Abstract: Introduction: Burns, depending on the degree of severity, induce a significant pathophysiological response in the body. The complement system participates in the body's defenses as well as in immune responses after burn-induced trauma.

Objectives: The main objective of the study was to examine how burn severity affects serum C3 and serum C4 complement values; whether burn severity correlates with serum C3 and C4 complement, and establish the predictive value of the serum C3 complement and serum C4 complement for assessing the severity of the burn.

Patients and methods: According to the degree of TBSA, patients were classified into three groups: group with %TBSA < 15% (30 patients), group with %TBSA > 15%-25% (30 patients), and group with %TBSA > 25% to 40% (30 patients). According to the depth of burns, patients were classified into two groups: partial-thickness burns (39 patients) and full-thickness burns (51 patients). We followed laboratory parameters: value serum C3 complement and serum C4 complement on the first and seventh day after burn trauma.

Results: Serum C3 complement was significantly lower in patients with %TBSA > 25%-40% and in the group with %TBSA > 15%-25% compared to patients with %TBSA < 15% on the first and seventh day after burn trauma. Serum C3 complement was significantly lower in patients with %TBSA > 15%-25% compared to patients with %TBSA < 15% on day one and day seven after burn trauma. Serum complement C4 was not significantly different between burn groups on the first and seventh day after burn trauma. Full-thickness burns have significantly lower levels of serum complement C3, compared to partial-thickness burns, on the 1st and 7th day. Full-thickness burns result in a decrease in serum C4 complement compared to partial-thickness burns on the 7th day after burn trauma, but this decrease is not significant. On the 1st day after burn trauma, we found a negative correlation between %TBSA with serum C3 complement. Serum C4 complement was not correlated with %TBSA on the day 1st.

Conclusions: %TBSA and depth of burn result in a significant decrease in serum C3 complement but not serum C4 complement. There is a negative correlation of %TBSA and C3 complement but not serum C4 complement on the 1st day after burn trauma. Serum C3 complement is a significant predictor of burn severity. The predictive significance of the C4 complement is not statistically significant.

Keywords: burns, %TBSA, depth of burns, serum C3 complement, serum C4 complement, predictive significance.

INTRODUCTION

In the daily clinical practice of treating burnt patients, it is crucial to accurately assess the severity of the burn based on exact laboratory parameters. One of these parameters is the level of serum complement.

The complement system participates in the body’s defenses as well as in immune responses after burn-induced trauma. Complement activation can result in opsonization, then activation of leukocytes, which have receptors for complement components, and finally lysis of the target cell, e.g. burn-damaged tissue cells. Serum C3 complement is involved in the process of complex formation and apoptosis as well as the defense against microorganisms, and thus in the creation of a desirable immune response. These components of complements are involved in the defense against microorganisms and the process of creating immune complexes and apoptosis.
RESEARCH OBJECTIVES

The objectives of the study are to examine how different degrees of %TBSA (Total Body Surface Area) and depth of burns affect serum C3 and serum C4 complement; to monitor the dynamics of these parameters in the 1st and 7th days after burns and monitor how serum C3 and serum C4 component of complement correlate with %TBSA. The objectives of the study are to determine the predictor significance of serum C3 and serum C4 components for assessing the severity of the burn.

PATIENTS AND METHODS

The study is a prospective clinical study of patients with burns conducted at the Clinic for Reconstructive and Plastic Surgery of the University Medical Center (UCC) in Sarajevo.

The study included 90 patients with varying degrees of severity of thermal trauma, aged 18 to 65 years, of both sexes, with %TBSA to 40%. The study was conducted from 2010 to 2017. The study did not include patients younger than 18 or older than 65 or patients with other acute and chronic diseases.

Patients with burns were classified according to the generally accepted classification of burns by the American Burns Association (1). According to the %TBSA of the burn, patients were classified into three groups: group with %TBSA < 15% (30 patients), group with %TBSA > 15%-25% (30 patients) and group with %TBSA > 25% to 40% (30 patients); according to the depth of the burn, the group I, partial-thickness burns (39 patients), and in group II, full-thickness burns, (51 patients). We did not have patients with grade IV in this study.

Serum complement C3 and serum C4 complement were laboratory determined on the 1st and 7th day after burn trauma at the Institute of Clinical Immunology, UCC in Sarajevo. Serum C3 complement and serum C4 complement were determined by nephelometry by measuring the turbidity of a water sample by passing a beam of light through the measured sample. A Siemens BN II nephelometer was used for this study Ser. Br. 292714, year of manufacture 2010.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration.

