76%	30.67%	43.02%	36.48%	18,3
Females with osteoporosis	29.52%	33.41%	30.91%	29.67%	34.17%	31.27%	16,9

Table 3. *Average values of measured densities, expressed in U/mm^2, within spongy parts of metacarpal bones*

Group	Number of patients N	Right side		Left side	
		Average value $\bar{x}$	Standard deviation SD	Average value $\bar{x}$	Standard deviation SD
Male patients with normal bone density	25	1.94	1.23	1.98	1.14
Female patients with normal bone density	18	1.90	1.11	1.89	1.08
Male patients with osteopenia	14	1.19	0.91	1.21	0.88
Female patients with osteopenia	20	1.15	0.89	1.11	0.92
Male patients with osteoporosis	25	0.77	0.71	0.82	0.69
Female patients with osteoporosis	30	0.68	0.74	0.65	0.72

Table 4. *Average values of measured densities, expressed in U/mm^2, within spongy parts of proximal phalanx*

Group	Number of patients N	Right side		Left side	
		Average value $\bar{x}$	Standard deviation SD	Average value $\bar{x}$	Standard deviation SD
Male patients with normal bone density	25	1.71	1.12	1.78	1.14
Female patients with normal bone density	18	1.69	1.14	1.69	1.13
Male patients with osteopenia	14	0.98	0.82	0.99	0.80
Female patients with osteopenia	20	0.95	0.78	0.98	0.79
Male patients with osteoporosis	25	0.56	0.71	0.53	0.69
Female patients with osteoporosis	30	0.51	0.74	0.52	0.72

Table 5. *Average values of measured densities, expressed in U/mm^2, of edentulous ridges in regions of missing of the first lower molars*

Group	Number of patients *N*	Right side		Left side	
		Average value $\bar{x}$	Standard deviation SD	Average value $\bar{x}$	Standard deviation SD
Male patients with normal bone density	25	2.02	1.12	2.04	1.14
Female patients with normal bone density	18	1.97	1.09	2.01	1.07
Male patients with osteopenia	14	– 0.85	0.82	– 0.82	0.84
Female patients with osteopenia	20	– 0.95	0.79	– 0.89	0.81
Male patients with osteoporosis	25	– 1.27	0.69	– 1.19	0.63

Table 6. *Measured heights of edentulous alveolar ridges of mandibles (in mm)*

Group	Number of patients *N*	Right side			Left side		
		Average value $\bar{x}$	Standard deviation SD	KV	Average value $\bar{x}$	Standard deviation SD	KV
Male patients with normal bone density	25	26.22	2.28	8.31	26.24	3.06	8.39
Female patients with normal bone density	18	24.62	2.09	8.87	24.65	2.12	8.91
Male patients with osteopenia	14	25.03	2.82	8.24	25.08	2.84	8.29
Female patients with osteopenia	20	25.29	2.79	8.39	25.31	2.81	8.41
Male patients with osteoporosis	25	24.96	0.62	2.43	24.90	2.68	2.19
Female patients with osteoporosis	30	21.84	2.28	12.21	22.39	1.91	7.51

Table 7. *Results of the degree of mandibular residual ridge resorption and height of edentulous residual ridge (p = 0.752)*

	Reduction of edentulous alveolar ridge of mandible			Height of ridge of mandible Right	Height of ridge of mandible Left
	small	moderate	intensive		
Normal density of bone	33	10	0	25,24 mm	25,44 mm
Osteopenia	5	13	5	25,16 mm	25,19 mm
Osteoporosis	2	12	41	23,41 mm	23,64 mm

Table 8. *Correlation between radiological measurement of mandibular edentulous ridge Y1 and optical density of segment of edentulous mandible*

		Y1
Optical Density	Pearson correlation	– 0.049 (**)
	P value	0.006
	Number of patients	89

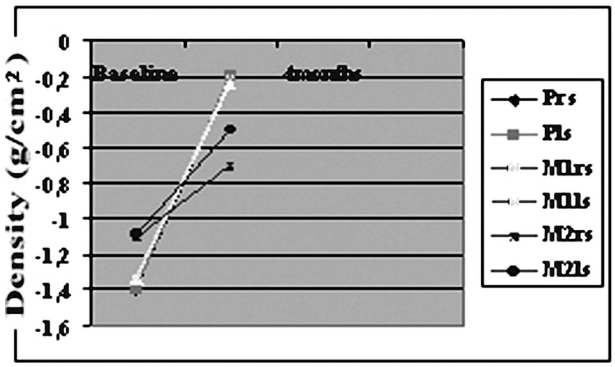
** Correlation was estimated for probability p = 0.01

Table 9. *Correlation between radiological measurement of mandibular edentulous ridge Y1 and body mass index*

		Y1
Body mass index	Pearson correlation	– 0.038 (**)
	P value	0.009
	Number of patients	89

** Correlation was estimated for probability p = 0.01

***Table 10**. Increment of density of mandibles of patients of the experimental group, in observation period of up-to 4 months*



study panoramic radiographs of patients were used, considered as real and meaningful in monitoring and display of height edentulous ridges, although respecting the literature data the measurements of an edentulous ridge height could be performed using a lateral cephalometric radiographs (7). Mandibular bone layers were showed for the purposes of this present study by the panoramic radiographs (5, 7, 9, 17, 34, 36–43), selecting and marking at the different points of reference, such as median jaw line-"symphysis", lines around the region of the mental foramen, the ridge where the missing first molars or distal point of the retromolar bulge. According to literature data there were not statistically significant difference between the numerical

***Table 11.** The results of a questionnaire on patients' opinions about their old dentures before therapy with calcitonin and calcium*

	Very satisfied (Good)		Fairly satisfied (Fair)		Dissatisfied (Poor)	
	Number	Percent	Number	Percent	Number	Percent
Fit (upper)	3	5%	27	45%	29	49%
Fit (lower)	1	1%	27	45%	31	53%
Ability to eat foods	1	1%	25	42%	33	56
Comfort	0	0%	35	59%	24	41%

***Table 12.** The results of a questionnaire on patients' opinions about their old dentures after therapy with calcitonin and calcium*

	Very satisfied (Good)		Fairly satisfied (Fair)		Dissatisfied (Poor)	
	Number	Percent	Number	Percent	Number	Percent
Fit (upper)	15	25%	45	75%	0	0%
Fit (lower)	16	27%	44	73%	0	0%
Ability to eat foods	16	27%	44	73%	0	0%
Comfort	19	32%	40	68%	0	0%

***Table 13.** Evaluation of the technical quality of new complete dentures positioned after therapy with calcitonin and calcium*

Evaluation of retention of base of compete denture	Number of patients
Upper complete denture	
Good Fair Poor	21 38 0
Lower complete denture	
Good Fair Poor	19 40 0

Table 14. *The correlation of functional assessment of a complete denture by a dentist and patient satisfaction with increased mandibular density*

Factors	Increased density in segments of mandible after therapy with calcitonin and calcium
Fit (upper)	0.317†
Fit (lower)	0.624†
Ability to eat foods	0.653†
Comfort	0.748†
Good retention of base of lower complete denture	0.639‡

† Pearson's correlation coefficient
‡ Spearman's correlation coefficient

values of edentulous ridge height measured at the center line of the jaw of a digital panoramic radiograph and the lateral-profiled cephalometric radiographs (44). Lateral cephalometric tele-type image, however, does not provide simultaneous insight into the left and right side of the jaw, because of superimposed surfaces to measure the height of the ridge in the lateral-lateral region, particularly on the right, as well as especially on the left side (5, 17).

Region where the first lower molar was missing in the lower jaw was chosen in this study for measuring bone density in the jaw as well as to measure the height of the edentulous ridge because it is in everyday professional practice, the biggest problem in the stabilization of lower jaw dentures at present exactly when the absorption of bone around the molars was expressed, or additionally reduced after tooth extraction. There are indications that the region of the first lower molar, among other predictors of the presence of osteoporosis, which may be related to a reduction in the edentulous ridge (17, 40, 41). The results of this research have just shown that in the bone damaged by osteoporosis, the reduction in the edentulous alveolar ridge is larger and more intense (Table 6, Table 7 and Table 9), which is consistent with the assertions by some authors that in the case of diseases such as systemic osteoporosis, the first signs displayed in the jaws were the reductions of the edentulous ridges (5, 8, 9, 34, 37), but in contrast with the results of some recent study (25).

In terms of correlating the edentulous ridge atrophy, reduction and the resorption in the jaw with osteoporosis, there are data to support that osteoporosis leads to specific osteoporotic reduction along the buccal-lingual direction, which in the late stages is revealed as a reduced ridge in the form of a "knife edge" (32, 45). This interpretation, however, cannot be accurately compared with the results of this study, given the fact that suitable form of alveolar ridges would be studied based on a 3-D display of the jaw.

Despite many efforts to prove the causal relationship between the residual edentulous ridge resorption and osteoporosis, a number of the existing situation in everyday dental prosthetic clinical practice can be objectively lead therapist in doubt as to comprehend and interpret the finding that patients with a marked reduction in the jaw ridge had normal bone density or vice versa. This has prompted researchers and the scientific community to try to identify the interconnections of development and intensity of reduction of residual ridges (17, 22, 23, 28, 47) with body mass index. In a study conducted on 128 women without teeth, a statistically significant association between high body mass index value and not-reduced edentulous ridges with significantly high values of the vertical dimension was found (22).

Based on the results of the study of 96 edentulous women, a statistically significant difference between the different measurements of height of radiographically presented edentulous ridges in the lower jaw and body mass index were showed (23).

In this study, 99 of the respondents also showed a statistically significant correlation between body mass index and resorption of the edentulous ridge in the sense that edentulous patients with low body mass indexes-BMI also had a small amount of edentulous ridges in advanced reduction process (Table 9).

The metacarpal index is an objective parameter in determining the existence of osteoporosis and assessing the degree of involvement of osteoporosis into the skeleton (36, 40, 47). In this present study, it was found that the MI values were significantly decreased in osteoporotic patients.

Results on medication of osteoporotic oral bones were favourable in this study. Repeated application of calcitonin and calcium in solutions to oral bone layers, may significantly improve oral bone's condition and denture-supporting area's potential. There should be also prerequisites to assume that therapy by calcitonin and calcium could increase position of remaining teeth of the osteoporotic jaws.

Osteoporotic edentulous mandible should be the first of all oral bones to implicate the solution of calcitonin and calcium. Additionally, respecting the levels of concentration of calcium ions applied, osteoporotic maxillary bone could be treated with the solution of the same kind, in the prolonged period of remedial use. Probable contraindication for local oral application of calcitonin and ionized calcium in solutions should be very intensive reduction of edentulous ridges with alveolar crest positioned under the superficial surface of mylohyoid muscle of the floor of an edentulous mouth.

It has been suggested in the literature that well-designed questionnaires can be used for assessment of patients' subjective and overall satisfaction with complete dentures (48–51). For the purposes of the present study, it seemed that patient satisfaction with local therapy with calcitonin and calcium, and the wearing of new conventional complete dentures, was equally as important as the validity of the increment of mineral content that is, the solidity of the osteoporotic jawbones after the application of calcitonin and calcium in solution.

The results of this study revealed that more than 27% of the patients in the experimental group were extremely satisfied with their new complete dentures after the calcitonin/calcium therapy, despite their extensive experience in denture-wearing. Additionally, significant correlations were observed between the retention of lower dentures, ability to eat foods, comfort, and increased density of mandibular segments and local calcitonin/calcium therapy.

In this study, densities of segments of mandibles under surfaces of edentulous alveolar ridges were measured and analyzed. Authors of certain studies advocated for assessment of osteoporosis of mandibles on the basis of thickness of mandibular cortex layers within the bases of mandibles and in the distal angle of mandible-gonion. From diagnostic point of a view it is accepted to judge the proof for diagnosing of osteoporosis in a jaw on the basis of considerations of densities in basal layers which are hard to decompose (52, 53). However, from an aspect of clinical treatments and therapy of osteoporotic jaws, it is necessary, above all, to provide data and parameters of layers of jaw-bones which will be in function, and which would be at the forefront, loaded, sooner or later by bases of dentures during chewing. Because of that, the approach applied in this study to focus and to diagnose osteoporotic degradations in segments of missing premolars and molars of mandibles-i.e. in superficial parts of edentulous alveolar ridges, is absolutely approved (28).

CONCLUSION

Systemic osteoporosis leads to decrease of densities of bones of mandibles and causes reduction of edentulous ridges. Therapy by calcitonin and calcium causes increments of density in jaw-bones and deliberate reduction of edentulous alveolar ridges.

Abbreviations

BMI — body mass index
BMD —bone mineral density
MI — metacarpal index
VBG — heights (in mm) of edentulous mandibular ridges
DXA — dual x-ray absorbtiometry
P — area of mandible where roots of the second premolars were missing
M1 — area in which roots of the first molars were missing after extractions
M2 — area without roots of the second molars.
3-D display — three dimensional display

Sažetak

SISTEMSKA NEMALIGNA OSTEOPOROZA SKELETA I RESORPCIJA BEZUBIH GREBENOVA VILICA

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Uvod. Sistemska osteoporoza oštećuje kosti humanih skeleta u različitoj meri.

Cilj ove studije je bio da se utvrde intenzitet i povezanost promena u gustini skeleta usled osteoporoze i indeks telesne mase (BMI) sa redukcijom bezube mandibule, i da se proceni mogućnost reparacije slojeva u mandibuli praćena porastom mineralnog sadržaja u vilicama pacijenata obolelih usled osteoporoze.

Materijal i metode. U ovoj studiji, 99 bezubih pacijenata sa smanjenom gustinom kosti su sačinjavali eksperimentalnu grupu, a 48 bezubih pacijenata sa normalnom gustinom kosti su bili kontrolna grupa. Godine starosti ispitanih pacijenata i pacijentkinja su iznosile 69,02 ± 7,9, u granicama od 53 do74 godine kod žena, i 69,11 ± 7,1, u granicama od 59 do76 godina kod muškaraca. Radiografije šaka i ortopantomogrami su

načinjeni kod svih ispitanih pacijenata. Vrednosti BMI, metakarpalnih indeksa, gustine tela lumbalnih pršljenova (L2–L4), u falangama i u segmentima donjih vilica, a takođe i visine bezubih alveolarnih grebenova su bile ispitane, izmerene i izračunate.

Rezultati. Najmanja vrednost ukupne gustine skeleta je utvrđena kod pacijenata obolelih od osteoporoze na osnovu T veličine od –2,5 kod muškaraca, i –2,6 kod žena. Minimalne vrednosti visina bezubih grebenova (desno/levo, u mm) su bile izmerene i kod žena (21,84/22,39) i kod muškaraca (24,90/24,96) obolelih od osteoporoze. Upoređivanjima gustina metakarpalnih kostiju, proksimalnih falangi, segmenata (prostora) bezubih mandibula, i na osnovu numeričkih vrednosti izmerenih visina bezubih grebenova, $\chi^2 = 3,81$ je izračunato kod muškaraca, a $\chi^2 = 4,03$ kod žena sa normalnom koštanom gustinom; $\chi^2 = 5.92$ je izračunato kod muškaraca, a $\chi^2 = 6,25$ kod žena sa osteopenijom; $\chi^2 = 2,63$ je izračunato kod muškaraca, a $\chi^2 = 3,85$ je izračunato kod žena sa osteoporozom, na osnovu nivoa verovatnoće od 0,05. Posle aplikovanja kalcijuma i kalcitonina u rastvoru, umeren porast gustine ($p < 0,05$; $p < 0,01$) je zabeležen, nadoknađujući, na taj način ukupno do 4% gubitka koštane mase i mineralnog sadržaja u nosećim i potpornim tkivima osteoporoznih donjih vilica.

Zaključak. Sistemska osteoporoza dovodi do smanjenja gustine kosti donje vilice i uzrokuje resorpciju bezubih grebenova.

Ključne reči: osteoporoza; donja vilica; stomatognati sistem; gustina; mineralni sadržaj; kosti.

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COMPARISON OF CLINICAL AND ARTHROSCOPIC FINDINGS IN MENISCAL TEARS

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Abstract: **Introduction:** This study was conducted to compare the clinical and arthroscopic findings in lateral and medial meniscal tear injuries in order to assess the diagnostic significance of the clinical examinations findings. **Patients and methods:** All patients attending our clinic with knee pain from 2009. to 2013. underwent systematic and thorough clinical assessment. From one hundred and three patients with knee problems in 40 were diagnosed LM (lateral meniscus) tears and in 45 MM (medial meniscus) tears arthroscopically. In this study meniscal tears were clinically diagnosed by positive McMurray and Apley test. All clinically diagnosed patients underwent diagnostic and therapeutic knee arthroscopy to assess the accuracy of clinical diagnosis. The accuracy, PPV, NPV, sensitivity and specificity were calculated based on clinical examinations and arthroscopic findings. **Results:** Identification of meniscal tears in our study was presented with 85% accuracy of "McMurray" clinical examination test for LM tears and 80% accuracy for the MM tears, and for "Apley" clinical examination test for LM tears was obtained with 73% accuracy and 63% accuracy for MM tears. **Conclusion:** According to our findings we can conclude that McMurry clinical examination test is more accurate for predicting i.e. diagnosing of meniscal tears. Contrary, Apley clinical examination test showed less accuracy for predicting i.e. diagnosing of meniscal tears.

Key words: arthroscopy, meniscus tear, knee, clinical diagnosis.

INTRODUCTION

The major meniscal functions are to distribute stress across the knee during weight bearing, provide shock absorption, provide articular cartilage nutrition and lubrication, facilitate joint gliding, prevent hyperextension, and protect the joint margins (1, 2). They may also function as secondary stabilizers (particularly in the absence of a functioning anterior cruciate ligament), have a proprioceptive role and aid in the lubrication and nutrition of the articular cartilage (3, 4).

Since the condyles of the femur and tibia meet at one point (which changes during flexion and extension), the menisci spread the load of the body's weight (5). Meniscal motion allows maximal congruency during knee flexion and helps to protect the menisci from injury (6, 7).

A commonly used surgical classification of meniscal tears includes the following types: horizontal, longitudinal, radial, bucket handle, displaced flap and complex (8).

In this study meniscal tears were clinically diagnosed by positive McMurray test and Aplay test. Arthroscopy was used to assess there liability of clinical diagnosis.

Advanced modality in the management of meniscal tears is arthroscopy, which can be used in its dual mode, either as diagnostic and/or as therapeutic tool. Arthroscopy offers direct visualization of all intra-articular structures with high diagnostic accuracy, the possibility to examine the stability of the knee under anesthesia and the possibility to perform a therapeutic procedure in the samesession (9).

The aim of this study was to compare findings from clinical examinations and arthroscopy inlateral and medial meniscal tears in order to assess the diagnostic significance of the clinical examinations findings.

PATIENTS AND METHODS

In our study we involved 103 patients with history of knee injuries who were admitted in the Clinic of Tra-

umatology, Clinical Center-Majka Tereza, Skopje. MRI of the knee joint was done before the admission and some of them before the clinical examination.

In this study meniscal tears were clinically diagnosed by:

McMurray's test. At various stages of knee flexion internal and external tibial rotation is performed. A palpable click and pain is considered as a positive test (10).

Apley grinding test. The patient is in prone position, the hip is extended and the knee flexed in 90°. The examiner applies axial pressure on to the foot and rotates the tibia. There sulting knee joint pain is regarded as a positive test (11).

The same surgeon has performed clinical as well as arthroscopic examination. Though this reduces the chance of inter observer variability, it creates possibility of obser-vational bias.

In patients with positive clinical examination findings for meniscal and anterior cruciate ligament (ACL) tears of the knee, who admitted in the Clinic of Traumatology, Clinical Center-Majka Tereza, Skopje, during the period from September 2009 to May 2013, diagnostic and therapeutic knee arthroscopy was performed to assess the accuracy of clinical diagnosis.

All arthroscopic procedures were performed in a standard manner by experienced arthroscopic surgeon carried out underregional or general anaesthesia with tourniquet, using standard anteromedial and anterolateralportals. Additional portals were used when required. Operative findings were documented in the official patient's document, which included the survey of the entire joint and anatomical structure, lesions involved with the presence or absence of tear, its location, status of the articular cartilage and others. The composite data was tabulated and studied for correlation with clinical examinations findings and grouped into four categories:

1. True-positive — if the clinical examinations findings were confirmed by arthroscopic evaluation.

2. True-negative — when clinical examinations findings only for meniscal lesion, (McMurray and Apley) were negative and the same was confirmed by arthroscopy.

3. False-positive — when clinical examinations findings shows lesion but the arthroscopy was negative.

4. False-negative-result when arthroscopy was positive but the clinical examinations findings showed negative findings.

Statistical analysis

Statistical analysis was used to calculate the sensitivity, specificity, accuracy, positive predictive value (PPV) and the negative predictive value (NPV), in order to assess the diagnostic significance of the clinical examinations findings.

Categorical variables were summarised using frequency and were comparedusing the chi-square or McNemar test as appropriate, p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The study group of 103 patients consisted of 81 men (79%) and 22 women (21%). The average age of patients was 29.7 ± 10.77 years, range 16 to 58. We found statistical significant difference in distribution of frequencies, male patients were dominant ($x^2 = 33.79$, DF = 1, $p < 0.01$). The males average age was 30.26 ± 10.26 ranged 16–58, and females average age was 27.68 ± 12.52 ranged 16–53, Student's t-test for two large unrelated samples showed t = 0.99, DF = 101, $p > 0.05$, i.e. perceived difference is not statistically significant in average ages between males and females. All patients underwent arthroscopic kneesurgery. Maximum number of patients (n = 34) who suffered knee injuries were in the age group of 21–30 years (Figure 1). The right knee was involved in 56 cases (54.4%) and the left knee in 47 (45.6%).

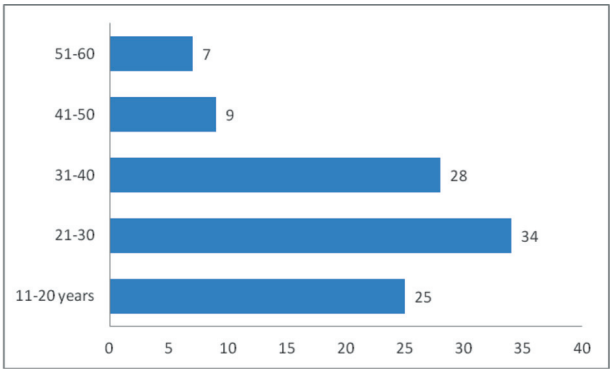

Figure 1. *Number of patients according age distribution*

Table 1 show the methods and formulas used to calculate there liability of clinical diagnosis. Clinical diagnostic test characteristics:

— Sensitivity: how good the clinical examination test is at detecting meniscal tears

— Specificity: how good the clinical examination test is at identifying normal knee

— Positive predictive value: how often a patient with apositive clinical examination test has the meniscal tears

— Negative predictive value: how often a patient with anegative clinical examination test does not have meniscal tears

— Accuracy: proportion of clinical examination test which correctly identifies meniscal tears

Comparison of the arthroscopic and clinical "McMurray test" findings yielded the following results.

Clinical "McMurray" test findings for the lateral meniscus (LM) yielded 24 true-positives (were confirmed on arthroscopy) and 63 true-negatives (without evidence of LM tears) with 0 false positive (were misinterpreted to have LM tears) and 16 false negative (were not diagnosed clinically) (Table 1), which resulted in 60% sensitivity, 100% specificity, 100% PPV, 84% NPVand 85% accuracy (Table 5).

Table 1. *McNemar matching for arthroscopy and clinical McMurray test findings in LM*

	Arthroscopy findings in LM		
	Positive findings	Negative findings	Total
Clinical "McMurray test" Positive findings	24 (TP)	0 (FP)	**24**
Clinical "McMurray test" Negative findings	16 (FN)	63 (TN)	**79**
	40	**63**	103

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

Comparison of the arthroscopic and clinical "Apley" test findings yielded the following results.

Clinical "Apley" test findings for the LM yielded 29 true-positives (were confirmed on arthroscopy) and 11 true-negatives (without evidence of LM tears) with 17 false positive (were misinterpreted to have LM tears) and 11 false negative (were not diagnosed clinically) (Table 2), which resulted in 81% sensitivity, 77% specificity, 73% PPV, 84% NPVand 73% accuracy (Table 5).

Table 2. *McNemar matching for arthroscopy and clinical Apley test findings in LM*

	Arthroscopy findings in LM		
	Positive findings	Negative findings	Total
Clinical "Apley test" Positive findings	29 (TP)	17 (FP)	**46**
Clinical "Apley test" Negative findings	11 (FN)	11 (TN)	**57**
	40	**63**	103

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

Clinical "McMurray" test findings for the medial meniscus (MM) tears yielded 37 true-positives (were confirmed on arthroscopy) and 45 true-negatives (without evidence of MM tears) with 13 false positive (were misinterpreted to have MM tears) and 8 false negative (were not diagnosed clinically) (Table 3), which resulted in 86% sensitivity, 80% specificity, 79% PPV, 87% NPV and 80% accuracy (Table 5).

Table 3. *McNemar matching for arthroscopy and clinical McMurray test findings in MM*

	Arthroscopy findings in MM		
	Positive findings	Negative findings	Total
Clinical "McMurray test" Positive findings	37 (TP)	13 (FP)	**50**
Clinical "McMurray test" Negative findings	8 (FN)	45 (TN)	**53**
	45	**58**	103

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

Clinical "Apley"test findings for the MMtears yielded 39 true-positives (were confirmed on arthroscopy) and 26 true-negatives (without evidence of MM tears) with 32 false positive (were misinterpreted to have MM tears) and 6 false negative (were not diagnosed clinically) (Table 4), which resulted in 92% sensitivity, 50% specificity, 69% PPV, 84% NPVand 63% accuracy (Table 5).

Table 4. *McNemar matching for arthroscopy and clinical Apley test findings in MM*

	Arthroscopy findings in MM		
	Positive findings	Negative findings	Total
Clinical "Apley test" Positive findings	39 (TP)	32 (FP)	**71**
Clinical "Apley test" Negative findings	6 (FN)	26 (TN)	**32**
	45	**58**	103

TP (true positive); TN (true negative); FP (false positive); FN (false negative)

Table 5. Reliability of clinical examinations tests for medial and lateralmeniscal tears

	McMurray Test LM (%)	Apley Test LM (%)	McMurray Test MM (%)	Apley Test MM (%)
Sensitivity	60	81	86	92
Specificity	100	77	80	50
Positive predictive value (PPV)	100	73	79	69
Negative predictive value (NPV)	84	84	87	84
Accuracy	85	73	80	63

McNemar test showed that $x^2 = 16.56$, DF = 1, $p < 0.01$, i.e. there are statistical significant differences in distribution of frequencies in positive and negative findings of LM tears in clinical examination "McMurray"test and arthroscopy or perceived difference is statistically significant and **they do not match among themselves**. Contrary, we found nostatistical significant difference in distribution of frequencies in positive and negative findings of LM tears in clinical examination "Apley" test and arthroscopy ($x^2 = 3.4$, DF = 1, $p > 0.05$) (Figure 2) or perceived difference is statistical not significant and **they match among themselves**.

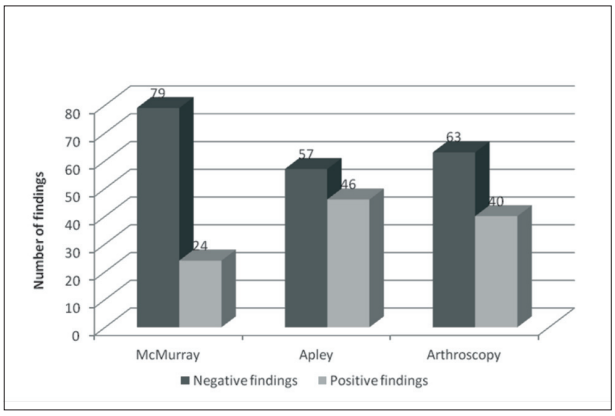

Figure 2. *McMurray and Apley tests findings versus arthroscopic findings in LM tears*

McNemar test showed that $x^2 = 3.5$, DF = 1, $p > 0.05$, i.e. there are no statistical significant differences in distribution of frequencies in positive and negative findings of MM tears in clinical examination "McMurray" test and arthroscopy or perceived difference is not statistically significant and **they match among themselves**. Contrary, we found statistical significant difference in distribution of frequencies in positive and negative findings of MM tears in clinical examination" Apley" test and arthroscopy ($x^2 = 26$, DF = 1, $p < 0.01$) (Figure 3) or perceived difference is statistical significant and **they do not match among themselves**.

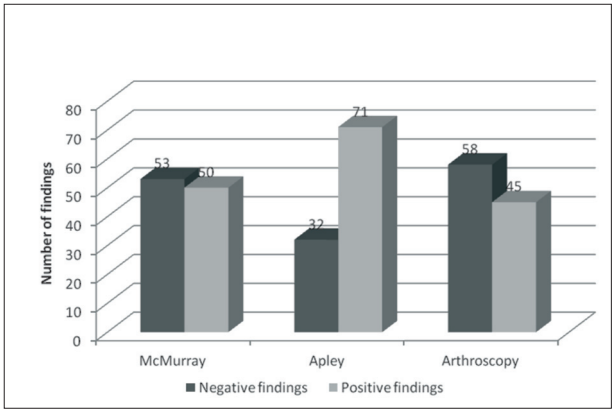

Figure 3. *McMurray and Apley tests findings versus arthroscopic findings in MM tears*

DISCUSSION

The purpose of this study was to compare the findings from clinical examination and arthroscopy in order to evaluate the significance of these findings in diagnosis of medial and lateral meniscal tears. In the study we evaluated 103 patients with history of knee injuries (sports injury was the most common mode) who were admitted at our hospital.

The age group ranging from 16 to 58 years. Maximum number of patients (n = 34; n = 28) who suffered knee injuries were in the age group of 21–30 years and 31–40 years (Figure 1). Sports injury was the most common mode of injury. This showed that there was a tendency of males getting injured and operated at the earlier age since they are sports active. A study done by Avcu et al. showed males are most likely to suffer knee injuries since they are active in sports and the right knee was more frequently injured than left (12).

Our results were 60% sensitivity, 100% specificity, 100% PPV and 84% NPV of "McMurray"clinical examination test with respect to fair correlation with arthroscopy in LM tears.

Identification of meniscal tears in our study was presented with 85% accuracy of "McMurray" clinical examination test for LM tears and 80% accuracy for the MM tears correlated with arthroscopy and they belong together in range of very good (80–90%) accurate group according many studies of "McMurray" clinical examination test accuracy of predicting meniscal tears.

For the "Apley" clinical examination test we obtained 81% sensitivity, 77% specificity, 73% PPV and 84% NPV compared arthroscopy in diagnosing LM tears.

Identification of meniscal tears in our study presented with "Apley" clinical examination test for LM tears was obtained with 73% accuracy and 63% accuracy for MM tears correlated with arthroscopy were ranged together in good accurate group. According many studies of clinical examination tests compared (correlated) with arthroscopy, the accuracy of predicting meniscal tears depend from the level of skilled orthopaedic surgeon's hands.

For the MM tears in our study we obtained 86% sensitivity, 80% specificity, PPV 79% and NPV 87% of "McMurray" clinical examination test in comparing with arthroscopic findings. Identification of MM tears in our study presented with "Apley" clinical examination test correlated with arthroscopy was obtained with 92% sensitivity, 50% specificity, PPV 69% and NPV 84%.

Miller found overall clinical diagnosis accuracy of meniscal tears of 80.7% and the corresponding accuracy for MRI was 73.7% (13).

Rose and Gold found the clinical examination to be correct more often than MRI diagnosis. They found no

significant difference in accuracy between clinical examination and MRI in both medial and lateral meniscal tears or anterior cruciate ligament (ACL) tears (14).

Akseki et al. suggested that the accuracy of clinical diagnosis of a meniscal tear is decreased by the presence of an ACL tear and the presence of both these injuries requires more frequent magnetic resonance imaging (15).

The results are close to the ones given in the professional literature which refers to the standardized approach in taking the anamnestic data and realization of the physical signs and test examination technique (16, 17, 18).

Kocabey et al. stated that clinical examination is as accurate as MRI in the skilled orthopaedic surgeon's hands and MRI should be reserved for more complicated and confusing cases (19). Bohnsack et al. also concluded that an experienced examiner can diagnose adequately by clinical examination alone (20).

At the same time, reliable statistical data of the diagnostic value of the MRI are also related to the independent base of reference. A meniscal tear was correctly diagnosed in 76% of cases with conventional MRI and in 88% of cases with high-resolution MRI (21).

Regarding knee MRI, in most of the studies and in our study as well, the base of reference is arthroscopy (22, 23). This presupposes that arthroscopy is 100% accurate allows for the diagnosis of every possible knee pathology. This is not always the case (24).

Arthroscopy should be considered a diagnostic aid used in conjunctions with a good history, complete physical examination and appropriate radiographs. With increased proficiency in examination of extremities and more accurate adjuvant tests, including MRI, we rarely perform simple "diagnostic arthroscopy." Surgical alternatives are discussed thoroughly with the patient before the procedure, and the definitive surgical procedure is performed at the time of an arthroscopic examination.

CONCLUSION

The results of our study suggest that McMurray clinical examination test is more accurate for predicting i.e. diagnosting of meniscal tears. Contrary, Apley clinical examination test showed less accuracy for predicting i.e. diagnosting of meniscal tears. According many studies of clinical examination tests compared (correlated) with arthroscopy, the accuracy of predicting meniscal tearsdepend from the arthroscopic skill level of the surgeon hands.

Abbreviations

ACL — anterior cruciate ligament
MRI — magnetic resonance imaging
LM — lateral meniscus
MM — medial meniscus
PPV — positive predictive value
NPV — negative predictive value
TP — true positive
TN — true negative
FP — false positive
FN — false negative
DF — distribution of frequencies
x^2 — chi-square

Sažetak

POREĐENJE KLINIČKIH I ARTROSKOPSKIH NALAZA POVREDA MENISKUSA

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Uvod: Ova studija je sprovedena kako bi uporedila kliničke i artroskopske nalaze povreda u lateralnim i medijalnim meniskusa u cilju procene dijagnostičkog značaja kliničkih ispitivanja nalaza. **Pacijenti, metode i rezultati:** Svi pacijenti s bolom u kolenu, koji su posetili našu Kliniku, od 2009. do 2013. god. prošli su sistematsku i temeljnu kliničku procenu. Od stotinu i tri pacijenta s problemima kolena kod 40 su artroskopski dijagnostifikovane povrede kod LM (lateralnog meniskusa) a kod 45 povrede MM (medijalnog meniskusa). U ovoj studiji meniskusne povrede klinički su dijagnostifikovane pozitivnim McMurray i Apley testom. Svi klinički dijagnostikovani pacijenti bili su podvrgnuti dijagnostičkoj i terapijskoj artroskopiji kolena kako bi se procenila tačnost kliničke dijagnoze. Tačnost, PPV, NPV, osetljivost i specifičnost su izračunate na temelju kliničkih ispitivanja i artroskopskih nalaza. Identifikacija meniskusnih povreda u našoj studiji je predstavljena sa 85% tačnosti „McMurray" kliničko-preglednog testa za povrede LM i 80 % tačnosti za povrede MM, a

za „Apley“ test kliničkog ispitivanja za povrede LM dobili smo 73 % tačnosti i 63% za povrede MM. **Zaključak:** Prema našim saznanjima, možemo zaključiti da McMurray kliničko-pregledni test je tačniji za predviđanje odnosno dijagnozu povrede meniskusa. Suprotno, Apley kliničko-pregledni test je pokazao manju tačnost u predviđanju odnosno u dijagnozi meniskusne povrede.

Ključne reči: artroskopija, povreda meniskusa, koleno, klinička dijagnoza.

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CORRELATION ANALYSIS BETWEEN DEPRESSIVE MANIFESTATIONS AND MORPHOLOGICAL LESION CHARACTERISTICS IN PATIENTS WITH STROKE

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Abstract: **Introduction:** Knowledge of etiopathogenesis of post-stroke depressive phenomena contributes to early diagnostics which shortens recovery to a great extent and suits the social and professional rehabilitation of patients, if followed by proper psycho/pharmacotherapy. The aim of this work is to research dependence of depressive manifestations considering the size and anatomical localization of lesion. **Subjects and Methods:** The research included 118 patients with stroke. Lesion localization was defined on computerized axial tomography records, whereas the area and perimeter of lesion were measured by AutoCAD 2004 software. Examinations by means of Hamilton Rating Scale for Depression were carried out by the method of random selection 11–40 days after stroke. Correlation analysis was made by simple linear/non-linear regression and Cox's hazard regression model. **Results:** Negative correlation was observed between the intensity of depressive manifestations and the size of cerebrovascular lesion (Spearman's $r = -0.263$, $P = 0.004$). By means of Cox's regression model we determined 4.389 times higher risk for depression occurrence in female patients ($P < 0.001$), as well as higher risk due to lobus limbicus structure damages (hazard ratio e^b (HR) = 2.661, $P = 0.019$). **Conclusion:** Lower intensity of depressive manifestations with larger cerebrovascular lesions, we have explained by activation of reparation mechanisms with energy savings and decrease (due to neurological deficits) of afferent peripheral sensations which antecedent the occurrence of emotions (James-Lange peripheral theory of emotions).

Key words: stroke; lesion; depression; correlation; analysis.

INTRODUCTION

Disorders in the form of depression and anxiety represent the most often clinical psychiatric entities that occur in persons suffering from cerebrovascular stroke. It has been confirmed that anxiety and depression significantly inhibit physical and cognitive recovery and the quality of life of these patients. It is similar with other disorders of psychic functions too (1, 2). Knowledge of etiopathogenesis of post-stroke depressive phenomena contributes to early diagnostics which shortens recovery to a great extent and suits the social and professional rehabilitation of patients, if followed by proper psycho/pharmacotherapy. The aim of this work is to examine dependence of depressive manifestations on organic brain damages, since they are explained by disorders of dynamic mechanisms; also, to estimate the influence of gender and dominancy of hemisphere in the pathoplasticity of depressive disorders. Work hypothesis: H1a. There is statistically significant correlation between the size of cerebrovascular lesion and intensity of depressive manifestations; H1b. Lesion localization depending on affected morpho-anatomical structures (damages of lobus frontalis, corpus striatum, lobus limbicus and diencephalon) shall condition specific psycho-pathological picture.

SUBJECTS AND METHODS

Participants

The research included the total of 118 persons suffering from cerebrovascular stroke (of ischemic and hemorrhagic origin) who had no previously diagnosed psychiatric disorders: 59 male persons and 59 female persons at the age span 44–87 years. The patients were inquired at Neurological Department of the Institute for Physical Medicine, Rehabilitation and Orthopaedic Surgery "Dr. Miroslav Zotovic" Banja Luka. The study had two phases. In the first phase we have assessed inclusion criteria, in the second phase we carried out psy-

chological testing. The study included patients with first stroke and macroscopic lesions of prosencephalon on computerized axial tomography (CAT) records. CAT records were done in the period of 72 hours after stroke. Because we were interested in emotional changes of patients in subacute phase of stroke we have decided to perform psychometric examination in the period 11–40 days after stroke. Patients were assessed once, and the exact day of psychometric testing for each patient was defined by means of the method of random selection. Study has been approved by the Faculty of Medicine Ethic Committee and participants gave informed consent prior to their inclusion in the study. Details that disclose the identity of the participants were omitted.

Criteria of including patients into second phase of study

Due to significant mixture of influences, patients in heavier, comorbid states (heart decompensation, unstable angina, infarctus myocardii in the previous year and the year of examination, infective diseases, malign and chronic immunological diseases) were excluded. Also, the study included only patients with baseline NIHSS (National Institute of Health Stroke Scale) score at the moment of psychological testing $2 \leq X \leq 10$. Total score on NIHSS scale ranges between 0–42, where higher values reflect greater weight of cerebral infarction. According to Brott et al. NIHSS score of less than 10 includes patients with mild and adequately severe neurological deficit (3). Among patients with mild neurological deficit, those were included with whom "drift test" was positive on both same sided extremities (NIHSS = 1 + 1) or NIHSS score had the value of minimum 2 on one of the extremities. Exclusion criteria were also moderate and severe sensory and motor dysphasia in the first phase of study since they complicate to a great extent the carrying out of neuro-psychological testing, since we have used verbal neuropsychological tests.

Research instruments — CAT brain records

Morphometric research comprised superacute (up to 24h) and acute ischemic/hemorrhagic lesions (24h up to 3 days). Sensitivity of CAT scanner in detection of early ischemic lesions is limited, and only one half of all strokes are visualised within 48h after the stroke (4, 5). Acute phase of stroke (the first week after the stroke) is characterised by intensified hypodensity of affected brain tissue (gray and white matter). Brain oedema and the mass effect reach their maximum values usually 3 to 5 days after the stroke (4). Given that in this case pathological process spreads more and more into the healthy tissue, in our study morphological research was limited to lesions that appeared up to 72h after the stroke. The surface of hemorrhagic lesions being defined (in 13 patients) included the zone of cytotoxic oedema too.

AutoCAD digital planimetry of cerebrovascular lesions

Localization of lesions with clearly stated affected morpho-anatomic structures (cortex, basal ganglia, structures of diencephalon, white matter) (6, 7) was defined on non-contrast CAT records (5 mm layer thickness) on the surface of the biggest lesion cross section. Cerebral lesions were classified into the following categories: 1. frontal lobe/other forebrain segments damages, 2. striate body damages (yes/no), 3. limbic lobe i.e. limbic cortex, adjacent white matter, limbic nuclei damages (yes/no), and 4. interbrain damages (thalamus and/or hypothalamus) (yes/no). The aforementioned lobe categories have included both cortical and subcortical lesions. To define deep (subcortical) frontal lesions, the border of the frontal lobe at the level of insular cortex and para-insular structure sections was the orthogonal line drawn through the front end of sulcus circularis insulae on the axis of neuraxis (mediosagittal plane), thus comprising precaudate structures. However, the most of frontal lobe lesions were mixed and they caught adjacent lobes (25 of 35 lesions).

Area and perimeter of lesions were measured by AutoCAD digital planimetry (Figure 1) with previous transformation of CAT records into the digital format by means of digital camera with resolution 8 Mpx. AutoCAD version 2004 for PC Windows (developed

Figure 1. AutoCAD digital planimetry

*Morphometry of cerebrovascular lesion affecting the anterior limb of capsula interna and the left corpus striatum (caput nc. caudati, putamen and the lateral segment of globus pallidus). Area = 766.13 mm^2, perimeter = 14.044 cm

by Autodesk, Inc. San Rafael, California, USA; see http://usa.autodesk.com/autocad/) belongs to programme package groups meant for drawing, projecting and other forms of computer application in engineering practice. This programme package can be used for measuring of surfaces having irregular geometric forms, such as structures of central nervous system (8).

Psychometric tests

The following psychometric tests were used to test disorders in psychic functions:

1. Hamilton Rating Scale for Depression (HRSD); 21 items, application time 15–20 min (9). Although the Hamilton scale consists of twenty one items, only the first seventeen are being scored. Values 8–13 indicate mild depression, 14–18 moderate depression, 19–22 severe depression, whereas values ≥ 23 indicate very severe depression.

2. Questionnaire for qualitative evaluation of object relations in etiopathogenesis of post-stroke behavioural and emotional disturbances (10). For the purpose of an orientation insight into quality of object relations in patients affected by cerebrovascular stroke (in childhood- up to age 18), the following parameters were tested: The patient's primary family profile compared to its integrity: presence of divorce, or death of a parent; Continuous separation of the patient from his/her mother: death of mother during childbirth, custody of a child given to father after divorce, the adopted child, woman immature for the role of a mother gives her child to someone else; Discontinuous separation of mother from the patient: prolonged hospitalisation of mother due to mental illness, prolonged hospitalisation of mother due to somatic illness, parental substitutes — "weekend" mother, which was justified with housing and economic reasons. Patients who presented one or more positive answers were classified into category: detachment from parents = yes, which was used for further statistical analysis. 3. To evaluate dominance of brain hemisphere in sensory-motor functions, Handedness Questionnaire was used (11, 12).

Statistic data processing

The size of focal lesion was brought in connection with the intensity of depressive manifestations by applying Pearson's coefficient of linear correlation. Basic assumptions of the linear model (normality, homoscedasticity) were tested (13). In order to make a difference between whether depressive symptomatology is a reaction on the very disease or to a specific morpho-anatomic lesion localization, the significance of difference in dependence of affected structure of the central nervous system was examined (the significant differences would indicate specific locus deficiencies of psychic functions). The significance of difference was examined by means of non-parametric Fisher's exact test. The dependence of depressive reactions on the surface of lesion was also examined via simple non-linear regression as well as Spearman's rank correlation. To estimate the risk of psycho-pathological manifestations, besides classical parameters such odds ratio (OR) and relative risk (RR), Kaplan-Meier's and Cox's hazard model were used. Analyses were performed using SPSS version 16.0 for Windows. Statistic conclusions were derived on the basis of 2-tailed P values and the level of significance $P < 0.05$.

RESULTS

The frequency of cerebrovascular lesion localization in dependence of affected brain structures is presented in Table 1.

Table 1. *Distribution of cerebrovascular lesions in dependence of forebrain's affected structures*

Affected structure	*n*[a]	%
Frontal lobe	35	29.7
Striate body	33	28.0
Limbic lobe	19	16.1
Interbrain (thalamus and/or hypothalamus)	15	12.7

[a] Note: The total number of lesions in Table 1 is not 118, because for instance frontal lobe lesions overlap with limbic lobe lesions (e.g. limbic structures such as anterior segment of gyrus cinguli are positioned on frontal lobe). Also, mixed lesions have influenced the result.

Depression (HRSD positive) on the examined sample (n = 118) was found in 28.8% of the patients. Descriptive values of HRSD score of the examined group of patients are presented in Table 2.

Table 2. *Hamilton Rating Scale for Depression (HRSD) score values of patients with stroke*

HRSD score	
n	118
Mean	5.45
Median	5
Mode	4
Standard deviation	3.327
Minimum	1
Maximum	15

Table 3. *Determination coefficient (r^2) of the largest cross-section area of cerebrovascular lesions and the level intensity of depressive manifestations (HRSD score values)*

Model summary and parameter estimates							
Dependent variable: Hamilton Rating Scale for Depression (HRSD) score							
Model summary						Parameter estimates	
Equation	r^2	F	d.f.1	d.f.2	P	Regression constant	Regression coefficient b1
Linear	0.056	6.896	1	116	0.010	6.384	– 0.002
Logarithmic	0.046	5.591	1	116	0.020	11.312	– 0.966
Power	0.063	7.830	1	116	0.006	18.734	– 0.239
Exponential	0.069	8.560	1	116	0.004	5.473	– 0.0004
The independent variable: lesion area (mm^2) Linear $– 0.002 \cdot x + 6.384$ Exponential $5.473 \cdot e^{(– 0.0004 \cdot x)}$ Euler's constant $e \approx 2.718$							

Table 4. *Spearman rank correlation between the largest cross-section area of cerebrovascular lesions and the level intensity of depressive manifestations (HRSD score values)*

		Value	Asymptotic s.e.[a]	Approximate t[b]	Approximate P
Interval by interval	Pearson's r	– 0.237	0.073	– 2.626	0.010[c]
Ordinal by ordinal	Spearman correlation	– 0.263	0.088	– 2.930	0.004[c]
Total Sample n		118			

[a] Not assuming the null hypothesis.
[b] Using the asymptotic standard error assuming the null hypothesis.
[c] Based on normal approximation.
HRSD, Hamilton Rating Scale for Depression.

Table 5. *Odds ratio and a relative risk of depression occurrence in patients with stroke*

Variable	Odds ratio (OR)	Relative risk (RR)	Fisher's exact test (2-sided) P
Gender (female/male)	2.788	2.091	0.025
Detachment from parents (e.g. death of parent or divorce before age 18) (yes/no)	3.472	2.171	0.024
Hand-dominant hemisphere (yes/no)	1.125	1.087	0.839
Frontal lobe/other forebrain segments	1.745	1.468	0.266
Striate body (yes/no)	0.900	0.927	0.999
Limbic lobe (limbic cortex, adjacent white matter, limbic nuclei) (yes/no)	2.664	1.876	0.094
Interbrain (yes/no)	1.276	1.184	0.762
Hemorrhagic lesion (yes/no)	1.111	1.077	0.999

Table 6a. *Cox regression analysis of depression occurrence in patients with stroke — affected limbic lobe*

Categorical variable codings			
	Frequency (1)		
Gender	Female	59	1
	Male	59	0
Hand-dominant hemisphere	Yes	67	1
	No	51	0
Limbic lobe	Yes	19	1
	No	99	0
Detachment from parents	Yes	19	1
	No	99	0

Table 6b. *Variables in the equation*

	Regression coefficient b	s.e.	d.f.	*P*	Hazard ratio e^b (HR)	95.0% CI for HR	
						Lower	Upper
Gender	1.479	0.414	1	< 0.001	4.389	1.951	9.876
Detachment from parents	0.518	0.384	1	0.178	1.678	0.790	3.564
Lesion area (mm^2)	– 0.0002	0.00047	1	0.742	0.9998	0.9989	1.001
Hand-dominant hemisphere	– 0.092	0.353	1	0.795	0.912	0.457	1.822
Limbic lobe	0.979	0.419	1	0.019	2.661	1.171	6.046

By means of regressive analysis of the coefficient of determination (r^2), a statistically significant linear dependence of the surface of the highest cross section of cerebrovascular lesion and the intensity of depressive manifestations (HRSD values) was determined ($P = 0.010$) (Figure 2, Table 3).

Due to distortion of the basic assumptions of linear regression model (normality and homoscedasticity), to estimate the intensity and direction of correlation Spearman's rank correlation was used (Table 4).

Negative correlation of the values of HRSD score and the surface of the biggest cross section of cerebrovascular lesion with a high level of significance was found ($P < 0.01$) (Table 4).

Excluding the high leverage values and values with large Cook's distance from regression model (lesion area: 2386.58 mm^2, 2645.20 mm^2, and 2780.08 mm^2) changed Pearson's (linear) correlation coefficient from $r = -0.237$ ($P = 0.010$) to $r = -0.303$ ($P = 0.001$), and Spearman's from $r = -0.263$ ($P = 0.004$) to $r = -0.274$ ($P = 0.003$). Spearman's r, as expected, showed greater stability.

In Table 5 we presented the analysis of occurrence of depression (according to HRSD criteria) in dependence on the affected brain regions of interest.

Kaplan-Meier's analysis (Figure 3) confirmed a greater hazard of the occurrence of depression in female persons (Log Rank, $P < 0.001$).

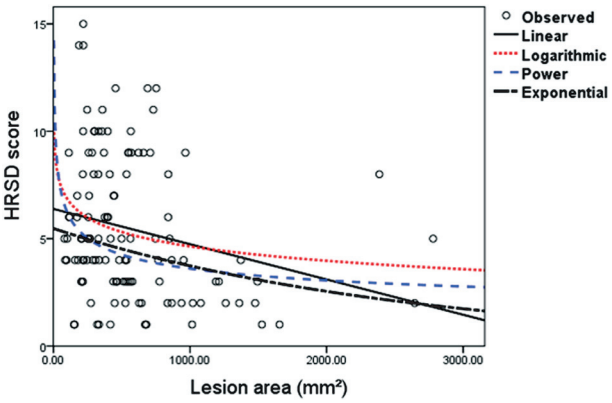

Figure 2. *Regression analysis between the largest cross-section area of cerebrovascular lesions and the level intensity of depressive manifestations in patients with stroke*

*X-axis shows values of the area of cerebrovascular lesions (in mm^2) measured through the largest cross-section, while Y-axis shows observed Hamilton Rating Scale for Depression (HRSD) score values of patients included in the study. Using the method of least squares line and curves (logarithmic, power, and exponential) which best fit the observed data are plotted.

Hazard ratio e^b (HR) represents a numerical expression of Cox hazard. The value > 1 indicates higher, and the values < 1 lower risk of explanatory variable (e.g. gender) on the occurrence of depression. In the Table 6b, the risk of that modality of used explanatory

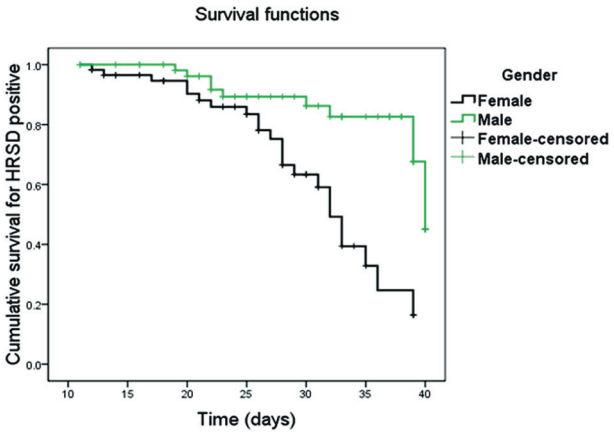

Figure 3. *Kaplan-Meier hazard analysis of depression in patients with stroke depending on the patients' gender*

*Survival functions is the common name for presented functions, since the initial tests were used to assess the risk of death from the diseases. The lower the curve of an event (in our example the occurrence of depression) for the modality of tested characteristics (gender of respondents), the higher is the risk. HRSD, Hamilton Rating Scale for Depression.

variable which is coded in the Table 6a with the value 1 (e.g. female gender) is presented. Via Cox's analysis, the dependence of the occurrence of depression on the person's gender was determined. Higher risk of women (HR = 4.389, $P < 0.001$) was found. Also, higher risk of the occurrence of depression due to the affected lobus limbicus structures (HR = 2.661, $P = 0.019$) is seen. The risk of occurrence of depression in the case of 200 mm^2 bigger surface is 3.92% lower (P = 0.742), which was calculated via the formula 100% x (1 – $(0.9998)^{200}$). By including only two explanatory variables (gender and lobus limbicus affectedness) in the model the results did not change statistical significance (female: HR = 4.256, $P < 0.001$; lobus limbicus: HR = 2.704, $P = 0.015$). After adjustment to fit, for the same explanatory variables as in Table 6b, the risk of occurrence of depression due to the damages of other regions of interest did not show statistically significant differences (lobus frontalis: HR = 1.284, $P = 0.535$; corpus striatum: HR = 0.853, $P = 0.695$).

DISCUSSION

Incidence of depressive manifestations in patients with stroke

The frequency of depression in patients with stroke depends to a great extent on the time of psycho-metric examinations after stroke as well as on psycho-metric test used thereby. This complicates the comparison of results between studies, although the subject of research is the same. Starkstein et al. discover apathy in the first ten days after stroke in 22.5% of cases, and depression in 33.8% (14). Brodaty et al. describe apathy as an analogue to depression three to six months after stroke in 26.7% of patients (15). Some authors (16) point to a higher frequency of apathy (50%) as well as to dependence of this emotional disorder on lower blood flow in the right dorsolateral parts of frontal lobe and left fronttemporal regions. Aström et al. find major depression in 25% of patients in the first month after stroke, in 31% three months after stroke, in 16% twelve months after, in 19% two years after and in 29% three years after (17). The study Starkstein et al. determines major depression in acute phase of stroke in 18.3% of patients, and minor depression in 11.8% of cases (18). It is interesting to mention the study Schwartz et al. which finds depression in men in the period of acute post-stroke rehabilitation in 40% of patients (19). One year (fifteen months) after stroke Brodaty et al. determine depression in 20.7% of patients (20). Three to five years after stroke the frequency of depression is 18.3%, and seven years after stroke 20% (21, 22). Some studies point to the significance of passivity and indifference of patients with acute stroke. Aybek et al. notice passivity in acute stroke in 49% of cases, and emotional indifference in 53% (23). The frequency of depressive manifestation in patients suffering from cerebrovascular stroke amounted in our research to 28.8%. This frequency approximates to the frequency of study Starkstein et al. who determines depression in acute phase of stroke (first month) in 30.1% of patients ($n = 93$) (18).

Correlation between depression intensity and the size of a cerebrovascular lesion

By applying a regression analysis of dependence between the highest cross-section of the lesion area and the level of the depression intensity we found linear determination coefficient $r^2 = 0.056$ ($P = 0.010$). The violation of normality and homoscedasticity parameters of the linear model was observed. The observed HRSD values significantly deviate from normal distribution (Shapiro-Wilk, $P < 0.000$). The heteroscedasticity i.e. dispersion of the standardized residuals of the HRSD score to the right side — "fan out" is present. Due to violation of the model's hypotheses and low values of Pearson (linear) correlation coefficient ($r = – 0.237$, $P = 0.010$), which indicates a weak correlation between the examined phenomena, and to avoid false positive results, the Spearman rank correlation was applied. The Spearman correlation coefficient proved a monotonously decreasing relationship ($r = – 0.263$, $P = 0.004$).

The positive correlation between depression and the size of the right hemisphere lesion and the intensity

of the neurological deficit was confirmed by various studies (19, 24, 25). The studies (21, 26, 27) also confirmed the existence of the correlation between the lesion extent, neurological deficit and intensity of depression, while the studies (20, 28) deny it. In contrast with the mentioned studies, the negative correlation has been confirmed within our study. We explain our finding by differences in period of assessment of depressive phenomena. In our study we have examined these phenomena in earlier- subacute phase (11–40 days) after stroke, where heavier general medical condition plausibly altered the finding. From the point of evolutionary psychology and work of some behaviourists, e.g. Engel, the role of depression withdrawal is the preservation of bodily energy (29). In accordance with that, large brain lesions may as well activate defence mechanisms resulting in depression inactivation and energy savings. Lower intensity of depressive manifestations with larger cerebrovascular lesions, is explained by the fact that afferent sensations, which precede emotions, are diminished due to a neurological deficit (based on the James-Lange peripheral theory of emotions). Although this concept was abandoned after McLean-Papez central theory of emotions, Damasio (2000) used similar peripheral mechanisms to explain the origin of the consciousness (30). According to the ICD-10 classification, depression disorders refer to hyperthymia, which support the aforementioned statement. Inactivation of depressive manifestations spectrum and indifference are defence mechanisms. Therefore, it is not surprising that Aybek et al. find that 53% of people suffering from cerebrovascular stroke are indifferent (23). Statistically insignificant difference of correlation between lesion size and depression by Cox model (Table 6b) compared to Spearman model is the result of comparison between lesion area and positive cases of depression, rather than the range of HRSD scores. It is observed that the risk of depression is lower by 3.92% if a lesion is by 200 mm^2 larger ($P > 0.05$).

Significance of stroke lesion localization for the consequent depression

Beblo et al. (28) came with the conclusion that post-stroke depression is in relation with the basal ganglia lesions; for Finset et al. (31) it is related to deep retrorolandic lesions, while Starkstein et al. (18) connect post-stroke depression with the parietal cortex lesions. Zhang et al. (32) associate depression with lesions at posterior limb and genu of internal capsule and cortical-subcortical area of the temporal lobe, while Tham et al. (33) have highlighted pathology of white matter in prefrontal brain region. In our study, the correlation between lesions of the limbic system (lobus limbicus) and depressive manifestations is observed by applying Cox's regression model (HR = 2.661, $P = 0.019$). The lobus limbicus category has included lesions of both medial and basolateral limbic cortical regions, as well as lesions of subcortical limbic nuclei (e.g. corpus amygdaloideum). Similar results have been obtained by Terroni et al. (34) who indicate that depression due to stroke is etiologically related to the disruption of the limbic-cortical-striatal-pallidal-thalamic circuit, and Farinelli et al. (35) who emphasize the damage of the anterior subcortical-cortical midline system (as core of the limbic system) and its relationships to depression. The risk of depression in our study is by 1.284 times higher when frontal lobe is affected, compared to other regions of prosencephalon and by 14.7% lower if corpus striatum is affected, but these are without statistical significances ($P > 0.05$).