Statistical data

All data collected by this survey were prepared for statistical evaluation and stored in the Excel 2010 program, Microsoft Office software package (Microsoft USA), while statistical processing was done in the statistical data processing program SPSS ver.20.

The following tests were performed for inferential statistics depending on the sample distribution: Student T-test, Mann-Whitney U test, AVENE test, and Kruskal-Wallis test. The Pearson test or Spearman’s test was used in the correlation analysis depending on the distribution of the samples.

A linear multivariate regression model was used to assess the predictor effect of individual variables on the dependent outcome variable (%TBSA). Values of p < 0.05 were accepted as statistically significant.

RESULTS

We statistically analyzed the values of serum C3 complement individually in each group with different %TBSA (%TBSA < 15%; %TBSA > 15%-25%; %TBSA > 25-40%) on the 1st and 7th day. Serum C3 complement was significantly lower in the patients with %TBSA < 15% on day 7th, compared to day 1, p = 0.043. In the group with %TBSA > 15%-25% serum C3 complement was significantly lower on the 7th day, compared to the 1st day, p = 0.038. In the group of patients with %TBSA > 25%-40%, serum C3 complement was significantly lower on the day 7th compared to the day 1st after burn trauma, p < 0.005 (Table 1).

Statistical analysis of serum C3 complement between 3 groups of patients with different %TBSA (%TBSA < 15%; %TBSA > 15%-25%; %TBSA > 25-40%) on the first day, we found a significant difference between the groups, p < 0.005. Seventh day after the burn we found a significant difference between the groups (%TBSA < 15%; %TBSA > 15%-25%; %TBSA > 25-40%), p < 0.005.

Table 1. Serum C3 complement and %TBSA

| %TBSA     | 1st day X ± SD | 7th day X ± SD | P    |
|-----------|----------------|---------------|------|
| %TBSA < 15% | 1.44 ± 0.19  | 1.27 ± 0.26   | p = 0.043 |
| %TBSA > 15-25% | 1.02 ± 0.22  | 0.87 ± 0.21   | p = 0.038 |
| %TBSA > 25-40% | 0.83 ± 0.19  | 0.59 ± 0.12   | p < 0.005 |

Legenda: X - mean value, SD - standard deviation
We analyzed the behavior of serum C3 complement between groups on the 1st and 7th day. By statistical analysis, we obtained the data that serum C3 complement on day 1st after burn trauma was significantly lower in the patients with %TBSA > 25-40% compared to the patients with %TBSA > 15%-25%, p = 0.004. Serum C3 complement on the 1st day after burn trauma were significantly lower in patients with %TBSA > 25-40% compared to patients with %TBSA < 15%, p < 0.005. On the 1st day, serum C3 complement were significantly lower in the patients with %TBSA > 15%-25% compared to the patients with %TBSA < 15%, p < 0.005.

On the 7th day, the serum C3 complement were significantly lower in the patients with %TBSA > 25-40% compared to patients with %TBSA < 15%, p < 0.005. Serum C3 complement values decreased on the 7th day compared to the 1st day in all groups of burns, but this decrease was not significant (in the group with %TBSA < 15%, p = 0.976; in the group with %TBSA > 15%-25%, p = 0.977; in the group with %TBSA > 25%-40%, p = 0.331) (Table 2).

On the 7th day after burn trauma, full-thickness burns resulted in a significant decrease in serum C3 complement compared to partial-thickness burns, p < 0.005. Full-thickness burns result in a decreased serum C4 complement compared to partial-thickness burns on the 7th day after burn trauma, but this decrease is not significant, p = 0.497 (Table 3).

### Table 2. Serum C4 complement and %TBSA

| % TBSA      | DAYS  | p     |
|-------------|-------|-------|
|             | 1st day | 7th day |
|             | M  | IQR | M  | IQR |
| %TBSA < 15%| 0.24   | 0.17-0.32 | 0.23  | 0.19-0.32 | p = 0.976 |
| %TBSA > 15-25% | 0.23   | 0.19-0.30 | 0.2   | 0.16-0.30 | p = 0.977 |
| %TBSA > 25-40% | 0.21   | 0.16-0.26 | 0.18  | 0.15-0.30 | p = 0.331 |

Legend: M - median, IQR - interquartile range

### Table 3. Serum C3 and serum C4 complement on the 7th day

| Serum complement | BURN DEPTH | p     |
|------------------|------------|-------|
|                  | Partial thickness burn | Full thickness burn |
|                  | M (IQR)    | M (IQR) |
| C3               | 1.2 (1.02-1.52) | 0.7 (0.54-0.90) | p < 0.005 |
| C4               | 0.25 (0.18-0.31) | 0.23 (0.18-0.31) | p = 0.497 |