Gender and hemisphere dominance as variables in pathoplasticity of depressive phenomena

Considering the gender as a risk factor, Pohjasvaara et al. did not find statistically significant differences in depressive disorders frequency (27). Kishi et al. identified the differences and described more frequent depressive disorders with women (36). In our study, the female gender was accompanied by a higher risk of depression (OR = 2.788, $P = 0.025$), and also the dependence is established on the object's relations structure distortion, that is, detachment from parents — death or divorce of the patient's parents before the age of eighteen (OR = 3.472, $P = 0.024$). Kaplan-Meier's analysis confirmed a higher hazard with women (Log Rank, $P = 0.000$). The same hazard is confirmed by Cox's regression model. In case of *risk classification lobus limbicus/other regions of prosencephalon*, the risk of the development of depression with women is by 4.389 times higher than with men (Table 6b). Statistical significance of the correlation between occurrence of depression and gender in the Kaplan-Meier and Cox model is explained by an earlier period of emotional reactions of women to stress since for the calculation of risk these models take into account the time of the observed event occurrence. If we take into account the specific characteristics of the emotions: time accumulation of affects/abreactions, we can conclude that men are more tolerant to stress than women and have a higher threshold of emotional reaction than women. By changing the threshold of emotional reaction, lesions of anatomic structures (lobus frontalis, corpus striatum and lobus limbicus) could also influence the occurrence and frequency of depressive disorders in patients with stroke.

One of the risk factors in the etiopathogenesis of post-stroke depressive disorders is frontal left hemisphere lesions (17, 37–41). Terroni et al. (34) also point to left side of lesions. Kishi et al. suggest that left-sided lesions are more frequent with patients with self-acknowledged depression than in case of patients whose depression is observed by others (36). Vataja et al. do not correlate depression-dysexecutive syndrome (DES) with hemisphere side, but stress the importance of frontal-subcortical ischemic lesions (42). Brodaty et al. (20) also deny correlation between depression and the side of hemisphere, while Sharpe et al., Aben et al. and House et al. exclude the possibility of correlation of post-stroke depression with frontal left lesions (21, 26, 43). In order to assess the pathoplasticity of psychological functions and due to higher functional deficit, we have analysed the correlation between depression and motor-dominant hemisphere damage. By application of the Cox's model, higher risk of motor-dominant vs. non-dominant hemisphere damage for depression occurrence isn't confirmed in our study.

Reactivity vs. organic changes

It is interesting to note that patients in psychometric tests didn't express feelings of guilt, which is, according to psychoanalytic teachings, the basis of dynamic mechanisms. This supports the reactivity of the depression in patients with stroke, which largely increases the importance of implementing measures of mental hygiene, not only by psychiatrists but also other services and health workers.

Strength and limitations

The strength of this study in comparison to the aforementioned researches is reflected in a more precise definition of anatomic localization of lesion. Specific design of study enabled application of Cox multiple regression model which attenuates confounding effects. A relatively small number of positive cases of depression as well as positive frequencies of some explanatory variables on the examined sample of patients can be considered as a limitation. However, it should be noted that achieving the statistical significance on a smaller sample requires greater numerical differences, which may indicate a more powerful influence of explanatory variable on the observed disorder. We emphasize that our study is applicable only to the population of patients with subacute stroke (11–40 days after stroke), because associated heavier somatic condition of patients had significant influence on the results. Also, for assessment of correlation between lesion size and depression we used all values of HRSD score, and hence indicated to symptoms rather then depression itself. Results considering the risk for depression occurrence on frontal lobe damages must be taken with precaution because high frequency of mixed lesions disabled adequate distinction between frontal lobe lesions and lesions of other forebrain segments.

CONCLUSION

Our conclusions, as well as the overall results, have confirmed the working hypothesis H1a. The H1b hypothesis has been confirmed in case of depression caused by lesions of limbic lobe structures. The identified risk factors of depressive phenomena development in patients with stroke (female gender, size and localization of cerebrovascular lesion) obliges us to timely identify vulnerable groups of patients and implement an early treatment of mental disorders, bearing always in mind that the word is cure as well.

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Conflict of interest

None to declare.

Abbreviations

CAT — computerized axial tomography
HR — hazard ratio
HRSD — Hamilton Rating Scale for Depression
NIHSS — National Institute of Health Stroke Scale
OR — odds ratio
RR — relative risk

Sažetak

KORELACIONA ANALIZA DEPRESIVNIH ISPOLJAVANJA I MORFOLOŠKIH KARAKTERISTIKA MOŽDANIH LEZIJA KOD BOLESNIKA SA INZULTOM

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Uvod: Poznavanje etiopatogeneze postinzultnih depresivnih fenomena doprinosi ranoj dijagnostici koja ukoliko je praćena adekvatnom psiho/farmakoterapijom u velikoj meri skraćuje oporavak, i pogoduje socijalnoj i profesionalnoj rehabilitaciji pacijenata. Cilj ovog rada je da se istraži zavisnost pojave depresivnih ispoljavanja od veličine i anatomske lokalizacije lezije. **Ispitanici i metode:** Istraživanje je obuhvatilo 118 osoba oboljelih od cerebrovaskularnog inzulta. Lokalizacija lezije određivana je na aksijalnim nekontrasnim CT snimcima, a površina i obim lezije primenom AutoCAD digitalne planimetrije. Psihometrijsko ispitivanje pomoću Hamiltonove skale za depresiju izvođeno je metodom slučajnog odabira 11–40 dana nakon inzulta. Korelaciona analiza vršena je prostom linearnom/nelinearnom regresijom, i Coxovim hazardnim regresionim modelom. **Rezultati:** Uočena je negativna korelacija između intenziteta depresivnog ispoljavanja i veličine cerebrovaskularne lezije (Spearman $r = -0.263$; $P = 0.004$). Coxovim regresionim modelom utvrdili smo 4.389 puta veći rizik za pojavu depresije kod osoba ženskog pola, kao i veći rizik usled oštećenja struktura lobus limbicusa (hazard ratio e^b (HR) = 2.661, $P = 0.019$). **Zaključak:** Manji intenzitet depresivnog ispoljavanja kod većih cerebrovaskularnih lezija objasnili smo aktivacijom reparacionih mehanizama sa uštedom energije i smanjenjem (usled neuroloških ispada) aferentnih perifernih senzacija koje prethode pojavi emocija (James-Langeova periferna teorija emocija).

Ključne reči: inzult, lezija, depresija, korelacija, analiza.

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EARLY SIGNS OF DIABETIC NEPHROPATHY AND ULTRASOUND CHARACTERISTICS OF KIDNEYS IN CHILDREN AND YOUTH WITH DIABETES MELLITUS TYPE 1

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Abstract: **Introduction:** Diabetic nephropathy is a chronic complication in patients with diabetes mellitus typ 1, which leads to kidney disfunction.

Aim: The aim of this study was to compare the metabolic control and albuminuria with ultrasound findings of morphometric measurements and registration Doppler signals of kidneys between children and youth with diabetes mellitus type 1 according to the duration of illness.

Material and Methods: The retrospective-prospective study included 69 patients of both genders, that got diabetes mellitus type 1 when they were in the age from 2. to 25. years. Patients were divided into two groups according to the length of diabetes mellitus type 1: the first group was those whose illness had lasted for more than 10 years, and second group with duration of diabetes mellitus typ 1 less than 10 years.

Results: No significant difference was registered between the groups regarding frequency of albuminuria, but the chance of it occurring are greater in patients with longer duration of diabetes mellitus type 1. Patients with albuminuria and diabetes mellitus type 1 duration over 10 years had higher glycated hemoglobin A1C, blood pressure, body mass index followed by enlarged volume of both kidneys. Patients with albuminuria and diabetes mellitus type 1 for less than 10 years had a higher creatinine clearance.

Conclusion: Ultrasound dimensions and volume of the kidneys in patients with metabolic control parameters are useful for monitoring especially in the early stages of diabetic nephropathy.

Keywords: metabolic control, diabetes mellitus typ 1, diabetic nephropathy, albuminuria, ultrasound parameters.

INTRODUCTION

Diabetic nephropathy (DN) is a chronic complication in patients with diabetes mellitus typ 1(DMT1), which leads to progressive decay of the parenchyma and kidney disfunction (1). First stage of DN, marked as hypertrophic hyperfiltration, varies in duration, occurs within the first five years after diagnosing DMT1 (1, 2). Size of kidneys enlarges 20%, renal blood flow increases 10–15%, while albuminuria (AER) and blood pressure are within referential values. Second stage of DN is characterized by occurrence of albuminuria (AER) (2). Albuminuria is clinically the first detectible sign of DN and in following years has a tendency to progress into manifested proteinuria. In this stage the size of kidneys and glomerular filtration are elevated with pronounced AER. Blood pressure may be elevated or within normal values. Manifested AER is pronounced in the period of 10–15 years upon diagnosing DMT1 (3). Size of kidneys and glomerular filtration (GF) may be within referential values, but AER and blood pressure are elevated (4, 5). In initial stages of DN in children with normal renal function, ultrasound exam showing enlarged dimensions of kidneys in relation to standards may be an early pathomorphological sign of parenchymal kidney lesion. It has been noticed that, besides kidneys, other organs were also enlarged in the initial stage of DM (1, 5, 6). Maintaining appropriate metabolic control with regular ultrasound examinations of kidneys and controlling AER might affect the appearance of and inhibit the occurrence of progression of DN in children and youth.

The aim of this study was to compare the metabolic control and albuminuria with ultrasound findings of

morphometric measurements and registration Doppler signals of kidneys between children and youth with diabetes mellitus type 1 according to the duration of illness.

MATERIAL AND METHODS

The analysis included 69 patients of both genders whose DMT1 occurred when they were in age from 2. to 25. years, and regularly have controls at Endocrinology ambulance in the Clinic for children and Clinic for internal diseases of the University Clinical Center Tuzla (UCC Tuzla). Patients have DMT1 for various lengths of time, they were of different ages when DMT1 occurred. All data of our patients were acquired based on available medical documentation (illness history and medical charts), questionnaire, physical examination and laboratory findings. Data for questionnaires was gathered from parents of patients younger than 18 and from patients themselves if aged over 18, with their consent in accordance with procedure in UCC Tuzla.

The major part of laboratory testing was conducted within previous hospitalizations at the Clinic for children and Clinic for internal diseases, and performed at the Institute for Biochemistry of the Polyclinic for laboratory diagnostics of UCC Tuzla (4, 6). AER was determined in native urine via nephelometric method with reagents from Dade Behring Company. Creatinine concentration in urine was determined through auto-analyzer using a kinetic method by Dade Behring Company.

Afterwards, the relation between albumin and creatinine in urine was determined (5). Before the exam, the blood pressure was measured by the patients while sitting and laying down, on both hands, using a mercury manometer instrument. Information about the duration of DMT1 and metabolic control was analyzed from patient's medical history and medical charts.

The clearance of endogenous creatinine was determined through Schwartz equation, and based on Counahan-Barrat equation glomerular filtration (GF). To apply Schwartz equation, values of creatinine clearance, height of the patients and serum creatinine values were necessary, along with constant equations adjusted in accordance with age groups and gender (5).

By employing real-time technique on Toshiba Corevision 350 and Logiq 3 machines and using a multi-frequency convex probe of 3.75 mHz, dimensions of both kidneys individually were measured, first in a lying position on the back of patients, and then in the right and left "semi-oblique decubitus-position", also the longitudinal diameter of the kidney, width (expressed in mm) were measured, parenchyma echogenity, sinus appearance was monitored and kidney volume in milliliters (ml) was determined (7, 8). Analyzed also were dimensions of kidneys, acquired from the maximum longitudinal so-called central medium longest kidney diameter. The width of parenchyma was measured through the previous intersection located at the junction of medial and peripheral third. All values of acquired parameters were compared to the values of nomogram (percentiles) officially used in standard measurements with children, related to decimal age, body height, mass and body surface (7, 8). Upon colorization of arterial and vein flux, the Doppler spectrum was monitored on renal and interlobar arteries, and expressed as index of resistens (IR) (9, 10). It was compared to official nomograms for children in accordance to decimal age, body height, mass and body surface (9).

Statistical analysis was performed with biomedical application software called "MedCalc for Windows, version 114.4". For statistical significance of value *p* the usual level of significance, $p < 0.05$, was chosen (11).

RESULTS

The entire sample encompassed 69 patients, 36 (52.17%) had DMT1 longer than 10 years (first group), and 33 (47.83%) less than 10 years (second group). No statistically significant difference was found in proportions of patients between the both groups ($p = 0.609$).

From 69 patients, 41 (59.42%) were male and 28 (40.58%) were female, with male:female ratio 2.28:1. Male patients were significantly more represented (59.42 vs. 40.58; $\chi^2 = 4.89$; $p = 0.02$).

Table 1 shows the demographic date of patients with DMT1 related to illness duration. There were statistically significant differences among means of age, and the age when DMT1 occurred in first group, compared to the second group ($p = 0.002$; $p = 0.004$). Statistically significant difference was found in the mean of duration of DMT1 in patients in first group compared to patients from second group ($p < 0.001$). Statistically significant difference in gender distribution in the both groups was not found.

There was no statistically significant difference among the groups considering the frequency of AER ($p = 1.0$), but there are greater chances of occurrence of AER in the first group, i.e. with longer duration of DMT1 (> 10 years) [OR = 1.062 95% CI (0.413 – 2.733)], it is shown in Table 2.

Elevated albumin/creatinine (urine) ratio was documented in 18 (30.51%) patients with DMT1 >10 years, while in second group with shorter DMT1 duration (< 10 years) it was documented in 16 (44.4%) patients, without significant differences in frequency, as shown in Table 3.

Table 1. *Demographic data of patients with DMT1*

Demographic date of patients	Patients with DMT1			
	> 10 years	< 10 years	t-test	P
Number	36	33	–	–
Male/Female	22/14	19/14	2.8	0.765
Age (years; $\bar{x}$ ± SD)	19.2 ± 4.6	15.3 ± 5.2	3.3	0.002
Age of occurrence DMT1 (years; $\bar{x}$ ± SD)	5.8 ± 4.2	9.5 ± 5.9	3.0	0.004
Duration of DMT1 (years; $\bar{x}$ ± SD)	13.3 ± 3.0	5.8 ± 2.4	11.4	< 0.001

DMT1 = diabetes mellitus type 1

Table 2. *Frequency of albuminuria in patients according to duration of DMT1*

Albuminuria	Duration of DMT1			
	> 10 years N (%)	< 10 years N (%)	χ^2	p
Microalbuminuria	18 (50)	16 (48.5)	0.0	1.0
Proteinuria	1 (2.8)	1 (3)		
No microalbuminuria	17 (47.2)	16 (48.5)	0.0	1.0
Total	36 (100%)	33 (100%)		

Table 3. *Elevated albumin/creatinine (urine) in patients with DMT1 according to the duration of DMT1.*

Diabetic nephropathy	Patients with DMT1			
	> 10 years *N = 36 (52%)	< 10 years N = 33 (48%)	χ^2	p
Elevated albumin/creatinine in urine	18 (30.5%)	16 (44.4%)	1.3	0.24

N = number of patients

Table 4. *Parameters of metabolic control of patients with albuminuria according to duration of DMT1*

Parameters of metabolic control	Patients with DMT1			
	> 10 years	< 10 years	t-test	P
BGL* (mmol/l; $\bar{x}$ ± SD)	10.1 ± 2.0	10.1 ± 1.4	0.14	0.89
HbA1C*(%; $\bar{x}$ ± SD)	11.0 ± 2.1	9.6 ± 2.3	2.87	0.005
Blood pressure (systolic) (mmHg; $\bar{x}$ ± SD)	115 ± 2.3	108 ± 1.3	15.67	< 0.001
Blood pressure (diastolic) (mmHg; $\bar{x}$ ± SD)	80 ± 0.9	70 ± 1.7	30.76	< 0.001
BMI* (kg/cm^2; $\bar{x}$ ± SD)	20.1 ± 3.3	19.6 ± 2.9	1.75	0.04

*** BGL — blood glucose level; HbA1C — hemoglobin A1C; BMI — body mass index**

Table 5. *Creatinine clearance in patients with albuminuria according to the duration of DMT1*

Creatinine clearance (ml/min; $\bar{x}$ ± SD)			
Patients with *DMT1 > 10 years (N = 36)	Patients with DMT1 < 10 years (N = 33)	t-test	P
80.4 ± 4.5	87.5 ± 5.5	2.08	< 0.008

*** DMT1 = diabetes mellitus type 1**

Table 6. *Frequency of ultrasounds pathological finding of longitudinal diameter of the right kidney related to DMT1 duration, age and anthropometric parameters in our patients*

Decimal age and anthropometric parameters	*US finding of longitudinal diameter of the right kidney our patients related to DMT1 duration				Z	P
	> 10 years (n = 36)		< 10 years (n = 33)			
	N*	P*	N	P		
	*n (%)	n (%)	n (%)	n (%)		
Age	34 (49.1)	2 (2.9)	29 (42.2)	4 (5.8)	– 0.97	0.33
Body height	32 (46.2)	4 (5.8)	28 (40.1)	5 (7.9)	– 0.50	0.62
Body mass	32 (46.2)	4 (5.8)	32 (46.2)	1 (1.8)	1.29	0.20
Body surface	32 (46.2)	4 (5.8)	30 (43.6)	3 (4.4)	0.28	0.78

***US = ultrasound, N = normal finding, P = pathological finding, n = number of patient.**

Table 7. *Frequency of US pathologic findings of longitudinal diameter of left kidney, related to illness duration, age and anthropometric parameters in our patients*

Decimal age and anthropometric parameters	*US findings of longitudinal diameter of left kidney our patients related to DMT1 duration				Z	p
	> 10 years (n = 36)		< 10 years (n = 33)			
	N*	P*	N*	P*		
	*n (%)	n (%)	n (%)	n (%)		
Age	34 (49)	2 (2.8)	32 (46)	1 (2.2)	0.51	0.61
Body height	32 (46)	4 (5.7)	32 (46)	1 (2.2)	1.29	0.20
Body mass	32 (46)	4 (5.7)	32 (46)	1 (2.2)	1.29	0.20
Body surface	32 (46)	4 (5.7)	32 (46)	1 (2.2)	1.29	0.20

***US = ultrasound, N = normal finding, P = pathological finding, n = number of patient.**

Table 8. *Frequency of US pathologic findings of right kidney volume in relation to duration of DMT1, age and anthropometric parameters in our patients*

Decimal age and anthropometric parameters	*US findings of right kidney volume in relation to duration of DMT1 in our patients				Z	P
	> 10 years (n = 36)		< 10 years (n = 33)			
	*N	*P	N	P		
	*n (%)	n (%)	n (%)	n (%)		
Age	33 (47.6)	3 (4.5)	32 (46.5)	1 (1.4)	0.94	0.35
Body height	30 (43.3)	6 (8.7)	32 (46.5)	1 (1.4)	1.87	0.03
Body mass	30 (43.3)	6 (8.7)	32 (46.5)	1 (1.4)	1.87	0.03
Body surface	29 (41.8)	7 (10.1)	30 (43.6)	3 (4.5)	1.22	0.22

***US = ultrasound, N = normal finding, P = pathological finding, n = number of patient.**

Table 9. *Frequency of US pathologic findings of left kidney volume in relation to duration of DM T1, age and anthropometric parameters in our patients*

Decimal age and anthropometric parameters	*US findings of left kidney volume in relation to duration of DM T1 in our patients				Z	P
	> 10 years (n = 36)		> 10 years (n = 33)			
	*N	*P	N	P		
	*n (%)	n (%)	n (%)	n (%)		
Age	33 (47.6)	3 (4.3)	32 (46.5)	1 (1.6)	0.94	0.35
Body height	31 (44.7)	5 (7.2)	32 (46.5)	1 (1.6)	1.6	0.05
Body mass	31 (44.77)	5 (7.2)	31 (86.1)	2 (13.9)	1.6	0.05
Body surface	29 (41.8)	7 (10.1)	28 (40.7)	5 (7.4)	0.47	0.64

***US = ultrasound, N = normal finding, P = pathological finding, n = number of patient.**

***Table 10**. Frequency of pathological findings of IR in renal and interlobar arteries of the right kidney in patients with DM T1*

Artery	*IR of renal and interlobar arteries of the right kidney related to duration of DM T1					
	> 10 years (n = 36)		< 10 years (n = 33)		Z	P
	*N	*P	N	P		
	*n (%)	n (%)	n (%)	n (%)		
Renal	28 (43.5)	8 (11.5)	30 (43.6)	3 (4.4)	1.49	0.14
Interlobar	28 (43.5)	8 (11.5)	28 (40.7)	5 (7.3)	0.75	0.45

***IR = index of resistence, N = normal finding, P = pathological finding, n = number of patient.**

***Table 11.** Frequency of pathological findings of IR in renal and interlobar arteries of the left kidney in patients with DMT1*

Artery	*IR of renal and interlobar arteries of the left kidney related to duration of DMT1					
	> 10 years (n = 36)		< 10 years (n = 33)		Z	P
	*N	*P	N	P		
	*n (%)	n (%)	n (%)	n (%)		
Renal	28 (43.5)	8 (11.5)	30 (43.6)	3 (4.4)	1.49	0.14
Interlobar	28 (43.5)	8 (11.5)	28 (40.7)	5 (7.3)	1.27	0.20

***IR = index of resistence, N = normal finding, P = pathological finding, n = number of patient.**

Table 4 provides an overview of statistically significant higher value of glikolizated hemoglobin A1C (HbA1C), systolic and diastolic blood pressure, body mass index (BMI) in patients with AER and DMT1 lasting longer than 10 years, in relation to patients with shorter DMT1 duration. Statistically significant difference in glycaemia between the both groups was not registered.

Statistically significant higher creatinine clearance was present in patients with AER, whose DMT1 has lasted less than 10 years, in comparison to patients who have had DMT1 longer ($t = 2.08$ $df = 17$; $P = 0.008$), as shown in Table 5.

Figure 1 shows ROC curve of relation between duration of DMT1 and AER, with calculated duration of DMT1 of 11.6 years in children and youth, time necessary for development of AER. After 11.6 years, 50% of patients with AER will develop other form of DN, and in the group with no AER, 77% will not develop DN (12).

Table 6 and 7 present the frequency of ultrasound (US) pathological findings of longitudinal diameter of the right and left kidney in patients with DMT1 > 10 years, related to decimal age, body height, mass and BMI, with no statistically significant difference compared to patients with DMT1 < 10 years.

The frequency of US pathological findings of the volume of right kidney in the group of patients with DMT1 > 10 years, related to decimal age and BMI, is not statistically significantly different from the second group, while according to body mass and height are a significant different, as shown in Table 8.

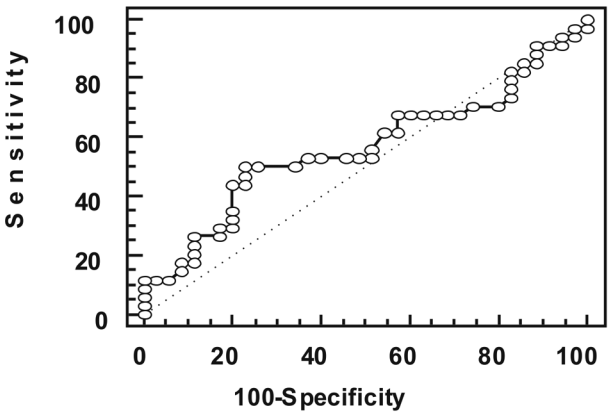

***Figure 1.** ROC curve of relation between DMT1 duration and microalbuminuria*

Table 9 shows in relation to patients with DMT1, frequency of US pathological findings for left kidney volume is statistically significantly higher for group of patients who have had DMT1 > 10 years, in accordance to their body height and mass.

Proportion test determined that there is no statistically significant difference in frequency of pathological findings of IR in renal and interlobar arteries in the right and left kidney, in relation to duration of DMT1, as shown in Tables 10 and 11.

DISCUSSION

Average age of our patients in the first group was 19.2 years and statistically significantly differed from the age of patients in the second group. DMT1 is the most common illness in elementary school-aged children and over 80% of children and adolescents is aged under 20 when DMT1 is diagnosed [13]. The average age of occurrence DMT1 in our patients was preschool and elementary-school years, with emphasized statistical difference beetwen the both groups. DMT1 may occur at any age of life, although most patients had it diagnosed in childhood and early adolescence (13, 14).

In the group of our patients with AER, statistically significant higher values of HbA1C, systolic and diastolic blood pressure and BMI were found. Inadequate metabolic control directly correlated with kidney hyperfiltration and hyperperfusion. It presents one of the major risk factors for occurrence of AER among children and youth with DMT1 (15, 16).

High levels of HbA1C as a marker of chronic hyperglycemia are the most commonly determined risk factor for appearance of AER and development of DN in children and youth with DMT1 (16). Elevated values of systolic and diastolic blood pressure, according to some authors, appear independently from AER (16, 17, 18). Despite the high prevalence of elevated blood pressure in children with DM and AER, prospective longitudinal studies shows the great influence of other risk factors, especially those that are inherent (17). BMI values above 95 percentiles are noticeable at the onset of DMT1, while after a long time of duration of DMT1, it may occur in children due to irregular or misused insulin therapy (19). Higher or lower BMI values of our patients are possibly explained by various ages during this research.

Duration DMT1 in our children and youth necessary for development of AER was 11.6 years (Figure 1). Sensitivity in one half of our patients will confirm the presence of AER, while higher specificity in three quarters of patients will exclude those who do not have AER (Figure 3). It means that probably 50% of all patients will show some signs of nephropathy after 11.6 years duration of DMT1. A more reliable evidence is the fact that 77% of patients will not have AER, after 11.6 years with the illness, but it is possible that they will exhibit certain distorted parameters of metabolic control, such as HbA1C, elevated blood pressure or BMI.

This crucial information, that 11.6 years of DMT1 duration is necessary for development of AER as an early sign of nephropathy, opens up a possibility and chance to detect early morphologic and functional changes on kidneys in ill children and youth before. For prevention, further progression of DN, development of hypertension and other chronic complications, time period is of essence. This is the so-called intervention point, critical time for prevention of most important risk factors in renal weakening progression: hypertension, infections and distorted metabolic control (20). DN itself and factors of its appearance can not be prevented and can be influenced very little, but side effects, such as infection, hypertension, disturbed metabolic control, as well as obesity, can be regulated with a systematic and scientifically based manner.

Hyperfiltration and kidney hyperperfusion are difficult to determine, so reliable measurement (quantification) of AER remains one of the most reliable tests (17, 19, 20).

In the research by Turkish authors, AER prevalence was higher in their patients, by whom DMT1 were diagnosed before the age of 15, but also significantly higher in patients with longer duration of DMT1 (20). Statistically, there was no significant difference between the groups of our patients in frequency of AER ($p = 1.0$), but chances of AER occurrence are higher in the patients with longer duration of DMT1 > 10 years. Nowadays, the duration of DMT1, blood pressure, and poor metabolic control are the major risk factors for the development and progression of DN. Some of habits, just as cigarette smoking and eating fast food have big influence to metabolic control and progression DN. Positive family history of renal disease with low socio-economic status with longer duration of DMT1 may accelerate progression of DN. Elevated relation albumin/creatinine in urine does not significantly differ in both groups, while the possibility of occurrence of elevated values of albumin/creatinine in urine is equal in both groups of our patients. Considering the limitations of this study with a fewer number of patients, a more decisive conclusion could be drawn on samples with higher number of patients (1, 16, 20). Nevertheless, statistically significant lower value of creatinine clearance occurred in our patients with DMT1 > 10 yeras, related to patients with shorter period of duration DMT1 ($P = 0.008$). Elevated values of creatinine clearance in our patients with shorter DMT1 history can be explained with hyperfiltration in the initial stage of DN (15, 16). In the group of our patients who have had DMT1 > 10 years, longitudinal diameter of both kidneys was not significantly changed in relation to applied standards for age and anthropometry parameters. Other authors as well, in similar research, gathered almost identical results (9, 13). A more significant aberration of volume of right and left kidney in relation to standards for body height and mass was found in the group of patients who have DMT1 > 10 years. Enlargement of kidney volume in DM appears with retaining

salt and water, as well as complex pathophysiological processes on the very small blood vessels (14,16). Enlargement of dimensions and volume of kidneys is one characteristics of DMT1 in the stage of hypertrophic hyperfiltration (13, 16).

In our study, no significant differences were noticed in frequency of pathology result for IR in renal and interlobar arteries in the right and left kidney among patients with various length of DMT1. Higher values of IR follow the progression of DN only in clinically manifested and advanced stadia of DMT1 (23). In the stage of hyperfiltration, IR values may be lower than normal. Hyperfiltration, as a hemodynamic change, cannot be diagnosed based only on IR in most patients with DMT1 (23, 24). Color Doppler ultrasonography has not proved to be a sensitive method in early stages of detecting complications of DMT1 at the small kidney blood vessels (24). It is difficult to explain that the dimension and volume of kidneys in certain stages of DMT1 change, they enlarge, while Doppler spectrums above blood vessels remain unchanged.

CONCLUSION

Early lesions of kidney parenchyma with following metabolic control appear after 10 years of duration of diabetes and are rarely registered earlier. More intense surveillance over metabolic control, along with following albuminuria, could be useful for defining optimum time frame for screening and prevention of diabetic nephropathy.

Ultrasound exams of dimensions and volume of kidneys are useful parameters since they can be applied and analyzed with parameters of metabolic control, especially in early stages of DN (hypertrophic hyperfiltration). Changes on blood vessels in early stages of diabetic nephropathy are not clear and evident, so pathologic values could not be registered using Doppler spectrum.

Abbreviations

DN — diabetic nephropathy
DMT1 — diabetes mellitus type 1
GF — glomerular filtration
AER — albuminuria
UCC Tuzla — University Clinical Center Tuzla
ml — milliliters
IR — index of resistens
US — ultrasound
BMI — body mass index
HbA1C — glikolizated hemoglobin A1C

Sažetak

RANI ZNACI DIABETIČKE NEFROPATIJE I ULTRAZVUČNE KARAKTERISTIKE BUBREGA KOD DECE I MLADIH SA DIABETESOM MELITUSOM TIP I

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Uvod: Diabetička nefropatija je hronična komplikacija diabetesa Tip I koja dovodi do difunkcije bubrega.

Cilj: Cilj ove studije bio je poređenje metaboličke kontrole i albuminurije sa ultrazvučnim nalazom mofrometrijskih karakteristika i Doppler signala bubrega medju decom i mladima obolelim od diabetesa Tip I u zavisnosti od trajanja bolesti.

Materijal i metode: Retrospektivno-prospektivna studija obuhvata 69 pacijenata oba pola koji su dobili diabetes Tip I između 2. i 25. godine. Pacijenti su podeljeni u dve grupe prema dužini trajanj bolesti na one kod kojih je bolest trajala duže od 10 godina i one kod kojih je bolest traja manje od 10 godina.

Rezultati: Nije registrovana statistički značajna razlika u postojanju albuminurije ali je šansa o pojavi ista među pacijentima sa dužim trajanjem bolesti. Pacijenti sa albuminurijom i trajanjem diabetesa Tip I dužim od 10 godina imali su veći glikolizirajući hemoglobin HbA1C, krvni pritisak, BMI (body mass index) i volumen oba bubrega. Pacijenti sa albuminurijom i trajanjem diabatesa Tip I manjim od 10 godina imali su veći klirens kreatinina.

Zaključak: Utrazvučne dimenzije i volumen bubrega sa parametrima metaboličke kontrole su korisni za monitoring posebno u ranim stadijumim a diabetičke nefropatije.

Ključne reči: metabolička kontrola, diabetes melitus Tip I, diabetička nefropatija, albuminurija, ultrazvučni parametri.

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SEPSIS BIOMARKERS AND PATHOGEN DETECTION METHODS — STATE OF THE ART

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Abstract: Evidence-based blood culture testing is of utmost importance for ICU patients with suspected sepsis or organ infection. Knowledge of the etiologic agent (bacteria or fungi) and their susceptibility against antimicrobials enables the clinician to initiate an appropriate antimicrobial therapy and guides diagnostic procedures. This has been shown to reduce mortality, ICU--stay and antibiotic overuse. Whereas microbiological laboratory practice has been highly standardized, shortfalls in the preanalytic procedures in the ICU (indication, timing, volume, numbers, collection of blood cultures) have a significant effect on the diagnostic yield.

Due to system-related drawbacks of molecular diagnostics, i.e. PCR-based pathogen detection, which are arguable sensitivities, the failing of the 'fast time-to-result argument', no solution to establish a comprising antibiogram, still ongoing discussions on the coverage of the target panel, high overall costs, and the lacking of resilient data on clinical utility, non-culture-based NATs do currently not represent an alternative to blood culture testing.

Inflammatory markers are recognized to play an increasingly important role in the diagnosis and monitoring of sepsis. This is partly due to low specificity of clinical symptoms and conventional inflammatory signs for the diagnosis of sepsis but also to a lack of correlation with the severity of the inflammatory response. Elevated serum PCT levels indicate systemic inflammation reliably. PCT is the only sepsis marker that is helpful in the differentiation between infectious and non-infectious causes of organ dysfunction and shock and might support antibiotic therapy.

Keywords: Blood stream infection, blood cultures, sepsis, guidelines, antimicrobial therapy, molecular diagnostics.

INTRODUCTION

According to the latest data from the Center for Sepsis Control and Care (CSCC) funded by the Federal Ministry for Science and Research (BMBF), a total of 174,589 patients developed septic conditions in Germany in 2011 (1). Of these, 87,152 patients had sepsis, 68,551 severe sepsis, and 18,886 septic shock. These figures are based on the new ICD-10 encodings introduced by the German Sepsis Society (DSG) in 2007. The peak age was between 70 and 75 years and 49,198 patients died in hospital. Hospital mortality rate for sepsis was 10.5%, for severe sepsis 41.5% and 60.5% for septic shock. These latest figures exceed data on incidence and mortality reported by the German Competence Network SepNet in 2004. Given the increasing age of the population, figures can be expected to continue rising during the coming years. The previous failure of new approaches to the treatment of sepsis is closely related to diagnostic deficits. The time of diagnosis, and thus the early initiation of therapeutic measures is the crucial determinant of the high mortality (2). In both the pre- and intrahospital course of the disease often takes several hours to days to diagnosis and ultimately adequate treatment, since there is currently no reasonable means to predict the transition of a localized infection to severe sepsis. An earlier diagnosis by means of sensitive and specific biomarkers could help to reduce the high mortality and morbidity.

Microbiological diagnosis of sepsis using blood cultures

The detection of microorganisms using what has recently and rather conspicuously been termed "conventional" or "traditional" BC-testing (in contrast to what is referred to as "modern" molecular biological testing for the pathogen causing sepsis using DNA-testing) is essential for implementation of a causal therapy for infectious diseases brought about by bacteria or fungi. Knowledge of the pathogen and its antibiotic susceptibility enables a targeted antimicrobial therapy to

Table 1. *Guideline-based blood culture testing (acc. to Brunkhorst FM, Seifert H, Kaasch A, Welte T: Shortfalls in the application of blood culture testing in ICU patients with suspected sepsis. DIVI 2010, 1:23)*

1. Point of time of blood withdrawal: • Withdrawal of in minimum two BCs prior to start of antimicrobial therapy otherwise at the end of dosing interval. • BC should be withdrawn in parallel from 2 different sites or with a gap of several minutes. • Time point and sampling sites have to be marked onto the data sheet. • Drawing of double blood volumes and distribution onto 2 BC sets (4 bottles) in case of venipuncture is not recommended. Fresh puncture is obligate.
2. Aseptic puncture: • Hygienic hand disinfection. • Disposable gloves (not sterile). • Skin disinfection (e.g., with 70% alcohol for in minimum 1 minute). • Puncture without new vein palpation. • Puncture of peripheral veins. • No withdrawal from intravenous catheters (risk of contamination). • Exception: withdrawal of one BC set (anaerobic/aerobic, each) from a catheter suspected for infection and from a peripheral vein.
3. Contamination-free inoculation of BC bottles: • Cover removal • Disinfection of the septum with alcoholic solution (alcohol must not enter the bottle). • Storage of not inoculated BC bottles at room temperature. Bottles must not be inoculated if cooled.
4. Blood volume required: • 8–10 ml per bottle. • Inoculation of the anaerobic flask at first (prevents entry of air bubbles from the tip pf the syringe), aerobic flask at second. • Special media for pedriatric applications may be inoculated with 1–3 ml per bottle. • BC bottles must not be ventilated; bottle barcodes should not be pasted over.
5. Number of BCs required: • 1 BC consists of 2 BC flasks with aerobic and anaerobic culture media, respectively. If applicable, special media may be used (e.g., for the detection of fungi) additionally. • Withdrawal of 2 to 4 BCs are recommended. Taking of only 1 BC is not sufficient, given that a negative result permits no preclusion of the presumed infection. A single detection of facultative pathogens (e.g., coagulase-negative *Staphylococcus* spp.) affords no certain discrimination between contamination and infection.

be conducted and guides the subsequent patient management. This not only improves the prognosis and reduces mortality rates (3, 4, 5), but also shortens the duration of hospital stay and helps to prevent the overuse of antimicrobials. Blood culture testing is thus clearly the most important evidence-based detection procedure in intensive care. Commercially available BC media permit the growth of almost all microorganisms. Nowadays, it is also customary that BC-testing is performed using equipment with an automated detection system that offers improved susceptibility and shorter detection times. The majority of BCs are positive within the first 12–24 hours. After the detection of a positive BC bottle, gram staining is immediately carried out, subcultures and preliminary susceptibility testing performed. Definitive susceptibility testing to determine the minimum inhibitory concentrations requires a standardized inoculum and is usually performed after overnight incubation resulting in sufficient growth. Subcultures are incubated in aerobic and anaerobic BC-bottles for at least 72 hours. If no bacterial growth can be detected despite positive microscopic identification of bacteria on Gram staining, the incubation time for agar media is increased to 5–7 days in order to enable the identification of slow-growing microorganisms. As procedures for processing BC-bottles in the microbiological laboratory have been standardized to a large degree, guideline-based pre-analytic procedures in intensive care units (indication for obtaining blood cultures, blood collection techniques, transport times) have enormous impact on the diagnostic yield (see Table 1).

Current shortcomings in BC testing

The reliability of BC-testing for the identification of cultivable microorganisms in blood is high and the contamination rate is below 3% when guideline-based collection techniques are followed (6). However, the proportion of positive BCs compared to the total number of BCs varies substantially in different retrospective studies but usually ranges between 10 und 25%. In controlled trials, the detection rate for patients with severe sepsis and septic shock is 20–40% and thus significantly higher. These fluctuations are mainly depend-

ent on the group of patients being examined, how freely a diagnosis is made, any pre-treatment with antibiotics, the number of blood cultures performed and on adherence to standardized test conditions.

According to a representative study performed by the German National Reference Center for the Surveillance of Nosocomial Infections (NRZ) a total of 223 ICUs provided data on blood culture practice (7). BC pairs taken in 2006 have been 60 (with a huge variation from 3.2 to 680) per 1,000 patient days. The mean primary blood stream infection (BSI) rate was 0.90 per 1,000 patient days and 0.25 BSIs per 1,000 patient days were caused by coagulase negative staphylococci (CNS). The mean central venous catheter (CVC)-BSI rate was 1.40 per 1,000 CVC days. In the univariable analysis the number of blood cultures obtained per 1,000 patients day (referred to as blood culture frequency below) had a significant influence on the CVC-associated BSI-rate, considering either all pathogens ($p = 0.001$) or only the subgroup of CNS-related cases ($p = 0.019$). There was also a significant influence of the BC-frequency on the CVC-BSI-rate considering all pathogens ($p=0.004$) as well as the subgroup of CNS ($p = 0.018$). According to the multivariable analysis an increase of the BC-frequency of 100 BCs per 1,000 patient days leads to a 1.27-fold higher incidence density of CVC-BSI with a 95% confidence interval (95% CI) of 1.01–1.26. A further significant risk factor for CVC-BSI was the length of stay in the ICU with an adjusted incidence rate ratio (IRR) of 1.25 (95% CI 1.15–1.35). The authors concluded that if an external benchmarking of CVC-BSI-rates on ICUs is intended an adjustment according to the BC-frequency is necessary. The BC-frequency itself should be established as a quality indicator in intensive care management.

A survey on blood culture practice performed in 2009 in Germany, the United Kingdom, Italy, and France in 80 microbiological laboratories and 60 ICUs revealed differing estimations on guideline adherence (8). 62% of the german ICU clinicians saw no problems in handling blood cultures, 8% criticized transport times bedside to laboratory (guideline recommendation < 12 hours), which was significantly increased in comparison with the other countries. In contrast, at least 41% of microbiologists of the same facilities criticized the transport times and 49% saw shortcomings in pre-analytical quality, e.g. due to false positives by contamination (guideline recommendation < 5%). 47% of questioned microbiologists criticized the low numbers of blood culture sets coming in from the clinic. Cost pressure has been valued rather comparable (Figure 1).

These figures point to a substantial and generally underestimated shortcoming as far as adequate microbiological testing is concerned, and indicate in particular that blood culture testing in German intensive care units is not conducted in accordance with guidelines.

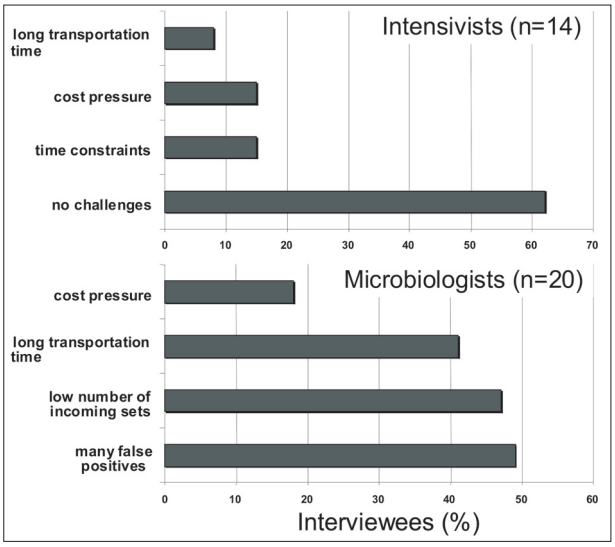

***Figure 1**. Differences in perceptions of blood culture testing practices between intensivists and microbiologists of the same institutions (according to (8))*

Data on the incidence of bacterial infections vary depending on the patient population and results are dependent on age, underlying illness and a large number of further risk factors, as well as on the clinical department and the size, type and level of healthcare provided by the hospital. In relation to the number of days spent in hospital, the incidence totals 1–3 cases/1,000 days in hospital (9–13), corresponding to 10–20 cases/1,000 hospital admissions. For patients in intensive care, the incidence is 30–40 cases/1,000 admissions (14, 15). Population-based studies show the incidence of severe bacteremic infections — i.e. those requiring intensive care — to be about 2 cases/10,000 (16).

Despite bacteremic infection, criteria for sepsis are not always fulfilled in elderly persons, patients undergoing immunosuppressive therapy, patients in intensive care (e.g. following polytrauma or burns), in patients with intravascular implants (e.g. prosthetic cardiac valves or stents) and in neonates. In these cases, the indication for blood cultures should be viewed generously. In elderly patients, it should be taken into consideration that acute encephalopathy is often the first sign of sepsis and that patients may remain afebrile. Blood culture should be obtained — in these cases too, as well as in the case of uncontrolled diabetes in an otherwise well-adjusted diabetic patient. In patients with neutropenia, every fever (temperature > 38°C) should be regarded as a possible sign of sepsis and blood cultures should be obtained in this group of patients, the rate of bacteraemia is 20 to 30%.

Table 2. *Drawbacks in routine NAT-based pathogen detection of sepsis pathogens without pre-culturing. BC: blood culture, cfu: colony forming units, MIC: minimal inhibition concentration*

- No information on most antimicrobial **susceptibility patterns** (only available for a small selection, incl. *mecA*, *vanA/B*, few ß-lactamases)
- No information on **expression status** after detection of resistance targets
- No information on drug **MIC data**
- **Sensitivity** ≥ **3 cfu/ml** whole blood; expedient target concentration: < 1 to 5 cfu/ml
- **High workload / hands-on-time** (depending on assay system)
- Mostly **experienced personnel required** (depending on assay system)
- Results are not available at start of therapeutic measures (like BC)
- **Transport time** to lab assumed as long as time-to-incubation for BC — in Germany up to 20 h to off-site labs
- **Clinical significance** of targeting nucleic acids vs. whole / viable pathogen cells still under discussion; sensitivity for free / phagocytozed (microbial) DNA not defined
- **Origin and clinical significance** of targeted microbes ('true positives') arguable; verification by virtual clinical gold standard (sum of clinical plus microbiological evidence) required
- Ongoing discussion on the usefulness of broad-range vs. specific PCR primers; restricted target panel achieved with specific primers (only detection of the most prevalent pathogens) — broad-range primers prone for detection of non-disease associated germs
- **High initial investment** for devices / lab equipment, **high running costs** — 1 sample currently >> 100, – € ≥ 2 samples are recommended according to sepsis guidelines for blood withdrawal BC
- **No replacement of BC feasible** -> additional costs / work load
- **Only restricted data on clinical utility** / patients' benefits available.

Pathogen detection by means of molecular diagnostics

The non-culture-based identification of pathogens by means of nucleic acid amplification techniques (NAT), such as multiplex-PCR (identification of a limited number of specified pathogens by a defined set of oligonucleotide primers) and broad-range PCR (unrestricted detection of fungal and bacterial DNA by few taxon-specific primers) (Figure 2) is advertised as promising approach to support adequate anti-infective measures at an early stage of sepsis. Amongst most conversant assays for detection of bacterial and fungal DNA in whole blood which are approved for clinical use by European regulatory authorities (i.e. CE-certified) are two multiplex real-time PCR (qPCR) assays. SeptiFast® (Novartis, Basel, Switzerland) simultaneously detects a predefined rather narrow panel of the most important sepsis pathogens by species- and genus-specific primers and subsequent amplicon detection with fluorescent probes. SepsiTest™ (Molzym, Bremen, Germany) aims at the broad-range detection of bacterial and fungal genomic DNA via 16S and 18S rRNA gene-based universal primers followed by sequencing of amplicons and gene bank species identification. Abbott (Wiesbaden, Germany) also offers a broad-range system followed by mass spectrometry for species identification (Plex-ID).

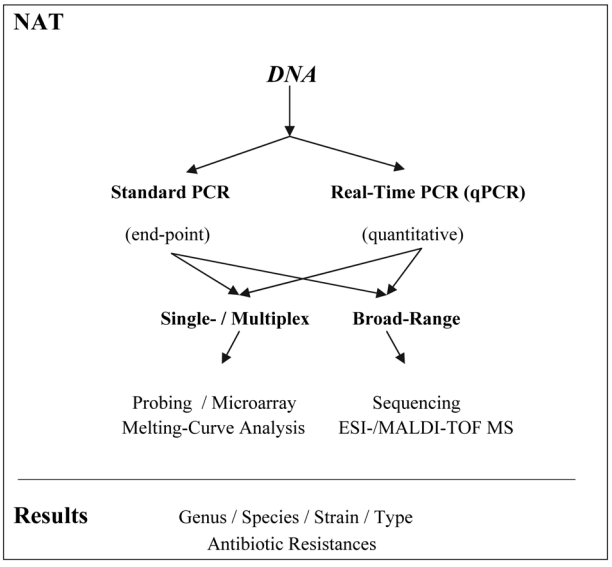

Figure 2. *Basic principles of NAT-based applications in bacterial/fungal pathogen detection. ESI-/MALDI-TOF: electrospray ionization time-/ matrix-assisted laser desorption-ionization time-of-flight mass spectrometry*

Current limitations in PCR-based sepsis pathogen detection

Sensitivity & specificity

Crucial for pathogen detection is the low abundance of pathogens within the blood stream of patients with BSIs. In cases of severe sepsis/septic shock, an about 10 colony forming units (cfu) per ml of whole blood are to be expected in adults (17, 18), within a

range of 1 to 30 cfu per ml (19). In contrast, 100 to 300 cfu per ml have been detected in newborns (17, 20). In case of severe sepsis or septic shock, only reduced benefit for the patient's outcome will be achieved by the causative pathogen's identification at the later stages of the disease. Thus, pathogen detection should be initiated as soon as possible at lower bacterial loads ideally down to < 1 cfu/ml whole blood. Even 3 cfu/ml marks the lowest limit of detection of the most sensitive NAT-based assays (3 to 100 cfu/ml detection range (21)) which unfortunately is absolutely in range with frequent contamination loads.

Sensitivity mainly depends on the ratio of target sequence content to background DNA, i.e., particularly human leukocyte DNA. This bulk DNA, co-isolated with microbial pathogen nucleic acids, is the cause for a minute pathogen to human DNA ratio by a factor of ~2 x 10^{-9} cfu/ml whole blood, roughly given that 10 genome copies of ~ 5 fg each are present within 100 µg of total DNA isolated from 1 ml whole blood, the reason for low assay sensitivities in the presence of bulk DNA (22). Those drawbacks can be restrictedly conquered by using higher starting material volumes combined with effective background DNA reduction while maintaining the total DNA load per PCR reaction (23, 24, 25) — which in other words means to increase the target number per total DNA load of the amplification reaction. However, current tools for bulk DNA degradation have not revised the situation: the lowest assay sensitivity stated remains at 3 cfu/ml whole blood.

Assays using universal primers are in part compromised by their high sensitivities (within constrains mentioned above) due to the fact, that the addressed targets are often present in multiple copies (e.g., panbacterial 16S, 5S, 23S rDNA/RNA or panfungal 8S, 18S, 5.8S, 28S rDNA / RNA targeting) but more or less without narrowing possible outcomes. Respective (PCR) primers have been proven to be suitable even for nucleic acid trace detection in diagnostic consumables (26–29), contaminated disinfectants, antiseptics, and BC media (30), or introduced via applying routine sample withdrawal techniques, causing false-positive results. Of major concern in sepsis diagnostics are clinically irrelevant results of non-disease-associated transient bacteraemia, the inability to distinguish between DNA from non-viable, non-active, or dead pathogen cells, or free circulating versus phagocytised DNA in immuno-competent host cells in cases of microbial DNAemia.

Circulation of translocated microbial DNA or the transient presence of bacteria in blood not associated with the disease have been proven in vivo (31). Culture-positive bacteraemia was reported after tooth brushing and other dental measures as transient translocations of mucosal colonizing microorganisms due to minimal manipulations already decades ago (32, 33). Moreover, isolation of bacteria in asymptomatic blood donors and even transient fungaemia without apparent clinical significance has been repeatedly reported (34, 35). Pure microbial background DNA has been detected in the blood of healthy individuals (36, 37) and asymptomatic blood cell donors (38) but no resilient information is available for the mean value of whole blood microbial background DNA load of healthy individuals. This indicates the range of risks to obtain false-positives and the difficulties to distinguish them from true-positives. However, origin and clinical significance of 'false-positive' samples are often ambiguous and might belong to yet unbeknown host-pathogen interactions (39). Concluding, non-specific PCR protocols are prone for the detection of non-disease associated germs but also promise to facilitate the finding of microorganisms that are found less frequently, even yet unknown, or generally non-cultivable (17) and might therefore be of high scientific relevance but rather objectionable in sepsis causative species verification.

The panel of multiplex PCR assays with specific primers is limited due to primer interactions / heteromer formation and technical limitations (e.g., fluorescence dyes), which sets margins to the amount of targets detectable within one PCR tube or primer pool. Otherwise, a setting of too many individual PCR reactions for investigation of a single specimen would lead to high costs and workload. It has been consequently proposed, that PCR detection in clinical diagnostics should focus only on those pathogens or resistance determinants that are not covered by guideline recommended treatment regimens and that have been identified as the major cause of inappropriate treatment (40). The authors stated that multiplex panels should be scaled down by high conservation of its individual primers. Such a narrower assay would be more cost effective, may achieve higher accuracy due to reduced intra-test interference, and would better address current and emerging clinical needs. However, clinicians rightly call for all-embracing diagnostic approaches: what would be the benefit of an assay which gives only an aperture of the targets of relevance and necessitates continuation of the traditional clinical course in parallel to cover all diagnostic outcomes?

Shortfalls in the detection of antibiotic resistances

Clinical and commercial success of NAT-based assays mainly depend on their ability to compete with the standard (i.e., cultural) methods in antibiotic resistance detection. This becomes increasingly important in the context of rising broad-spectrum resistance in Gram-ne-

gative rods, particularly for carbapenemase-expressing pathogens, like *Klebsiella* spp. and multi-drug resistant *Acinetobacter baumannii* (41, 42). Fast identification of MRSA or VRE may be feasible by molecular methods since methicillin and vancomycin resistances are encoded by a limited number of known genotypes. However, many types of bacterial resistance base on several distinct point mutations, e.g. Penicillin-resistance in *Pneumococcus* spp., and more than 300 extended-spectrum ß-lactamases (ESBL) described so far for distinct Gram-negatives are encoded by a huge and still evolving variety of genotypes. Moreover, phenotypic resistances and the number of various regulatory genes, whose role in resistance expression remain elusive, precludes simple molecular- (or proteomic-) based economic detection approaches for routine purposes. Therefore, molecular detection of the most prominent bacterial antibiotic resistance-mediating target genes, one of the most relevant aspects for a timely confirmation or correction of the initial therapeutic regimen, will be restricted to a limited number of resistance types in the foreseeable future and broad antibiograms remain subject only to the cultural approach convicting the molecular methods to be costly adjuncts to the traditional way of action.

Furthermore, add-on information on antibiotic minimal inhibition concentrations (MIC) becomes increasingly important. Therapeutic drug monitoring (TDM) is discussed as a key intervention to improve outcomes in patients with sepsis and septic shock. In these patients, pharmacokinetics of ß-lactam antibiotics may be altered due to dynamic, unpredictable pathophysiological changes and significant increases in the volume of distribution and/or augmented renal (drug) clearance (ARC) (43, 44, 45). When ß-lactam antibiotics are applied uncontrolled, pharmacokinetic changes can result in sub-therapeutic plasma concentrations, treatment failure, and the development of antibiotic resistance. Individualized dose optimization to achieve drug concentrations above the MIC of the infecting pathogen is demanded. Changes in plasma concentration were also monitored in a recent meta-analysis on the outcomes of extended or continuous versus short-term intravenous application of first-line drugs (46) and in a multicenter trial on continuous infusion versus intermittent bolus therapy (47). Aside from the respective findings there is growing evidence for a high potential in an individualized modification of the anti-microbial therapy. Knowledge of the MIC data of the underlying presumed pathogen could substantially improve the patient's outcome but is only achievable via culturing and not by NAT.

Transport vs. time-to-results

A weighty argument to adopt molecular tools in sepsis diagnostic routine is the promise to deliver therapy-relevant results much faster than via cultural workflows. Beside the fact that patients with advanced community-acquired sepsis (about 30% of sepsis incidences) are in part already empirically treated with broad-spectrum antibiotics when they enter the emergency units (48), already a (e.g.) 2 hour time-to-result, which is currently not achievable at all directly from whole blood, would deliver NAT results too late to direct immediate anti-infective measures with first-line drugs. The physician's attendance to change an initiated therapeutic measure then decreases steadily within the first days. In most cases, hospital-acquired (nosocomial) early-stage and primary sepsis (e.g. catheter-associated sepsis) is handled under constant surveillance by cultural methods and timely pressure is lower to anticipate a meaningful cultural or NAT-based result on the underlying infective agent. However, NAT could positively impact pathogen detection in cases of nosocomial septic infections where patients at risk, i.e. patients with artificial ventilation, urinary or central-venous catheters, peripheral venous cannulation, immunosuppression, or surgery, are monitored broadly from the beginning of the period of risk where sepsis could be diagnosed at an early stage with a higher likelihood of recovery (if other premises would be solved).

Achievements in time-to-result are often biased by limitations in sample transport between bedside and lab as revealed by the international survey on BC diagnostics cited above (8). Time-to-incubation for BC depends on the way of sample transportation, opening hours of the microbiological labs, BC management outside these time frames, and ranges from 2 h in the UK and German on-site laboratories to 20 h in German remote labs. The benefit from shortening the time-to-result due to technical accomplishments is hampered due to opposing sample logistics. This without regard to an aspired batch-wise analysis of samples, still high turnaround times, in part low sample throughput, high technical complexity, and (for most clinical facilities) prohibitive overall costs (>100 €/sample). Expectedly, even for the as to be rapid advertised assay SeptiFast®, the median time-to-results in a 2009 retrospective clinical trial were 18 hours, with a minimum of 6.75 hours and a maximum of 74 h (samples were collected at the beginning of the weekend) when samples were taken twice a day, and with a median time of 26.25 hours, a minimum of 6.75 hours and a maximum of 79 hours with a once daily analysis (49). Notably, the setting corresponds to on-site lab availability. However, these observations challenge recent data on clinical utility of the assay (50). To give a further example, Bloos and colleagues revealed a median time-to-positivity of 24.2 hours for their NAT and 68 hours for BC, also with a lab on-site (51). Depending on technician availability, the

Table 3. *Clinical markers for the enhancement of sepsis diagnosis. ATG: anti-human T-lymphocyte globulin; TNF-α: tumor necrosis factor α*

	Specificity for infection	Sensitivity for non-infectious inflammation	Clinical utility as sepsis marker	
			Pros	Cons
Procalcitonin (PCT)	++++	+	— high specificity and sensitivity for sepsis — good correlation with severity code — fast induction (< 2 h) — high stability — half-live period 24 h — broad biological time span	— possibly increased in cases of severe sepsis due to: • comprehensive surgical operations • poly-trauma • hemorrhagic shock • cardiogenic shock • cardiopulmonary bypass • birth stress • severe burns • immunosuppressants (e.g., TNF-α, ATG) • C-cell cancer — cost-intensive — slow induction in neonatal sepsis
C-reactive protein (CRP)	+	++	— relatively cost-effective	— low specificity — slow induction (peak after 48 h) — small biological span — no correlation with severety
Interleukin-6 (IL-6)	+	++++	— high sensitivity — fast induction (range of minutes) — broad biological time span	— low specificity — short half-live time (min) — low biostability — cost-intensive
Lipopolysaccharide-binding protein (LBP)	+	++	— high sensitivity — long half-live time (> 48 h)	— low specificity — slow induction — cost-intensive
Leukocyte count	+	+	— simple — cost-effective	— low specificity
Temperature	+	+	— simple — cost-effective	— low specificity

NAT median time-to-positivity increased up to 53.5 hours on weekends.

CONCLUSION

Over the last years, growing evidence appeared which supported the add-on value of NAT-based commercials as adjuncts to culture methods in an early but even faster detection of true positives and designated antibiotic resistance markers. Positive influence on time-to-initiation of directed anti-infective measures, mortality, length-of-stay in hospital, overall costs and administration of broad-spectrum antibiotics have also been stated. Selected assays might have gained significant added value in enabling evidence-based antibiotic stewardship in exceptional cases. However, due to system-related drawbacks like arguable sensitivities in adult sepsis, the failing of the 'fast time-to-result argument' (especially in Germany), no solution to establish a comprising antibiogram with additional information on MIC data, the ongoing discussions on the coverage of the target panel, significantly too high overall costs, and the lacking of resilient data on clinical utility,(for a summary on drawbacks see Table 2) non-culture-based NATs do currently not represent an alternative to BC testing and it is not yet possible to derive any recommendations for clinical practice.

Sepsis markers — a gain of time!

An early diagnosis using sensitive and specific biochemical or immunological markers could help to reduce the high mortality and morbidity associated with

sepsis. Conventional parameters such as body temperature, heart rate and white blood cells (WBCs) are inadequate parameters to describe the complexity and the magnitude of the inflammatory host response. Apart from insufficient tissue oxygenation, a major risk factor responsible for organ dysfunction is systemic inflammation. This leads to various alterations in cellular metabolism of immune competent and parenchymatous cells (induction of pro- and anti-inflammatory mediators (interleukins IL-1, IL-2, IL-6, IL-8, IL-10, as well as factors like tumor necrosis factor α, (TNF-α)), metabolites (NO, neopterin, formation of oxidative radicals, alteration of immunologic reactivity (HLA-DR), synthesis of proteins and functional proteins (procalcitonin (PCT), C-reactive protein (CRP), other acute phase proteins), induction of apoptosis). To determine the course and extent of systemic inflammation is therefore crucial to assess the risk profile of a patient, therapeutic success of such measures and prognostic significance of the illness. Without additional parameters, the assessment of the degree of inflammation based simply on clinical symptoms or on the development of organ dysfunction is often unreliable. There are few parameters used in clinical diagnostics that are closely associated with the degree of inflammation including interleukin-6, interleukin-8, PCT and with certain limitations also C-reactive protein (see Table 3). To date, most of the investigations are limited to case reports or cohort studies with underpowered sample size, thus the quality of the studies are often compromised. The use of different definitions for sepsis and lack of continuous daily parameters during the course of illness are partly accountable for conflicting findings. Well designed, controlled studies with convincing statements concerning sensitivity, specificity and receiver operating characteristics (ROC) are still rare. A variety of parameters are not currently used for diagnostic purposes in routine health care due to limitations such as very short biological half-life, lack of standardized testing, or accumulation in renal insufficiency. Although numerous bioactive peptides could possibly be used as bio-markers during the course of sepsis, many of them are not reliably detected by conventional assays due to their receptor-related protein-binding sites and degradation by proteases (in vivo and in vitro). For these reasons, many parameters have only a limited value e.g. markers of the endothelium, and endothelial dysfunction (adhesion molecules and their ligands), cytokines such as TNF-α and IL-1, various acute phase proteins (transferrin, LBP), PMN elastase, neopterin, phospholipase A2, platelet-activating factor acetylhydrolase (PAF-AH), complement C3a, and others. In clinical practice, some non-specific parameters may be helpful as part of a routine laboratory monitoring and should not be underestimated. These include clotting parameters (Quick, PTT, platelet count) and metabolic parameters and classical signs of inflammation (lactate, leucocytes, temperature).

Procalcitonin (PCT)

PCT (13kDa) is a precursor of the hormone calcitonin and is normally produced by the C-cells of the thyroid gland. In healthy individuals PCT is released into the blood stream in form of calcitonin only after endopeptide cleavage. Although plasma levels of PCT are very low under normal conditions (< 0.1 ng/ml), they may rise up to 5,000–10,000 fold in patients with severe sepsis while the plasma calcitonin concentrations remain within the normal range (52). Unlike calcitonin which has a half-life of about 10 minutes, PCT has an increased half-life of ~24 hours.

Although the exact biological role of PCT and its origin are still not clear, it appears that extrathyroid tissue is also able to secrete PCT systemically and secretion generally correlates well with the degree of sepsis. There are several conditions that may stimulate PCT release including bacterial infection and non-infectious stimuli, e.g. major surgery, multiple trauma and burns, though the increase is most pronounced in severe sepsis and septic shock. PCT is detectable in the blood as early as 2 hours after the microbial stimulus and therefore is "faster" than CRP, but "slower" than the cytokines (53). PCT has a very high biostability *ex vivo* with over 90% of activity detectable in plasma samples even after 12 hours storage at room temperature. Moreover, PCT obviously does not bind to receptors or other proteins, and so indeed can be captured by conventional immunoassays.

A comprehensive meta-analysis confirmed the role of PCT as a helpful biomarker for the differentiation of sepsis and systemic inflammatory response syndrome (SIRS) with non-infectious origin in critically ill in 2013. However, the confinement is the case history, comprising medical examination, and microbiological assessment (54). 30 individual studies with in total 3,244 patients were examined. Bivariate analysis resulted in a medium PCT sensitivity of 0.77 (95% CI 0.72–0.81) and a specificity of 0.79 (95% CI 0.74––0.84). ROC curve analysis unfolded an area under curve of 0.85 (95% CI 0.81–0.88). A further meta-analysis of seven studies with in total 1,075 patients suffering from sepsis and septic shock came to the conclusion, that PCT-driven therapy is suited to support antibiotic regimens and surgical interventions but without significantly influencing overall mortality (55): whether hospital nor 28-day mortality differed between the groups of patients with PCT-driven therapy and with standard care. However, length of antibiotic adminis-

tration was significantly shortened within the first group, although length of ICU- and hospital stay did not vary in both groups.

Record of PCT leves in serum at an early stage are recommended within the current sepsis guidelines to rule out severe sepsis and to consequently confirm diagnosis (56). Conversely, in case of localized infections without systemic inflammation, there is only a slight increase of PCT levels despite considerably increased CRP values. Likewise, PCT concentrations decrease in patients with severe sepsis following an adequate therapy even when the focus is not completely eradicated. In these patients, however, further antibiotic treatment or surgical restoration of the focus may be needed despite normal PCT concentrations. While the predictive value of PCT in critically ill patients has been demonstrated in several studies, this is not the case for TNF-α, IL-6, or CRP.

High PCT levels are closely associated with the occurrence of organ dysfunction distant from the site of infection. This marker seems able to distinguish an infectious from a non-infectious etiology of generalized inflammation, which in turn may have far-reaching therapeutic implications. PCT plasma concentrations are also increased in case of hemodynamic failure e.g. due to a translocation of endotoxin after major surgery, polytrauma, or cardiogenic shock. In this situation daily monitoring of PCT plasma concentrations can be helpful for early detection of possible septic complications.

Control of antimicrobial therapy and monitoring of therapeutic interventions

Strategies to reduce ICU mortality rate in patients with bacteraemia and sepsis should include an individual early risk profile and correct intravenous antibiotic therapy based on microbiological resistance patterns (57). Antibiotic therapy should be initiated rapidly, possibly within an hour after the onset of severe sepsis or septic shock (58). The German Sepsis Society (DSG) recently recommended a regular evaluation of antibiotic regimes every 48-72 hours based on clinical and microbiological criteria in order to narrow down the antimicrobial spectrum, the risk of resistance, the toxicity, and the duration of antibiotic therapy depending on clinical response and thus reduce costs (58). These measures are in practice often difficult to implement because the existing "clinical and microbiological" criteria are still inadequate. An improved diagnostic is therefore highly recommended. New and more sensitive PCT assays with a functional analytic sensitivity of 0.04 ng/ml allow for diagnosis and monitoring of non-septic weak infections in threshold ranges below 0.5 ng/ml. A randomised study with patients with community-acquired sepsis showed that length of antibiotic regimen could be decreased from 13 to 6 days by application of a PCT-driven algorithm without compromising the patient's outcome (59). The same authors proved a significant reduction of length of antibiotics administration and dosage after one-time PCT determination in 243 medium ill patients with ventilator-associated pneumonia (83% vs. 44% and 12.8 vs. 10.9 days, respectively) (60). The results may not be transferable onto critically ill but suggest the potential of such algorithms.

In 2009, the ProHOSP study confirmed a significant reduction of antibiotics usage and antibiotic-associated side-effects in the group of PCT-driven therapy in a study with patients suffering from infections of the lower respiratory tract (61). The algorithm used had provided defined threshold values for administration and stop of the anti-infective regime.

In another prospective randomized study conducted by the University Hospital Geneva, it was demonstrated for the first time that a protocol based on serial PCT measurement compared to a routine decision algorithm could reduce antibiotic treatment duration by 3.5 days (median) in patients with severe sepsis without apparent harm. Furthermore, the length of ICU stay in patients assigned to the PCT group was reduced by 2 days (62).

Interleukin-6

Interleukin-6 and interleukin-8 show positive correlation with severity and prognosis of patients with sepsis. In patients with sepsis, interleukin-6 (IL-6) concentrations increase 1,000-fold. Furthermore, increased IL-6 values over 1,000 pg/ml have been proposed as an indication for an immunomodulatory monoclonal antibody therapy. In the MONARCS study, a randomized controlled sepsis trial on monoclonal anti-TNF, increased serum IL-6 levels over 1,000 pg/ml were associated with a greater risk for organ dysfunction and mortality (mortality 47.7% in IL-6 > 1,000 pg/ml vs. 28.6% for IL-6 < 1,000 pg/ml) (63). Interestingly, in the PROWESS study that led the regulatory authority to license the recombinant human activated Protein C, high IL-6 levels (600 pg/ml) did not show prognostic significance in the post-hoc analysis (64).

Unlike TNF-α, the detection of IL-6 is not affected by the presence of a soluble receptor. The marked inter-individual variations in plasma IL-6 release due to phase-dependent activation of anti-inflammatory cytokines suggest that IL-6 measurements have relevance for the intra-individual assessments during the course of illness. Furthermore, the specificity of IL-6 is rather low in case of severe infections but the factor

may display advantages in neonatal sepsis due to a faster induction.

C-reactive protein (CRP)

C-reactive protein is an acute-phase protein produced by hepatocytes, which is activated by IL-6 or IL-1 and may act as a mediator and inhibitor of inflammation. It activates the complement cascade reaction after binding to bacterial polysaccharides or lysed cell membranes in response to infection and trauma. C-reactive protein prevents neutrophil adhesion to endothelial cells, inhibits the generation of superoxide anion and increases IL-1 receptor antagonist. CRP is the most commonly used biochemical marker to measure both the presence and amplitude of the inflammatory response. A high positive predictive value of CRP has been reported in a study of sepsis patients, e.g. SIRS due to an infection but without organ dysfunction (58). In other studies of patients with severe sepsis, e.g. with organ dysfunction, however, CRP failed to show meaningful correlation both for early diagnosis as well as in assessment of severity. In contrast to cytokines and PCT levels, the levels of CRP peaked only after 24 hours (62).

CRP concentration increases in response to mild microbial infections and in a variety of diseases. Elevated CRP concentrations over several days are usually observed even after successful eradication of the infectious focus. CRP has been shown to be a valuable enrichment for the monitoring of inflammatory change of local infections during effective antibiotic treatment.

CONCLUSION FOR PRACTICE

Inflammatory markers are recognized to play an increasingly important role in the diagnosis and monitoring of sepsis. This is partly due to low specificity of clinical symptoms and conventional inflammatory signs (high or low temperature, leukocytosis, low platelets) for the diagnosis of sepsis but also to a lack of correlation with the severity of the inflammatory response. At present, a combined approach can facilitate improved diagnosis and monitoring of sepsis. Clinical signs, scoring systems of organ dysfunction (e.g. SOFA score) and inflammatory markers can be used for diagnosis and follow-up. The potential clinical usefulness of current markers can be outlined as follows: (i) sensitivity and specificity of PCT for sepsis is better than that of CRP, IL-6, IL-8 and conventional parameters like leucocyte count and body temperature. (ii) to date it is the only sepsis marker that is helpful in the differentiation between infectious and non-infectious causes of organ dysfunction and shock. (iii) several studies indicate that PCT is much more elevated in bacterial infections compared to viral infections. (iv) a number of studies demonstrate that the course of PCT serum levels represent the success of infection or sepsis treatment early on and better than CRP. Furthermore, more recent studies strongly suggest that in patients with suspected respiratory tract infections and patients with community acquired pneumonia PCT is helpful to guide antibiotic therapy. PCT guided indication for antibiotics resulted in a 50% reduction in the use as well as in the duration of therapy with antibiotics.

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Abbreviations

BC — blood culture
cfu — colony forming units
CRP — C-reactive protein
ICU — intensive care unit
IL-6 — interleukin-6
NAT — nucleic acid amplification techniques
PCT — procalcitonin

Sažetak

BIOMARKERI SEPSE I METODE ZA DETEKCIJU PATOGENA

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Hemokultura je od velike važnosti za pacijente intenzivne nege kod kojih se sumnja na sepsu ili infekciju organa. Poznavanje etiološkog agensa (bakterija ili gljivica) i njegove osetljivosti na antimikrobne lekove

omogućava lekaru da inicira odgovarajuću antimikrobnu terapiju i sprovodi odgovarajuće dijagnostičke procedure. Ovo je pokazalo da smanjuje smrtnost, dužinu boravka na intenzivnoj nezi i preteranu upotrebu antibiotika. Dok je praksa mikrobiološke laboratorije visoko standardizovana, nedostaci u preanalitičkim procesima u intenzivnoj nezi (indikacija, pravovremensot, prikupljanje krvi) imaju značajan uticaj na dijagnostiku.

Zbog nedostataka molekularne dijagnostike, kao što je na primer PCR detekcija patogena čija je senzitivnost diskutabilna, dužeg čekanja rezultata, nepostojanja rešenja za utvrđivanje sveobuhvatnog antibiograma, još uvek aktuelne diskusije oko ovog panela, velikih troškova i nepostojanja podataka o kliničkom značaju, NAT testiranje još uvek ne predstavlja alternativu hemokulturi.

Inflamatorni markeri igraju značajnu ulogu u dijagnozi i praćenju sepse. Razlog tome je delimično nespecifičnost kliničkih simptoma i konvencionalnih znakova zapaljenja za dijagnozu sepse, ali takođe i nedostatak njihove korelacije sa težinom inflamatornog odgovora. Povišen nivo prokalcitonina (PCT) u serumu je siguran indikator sistemskog zapaljenja. PCT je jedini marker sepse koji je od pomoći u diferencijaciji između infektivnih i neinfektivnih uzroka disfunkcije organa i šoka i može podržati antibiotsku terapiju.

Ključne reči: Infekcija krvi, kultura krvi, sepsa, antimikrobna terapija, molekularna dijagnostika.

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THE ROLE OF IgM-ENRICHED INTRAVENOUS IMMUNOGLOBULIN IN TRANSPLANTATION

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Abstract: After organ transplantation, gamma globulin and intravenous immunoglobulin enriched with IgM are most frequently used in septic shock as early immune-support. If the explanted organ is infected, the transplantation, as a life-saving operation, can be performed if there is no systemic inflammation and the patient receives IgM enriched immunoglobulin prophylaxis during surgery. The period after transplantation can be divided into three parts from the infection point of view: the first month after transplantation, the first sixth months and the following six months. Infections within the first month are basically related to the surgical procedure. Because of the immunosuppressive therapy, the opportunistic and fungal infections are more common during the first sixth months. After this period, the occurrence and the type of infections are similar to that of the non-transplant population except for pulmonary infections. The latter is two to three times more frequent. This is explained by the secondary hypogammaglobulinaemia (lower blood levels of IgM and IgG) which is caused by the steroids but most of mycophenolate mofetil by inhibition of the T and B lymphocyte proliferation. Septic shock develops with a continuing fall of IgM levels. Under these circumstances additional intravenous immunoglobulin therapy with IgM can be lifesaving. Besides, immunoglobulin concentrates with IgM may also be used in the case of viral infections without prophylaxis and/or without etiological therapy such as in the case of West Nile virus infection. As a result of the increase in antibiotic resistance, the application of immunotherapy, including immunoglobulins may become the mainstream in the treatment of septic shock.

Key words: immunoglobulin, IgM, septic shock, transplantation.

INTRODUCTION

The immunoglobulin therapy started more than 100 years ago with the use of anti-diphtheria serum from horse and continued with the successful treatment of primary and secondary immunodeficiency with intravenous immunoglobulin (IVIG) products. Later on, this was augmented by the use of gamma globulin in the treatment of Guillan Barré syndrome, Kawasaki disease, chronic demyelinating polyneuropathy and different autoimmune diseases. Currently, the use of IVIG is integrating evidence-based practice in this field (1). The use of immunoglobulin in severe sepsis, septic shock is controversial (2, 3, 4), but a recent Cochrane analysis from 2013 highlighted the favorable effects of early treatment (5) (Table 1).

Monserrat et al. proved that the B lymphocytes reduced CD23 and elevated CD80 production in septic patients, which may result in high mortality rates (6). Moreover, the altered rate of immunoglobulin production also showed effects on outcome in sepsis: IgG1,

Table 1. *Effects of intravenous immunoglobulin*

Neutralisation of toxins
• Endotoxin, exotoxin, Gram negative bacterium: molecular patterns • Reception of endotoxin by the liver and the spleen • Decreases the bacterium's adherence to other organs
Leukocyte stimulation and increased bactericide effect
• Neutrophil: increased phagocytosis • Increased opsonization • Increased oxidative bactericide effect on T lymphocyte • Kuppfer cell increased phagocytosis
Inflammatory cytokine effect regulation
• decrease of pro-inflammatory cytokine • Increase of anti- inflammatory cytokine
Complement effect modulation
• C4, C3 effect reduction through leukocyte

total IgG, IgM and IgA have an independent protective role in severe sepsis and septic shock (7, 8).

During the last decade, the number of nosocomial infections caused by multiresistant pathogens increased significantly and further increase is expected. The development of antibiotics can only hardly or not at all keep up with the change of resistance, thus sooner or later the need for the wider use of immunoglobulins may become a reality (9, 10). It is important to note, that the guidelines in 2013 also recommend IVIG for the therapy resistant or severe Clostridium difficile infections (11).

In the field of transplantation, immunoglobulins are also used for the treatment of immunological diseases with infectious or non-infectious causes. One option for the treatment of non-infectious diseases is the therapy of antibody-mediated rejections or for example desensibilisation (12). The other option is the adjuvant treatment of transplanted patients, which is usually performed with the use of gamma globulin or intravenous immunoglobulin enriched with IgM. The early use of immunoglobulins in septic shock in transplanted patients is logical because of the immunosuppression (Figure 1).

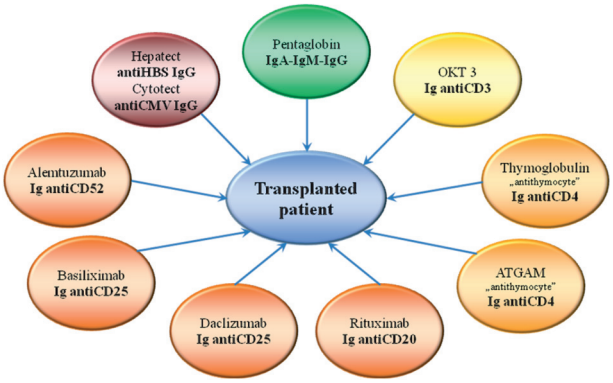

Figure 1. *Immunoglobulin products used with transplanted patients*

INFECTIONS DURING TRANSPLANTATION

Immunosuppression of the recipient can clearly be verified before transplantation based on the major, minor and laboratory criteria of immunodeficiency (13). Infections during transplantation occur because of the immunodeficient state due to the end-stage organ failure and the transitory ceasing of the barrier functions protecting the integrity of the body during the surgical procedure. Kidney failure and its treatment correlates with the reduced function of specific and non-specific protecting mechanisms. In case of peritoneal or hemodialysis the insufficiency of the non-specific protecting mechanisms may develop. The impairment of the specific protecting components occurs during the contact with the dialysis membrane (loss of complements, leukocyte dysfunction, IgG and IgM reduction due to the increased loss of proteins), but the reduction of the cellular immunity is due to the direct effects of uremia (14). Acute liver-failure as well as end-stage cirrhosis also implies immunodeficiency. The immunological role of the liver as we know is extremely important: the Kuppfer cells of the liver eliminate endogen bacteria from the portal vein, the liver synthetizes 90% of the complement cascade and the liver eliminates the extrahepatically opsonized bacteria. In liver-failure both the production of complements which take part in the opsonization, and the elimination of the opsonized organisms are severely impaired. In 80% of patient suffering from end-stage liver failure severe infections can be found, and in quarter of these patients have detectable bacteremia. The bacterial invasion's primary places of occurrence are the respiratory, urinary and peritoneal space.

In case of the intestinal flora-originated bacteremia (Escherichia coli, Klebsiella) the bacteria get through to the peritoneal space, where in the presence of ascites spontaneous bacterial peritonitis (SBP) can develop. The diagnosis is based on the increased number of leukocytes in the ascites (in ascites > 250 cell/μl) or the direct detection of the pathogen from the ascites (15).

The relationship between end-stage heart and lung disease and infection is a long known fact. There is weaker protection due to impaired circulation and oxygenation, which results in the disorder of the natural, not specific immunity which provides weaker protection against aerobic and anaerobic pathogens.

Infection occuring during the operation is caused by the breakthrough of bacteria through barriers protecting the body's integrity. The translocation is also helped by the transitory changes in the perfusion and oxygenation during the operation (14). Furthermore, there is a possibility of the donor organ carrying infection. When the focus of the infection is localized in the organ waiting to be removed and there is no systemic inflammation, hence the focus is inactive, under well-defined circumstances the transplantation as a live-saving operation can be performed. In this case the IgM-enriched immunoglobulin prophylactic therapy is recommended only during the time of the surgical procedure. A typical example is the cholangitis in patients suffering from ulcerative colitis causing cirrhosis and sometimes occurring with abscesses, or the primer biliary cirrhosis where in the recipients in the portal triads fragments of translocated bacteria and IgM can be identified together (16). Prior to transplantation the acute infection must be ruled out. In the literature there are case studies indicating that in certain cases it is pos-

sible to go astray from the guidelines. For example in a patient suffering from acute bacterial infection the transplantation can be performed, but it can only be done under the protection of IgM-enriched immunoglobulin. In addition the infection can be completely obliterated until the 5th postoperative day or until when the combined immunosuppression is being built up (7, 17). There are Hungarian experiences as well in this field. We recently treated a 30 year old mother of three children, whose antibiotic treatment for a right sided pneumonia lasted 3 months after the delivery of the third child, caused acute liver failure. Liver transplantation was performed along the specific antibiotic therapy and IgM-enriched immunoglobulin treatment, which was successful and the mother is still alive.

INFECTION AFTER TRANSPLANTATION

The period after solid organ transplantation (SOT) can be divided into three parts from the infections point of view: the first month after transplantation, the first and the second sixth months. The "golden rule" is that the development of rejection indicates development of infection and the development of infection indicates development of rejection (15, 18). The timescale of the transplanted patients' infections offers help in differential diagnosis and in planning the effective prophylaxis and empirical atibiotic therapy. It has a particular importance in the perioperative period, if there are other surgical procedures performed for other reasons (Figure 2).

The 1st month

In case of infections during the first month the possibility of transition from the donor arises, but the infections generally correlate to the surgical procedure as well. The use of complex immunosuppression therapy begins immediately after surgery and requires time, therefore the early postoperative infections are mainly unrelated to the immunosuppressed state. The prevalence and type of infections match the not immunosuppressed patient's infections associated to surgical procedures. In this period pneumonia, urinary and biliary tract infection related to catheter, drain or intravenous devices can occur. In case of liver-transplanted patients, due to the surgical interventions of choledochotomy, choledochojejunostomy, the ascending type biliary infections are the most frequent, in cases of kidney-transplanted patients the urinary tract infections, in heart-transplanted patients mediastinitis or the aorta suture's fungal infection, in cases of lung-transplanted patients the bronchial anastomotic insufficiency or mediastinitis can develop (14). During the first weeks af-

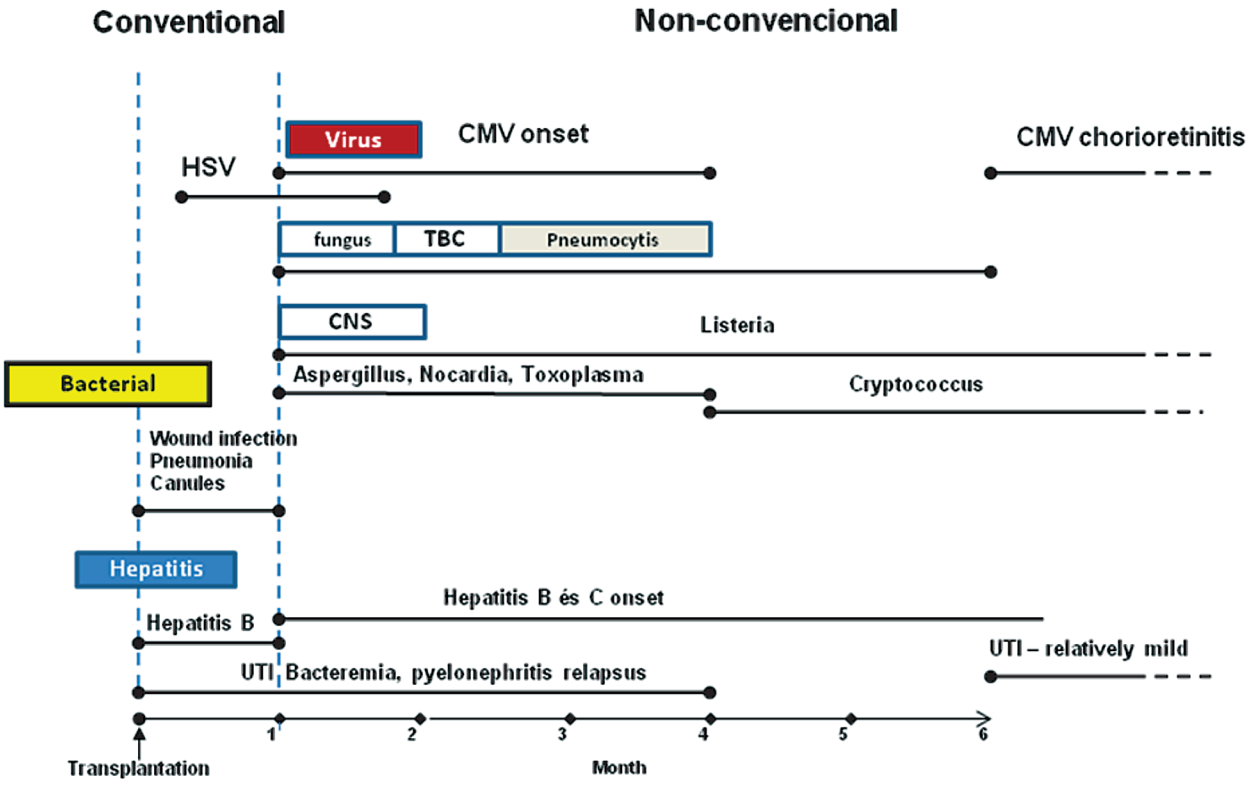

Figure 2. Occurrence of infections in timely order after transplantation

* CMV: cytomegalovirus; HSV: herpes simplex virus; CNS: central nervous system; TBC: tuberculosis; UTI: urinary tract infections; VZV: varicella zoster virus

ter transplantation acute rejection can occur, of which treatment consists of usually administration of large dose steroids or anti-lymphocyte globulin. In this case the "over" immunosuppression is in favor of the appearance of the more severe opportunistic infections. The chronic lymph loss in the first few days caused by the damage of the lymphatic vessels during the operation could play a distinct role in the development of reduced immunoglobulin levels in the immediate postoperative period. These can cause immunosuppressed state by themselves and with addition of the drug-induced immunosuppression. Together they surely cause "over" immunosuppression, although separately they don't (19, 20) (Table 2).

Moreover, steroids but mostly the T and B lymphocyte proliferation inhibitor mycophenolate mofetil also contributes to the reduced IgM and IgG blood levels. So it is logical that during the treatment of infections based on surgical complication, polytransfusion or systematic disease in the early stages of septic shock parallel with the application of the sepsis guidelines IgM-enriched immunoglobulin therapy can also be used.

From th 1st to the 6th months after transplantation

The period between the first and sixth months after transplantation is when the nature of the transplanted patients' infections changes. The immunosuppression applied during transplantation inhibits the acquired immunity and is heterogenic and its complexity is given by the possibility of the combinations. We inhibit the antigen recognition with the help of steroids, the proliferation with the help of mycophenolate mofetil. The inhibition of the cytokines' production is the most commonly used option to prevent rejection and lately it is often combined with co-stimulation blockade. The effect of the combined immunosuppression is strongest between the first and sixth months after transplantation, therefore the classic opportunist infections are to be expected during this period: they develop in 33–68% of liver-transplanted patients, in 54% of lung transplanted patients, in 47% of kidney transplanted patients, in 35% pancreas transplanted patients and in 21–30% of heart transplanted patients (Figure 1).

Out of the opportunistic viral infections the members of the herpes family have the highest significance, their main representative is the herpes type B cytomegalovirus infection (CMV). The CMV infection with a prolonged epidemic-like occurrence affects 10–50 % of the transplanted population. Its development depends on the donor's and the recipient's serological status, the characteristics of immunosuppression, the human leukocyte antigen (HLA) accordance and the

Table 2. *The protein and immunoglobulin content of ascites after liver-transplantation (laboratory test results, own material). Depending on the daily loss, this lack/deficit can cause immunosuppression*

Parameter	Value measured from ascites (g/L)	Serum normal value (g/L)
Total protein	44.3	60–85
IgA	0.83	0.7–4.0
IgG	2.84	7.0–16.0
IgM	0.41	0.4–2.3

type of the transplanted organ. It can appear as a mild or moderately severe "flu-like" syndrome, progressive colitis, hepatitis or gastritis, leukopenia, pneumonia or disseminated viral disease. Besides the direct effects of CMV the indirect effects are also very important. The CMV infection through its effects on the immune system raises the chance of opportunistic infections, e.g. in the presence of CMV infection the number of invasive fungal infections increases by 5–6–fold (21).

Among the other members of the human herpes virus family the HHV6 and HHV8 are the most important, which can cause for example pneumonia, encephalitis or myelosuppression. Their harmful effect as a cofactor is mostly set forth through immunomodulation, making the appearance of opportunist pathogens easier. One clear example for this is the role of Kaposi sarcoma-associated herpes virus (KSHV-HHV8) in the development of the transplantation-related late Kaposi sarcoma (21).

After transplantation in seronegative patients the varicella-zoster virus (VZV) can cause varicella, in seropositive patients it can cause herpes zoster. To prevent infection there is a blood test before the transplantation and depending on the result vaccination is advised. In bacterial or fungal superinfections immunoglobulin therapy should be considered (22).

Invasive fungal infections can originate from both endogen and exogen flora. The infection's progression is fast, therefore the initiation of the early empirical therapy is very important. Besides the adjuvant use of IgM-enriched immunoglobulins is an important and common sense part of the therapy. The aspecific clinical picture causes great difficulties in early diagnosis. The appearance of the non-albicans Candida, Aspergillus species and Fuzariums have become more and more common, which cause higher than average mortality among the immunosuppressed.

The incidence of Pneumocystis jiroveci is 3–11% without prophylactic treatment, it primarily causes pneumonia among the transplanted. It develops mainly in

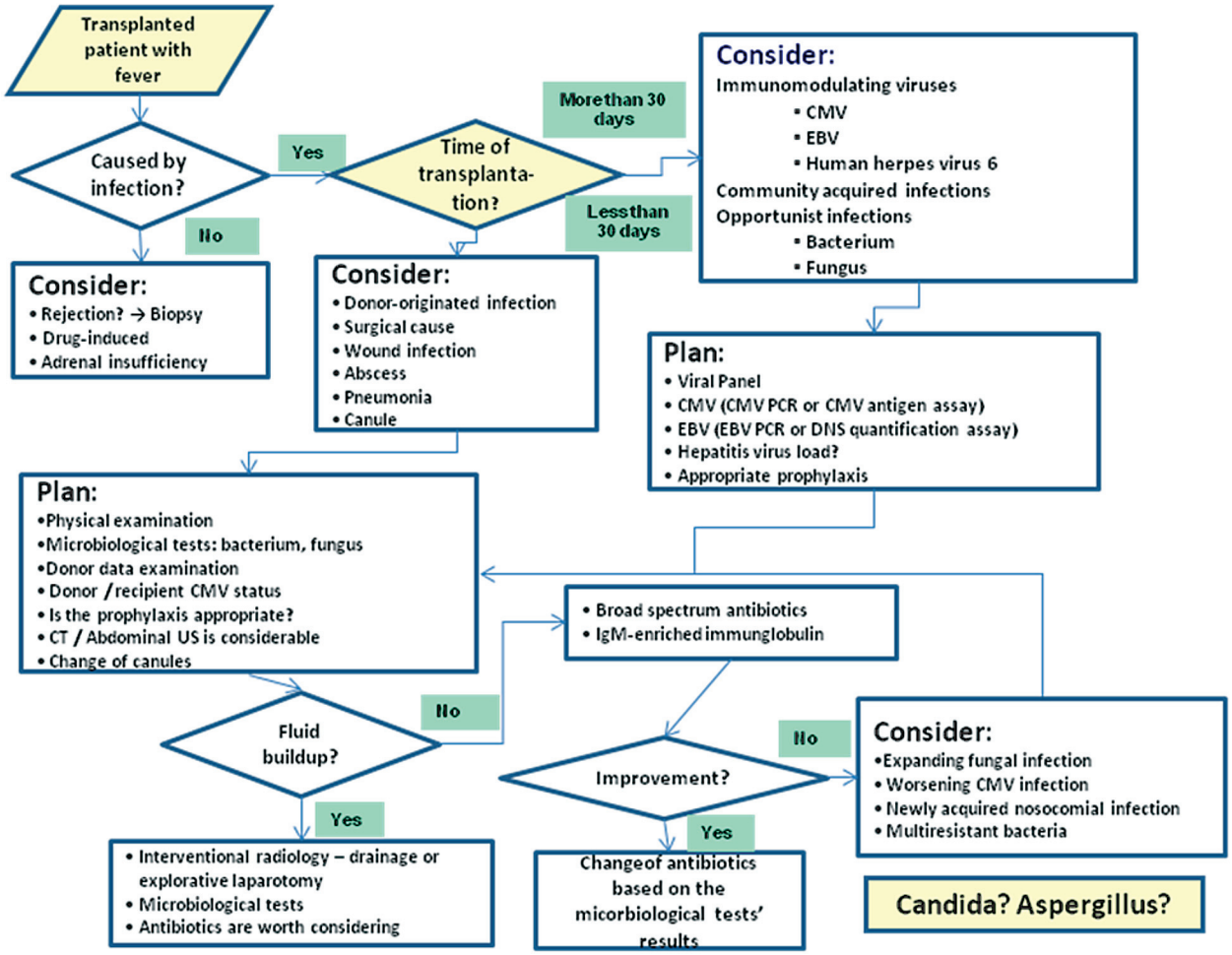

Figure 3. *Supposed infections' diagnostics and treatment after the transplantation of parenchymal organ*

*EBV: Epstein-Barr virus; CMV: cytomegalovirus; PCR: polymerase chain reaction

patients, who belong to the high risk group. The late diagnosis and late treatment causes high mortality here as well, but it can be easily prevented with prophylaxis (sulfonamid/ trimetoprim, in case of allergy pentamidin).

During the second period after transplantation (1–6 months) the opportunistic infections from the environment are also common (Nocardia, Listeria and Legionella infection), and unlike Mycobacterium, they don't cause infection through reactivation.

In severe sepsis and septic shock the use of early IgM-enriched immunoglobulins within the first 1–2 days is recommended. In patients suffering from severe sepsis, septic shock, the delay in the start of Pentaglobin® raises the mortality by 2.5% in every 24 hours (23, 24). This raises the mortality of transplanted patients with an even higher ratio.

Six months after transplantation

5–10% of the SOT patients develop rejection or chronic graft insufficiency on an immunological basis. Among these patients the immunosuppressive therapy is more pronounced and the therapy against rejection is also more common. It causes deeper, longer immunosuppression with all of its consequences, among which the prolonged presence of opportunistic infections are important to note. 10% of patients with chronic rejection suffer from some kind of chronic, progressive infection as well. Aspecific infections, e.g. nocardiosis, aspergillosis may develop only from a significant environment-originated exposition. On the other hand viral infections are also common (hepatitis C, B virus; cytomegalovirus; Epstein-Barr virus; papillomavirus), which cause damage to the implanted organ and have a role in the development of tumors. In the period following the sixth month after transplantation 80% of SOT patients have good graft function and receive immunosuppressive treatment at the lowest possible dose. In this case the type of infections and their incidence usually equals to the non-transplanted population, and only differs in respiratory tract infections. The cause of this is the secondary hypogammaglobulinemia (IgG: 400–700 mg%), which is caused by the mycophenolate mofetil (MMF) blood levels, which inhibit the T and B lymphocyte

proliferation (25). In these cases the MMF can be changed to an alternative drug, and monthly IVIG therapy can be given during the follow-up. Along the humoral "over" immunosuppression the prevalence of the pulmonary infections is 2–3–fold of the normal. Obviously if the same kind of patient arrives to the intensive care unit in the state of septic shock, the MMF therapy is to be stopped and a few days of IgM-enriched intravenous immunoglobulin therapy is recommended (26).

Although the course of bacterial infections look similar to that of in the non-immunosuppressed population, the course of certain viral infections can be more aggressive among the transplanted patients. In patients without prophylaxis or without definitive therapy (e.g. West Nile virus infection) the only treatment we can offer is the IgM-enriched immunoglobulin therapy as in addition to supportive therapy (27).

From a practical point of view it is important to be aware of the applied immunoglobulin product's characteristics: process of production, the method of conservation and most importantly, the pathogen specific antibody content. Regarding the patient, we have to know the plasma immunoglobulin content, and the answer to the three most important questions of the immunoglobulin therapy: when, how much, for how long. The details of a septic transplanted patient's immunosuppressed state must be known: its nature, its depth, its severity and for how long it has lasted, emphasizing the disorder of the cellular immunity. During the performance of microbiological tests we have to aim for the direct detection of the pathogen with histological, microbiological or with other antigen tests (punctions, bronchoalveolar lavage, biopsy). It is extremely important to start the combined broad spectrum antibiotic, antimycotic therapy immediately after the samples for microbiological tests are collected. With the use of laboratory tests in reasonable cases (MMF, steroids) the serum immunoglobulin levels and the levels of immunosuppressant must be determined and with its daily monitoring the doses must be reduced. The use of steroid monotherapy is possible if the graft function can be replaced with supportive therapy (e.g. in kidney-transplanted patients: hemodialysis). In case of a graft which stands for a vital organ the MMF is to be immediately stopped, other immunosuppression must be reduced to the border of rejection, and early use of IVIG or IgM-enriched immunoglobulin therapy must be started to strengthen immunity. Knowing the results of the microbiological tests (most likely the 3-5th day of therapy) antibiotics should be de-escalated, and the immunoglobulin therapy can be stopped (28, 29). The occurrence of potential side-effects, e.g. the kidney-failure, aberrant immunothrombosis, hemolysis, should be checked regularly (30) (Figure 2).

CONCLUSION

The use of intravenous immunoglobulins in patients undergoing SOT shows a growing tendency worldwide, and one of the main reasons is to aid the host response to infection. The general concept of organ support in the critically ill is valid in transplanted patients too, but the therapeutic window is smaller. Therefore, in cases of infections in transplanted, immunodeficient patients, the cornerstones of the treatment are reducing the degree of immunosuppression, starting empirical antimicrobial therapy as soon as possible and the use of IgM-enriched immunoglobulin therapy, besides the specific diagnostic and therapeutic interventions.

Abbreviations

CMV — Cytomegalovirus
CNS — Central nervous system
EBV — Epstein-Barr virus
HHV — Human herpes virus
HLA — Human leukocyte antigen
HSV — Herpes simplex virus
IVIG — Intravenous immunoglobulin
KSHV — Kaposi sarcoma-associated herpes virus
MMF — Mycophenolate mofetil
PCR — Polymerase chain reaction
SBP — Spontaneous bacterial peritonitis
SOT — Solid organ transplantation
TBC — Tuberculosis
UTI — Urinary tract infections
VZV — Varicella-zoster virus

Sažetak

ULOGA IgM-OBOGAĆENIH INTRAVENSKIH IMUNOGLOBULINA U TRANSPLANTACIJI

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Nakon transplantacije organa, gama globulini i IgM-obogaćeni intravenski imunoglubulini su najčešće korišćeni u terapiji septičkog šoka kao rana imuno podrška. Ako je izvadjeni organ inficiran, transplantacija, kao operacija od vitalnog značaja, može biti izvedena ukoliko nema sistemske upale i pacijent primi IgM obogaćene imunoglobuline kao profilaksu tokom hirurškog zahvata. Period nakon transplatancije organa može biti podeljen na tri perioda u odnosu na infekciju: prvi mesec nakon transplantacije, prvih šest meseci nakon transplantacije i sledećih šest meseci. Infekcije u prvih mesec dana su u osnovi povezane sa hirurškom procedurom. Zbog imuno-supresivne terapije, oprtunističke i gljivične infekcije su češće tokom prvih šest meseci. Nakon ovog perioda, učestalost i vrste infekcija su slične onim kod populacije koja nije imala transplantaciju, osim plućnih infekcija, koje su dva do tri puta češće. Objašnjenje je sekundarna hipogamaglobulinemija (niži nivo u krvi IgM i IgG antitela) koja je prouzrokovana steriodima, ali najčešće mikofenolat mofetilom usled inhibicije proliferacije T i B limfocita. Septički šok razvija se sa kontinuiranim padom nivoa IgM antitela. Usled ovih okolnosti dodatna intravenska imunoglobulinska terapija sa IgM antitelima može biti od životne važnosti. Osim toga, IgM obogaćeni imunoglobini mogu se koristiti kod virusnih infekcija bez profilakse i/ili bez etiološke terapije, kao što je slučaj kod infekcija izazvanih virusom Zapadnog Nila. Kako je aktuelan porast rezistencije na antibiotike, primena imunoterapije, uključujući i imunoglobuline, može biti osnova u lečenju septičkog šoka.

Ključne reci: imunoglobulini, IgM, septički šok, transplantacija.

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PROTECTIVE MECHANICAL VENTILATION IN PATIENTS WITHOUT OR WITH LUNG INJURY

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***Abstract*:** The mortality of Acute Respiratory Distress Syndrome (ARDS) is still high from 27 to 45% according to Berlin definition. Even in surgical patients without lung injury, the postoperative pulmonary complications (PPCs) are frequent. Mechanisms of ARDS, ventilator associated lung injury (VALI) and PPCs are better understood. In ARDS, protective ventilation with low tidal volume 6 ml/kg PBW and higher levels of positive end-expiratory pressure (PEEP) is widely accepted as routine practice. In no ARDS patients undergoing mechanical ventilation, protective ventilation with low tidal volume 6ml/kg PBW and low to moderate levels of PEEP has become the new challenge paradigm shift of supportive care mainly in ICU and perioperative patients. Respiratory monitoring is very helpful for optimizing mechanical ventilator setting to prevent VALI and early detect PPCs during the perioperative period. Several scores have been developed to stratify the risk of ARDS, VALI and PPCs. It's time to apply basic physiologic knowledge of respiratory function and evidence based practice to improve ARDS and PPCs outcomes.

Key words: Ventilator-Associated Lung Injury, Acute Respiratory Distress Syndrome, Transpulmonary pressure, Positive End Expiratory Pressure, Protective ventilation, Respiratory monitoring.

INTRODUCTION

Using the original American-European Consensus Conference (AECC) definition of the Acute Respiratory Distress Syndrome (ARDS), the incidence of ARDS was vary from 5–33.8 cases per 100,000 populations (1). Several studies have shown the improvement of outcome in selected groups of ARDS and the ARDS related mortality was gradually decreased from 70% to 40% in the last decades.

According to the limitations of AECC definition of ARDS i.e. the decrease in usage of Swan-Ganz catheter to distinguish the presence of hydrostatic edema and poor sensitivity of PaO2/FiO2 ratio in different levels of positive end expiratory pressure (PEEP), the Berlin definition has been developed in order to yield better predictability for mortality than AECC definition, to be easily for clinical research implementation as well as to exclude the term acute lung injury for avoiding possible misinterpretation (2). Recent studies have demonstrated that various severities of ARDS according to Berlin definition are associated with the degree extravascular lung water index, pulmonary vascular permeability index by using transpulmonary thermodilution method (3) and the finding of diffuse alveolar damage at autopsy (4, 5). The mortality of ARDS is still high from mild to severe as 27 to 45% by Berlin definition (2).

Not only ARDS but also pulmonary complications particularly during perioperative period and in intensive care unit (ICU) with previously non-injured lung became an interesting issue. A recent, large European cohort study involving 46,539 patients who un-

derwent non-cardiac surgery have shown unpredictably high mortality as 4% (6) which may resulted from the high incidence of postoperative pulmonary complications (PPCs) (7). The incidence of PPCs varies according to the definitions of each study. PPCs were classified as respiratory failure from pulmonary or cardiac origin, pneumonia, respiratory infection, pleural effusion, atelectasis, pneumothorax, bronchospasm, need of non-invasive respiratory support and re-intubation as well as mortality. These complications are associated with underlying status, types of surgery or anaesthesia as well as the mechanical ventilator settings (7).

Several studies have shown that mechanical ventilation (MV) not only preserves the life but can aggravate ventilator associated lung injury (VALI). Recent studies have focused on the new strategies for treatment and prevention of ARDS and VALI (8, 9, 10).

In ARDS, protective ventilation is now widely accepted as the routine practice. In non-ARDS patients with MV, protective ventilation has become the new challenge paradigm shift of supportive care mainly in ICU and perioperative patients as well as heart beating organ donors (11). In this article, we aim to describe: 1) the pathophysiology of ventilator associated lung injury in ARDS and the PPCs in non ARDS patients; 2) the bedside respiratory monitoring tools; 3) the recent evidence of protective ventilator strategies in both ARDS and non ARDS patients as well as adjunctive therapies; and 4) the predictive scores of ARDS, VALI and postoperative pulmonary complications.

1. Mechanisms of ventilator associated lung injury and postoperative pulmonary complications

1.1 Atelectrauma, volume trauma, barotrauma

Ventilation at high lung volume has been shown yielding to alveolar rupture and barotrauma. Several experimental studies have demonstrated that the high MV pressure setting induces rupture of air space not from the absolute pressure itself, but from the degree of lung over distension. Thus, the end inspiratory plateau and tidal volume (VT) per ideal body weight are inadequate parameters to determine the real lung stress and strain. The major determinants of VALI are lung stress or distending force (depend on the applied transpulmonary pressure [PL]) greater than 20–24 cm H2O during inspiration and lung strain defined by the proportion of applied tidal volume plus inflated lung volume due to PEEP application and the resting lung volume (functional residual capacity) greater than 1.5–2 (12, 13). Even in healthy lung, experimental study suggested that long term high tidal volume ventilation with lung strain of 2.5 caused the animals died from respiratory failure (14). If the lungs are inhomogeneous, the applied force will mainly stress on the regions around collapsed and consolidated part which joining to those of opened part. This leads to the increase in stress and strain at wall of opening alveoli particularly in higher alveolar volume (pressure multipliers or lung stress raiser) (12). Lung inhomogeneity is associated with overall mortality and severity of ARDS (12).

During ventilation at low lung volume, repetitive tidal-cyclic opening and closing of alveoli and distal airway leads to shear stress so called atelectrauma. The surfactant dysfunction results in airway collapse hence atelectrauma by the force that generated by surface tension and consequence to an increase of stress and strain. The maneuver which aiming to decrease the lung inhomogeneity such as higher PEEP improved oxygenation and tended to decrease in mortality (15).

In addition to mechanisms associated with VALI, others mechanisms producing PPCs are: 1) general anesthesia, sedation and muscle paralysis caused a reduction of respiratory muscle tone, oxygen reabsorption lead to an increase in atelectasis as well as peripheral airway closure; 2) reduction of functional residual capacity; 3) altering of diaphragmatic position (cephalic shift of diaphragm); 4) redistribution of thoracic blood volume and 6) surfactant inactivation.

1.2 Biotrauma

Lung cells have mechanisms that allow them to deal with physiological deformations during normal breathing. Nevertheless, under mechanical ventilation, such forces might be excessive and lead to cell injury. The concept of biotrauma arised in the early 1990s, when several experiments revealed the existence of a relevant biological response to mechanical forces (16, 17). Biotrauma involves the local release of inflammatory mediators that reach the systemic circulation spreading the damage, an excessive activation of the immune system, as well as other numerous cellular responses triggered by mechanical forces (18). The numerous effects of these multiple biological responses greatly account for the high mortality of patients with lung injury and ARDS from multiple organ dysfunction syndrome (19).

1.2.1 Effects on alveolar epithelium, endothelium and extracellular matrix

The force derived from mechanical ventilation and over inflation involve a rearrangement of the cellular shape of alveolar cells, types I or II, and endothelium. In this context, cytoskeleton plays a crucial role,

since it provides a physical basis for translating mechanical forces into biochemical responses (mechanotransduction). Cytoskeletal reorganization, integrins and ion channels are major players of this process, activating a complex network of intracellular pathways that eventually result in extracellular matrix remodelling, recruitment of white cells, and cytokine release (18, 20). If stress and strain reach the limit of rupture of the fibre system, mechanical failure may occur with direct rupture of alveolar walls and pulmonary capillaries (19).

Toll-like receptors (TLRs) are innate immune sensors expressed by a range of immune cells, and also by epithelial cells (21). TLRs activate the transcription factor nuclear factor kappa B (NF-kappaB), which eventually results in an increased expression of inflammatory genes. The activation of NF-kappaB, which has been established in VALI (22), induces the production of inflammatory mediators like tumor necrosis factor (TNF) alpha, interleukins (IL) 6, IL-8 or IL-1â (19), which, among others, have shown to be importantly involved in the pathophysiology of VALI (23, 24). Importantly, cytokines and chemokines released by epithelial cells and alveolar macrophages induce the recruitment of neutrophils to the lung via IL-8 (19), which amplify tissue inflammation.

Molecules that constitute the extracellular matrix (ECM), such as hyaluronan, biglycan, versican, heparan sulfate, fibronectin ortenascin-C are important activators of TLRs (25, 26). These molecules have essential functions in many lung pathophysiological processes, as they regulate tissue hydration, macromolecular structure and function, response to inflammatory agents, and tissue repair (27). Current evidence shows that mechanical ventilation, not only at injurious but also at physiologic tidal volume, may deeply affect ECM structure and function (28, 29). In this context, ECM modifications may influence the altered mechanical behavior of the lung parenchyma and also contribute to the subsequent inflammatory events, since ECM constituents are among TLR endogenous ligands (27).

Oxidative stress is another important mechanism during biotrauma. Evidence from animal models suggests that oxidative stress and redox imbalance contribute to enhance/perpetuate susceptibility to VALI (30, 31). Lung alveolar and epithelial cells have showed an increase on reactive oxygen species production in response to elevated stretch (32). Elevated oxidant release in serum as well as decreased lung glutathione, the major antioxidant in the lung, were observed in rats ventilated at high tidal volume (20 mL/kg) (33). An increased superoxide production has been observed in ventilated patients (34) and, importantly, antioxidant strategies have shown beneficial effects in experimental models both at the local and systemic levels, attenuating VALI-associated inflammation, apoptosis and oxidative responses (35, 36).

Mechanisms of inflammation, ECM disruption or oxidative stress are, to a greater or lesser extent, eventually associated with cell death. VALI has been shown to induce apoptosis of airway epithelial cells (37). Interestingly, experimental studies showed that hydrogen inhalation as a therapeutic approach provided cytoprotective effects against apoptotic and inflammatory signaling pathway activation during VALI (36, 38).

1.2.2 Mechanisms of repair

Membrane disruption acts as a mechanotransducer, whereby the influx of calcium after membrane injury leads to the up regulation of mediators as and NF-kappaB. Deformation-induced plasma membrane disruptions have been directly linked to the activation of pro-inflammatory signaling cascades including early stress response genes, chemokine receptors, and adhesion molecules regulation. Plasma membrane homeostasis is a dynamic process, and deformation-induced lipid trafficking is an important cytoprotective mechanism employed by the cell in the face of externally imposed shape change. The balance of these pathways may be influenced by certain conditions that shift the equilibrium towards the favoring of either a pro-injury or pro-repair state (39).

Processes of re-epithelization and collagen degradation are essential in lung repair, and inflammatory cells can modulate both ECM degradation and epithelial cell migration through different mechanisms (40): release of proteases that cleave collagen, gelatin, and elastin; modulation of inflammation toward an anti-inflammatory response; and by directly releasing growth factors that stimulate epithelial cell migration and proliferation (41). González-López et al. have demonstrated that some of these phenomena take place during the repair phase after VALI, concluding that an adequate inflammatory response and ECM remodeling are essential for recovery (42). However, ECM degradation and remodeling are complex mechanisms. If inflammation of pro-fibrotic responses are exaggerated and unbalanced, the process can lead to fibrosis and organ failure (43).

Apart from repair focused on inflammation or ECM, new approaches are emerging. In this sense, several studies have shown promising results using mesenchymal stem cells, which have shown beneficial results, reducing VALI in animal models (44, 45, 46).

1.2.3 Biomarkers for VALI

The majority of biological markers identified in plasma, serum, pulmonary edema fluid, and bronchoal-

veolar fluid in experimental studies are cytokines and chemokines. None of them distinguishes VALI from other etiologies of lung injury. However, the temporal association between changes in levels of these proteins and changes in tidal volume or PEEP suggests a causative role (47).

The study of a panel of biomarkers has shown superior performance to single biomarkers. Abnormal levels of five biomarkers in plasma provided excellent discrimination for diagnosis of ARDS in patients with severe sepsis, being three of them generated by lung epithelium (48). Interestingly, a recent study has found that VALI is characterized by a particular metabolic profile that suggests alterations in energy and membrane lipids (49).

Although the definition of ARDS is based on clinical criteria, altered levels of plasma biomarkers may be useful to assist in confirming the diagnosis in certain cases, to categorize them, to facilitate selection for clinical trials as well as a valuable tool for identifying patients at risk, determining prognosis and understanding pathogenesis (47).

2. Respiratory monitoring

2.1 Respiratory mechanics and Esophageal pressure measurement

Generally we can monitor patients receiving MV by observing static airway pressure, measuring respiratory system compliance and analyzing dynamic pressure-volume curve. Terragni et al assessed the accuracy of plateau pressure and stress index to identify the point associated with injurious ventilation. This study demonstrated that plateau pressure (Pplat) more than 25 cm H_2O and stress index more than 1.05 have been shown the best performance to define the injurious ventilation (50). In terms of lung strain, the reference value computed by the ventilator is still controversial. Furthermore, the reference volume at Functional residual capacity (FRC) is the lung volume at zero PEEP level and should be at resting lung (pre-stressed condition) (12). Some studies have proposed the reference volume should be the end expiratory lung volume during PEEP application, which considered as continuous strain (12).

The proportion of airway pressure is contributed by the elastance of chest wall. Some conditions that increase chest wall elastance i.e. obesity, abdominal surgery, intra-abdominal hypertension, pleural effusion or even ARDS (H1N1 associated ARDS) make the airway pressure not representing the real stress and strain of the lungs (51, 52). MV setting guided by transpulmonary pressure, not airway pressure, and intraabdominal pressure monitoring can promote appropriate alveolar recruitment and avoid lung hyperinflation and may prevent further pulmonary complications. In spontaneous breathing patients, during postoperative period, beside pulse oximetry (53) and capnography, esophageal pressure (Peso) can be used to monitor sedative drugs usage, patients-ventilator asynchrony and intrinsic PEEP during weaning from MV in high risk PPCs patients, i.e. COPD patients (52, 54).

Talmor et al. have demonstrated the value of Peso guiding for appropriate PEEP levels in ARDS patients to achieve better oxygenation at 72 hours compared with MV setting based on PEEP-FiO2 table according to ARDS Network. At end expiration, PEEP can be adjusted until the P_L become positive and should not exceed 10 cm H_2O to keep airway opening and avoid overstretch. At end inspiration, set tidal volume are limited to keep P_L less than 25 cm H_2O (12, 55). However, some authors have debated using the absolute pressure and preferred using the variation of the Peso rather than the absolute value to estimate lung stress namely elastance derived method calculating chest wall elastance (Ecw) which is the ratio of Pressure difference across chest wall (ΔPcw)/VT. Under static condition, $P_L = Paw \times (E_L/E_{RS})$ in which $E_L = \Delta P_L/VT$ and $E_{RS} = \Delta Paw/V_T$ (52). Peso can distinguish the fraction of airway pressure that overcome lung or chest wall elastance.

2.2 Ultrasound in ICU and Perioperative period

In ARDS, the presence of pulmonary vascular dysfunction and extravascular lung water are associated with higher mortality (56). Although some methods, i.e. pulse pattern analysis and transpulmonary thermodilution technique can provide the hemodynamic goal directed therapy and respiratory variables as well as extravascular lung water (EVLW) index in either ICU or high risk surgical patients. Nevertheless, these techniques are invasive. Regarding the availability and noninvasiveness of bedside ultrasound and echocardiography, lung ultrasound can estimate EVLW index and evaluate the response of PEEP titration in ARDS (57). Jambrik et al. have demonstrated that there is significant correlation between numbers of B-lines (58) and wet to dry ratio measured by gravimetric method ($r = 0.91$, $p < 0.001$). Corradi et al. have demonstrated that quantitative lung ultrasound, based on a video gray scale analysis correlated with the amount of EVLW (59). Furthermore, the pleural effusion could be defined by the presence of anechoic or hypoechoic homogenous structure (60). In moderate to severe ARDS, the prevalence of cor-pulmonale diagnosed by trans-

-esophageal echocardiography is 22% despite low level of PEEP. Bedside echocardiography can be used to evaluate the right ventricular and biventricular cardiac function. During perioperative period, we can early detect atelectasis by the presence of hyper echoic hepatization suggesting the presence of consolidation. Furthermore, the measurement of air column width of larynx during endotracheal cuff deflation is the good predictor of post-extubation laryngeal edema and may predict post-operative intubation (61).

3. Protective ventilation in non-injured lung

The beneficial effects of protective ventilation with lower tidal volume and higher PEEP were clearly established by ARDS Network 15 years ago (62). Protective ventilation (PV) for non-injured lung has been studied in a contest of ICU and perioperative period.

Lee et al. firstly studied the role of protective ventilations in 103 ICU patients with non-injured lungs (63). The main outcome of this study was the duration of mechanical ventilation that was lower in patients treated with protective ventilation (2.30 days vs. 3.90 days) (63). Gajic et al. evaluated the effect of PV in a cohort study on 166 patients without lung injury and admitted in ICU (64). The authors did not report significant differences in development of lung injury between the groups (64). Wolthuis et al. showed the effects of PV in lowering sedative use, as primary outcome, in non-injured lung patients (65). In this study the authors did not find significant difference between the groups (65). Yilmaz et al. performed a cohort study on 375 ICU patients to evaluate the role of PV on prevention of lung injury as primary outcome (66). The authors did not find difference between protective and conventional ventilation about the considered outcome (66). Determann et al. and Pinheiro de Oliveira et al.performed randomized controlled trials to assess the levels of cytokine in broncho-alveolar lavage (BAL) in patient treated with protective and conventional ventilation (67, 68). Interestingly, Determann et al. did not show any differences in cytokine in BAL, while Pinheiro de Oliveira et al. demonstrated a higher level of Cytokine in BAL in protective than in conservative ventilation (168 pg/ml vs.72 pg/ml) (67, 68).

Protective ventilation was also studied in a perioperative setting on healthy lungs. It has been reported that 5% to 10% of major abdominal and thoracic surgery developed postoperative pulmonary complications (69). Different randomized controlled trials (RCTs) have been performed in major abdominal surgery on PV with different outcomes. Determann et al. did not find any differences in BAL and plasma inflammation proteins between protective and conventional ventilation (70). Wolthuis at el. reported a reduction in BAL myeloperoxidase using PV in healthy patients underwent abdominal surgery (70). Weingarten et al. experienced a better oxygenations but no improvement of inflammatory biomarkers in a RCT on patients aged > 65 years (70). Treschan et al. evaluated the effectiveness of PV in 101 healthy lung patients (71). In this study, there was no difference in improving lung function between protective and conventional ventilation (71). Recently, Severgnini et al. performed a RCT evaluating the role of PV in major abdominal surgery (10). The authors reported an improvement in pulmonary function and modified clinical pulmonary infection score (mCPIS) and a reduction in X-ray chest findings in patients treated with PV during surgery (10). Futier et al. performed a large RCT on 400 patients submitted to major abdominal surgery with protective and conventional ventilation (9). The authors found a reduced incidence of pulmonary and extrapulmonary complications in PV group (9). Protective ventilation was also matter of study in cardiac surgeries. In coronary artery bypass surgery, three studies did not demonstrate any difference in plasma and BAL cytokine levels using PV (72, 73, 74). Sundar et al. performed a RCT on PV in cardiac surgery on 149 patients (75). The authors found less incidence of re-intubation and a faster weaning at 6–8 hours after surgery in patients treated with PV)75).

Protective ventilation in non-injured lung was also evaluated in different meta-analysis including ICU and perioperative studies. In meta-analysis by Serpa-Neto et al., the authors included 20 studies, but only 15 RCT, and 2833 ICU and surgical patients (76). In this meta-analysis, PV had a tidal volume of 6.5 ml/kg and PEEP of 6.4 cm H_2O while conventional ventilation with tidal volume of 10.6 ml/kg and PEEP of 3.4 cm H_2O. Among patients without lung injury, PV was associated with better outcomes as acute lung injury (RR 0.33; 95 %CI 0.23–0.47), pulmonary infection (RR 0.52; 95 %CI 0.33–0.82), atelectasis (RR 0.62; 95 %CI 0.41–0.95) and mortality (RR 0.64; 95 %CI 0.46–0.86) (76). In the meta-analysis by Hemmes et al., the authors included 8 studies. But 6 RCT, and 169 surgical patients without lung injury (77). PV was performed with 6.1 ml/kg mean tidal volume and 6.6 cm H_2O PEEP while conventional ventilation with 10.4 ml/kg tidal volume and 2.7 cm H_2O PEEP (77). In this study PV protected against postoperative complications as acute lung injury (RR 0.40; 95 % CI 0.22–0.70) pulmonary infection (RR 0.64; 95 % CI 0.43–0.97) and atelectasis (RR 0.67; 95 % CI 0.47–0.96) (77). A recent meta-analysis by Sutherasan et al. included 17 studies as RCT and 1,362 ICU and surgical patients (78). In this study PV was set with mean tidal volume of 6.1

ml/kg and PEEP of 7.6 cm H_2O while conventional ventilation with mean tidal volume of 10.7 ml/kg and PEEP of 2.5 cm H_2O (78). This meta-analysis suggested that among surgical and critically ill patients without lung injury, protective mechanical ventilation with use of lower VT, with or without PEEP, is associated with better clinical pulmonary outcomes in term of ARDS incidence (RR 0.27; 95 % CI 0.12–0.59) and pulmonary infection (RR 0.35; 95 % CI 0.25–0.63) but does not decrease atelectasis (RR 0.76; 95 % CI 0.33–1.37), mortality (RR 1.03; 95 % CI 0.67–1.58) or length of stay in ICU or hospital (WMD –0.40; 95 %CI –1.02; 0.22. WMD 0.13; 95 %CI –0.73; 0.08) (78).

4. Protective ventilation in ARDS

Protective ventilation is a supportive tool for patients with acute lung injury. In this setting, PV was extensively studied across the years. PV included low tidal volume, high PEEP and in some circumstances recruitment maneuvers.

In the 1998, Amato et al. performed a multicenter RCT to evaluate the effects of low tidal volume (6 ml/kg) on mortality, barotrauma and multiple organ failure (MOF) in a cohort of ARDS patients (79). PV, compared with conventional ventilation, was associated with improved survival at 28 days, higher rate of weaning from mechanical ventilation, and lower rate of barotrauma in patients with ARDS although it was not associated with higher rate of survival to hospital discharge (79). Brochard et al. and Brower et al. in prospective RCTs evaluated the effect of low tidal volume (7 ml/kg and 8 ml/kg) in ARDS patients in mortality, duration of mechanical ventilation and barotraumas (80, 81). Both studies concluded that there were no effects of PV on considered outcomes (80, 81). In the 2000, the ARDS network group performed a large RCT including 861 adult ARDS patients to assess the role of low tidal volume (6 m/kg) different outcomes as mortality, MOF, duration of mechanical ventilation, barotrauma and ventilator free days (62). In this study the authors concluded that in ARDS patients mechanical ventilation with a lower tidal volume, than is traditionally used, resulted in decreased mortality and increases the number of days without ventilator use (62). In the 2004, the ARDS network performed another study to evaluate the effect of higher versus lower PEEP in ARDS patients on the same previous outcomes (81). In this study, PV was set high PEEP 5–25 cm H_2O according FiO_2 while conventional ventilation with low PEEP 5 cm H_2O (82). This study found no difference in the considered outcome when high PEEP level was compared with low PEEP (82). PV with high and low PEEP levels was matter of study in three different RCTs performed in 2008 (55, 83, 84). In the study by Meade et al., high PEEP level was fixed between 5–25 cm H_2O according FiO_2 and low PEEP level at 5 cm H_2O (83). In the study by Mercat et al. high PEEP was chosen according to Plateau pressure of 28–30 cm H_2O and low PEEP between 5–9 cm H_2O (84). Talmor et al. fixed the high PEEP level at 17 cm H_2O and the low PEEP level at 10 cm H_2O (55, 29). In these studies high PEEP did not affect mortality (55, 83, 84). Interestingly, while Mercat et al. found an improvement in lung function and a reduction of MOF and duration of mechanical ventilation; Meade et al. did not confirm these findings (83, 84). Furthermore, the conclusion by Talmor et al, was very interesting because the authors suggested the use of mechanical ventilation guided by esophageal pressure to further improve oxygenation and compliance in ARDS patients (29). Recently a meta-analysis evaluated the effects of high versus low PEEP in ARDS patients considering 7 RCTs and 2565 ARDS patients (15). The outcomes were mortality, oxygenation, barotrauma and length of stay in ICU (15). High PEEP did not affect mortality, barotrauma and length of stay in ICU but improved the oxygenation of ARDS patients at 1, 3 and 7 days (15).

5. Other adjunctive rescue strategies for ARDS

5.1 Prone positioning

Prone position is the adjunctive treatment that can improve oxygenation in 70% of patients. The mechanisms are an increase in end-expiratory lung volume, improvement of ventilation-perfusion matching and decrease of mass effect of the heart at the lower lobes as well as an increased homogeneity of ventilation (11).

Recent randomized controlled trial has demonstrated that in patients with PaO_2/FiO_2 less than 150 when receiving FiO_2 of 60%, patients who received prone position showed the decrease in mortality than in supine position (28-day mortality; 16% vs. 32.8%, respectively) (85). Moreover, recent meta-analysis including this RCT have demonstrated that prone position with low tidal volume ventilation in severe ARDS patients significantly decrease 60-day mortality (86). In the subgroup which the duration of prone positioning was more than 10 hours per session showed markedly reduced in overall mortality compared with the shorter duration groups (87). However, the risk of pressure ulcer and major airways problems should be considered during implementing the prone positioning protocol (87).

5.2 Extracorporeal membrane oxygenation

Extracorporeal membrane oxygenation (ECMO) have shown the increase of survival in severe ARDS

Table 1. *Strategies for prevention and management patients with ARDS and without ARDS*

	Patients with ARDS	Patients without ARDS in ICU Patients undergoing high risk surgery i.e. intra-abdominal surgery
Risk factors for further VALI and postoperative pulmonary complications	– Sepsis – Fluid loading – Blood transfusion – Old age	– Underlying status – Type of anaesthesia – Type of surgery i.e. cardio-pulmonary surgery and abdominal surgery – Smoking status
Protective ventilation	– Tidal volume 6 ml/kg PBW – Plateau pressure = 30 cmH_2O – PEEP according to PEEP/FiO_2 table or titration by respiratory mechanics and/or oesophageal pressure – Recruitment maneuver	– Tidal volume 6–8 ml/kg PBW – Plateau pressure < 20 cm H_2O – PEEP of 6–8 cm H_2O – Recruitment maneuver every 30 minutes
Oesophageal pressure monitoring	– Obesity, pleural effusion, intra-abdominal hypertension, ARDS, massive ascites	– High risk abdominal surgery, obesity
Other effective strategies	– Prone position when PaO_2/FiO_2 < 150 mmHg and PEEP >10 cm H_2O – Extracorporeal membrane oxygenation – Neuromuscular blocking agents	– Perioperative physiotherapy – Noninvasive ventialtion
Predictive scores	LIPS score	ARISCAT, SLIP, PRF and SPORC

Abbreviations: ARDS: Acute Respiratory Distress Syndrome; ICU: intensive care unit; VALI: ventilator associated lung injury; PEEP: positive end expiratory pressure; PBW: Predicted body weight; LIPS: lung injury prediction score; PRF: postoperative respiratory failure index; ARISCAT: Postoperative pulmonary complications risk score; SLIP: Surgical lung injury prediction score; SPORC: Score for Prediction of Postoperative Respiratory Complications

($PaO_2/FIO_2 \leq 100$ and PEEP ≥ 5–10 cm H_2O). To avoid further VALI in ARDS and complications from ECMO itself, another approach is bridging MV with extracorporeal membrane oxygenation. During applying ECMO, the VT can be decreased until less than 6 ml/kg PBW ("ultra" protective MV) (85).

Strategies for prevention and management patients with ARDS and without ARDS are summarized in Table 1.

6. Methods for prevention of ventilator associated lung injury and postoperative pulmonary complications

6.1 Risk factors for ARDS and ventilator associated lung injury

In severe sepsis patients present at emergency department, high lactate, lung injury prediction scores and microbiological proven infection are independent risk factors for the development of ARDS (88). Other factors i.e. hypoalbuminemia, chemotherapy, obesity and diabetic mellitus have been proposed as the risk factors of ARDS. Gajic el al have validated lung injury prediction score (LIPS) to identify the high risk patients at the time of hospital admission and can discriminate the patients who developed ARDS from those who did not with an AUC of ROC at 0.80 (95%CI; 0.78–0.82) (89). The variables that were included in calculated worksheet composed of previously mentioned factors, type of surgery and trauma.

Several factors have been proven as the predisposing factors of VALI i.e. sepsis, fluid loading, blood transfusion and age. Old animals are more susceptible to VALI with high VT and constant PEEP compared with younger animals associated with the increase amount of lung lavage protein, IL-6 concentration, and increase of lung wet to dry ratios (90). Transfusion-related acute lung injury (TRALI) is the reaction of the

transfusion of antibodies against the recipients' antigen that accumulate during blood storage which aggravate lung injury. TRALI combined with injurious MV setting may aggravate further lung injury, particularly transfusion blood with longer storage duration in septic patients (91).

6.2 Role of recent predictive scores

PPCs are associated with various risk factors namely underlying status, type of anaesthesia and surgery particularly cardiopulmonary surgery and abdominal surgery as well as smoking status.

Several investigators have developed different scores to predict the risk of PPCs. The parameters required for score calculation can be assessed preoperatively and during bedside evaluation.

6.2.1 Post-operative respiratory failure risk index and pneumonia risk index

Based on National Surgical Quality Improvement Program (NSQIP) data, postoperative respiratory failure (PRF) index was developed by the investigators from veterans' affair in non-cardiac surgery patients. The risk factors were including the type of surgery, age, functional status and COPD. The laboratory data comprised albumin level less than 30 g/L, and blood urea nitrogen level more than 30 mg/dL in the model (92). The same group has constructed another index, postoperative pneumonia risk index that are added the data of impaired sensorium, cerebral vascular accident, transfusion, long-term steroid use, smoking, and alcohol use to previously mentioned index (93). These indexes have a limitation that the majority of populations included in the analysis are male veterans therefore these indexes may not be used generalizability.

6.2.2 Postoperative pulmonary complications risk score (ARISCAT score)

Canet et al. have identified the seven independent risk factors including low pre-operative arterial oxygen saturation, recent acute respiratory infection, age, anemia, upper abdominal or intra thoracic surgery, surgical duration of at least 2 h, and emergency surgery and showed that the AUCs under ROC curve of this score are 90% for the development of PPCs predictive index and 80% from the validation of this index (53). The PERISCOPE (Prospective Evaluation of a Risk Score for Postoperative Pulmonary Complications in Europe) study is completed and will show the data of external validation of this score (94).

6.2.3 Surgical lung injury prediction score (SLIP score)

The surgical lung injury prediction score (the SLIP score) were developed to classify the patients who at risk for postoperative ARDS in patients with high-risk surgery and require mechanical ventilation during general anaesthesia for longer than 3 hours with an AUC of 0.82 (95% CI 0.78–0.86). The score includes diabetes, COPD, GERD, alcohol abuse and type of surgery. However, this study is conducted in single centre and retrospective study (95).

6.2.4 Score for Prediction of Postoperative Respiratory Complications (SPORC)

Another recent score that developed to predict postoperative re-intubation is Score for Prediction of Postoperative Respiratory Complications (SPORC) within the first 3 postoperative days. Four independent predictors contribute to an American Society of Anesthesiologists (ASA) score ≥ 3: emergency surgery, high-risk surgical service, history of congestive heart failure and chronic pulmonary disease (96).

CONCLUSION

Protective ventilation has been proven preventing further complications i.e. VALI, postoperative pulmonary complications in either ARDS or non-injured lungs. Predictive scores and bedside respiratory monitoring i.e. ultrasound and esophageal pressure measurement are crucial tools to identify at risk patients and minimize further VALI as well as PPCs.

Abbreviations

AECC — American–European Consensus Conference
PPCs — Postoperative pulmonary complications
ARDS — Acute Respiratory Distress Syndrome
VALI — Ventilator associated lung injury
PEEP — Positive end expiratory pressure
ICU — Intensive care unit
MV — Mechanical ventilation
PL — Transpulmonary pressure
VT — Tidal volume
TLRs — Toll-like receptors
NF-kappaB — Nuclear factor kappa B
TNF — Tumor necrosis factor
IL — Interleukins
ECM — Extracellular matrix
Pplat — Plateau pressure
FRC — Functional residual capacity

Peso — Esophageal pressure
Ecw — Chest wall elastance
EL — Lung elastance
ERS — Respiratory system elastance
PL — Transpulmonary pressure
Paw — Mean airway pressure
Pcw — Pressure difference across the chest wall
EVLW — Extravascular lung water
PV — Protective ventilation
BAL — Broncho-alveolar lavage
RCTs — Randomized controlled trials
mCPIS — Modified clinical pulmonary infection score
MOF — Multiple organ failure
ECMO — Extracorporeal membrane oxygenation
LIPS — Lung injury prediction score
TRALI — Transfusion-related acute lung injury
NSQIP — National Surgical Quality Improvement Program
PRF — Postoperative respiratory failure
the SLIP score — Surgical lung injury prediction score
SPORC — Score for Prediction of Postoperative Respiratory Complications
ASA — American Society of Anesthesiologists

Sažetak

PROTEKTIVNA MEHANIČKA VENTILACIJA KOD PACIJENATA SA ILI BEZ POVREDE PLUĆA

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Smrtnost od Sindroma Akutnog Respiratornog Distresa (ARDS) je još uvek visoka i kreće se od 27 do 45% prema Berlinskoj definiciji. Čak i kod hirurških pacijenata bez povrede pluća, postoperativne plućne komplikacije (PPC) su česte. Kod ARDS-a, protektivna ventilacija sa niskim „tidal" volumenom od 6 ml/kg PBW i višim nivoima pozitivnog end-ekspiratornog pritiska (PEEP) su široko prihvaćeni kao rutinska praksa. Kod pacijenata bez ARDS-a, podvrgnutih mehaničkoj ventilaciji, protektivna ventilacija sa niskim „tidal" volumenom od 6 ml/kg PBW i niskim do srednjim PEEPom su postali nov izazov suportivne nege posebno u jedinicama intenzivne nege i kod perioperativnih pacijenata. Respiratorni monitorig je od velike pomoći za podešavanje optimalne ventilacije kako bi se prevenirala povrede usled asistirane ventilacije i na vreme detektovale postoperativne plućne komplikacije u perioperativnom periodu. Nekoliko rezultata je usvojeno kako bi se smanjio rizik od ARDS, PPC i VALI (ventilator associated lung injury). Vreme je za primenu bazične fiziologije respiratorne funkcije i na dokazima zasnovane prakse kako bi se unapredili ishodi ARDS-a i PPC.

Ključne reči: asistirana ventilacija, Akutni Respiratorni Distres Sindrom, transpulmonarni pritisak, Positivni End Ekspiratorni Pritisak, Protektivna ventilacija, Respiratorni monitoring.

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INTRAVENOUS CLARITHROMYCIN: A VALUABLE IMMUNOMODULATOR FOR SEVERE INFECTIONS

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Abstract: There are many studies suggesting that intake of a macrolide in the treatment regimen is linked with improved outcomes for patients with community-acquired pneumonia. However this was never proved through one randomized clinical study (RCT). We reviewed all pre-clinical and clinical development from 2003 to 2014 for intravenous clarithromycin as adjunctive treatment of severe infections. This process ended with the conduct of two RCTs; the first in 200 patients with ventilator-associated pneumonia; and the second in 600 patients with proven or suspected Gram-negative infections of non-pulmonary origin. The two RCTs has similar findings; significant decrease of mortality of patients with septic shock and multiple organ dysfunctions; and shorter resolution of severe infections. The second RCT also showed significant savings for survivors. These data support the use of intravenous clarithromycin as adjunctive treatment of severe infections.

Key words*:* clarithromycin, cytokines, inflammation, sepsis

INTRODUCTION

Despite increasing antimicrobial resistance, intake of a macrolide is connected with improved outcomes of patients with community-acquired pneumonia (CAP) (1–5). Benefit is even pronounced among patients with bacteremia by macrolide-resistant *Streptococcus pneumonia* (5) leading people to consider that this comes from modulation of the immune response of the host by macrolides. However, the definite proof may come only from randomized clinical trials (RCTs). No RCT has ever been conducted to prove benefit of macrolides intake in the outcome of severe CAP. Despite the lack of such RCT, the updated Surviving Sepsis Campaign guidelines recommend the use of a macrolide for the management of CAP complicated by bacteremia from *Streptococcus pneumoniae* (6), giving emphasis to the validity of this intervention.

Our group has launched a research program since 2003 to explore the modulation of the immune response of the host and the benefit that is shown for patients with severe infections when intravenous clarithromycin is added in the treatment regimen. The present review outlines the main findings of these research efforts.

ANIMAL STUDIES

The efficacy of intravenously administered clarithromycin was studied in a series of pre-clinical animal studies. Results of these studies are summarized in Table 1 (7–11). The main infection models studied were pyelonephritis, pleuritis and peritonitis after cecal ligation and puncture. For pyelonephritis and pleuritis bacterial challenge was done by multidrug- and pandrug-resistant isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* against which macrolide do not possess any antimicrobial activity. Results from these animal studies revealed that clarithromycin, either alone or in co-administration with amikacin, prolonged survival considerably. This was accompanied by improvement in oxygen saturation and heart rate when administered in rabbits when signs of pulmonary edema appear (10). In pyelonephritis by multidrug-resistant *P.aeruginosa* and pandrug-resistant *K.pneumoniae*, histology showed that clarithromycin attenuated: a) peribronchial inflammation in the lung; b) mononuclear infiltration and necrosis in the liver and kidney; and c) activation of B- and T-cell rich areas in spleen (10, 11). The common denominator of all these studies was that monocytes and lymphocytes appeared to be the main target of action of clarithromycin.

In one model of pleuritis induced by multidrug-resistant *P.aeruginosa*, specimens of pleural fluid of rab-

Table 1. *Summary of pre-clinical studies assessing the efficacy of intravenous clarithromycin in acute infection models*

Reference	Experimental model	Microorganism	Survival	Bacterial growth	Circulating mediators	Mononuclears	Histology
7	Pyelonephritis	MDR *Pseudomonas aeruginosa*	Prolongation when co-administrated with amikacin	No effect	↓TNFα, MDA	N/A	N/A
8	Pyelonephritis	Susceptible *Escherichia coli*	Prolongation as pretreatment and treatment	No effect	↓ TNFα, MDA	↓monocytic caspase-3	N/A
9	Pyelonephritis	Susceptible *Escherichia coli*	Prolongation when co-administered with amikacin at signs of sepsis	No effect	No effect	N/A	N/A
10	Pyelonephritis	MDR *Pseudomonas aeruginosa*	Prolongation when co-administrated with amikacin at signs of sepsis	No effect	No effect	↓ ex vivo production of TNFα by monocytes	↓lung-spleen inflammation
11	Pyelonephritis	PDR *Klebsiellapneumoniae*	N/A	No effect	↓TNFα, MDA	↓ ex vivo production of TNFα by monocytes	↓kidney-liver inflammation
12	Peritonitis by CLP		Prolongation when co-administrated with piperacillin/ /tazobactam	No effect	N/A	↓ ex vivo production of TNFα, ↓ apoptosis lymphocytes and monocytes	N/A
13	Pleuritis	MDR *Pseudomonas aeruginosa*	N/A	↓ lung and pleural fluid	↓TNFα	↓ production of TNFα and IL-6	

Abbreviations: CLP: cecal ligation and puncture; IL: interleukin; MDA: malondialdehyde; MDR: multidrug-resistant; N/A: not assessed; PDR: pandrug-resistant, TNFα: tumor necrosis factor-alpha ;↓: decrease

bits were used to stimulate cytokine production from U937 monocytes. It was shown that those specimens coming from animals treated with a combination of clarithromycin and piperacillin/tazobactam produced much lower concentrations of tumour necrosis factor-alpha (TNFα) and of interleukin (IL)-6 compared with the amount of cytokines released from specimens coming from rabbits administered single piperacillin/tazobactam (13). This finding drives the hypothesis that part of the mode of action of clarithromycin is mediated through attenuated release of pathogen-associated molecular patterns by *P.aeruginosa*.

INTRAVENOUS CLARITHROMYCIN IN SEPSIS: CLINICAL EVIDENCE

The promising results of experimental studies led us to design and conduct two randomized clinical trials to explore the immunomodulatory effect of clarithromycin in patients with sepsis. It is suggested that part of the failure of previous clinical trials with immunomodulators was due to the inclusion of heterogenous groups of patients, namely patients with sepsis caused by different types of infections. To overcome this, the first trial enrolled patients suffering from the same underlying infection, namely ventilator-associated pneumonia (VAP). Two hundred patients with sepsis and VAP were enrolled in a double-blind, randomized, multicenter trial from June 2004 until November 2005 (14). Clarithromycin (1 g) was administered intravenously once daily for 3 consecutive days in 100 patients; another 100 patients were treated with placebo. Main outcomes were resolution of VAP, duration of mechanical ventilation, and sepsis-related mortality within 28 days. There were no differences between groups in baseline characteristics. Microbiology was also assessed

and Gram-negative bacteria growing at counts greater than 10^5cfu/ml of tracheobronchial secretions (TBS) were considered. Gram-negative bacteria were identified as underlying pathogens in 68 placebo-treated and 66 clarithromycin-treated patients. The most frequent pathogens were *Acinetobacter baumannii*in 43 and 36 patients and *P.aeruginosa* in 12 and 17 patients. Initial empirical antimicrobial coverage was appropriate against 62.7% of pathogens isolated from placebo-treated patients and against 75.4% of pathogens isolated from clarithromycin-treated patients (p = 0.44 between groups). TBS were sampled again at follow-up. Eradication of the pathogen was achieved in 25.4% and 33.8% of cases on day 5 (p = 0.31 between groups) and in 31.3% and 29.2% of cases on day 10(p = 0.82 between groups). Sepsis-related mortality was 25% in the placebo group and 23.3% in the clarithromycin group. Odds ratio for death from septic shock and multiple organ failure was 19.00 in placebo-treated patients; it was reduced to 3.78 in clarithromycin treated patients (p = 0.043 between groups). VAP resolved in 72.2% of survivors treated with placebo and in 79.9% of survivors treated with clarithromycin. Median time to resolution of VAP was 15.5 days in the placebo group and 10.0 days in the clarithromycin group scores (p = 0.011). Mean clinical pulmonary infection score for the placebo and the clarithromycin group on study enrolment were 7.92 and 7.62, respectively (p = 0.29). It was decreased to 6.10 and 5.23 respectively on day 5 of follow-up (p = 0.016); and to 5.88 and 5.09 respectively on day 10 of follow-up (p = 0.032).Weaning from mechanical ventilation was performed in 58.6% of placebo-treated patients within a median period of 22.5 days, and in 72.5% of clarithromycin treated patients within a median period of 16.0 days (p = 0.049).

To confirm these findings in another patient population, a second RCT was conducted in 600 patients with proved or suspected Gram-negative infections i.e. primary Gram-negative bacteremia, acute pyelonephritis and acute intrabdominal infections aggravated by systemic inflammatory response syndrome (15). Clarithromycin blind treatment was assigned to 302 patients; 298 patients were treated with placebo. In this RCT, period of treatment was prolonged from three to four days. The primary endpoint was mortality by severe sepsis/shock and MODS; resolution of infection and hospitalization costs were the secondary endpoints. Demographics and sepsis severity were similar between groups. The most common pathogens were Gram-negative bacteria namely *E.coli* (36.2% of bloodstream pathogens in the placebo arm and 43.8% of bloodstream pathogens in the clarithromycin arm), *K.pneumoniae* (21.2% and 11.2% respectively), *P.aeruginosa* (10.0% and 6.7% respectively) and *A.baumannii* (11.2% and 6.7% respectively). The time from sepsis onset to start of antimicrobials and appropriateness of antimicrobials did not differ between groups.

Overall 28-day mortality was similar between groups being 17.1% in the placebo group and 18.5% in the clarithromycin group. Mortality of placebo-treated patients with septic shock and MODS was 73.1% compared to 53.6% of clarithromycin-treated patients (p = 0.020). The median time until resolution of infection was 10 days among patients with severe sepsis/shock treated with placebo and 6 days among patients with severe sepsis/shock treated with clarithromycin (p = 0.037). The median cost of hospitalization was € 3383.5 for survivors of the placebo arm and € 2269.3 for survivors of the clarithromycin arm (p = 0.044). Serious adverse events were observed in 1.3% and 0.7% of placebo- and clarithromycin-treated patients, respectively (p = 0.502).

The findings of the second trial fully confirm results of the first trial. Intervention with clarithromycin decreased mortality by septic shock and MODS and shortened the time until resolution of Gram-negative infections worsened by severe sepsis or septic shock. As expected, earlier resolution of severe Gram-negative infections was accompanied by significant savings for survivors. More precisely, median savings for every survivor were € 1114.3. The studied intervention costs € 70 per patient.

WHAT IS THE MECHANISM OF ACTION OF MACROLIDES?

It is traditionally conceived that macrolides attenuate the inflammatory response of the host. Most of conducted studies in animals including those used for the pre-clinical development of intravenous clarithromycin in sepsis support this concept. Our animal studies indicate that treatment with intravenous clarithromycin leads to decreased circulating levels of pro-inflammatory mediators and to attenuation of the mononucleat tissue infiltrates (Table 1). To the same end, studies of infected mice show that pre-treatment with azithromycin is accompanied by decreased concentrations of pro-inflammatory cytokines in the systemic circulation and in the lung (16, 17, 18). It is well-known that macrolides attenuate pro-inflammatory phenomena in the lung where they accumulate. These properties may well explain the benefit shown in patients with VAP of the first RCT but not in patients of the second RCT who were not sufferers from any lung infection.

To explore the effect of intravenous clarithromycin in the inflammatory cascade in the first trial of patients with VAP (19), circulating cytokines were

measured before allocation to blind treatment and for six consecutive days. On the same time intervals, circulating monocytes were isolated and stimulated for cytokine production. Expression of the co-stimulatory molecule CD86 involved in antigen presentation was also measured on the cell membranes of monocytes. Contrary to what was expected, the ratio of circulating IL-10 to TNFα was decreased on day 4 in the clarithromycin arm compared to the placebo arm. On this same day, expression of CD86 on monocytes was increased as was also their ability for the production of IL-6 after stimulation with bacterial ligands. These findings were much pronounced among patients with septic shock and MODS for whom most of survival benefit was shown. This tight time connection between treatment and effect may indicate that clarithromycin is a modulator of the biological function of monocytes so as to corroborate animal studies. However these findings also suggest that in the clinical field intravenous clarithromycin modulated the function of monocytes towards reversal of sepsis-induced immunoparalysis of patients. The ratio of pro-inflammatory to anti-inflammatory cytokines was restored, antigen-presentation was more efficient and monocytes became fully functional for the production of pro-inflammatory cytokines.

It is now widely recognized that when severe sepsis and MODS develop, the host has entered into a state of immunoparalysis (20). The key components of sepsis-induced immunoparalysis are failure of monocytes for adequate cytokine production after stimulation and defective antigen presentation. These defects were fully restored in patients with VAP treated with clarithromycin particularly those with septic shock and MODS.

CONCLUSIONS

Despite the existence of many retrospective analysis showing improved outcome of severe CAP when a macrolide is added to the treatment regimen of CAP, no RCT is available to definitively prove this benefit. Despite the lack of such RCT, the updated Surviving Sepsis Campaign guidelines recommend the use of a macrolide for the management of CAP complicated by bacteremia from *Streptococcus pneumoniae*. The only available RCTs in critically ill patients compare the efficacy of intravenous clarithromycin over placebo when added to the standard of care of ventilator-associated pneumonia and of Gram-negative infections of non-pulmonary origin. Common findings of both RCTs are decrease of mortality and earlier resolution of infection of the most severe patients. These findings definitively support a novel therapeutic indication of intravenous clarithromycin for the severely ill patients.

Abbreviations

CAP — community-acquired pneumonia
IL — interleukin
MODS — multiple organ dysfunction syndrome
RCT — randomized clinical trial
TNFα — tumour necrosis factor-alpha
VAP — ventilator-associated pneumonia

Sažetak

INTRAVENSKA PRIMENA CLARITHROMYCIN-A: DRAGOCEN IMUNOMODULATOR KOD TEŠKIH INFEKCIJA

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Postoji mnogo studija koje sugerišu da je primena makrolida u lečenju upale pluća povezana sa boljim ishodom. Ipak, ovo nije nikada dokazano kroz jednu randomiziranu kliničku studiju. Pregledali smo sve prekliničke i kliničke rezultate od 2003. do 2014. godine, vezane za intravensku primenu klaritromicina kao dodatnog lečenja teskih infekcija. Ovaj proces se završio sprovođenjem dve randomizirane kliničke studije; prva je uključila 200 pacijenata na mehaničkoj ventilaciji, sa upalom pluća; druga je uključila 600 pacijenata sa dokazanom ili sumnjivom Gram-negativnom infekcijom koja nema plucno poreklo. Dve randomizirane kliničke studije su imale slične rezultate; značajno povećanje mortaliteta pacijenata sa septičkim šokom i multiorganskom disfunkcijom; i kraće lečenje teških infekcija. Druga randomizirana klinička studija je pokazala značajnu uštedu za preživele. Ovi podaci podržavaju intravensku primenu klaritromicina kao dodatno lečenje teških infekcija.

Ključne reci: klaritromicin, citokini, upala, sepsa.

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SAFE EXTUBATION AND REINTUBATION IN OPERATORY ROOM AND ICU PATIENTS

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Abstract: Each intubation should result in an extubation at end of procedure, and each extubation, theoretically, could represent a potential reintubation due to extubation manoeuvre failure.

There are many and different reasons for extubation to fail, starting from mechanical iatrogenic lesions due to difficult intubation up to patient's inhability to sustain spontaneous breathing and unassisted ventilation, going through extubation associated respiratory and cardiovascular complications, local mechanic or inflammatory factors, surgical techniques and accidental events.

Recent literature has clearly shown that if critical events associated with difficult intubation have lower incidence thanks to guidelines diffusion and implementation, extubation related accidents did not change incidence and consequences in last couple of decades.

This observation has led to developement of dedicated extubation guidelines and to attention from Scientific Community to promote culture of safety and anticipation, with predicted difficult extubation concept and protected extubation strategies, by using pharmacological and technical approach.

Aim of this paper is to review data for extubation related accidents, to provide overview with existing guidelines and description of approaches to predict difficult extubation, techniques to provide best extubation strategies including pharmacologic, non pharmacologic and instrumental techniques.

Key words: safe extubation, reintubation, operatory room, ICU.

INTRODUCTION

Extubation is a logical consequence of tracheal intubation. Despite greater attention is focused on intubation, and particularly on difficult intubation, airway control after extubation remains equally important as any other phase of securing the airway. Complications related to extubation are usually minor and less frequent if compared with intubation phase, nevertheless, in some cases, they can be very dangerous and life-threatening. Different reasons could explain such a condition: extubation is generally performed with lower attention level if compared with intubation, complications might be immediate but also delayed, and often at time of extubation the airway cart is far from where it is needed. These considerations might explain why postextubation respiratory complications are three times more frequent than those that occur during induction and intubation (1, 2). Recent literature suggests that during the last decade, Experts focused on process of tracheal intubation and related complications. Cornerstone paper from Peterson et Al (3) clearly showed a significant decline from 62% to 35% in mortality and fatal accidents related to airway management during induction phase and intubation, comparing data from closed Claims in ten years before and after introduction of ASA Guidelines: the Authors conclude that such a result comes from wide diffusion and applications of representative guidelines, but at same time their work clearly showed that incidence of deaths and serious brain damage related to extubation was almost unchanged on incidence of 12% over the last twenty years. As a result, different Experts groups focused their work and attention (4) on development of strategies and recommendations for periextubation period, aimed to increase safety of daily Anesthesia practice. Italian guidelines for difficult airway management (5) first suggested in 2005 some recommendation for protected extubation strategies in a dedicated paragraph, followed by official document (6) from SFAR (Societé Francai-

se Anesthésie et Réanimation) and recent publication from DAS (Difficult Airway Society) of dedicated guidelines for extubation (7).

Basing on these Literature contributions, this paper will review causes of difficult extubation, including mechanical and iatrogenic ones and considering cardiovascular and respiratory effects of extubation basing on comorbidities and patients characteristics. Strategies for safe extubation will be then discussed, including extubation planning and difficulty prediction, specific tests and monitoring, patient positioning and pharmacologic options. Finally devices and techniques for protected extubation will be considered, including environmental and pathophysiological differences with ICU patients.

Mechanical causes of difficult extubation

We should consider that intubation per se is not atraumatic, and many papers show that mimimal airway changes yet occurr three hours after intubation (8); this is more true whenever it is not performed correctly (9). If intubation is difficult, there is high possibility of developing oropharyngeal (19%), temporo-mandibular joint (10%), oesophageal (18%), tracheal (15%) and laryngeal (87.3%) trauma (3) and vocal cord palsy; this means that, per definition, any difficult intubation should be considered a difficult extubation. Whenever using double lumen tubes (DLT), special considerations should be assumed: extubation might rarely result in incidents such as the split on the posterior tracheal wall, or bronchial rupture, whereas it is more often associated with unsuccessful extubation. Extubation with DLT is much rigorous compared to extubation with single lumen endotracheal tube since these tubes are such bulky, stiffer and longer. Whenever extubation needs to be postponed, DLT should be replaced with single lumen endotracheal tube during anesthesia. This process can be facilitated by applying airway exchange catheters, preferably hollow ones, so to allow oxygen insufflation, or using fiberoptic devices with additional ports for oxygenation (10). Sometimes removing the tube out during extubation might be extremely difficult, if not impossible, resulting in potentially fatal situation; first cause to be considered should be in the inability to blow out one of the tube's cuffs because of: laryngeal trauma, cuff herniation or adhesion with tracheal wall or surgical ETT suturing with surrounding structures. In such a sudden and dramatic situations a slight rotation and tube drawling may be useful, and sometimes it is necessary to perform transtracheal cuff puncture. For these reasons, performing tube exchange under visual control with direct laryngoscope, videolaryngoscope or flexible fiberoptic is highly recommended, and any manoeuvre should be preceeded by generous preoxygenation (12).

Cardiovascular and respiratory response to extubation

Extubation is usually accompanied by an increase in blood pressure and heart rate in the range of 10 to 30% compared to baseline values. Depending on the intensity of hemodynamic stress response, this phenomenon can last up to 15 minutes after extubation, resulting in potential adverse effects on patients with low coronary reserve. Esmolol, lidocaine, glyceryl trinitrate, magnesium sulfate, remifentanil or propofol infusion might be used to minimize cardiovascular response to extubation (7), or using oral perioperative nimodipin or topical application of 10% lidocaine. Alternatively, in patients with reduced coronary reserve who are still anesthetized the ETT can be replaced with some supraglottic device. Tube change for LMA with patient in still deep anesthetic state has been described as Bailey maneuver (7, 13) aimed to minimize hemodynamic impact of extubation in high risk patient.

Incidence of postextubation cough, hoarseness, restlessness and sorethroat varies from 38% to 96%, and it has been shown to be reduced in the first 24 hours after extubation if using liquid instead of air to inflate tube cuff to reduce possibility of cuff overinflating due to rising of temperature or N_2O diffusion. Solution of 2% lidocaine in 1.4% Na-bicarbonate shows a greater diffusion profile and reduces possibility of serious incidents if endotracheal cuff rupture occurs. Use of liquid filled cuffs can be very useful in patients with increased intraocular or intracranial pressure, in cardiac patients and in patients with pulmonary hyperactivity, and should then be considered (14).

Respiratory complications after extubation

The appearance of bronchospasm after extubation is common in active smokers, patients with chronic obstructive pulmonary disease (COPD) and in children with recent respiratory tract infections. Intravenous use of magnesium and lidocaine can be very useful in preventing laryngospasm, and, in children, positioning of the not yet awake patient on left lateral side without further manipulation might be extremely useful too (15).

In everyday practice, postextubation airway obstruction can occur due to edema of larynx. Laryngeal edema is often associated with: traumatic intubation, unnecessarily using of large diameter tube, prolonged intubation and the changing position of the head and neck during surgery. Supraglottic edema can cause the

epiglottis backwards folding, resulting in glottic obstruction during inspiration. Subglottic edema results in dyspnea and dramatic stridor. This phenomenon can be especially serious in children, taking into account that the thickening of subglottic mucosa of only 1 mm reduces laryngeal diameter by 35%. Treatment involves use of warm, moist, oxygen-rich gas mixture, nebulized epinephrine or dexamethasone, heliox or re-intubation with smaller size tube as a final possibility. Surgery on the neck and thorax can also lead to postextubation airway obstruction due to direct compression on respiratory tract by the hematoma, or vocal cord palsy due to laryngeal nerves direct trauma. Unilateral vocal cords palsy after extubation typically results in hoarseness, and it usually disappears with conservative treatment in several weeks. Bilateral paralysis is a condition requiring urgent reintubation of the patient and tracheostomy. Among other, rarer cause of postextubation airway obstruction are tracheomalacia occurring after long-lasting external or internal pressure on tracheal rings: long-term intubation, presence of a large goiter, enlarged thymus, or hematoma. Softening and erosion of vulnerable trachea leads to the collapse of tracheal rings, and it is followed by difficult extubation symptoms, expiratory stridor and whistling. In those cases extubation procedure should be performed in deeper anesthesia in order to avoid coughing and straining. Rare postextubation respiratory complications include: postextubation vocal dysfunction and pulmonary edema (16).

Strategy for successful and safe extubation

Differently from intubation, extubation is an elective procedure, so it can be carefully planned and set in advance, allowing preparation of alternative plans and optimal conditions. DAS guidelines suggest planning of extubation after preliminary identification of low-risk or high-risk extubation, followed by careful strategy and devices setting before initiating any procedure (7).

How to recognize the difficult extubation?

Patients with severe cardiorespiratory disorders, obese patients, OSAS (Obstructive Sleep Apnea Syndrome) patients, full stomach emergency patients, and patients with known difficult airways who experienced numerous intubation attempts are certainly the candidates for problematic extubation and eventual reintubation. There are also surgical factors that include recurrent laryngeal nerve damages (thyroid surgery), presence of neck abscesses or hematoma (laryngeal and thyroid surgery, neck vascular surgery) and edema (neck surgery, posterior fossa cranial surgery, intermaxillar fixation and odontogenic abscess drainage), Trendelenburg position (3), prolonged cardiovascular, thoracic or orthopedic procedures (prone position, shoulder arthroscopic surgery) (17).

Prevention of unsuccessful extubation

In daily anesthesia practice unsuccessful extubation is not common, comparing to the mechanically ventilated and critically ill patients in intensive care units, among which this phenomenon is much more common and varies in the range of 0.4 to 25% of patients (18, 19). In order to identify patients with increased risk for potentially unsuccessful extubation, it is necessary to identify precise predictors, which might allow recognition of potentially reversible causes to be removed before the planned extubation. Successful extubation is defined as effective airway protection after removing the endotracheal airway, and it is determined by neuromuscular integrity and efficacy of breathing, effective cough and airway protection reflexes function and preserved mental status in absence of airway obstruction. As described before, many factors might result in airway obstruction, and detection of potential upper airway obstruction while tube is still in the trachea is a real challenge.

Gold standard is represented by direct fiberoptic (20) view or CT scan (21), which are not exams to be performed routinely; ultrasounds might represent interesting alternative, but there is too few evidence to support their use for glottic and subglottic diameter assessment (22).

Other clinical tests when postextubation stridor and upper airway obstruction are suspected include the absence of audible gas leak around endotracheal cuff while simple positive pressure is applied (qualitative cuff leak test). An alternative approach for detecting potential upper airway obstruction in intubated patients includes quantitative method of leaking gas volume measuring next to the tube after cuff volume reduction (quantitative cuff leak test). It is performed with patient in assisted-controlled ventilation and calculating the average difference between inspiratory and expiratory volume during six consequent breaths through spirometry (23). This quantitative test of gas leaking may be expressed either as an absolute value difference between the volume of inspiratory and expiratory volume or leakage can be expressed as a percentage of the inhaled volume. If the value of leakage near the cuff do not exceed more than 110–130 ml or below 10–15% of delivered respiratory volume the risk of threatening upper airway obstruction is significant (24, 25, 26).

Differently, a negative leakage test indicates that upper airway obstruction is probably not present.

Main problem with cuff leak test, including quantitative one with spirometry, is that it remains high sensible but with low specificity (7). Cheng and Coworkers (27) found the upper airway obstruction in only 5 out of 193 (2.6%) patients with quantitative leakage of inhaled respiratory volume (more than 24%), whereas the same study showed that in patients with a positive leakage test, postextubation stridor was present in 70% of patients and only 19% of respondents required reintubation. Interestingly, Authors attributed cause for high false positive rate (lack of specificity) to deposit of viscous secretions around the tube wall, which disabled gas leak.

Such a high rate of false positive could lead to excessive use of preventive steroids, unnecessary prolongation of time spent on mechanical ventilation, so it is necessary to identify particular risk groups where to apply leak test. Cheng and Co-authors, analyzing subgroups of patients, found that female and older than 80 years patients, without an adequate level of sedation and low Glasgow Coma Scale score (3 to 8) who were intubated in hospital rooms and not in the operating room, presented a higher risk for upper airway obstruction after extubation.

Results are actually controversial, so a reasonable conclusion is that if a positive test should encourage direct visualization of airway structures because of high obstruction suspicion, a negative test does not exclude extubation failure (28).

Other test is search for post extubation stridor (PES) which occurs in about 2–16% of extubations in ICU and which represents an easy to detect sign for diagnosis of airway obstruction and need for reintubation (24, 26). It can be detected with simple repeated neck auscultation representing an often underestimated tool to assess airway patency and PES early detection.

When and where to extubate?

Standard practice is to carry out extubation process at the end of inspiration when the glottis is fully opened in order to prevent trauma and laryngospasm development (29). Data shows that the threshold of laryngeal adductor neurons excitation involved in laryngospasm reflex varies during the spontaneous respiration cycle, showing the highest values in a middle of inspiratory part of a cycle (30), which supports extubation at end of inspiratory phase. As for intubation procedure, a generous preoxygenation should be performed even before extubation, so to increase safety time before desaturation occurrence in case of acute postextubation complications (17). Finally, careful, delicate and preferably under direct visual control suctioning should be performed before cuff deflation and extubation (7).

Most Anesthesiologists prefer extubation in the operating room, although data from the UK show that over 40% of all incidents that occur in intensive care immediately after the transfer of extubated patients is related to problems with airway patency. Such a finding is not strange if we look at results of recently published NAP4 report (31) and evidence that many ICUs do not have dedicated airway carts or $EtCO_2$ monitors. Extubation of the patient is the only responsibility of anesthesiologists despite the fact that many hospitals now have protocols that extubation can be done by any other appropriately trained personnel (32), and check for availability of devices, tools and strategies remains mandatory if considering extubation as elective maneuvre.

Neuromuscular blockade monitoring

Any strategy of successful extubation includes in the first place an adequate recovery from neuromuscular blockade, which is very important for maintaining a patency of patient airway and avoiding postextubation hypoxemia. The use of peripheral nerve stimulators to monitor the degree of neuromuscular block is preferred for any elective surgical procedure with the application of neuromuscular blocking agents, and it is absolutely mandatory if muscle relaxants are used in problematic airway. The last recommendations point out that TOFr ≥ 0.9 is the minimum required recovery for safe tracheal extubation. On other hand, pharmacological reversal with anticholesterases should not be initiated until at least two or three responses to TOF stimulation are present. In the absence of objective monitoring, clinical signs can be used to estimate neuromuscular block recovery, such as arms rising of the operating table ≥ 10 seconds and maintaining the head lifted ≥ 5 seconds (33, 34).

Recent findings have shown that postoperative residual curarization remains a still too often unrecognized problem (35), probably accounting for many causes of extubation failure; it appears mandatory, especially in case of predicted difficult extubation, to provide accurate instrumental monitoring of residual curarization, and development of pharmacological strategies which might include use of rocuronium-sugammadex to perform optimal intubation and extubation conditions in the critical airway patient.

The patient position during extubation

Many generations of Anesthesiologists learned the classic method of tracheal extubation with left lateral-head down position of the patient. Origins for this

traditional practice came from use of long lasting effect anesthetics with significant emetogenic potential, so that great attention was paid to prevention of aspiration and maintainance a patent airway after extubation. In such a position, in fact, tongue falling to the posterior wall of the pharynx could be avoided, and making maneuvres easier if patient reintubation was needed. Today, especially if considering non fasted patients, this practice resists the test of time (4, 32), whereas it cannot be considered as anesthetic practice based on evidence: accordingly to DAS guidelinest here is no evidence to support a universal patient position for extubation, while there is an increasing trend towards extubating in a head-up (reverse Trendelenburg) or semi-recumbent position (7). These indications promote important changes in anesthetic dogma, also considering that nowadays elective surgical patients are carefully prepared, newer molecules with fast pharmakokinetic profiles and lower emetogenic potential are available and more effective pharmacologic and fasting strategies are used to neutralize residual gastric content. Furtherly, modern and technologically innovative supraglottic airway devices have been widely introduced in recent years, with great advantage of being better tolerated after extubation and they do not lead to laryngeal incompentention. The application of these devices allows significantly greater level of vigilance during extubation, while on other hand they cannot be considered safe options for airway rescue in case of failed extubation due to laryngeal or sublaryngeal edema, so their role should be carefully considered for such a use (17).

Head-up tilt extubation is recommended for obese patients, in patients with obstructive lung disease, as well as in those in which difficult intubation was experienced. This position facilitates diaphragm motion and favours spontaneous respiration, increases the value of functional residual capacity, facilitates expectoration and improves lymphatic drainage by reducing evenctual airway edema.

Patients should be extubated awake or anesthetized?

The decision when the appropriate time for extubation is made in relation of two important facts: the first is a real risk of aspiration and the other is the presence or absence of previous difficulties with respiratory tract. As a general rule, patient should be extubated awake, because it grants better airway tone reflexes and respiratory drive, representing mandatory choice for full-stomach patients. On other hand, in general, the intensity of hemodynamic stress response, coughing and straining is significantly reduced when the patientis extubated in deep anesthesia. These advantages, which could be important in selected patients categories, must be counterbalanced with increased incidence of respiratory complications and upper airway obstruction (7). However, few studies (mainly in pediatric patients) pointed out significant conclusion about the incidence of respiratory complications when patients are extubated awake, because of the increased airway reactivity.

An interesting mediation could be represented by Laryngeal mask exchange (Bailey maneuver), consisting in replacement of a tracheal tube with a LMA to maintain a patent, unstimulated airway with stable physiological observations and protection of the airway from soiling secondary to blood and secretions in the mouth (7, 13), and it has been proven to allow better emergence profile if compared with both awake and deep extubation (36). It should thus be considered whenever hemodynamic response could be dangerous, but also in asthmatic or smokers patients to minimize airway stimulation, while it should be avoided in case of known difficult airways, high regurgitation risk and suspicion for laryngeal or sublaryngeal edema (7, 17).

Does the preventive use of steroids reduce the incidence of postextubation laryngeal edema and the need for tracheal reintubation?

The appearance of postextubation laryngeal edema and the need for tracheal re-intubation is relatively common after long-term surgery and several days of mechanical ventilation in intensive care units. Beginning of postextubation edema occurs within eight hours of extubation and it is caused by present mucosal edema in glottis region, and edema is caused by pressure or irritation of endotracheal tube, while mechanical edema is also result of repeated and traumatic laryngoscopic attempts in case of difficult intubation. In placebo controlled clinical studies, the incidence of laryngeal edema is in the range of 3–30% of respondents, and consecutive need for reintubation is present in 1 to 5% of respondents. Although the preventive use of steroids had been applied and reported before planned extubation yet since thirty years ago (37), there is today great controversial and lack of relevant evidence for clinical benefit (38–39).

If the patient is mechanically ventilated for more than 24 hours, the optimal time to initiate preventive treatment with steroids is 12 hours before the planned extubation with repeated doses depending on the steroid elimination time. It is believed that the use of steroids after extubation has no preventive effect on the occurrence of postextubation laryngeal edema. Howe-

ver, recent meta-analysis by Fan and Coworkers (40), which included six randomized, placebo-controlled clinical trials with 1923 patients, confirmed that intravenous giving of steroids immediately before extubation reduces the incidence of laryngeal edema for 62% and the need for reintubation for 71%. These figures raise to 86% and 81% if a second dose is repeated after extubation without significant side effects. Similar evidence is presented in a meta-analysis from Jaber et Al (41) and in recent paper by Rafii et Al (42). Further studies are requested to confirm real benefit of steroid administration, and above all to define precise indications, knowing that effect from this drugs can be different accordingly to cause and type of edema (differential between mechanical, vascular or inflammatory edema).

Airway exchange catheters and the concept of gradual extubation strategy

Airway exchange catheters (AEC) recently introduced into clinical practice are used primary for safe endotracheal tube replacement, and they were originally designed particularly for patients on long-term mechanical ventilation.

Their use has been extended to provide a continuous access to the patients airway after extubation allowing safe and fast tracheal reintubation, if necessary. Successful reintubation on airway exchange catheters is not a truly new practice, while it is probably understimated and poorly known, probably because of relatively low number of published studies and mainly focused to pediatric patients (43–45). Patients with difficult airways are best representative group to have potential greatest benefit from application of this technique, which has been elegantly and brilliantly described in observational analysis by Thomas Mort (46) on 9 years routine use of airway exchange catheters in 354 previously intubated patients with known or presumed difficult airway. After evaluation of extubation promptness, preoxygenation is provided via endotracheal tube (ETT). Some lubricant might be added to AEC, and some anesthetic solution can be instilled in trachea during this phase. AEC is then inserted through ETT following numbers marked on its surface, paying attention not to exceed more than 5 centimeters out of ETT tip. ETT is then withdrawn leaving AEC in site. Hollow AEC are preferable because they can be connected to oxygen source to provide further oxygenation. AEC is then safely fixed and left in position if re-intubation is needed or until when safety goal is achieved. In Mort's study method of gradual extubation strategy was applied in operating room, recovery room and intensive care units, and 47 of 51 patients (92%) were successfully reintubated with AEC, 41 (87%) of which at first attempt. Patients were reintubated with a mild sedative dose, and in the case of severe desaturation patients were ventilated directly through the exchanger tube or with masks and self-expanding balloons. In contrast, patients who required reintubation after AEC had been already removed from the trachea, had a significantly lower percentage of successful reintubation in the first attempt (14%) with frequent need for advanced method to secure airway and higher complications rate, showing safety and effectiveness of AEC as method of maintaining approach to the airway after extubation.

Such an evidence resulted in introduction on the market in last year of a dedicated set for staged extubation including teflon guidewire and dedicated AEC for long-lasting protected extubation strategies with minimal patient discomfort and maximal safety.

Extubation in ICU and extubation failure

The concept of difficult extubation in Intensive Care Unit is different from standard extubation in operatory room; in fact, apart from all observations and reasons we considered, in ICU many other factor make extubation (and decision to extubate) more difficult.

All mechanical factors involved in laryngeal trauma become more relevant in ICU, because the patient is typically longer time intubated (17, 19, 20), and many other patophysiologic parameters should be considered. Neural deficit, muscular weakness (including critically ill neuropathy — CIN) (47), ability to maintain oxygenation, cardiovascular stabilty, volume filling and acid-base status are only some of the many factors participating readiness to extubate. Postextubation respiratory failure is a common event after discontinuation of mechanical ventilation, reintubation being needed in about 10% of patients (48); patients who require reintubation after unsuccessful extubation usually do not have a good prognosis, with a mortality rate reaching 40% (49–50), whereas it remains unclear why unsuccessful extubation and the need for reintubation are associated with such a high mortality rate. Criteria defining unsuccessful extubation and the need for reintubation include: increasing $PaCO_2 \geq 10$ mm Hg, reduction of pH ≥ 0.1, $PaO_2 < 60$ mm Hg, $SaO_2 < 90\%$ with the use of $FiO_2 > 0.5$–1 as well as clinical signs of increased respiratory work (tachypnea, increased activity of axiliary respiratory muscles, paradoxycal abdominal breathing). Reintubation procedure is the invasive procedure which can often lead into serious, life-threatening complications such as sudden cardiac arrest, or esophageal intubation, aspiration of gastric contents, heart arrhythmia, pneumonia and pneumothorax (51): in this particular setting, although findings

have been contradictory and the technique is yet to be defined, great advantages might derive from use of noninvasive ventilation (NIV) not only to avoid intubation but also for some "prophylactic role" and post-extubation support in certain circumstances (52). Last but not least, it has been clearly shown that introduction of protocols and defined criteria and strategies, which inlude airway management and extubation, can improve survival rates and outcome (53). However, studies show that mortality rates associated with reintubation depends primarily on the causes that lead to unsuccessful extubation and duration of the time that has passed from unsuccessful extubation to reintubation. The causes of unsuccessful extubation are divided into two groups. In the first group the airway related causes (airway obstruction accompanied with stridor breathing and threatening aspirations and the inability to maintain airway patency due to the accumulation of pulmonary secretions). The causes which are not directly related to the very airway include: congestive heart failure, respiratory failure, encephalopathy, sepsis and gastrointestinal bleeding, and they are much more likely cause of the failure extubation and reintubation. In addition,this group of etiological factors is statistically more frequently associated with mortality of the patients who are reintubated. On the other hand, patients whose extubation was unsuccessful due ton theirs airway problems were reintubated within a short time interval, compared to patients who were reintubated due to other causes mentioned above. The time since extubation to reintubation is a strong predictor of mortality. In this sense, patients who were reintubated after unsuccessful extubation within 12 hours have a higher chance of survival (54).

For these reasons, particular care should be considered for extubation of ICU patients, with higher attention the longer the patient has been intubated and on mechanical ventilation. Visual direct control of larynx and vocal cords should be always considered before any maneuvre, and this migh include also use of video laryngoscope. Careful lubrication and generous preoxygenation should be provided before any extubation attempt and neuromuscular monitoring should be considered too if neuromuscular blocking agents have been used, especially if continuous infusion. A protected and staged extubation strategy should be adopted, with enlarged decisional criteria if compared with operatory room, taking account of not only anatomic and morphologic causes for difficult (re)intubation, but also for pathophysiological and environmental ones. It should be thus recommended for many extubations performed in ICU to provide use of dedicated kits or hollow tube exchangers, well lubricated and with possibility of oxygen connection. Safe time to remove AEC after extubation can be fixed accordingly to patients characteristics and extubation success possibilities, including cardiac and muscular reserve, repeated blood gas analysis and availability for NIV. If re-intubation is needed, sedation must be provided taking care of hemodynamic performance and preferably using laryngoscope or videolaryngoscope for safe and less traumatic procedure, and never bypassing mandatory post-intubation position control with auscultation and capnometry.

CONCLUSIONS

Extubation represents a crucial procedure for airway management, and despite representing last phase of the whole process, it is equally important as intubation and can be even much more challenging, as a failed extubation does not give any other opportunity if not taking back control of the airway. Also in psychologic terms, extubation can be tricky, as typically trouble occurs in moment in which attention is lower, when difficult airway cart is often far (closer to intubation sites) and without same anticipatory emotional tension which occurrs when expecting or facing a difficult intubation.

Starting from these considerations, and keeping in mind data from Literature, developement of safe extubation strategies is mandatory, obviously after a difficult intubations and not less importantly when facing conditions or patients in which difficulties might be encountered or suspected. Dedicated devices are today easily available, and despite controversial, different pharmacological strategies can be adopted. Choices for anesthetic plan for extubation, patient position and use of bridge techniques depend on individual case and should always be balanced preferring safety overextimation.

In the end, key message after twenty years of guidelines on difficult airway management, is that we need to look back at the problem, but starting from the end, to safely close the circle of airway management, for increased patient safety and better results for physicians and hospital staff.

Abbreviations:

AEC — Airway Exchange Catheter
ASA — American Society of Anesthesiologists
CIN — Critically Ill Neuropathy
COPD — Chronic Obstructive Pulmonary disease
CT scan — Computer Tomography scan
DAS — Difficult Airway Society
DLT — Double Lumen Tube
$EtCO_2$ — End Tidal Carbon Dioxyde

ETT — Endo Tracheal tube
FiO_2 — Fraction of Inspired Oxygen
ICU — Intensive Care Unit
LMA — Laryngeal Mask Airway
NAP4 — National Audit Project 4
NIV — Non Invasive Ventilation
OSAS — Obstructive Sleep Apnea Syndrome
$PaCO_2$ — Arterial Carbon Dioxyde partial pressure
PES — Post Extubation Stridor
SaO_2 — Arterial Oxygen Saturation
SFAR — Societé Francaise Anesthésie et Réanimation
TOFr — Train of Four ratio

Sažetak

BEZBEDNA EKSTUBACIJA I REINTUBACIJA PACIJENATA U JEDINICI INTENZIVNE NEGE I OPERACIONOJ SALI

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Svaka intubacija bi na kraju procedure trebalo da rezultira ekstubacijom, i svaka ekstubacija teoretski, može predstavljati potencijalnu reintubaciju zbog neuspeha ekstubacionog manevra.

Postoji mnogo najrazličitijih razloga za neuspešnu ekstubaciju, počev od mehaničkih, jatrogenih lezija usled teške intubacije, preko nemogućnosti pacijenta da održi spontano disanje bez ventilatorne potpore, pa do respiratornih i kardiovaskularnih komplikacija povezanih sa ekstubacijom, lokalnih mehaničkih ili inflamatornih faktora, hirurške tehnike ili pak slučajnih događaja.

Novija literatura je u poslednjih nekoliko decenija jasno pokazala da ukoliko kritični događaji, povezani sa teškom intubacijom, imaju nižu incidencu zahvaljujući vodičima difuzije i implementacije, ekstubacione nezgode ne menjaju incidencu i posledice.

Ovo zapažanje je dovelo do razvoja smernica posvećenih ekstubaciji i privuklo je pažnju naučnika da promovišu bezbednost i viši nivo obazrivosti, naročito kod predviđene teške ekstubacije, kao i primenu zaštitnih strategija ekstubacije pomoću farmakoloških i tehničkih sredstava.

Cilj ovog rada je pregled savremenih podataka o nezgodama vezanih za ekstubaciju, takođe i da omogući pregled postojećih smernica, opiše pristupe kojima se može predvideti teška ekstubacija, kao i tehnike koje obezbeđuju najbolju strategiju ekstubacije uključujući tu farmakološke, nefarmakološke i instrumentalne tehnike.

Ključne reči: sigurna ekstubacija, reintubacija, operaciona sala, jedinica intenzivne nege.

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UPUTSTVO AUTORIMA

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Latković Z. Tumori očnih kapaka. U: Litričin O i sar. Tumori oka. 1. izd. Beograd: Zavod za udžbenike i nastavna sredstva, 1998: 18–23.

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Trier JJ. Celiac sprue. In: Sleisenger MH, Fordtran J5, eds. Gastro-intestinal disease. 4 th ed. Philadelphia: WB Saunders Co, 1989: 1134–52.

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