Legend: M - median, IQR - interquartile range

### Table 4. Predictor importance of C3 and C4 complement for the assessment of %TBSA

| Model | Non-standard coeff. | Standard coefficient | t     | p     | 95% CI for B |
|-------|---------------------|----------------------|-------|-------|--------------|
|       | B       | Std. error | Beta |       | lower limit | upper limit |
| 1 cont. | 11.464   | 4.429 |       | 2.588 | 0.011 | 2.65 | 20.277 |
| C3    | -1.623   | 2.287 | -0.05 | -2.68 | 0.012 | -6.175 | 2.929 |
| C4    | -0.087   | 0.063 | -0.073 | -2.379 | 0.064 | -0.212 | 0.039 |

Legend: Dependent variable: %TBSA, Confidience interval (CI)

We analyzed the behavior of serum C3 complement between groups on the 1st and 7th day. By statistical analysis, we obtained the data that serum C3 complement on day 1st after burn trauma was significantly lower in the patients with %TBSA > 25-40% compared to the patients with %TBSA > 15%-25%, p = 0.004. Serum C3 complement on the 1st day after burn trauma were significantly lower in patients with %TBSA > 25-40% compared to patients with %TBSA < 15%, p < 0.005. On the 1st day, serum C3 complement were significantly lower in the patients with %TBSA > 15%-25% compared to the patients with %TBSA < 15%, p < 0.005.

On the 7th day, the serum C3 complement were significantly lower in the patients with %TBSA > 25%-40% compared to the patients with %TBSA > 15%-25%, p < 0.005, as well as the patients with %TBSA > 25%-40% to the patients with %TBSA < 15%, p < 0.005. On the 7th day, serum C3 complement were significantly lower in the patients with %TBSA > 15%-25% compared to the patients with %TBSA < 15%, p < 0.005.

We statistically analyzed the values of serum C4 complement individually in each group with different %TBSA (%TBSA < 15%; %TBSA > 15%-25%; %TBSA > 25-40%) on the 1st and 7th days. Serum C4 complement values decreased on the 7th day compared to the 1st day in all groups of burns, but this decrease was not significant (in the group with %TBSA < 15%, p = 0.976; in the group with %TBSA > 15%-25%, p = 0.977; in the group with %TBSA > 25%-40%, p = 0.331) (Table 2).

On the 1st day, serum C4 complement values did not differ significantly between group whit %TBSA < 15% compared to group whit %TBSA > 15%-25%, p > 0.05 , and compared to the patients whit %TBSA > 25%-40%, p = 0.077. Serum C4 complement values did not differ significantly between the patients with %TBSA < 15%, compared to the patients with %TBSA > 25%-40%, p = 0.318 on the day 7th after burn trauma.

On the 7th day after burn trauma, full-thickness burns resulted in a significant decrease in serum C3 complement compared to partial-thickness burns, p < 0.005. Full-thickness burns result in a decreased serum C4 complement compared to partial-thickness burns on the 7th day after burn trauma, but this decrease is not significant, p = 0.497 (Table 3).
On the 1st day after burn trauma, % TBSA negatively correlated with serum C3 complement, \( p < 0.005 \). On the 1st day after burn trauma, we found no correlation of %TBSA with serum C4 complement, \( p > 0.005 \).

On the 7th day after burn trauma, we proved that serum C3 complement is the greater predictor for the assessment of burn severity compared to serum C4 complement. The predictor importance of serum C4 complement is insignificant. The statistical model classified 84% of the variability of the dependent variable, which can be explained by independent parameters (Table 4).

**DISCUSSION**

The management of burn patients is always challenging for the clinician due to the high risk of bacterial sepsis, multi-organ failure, and death. Thermally devitalized tissue is a potent complement activator. Complement activation can be triggered by thermally damaged cell proteins, polysaccharides, bacterial endotoxins, fungi, or serum proteins.

This research proved that serum C3 complement is significantly activated on the first day after burn trauma. The intensity of serum C3 complement activation depends on the %TBSA and the duration of the burn trauma.

On the 1st and 7th day after burn trauma, C3 complement is more activated in burns with %TBSA > 25%-40% compared to groups with %TBSA > 15%-25% and also compared to patients with %TBSA < 15%. On the 1st and 7th day after burn serum C3 complement is more activated in a group with %TBSA > 15%-25% compared to patients with %TBSA < 15%.

Serum complement C4 is also activated on the first day after burn trauma. The activation of serum C4 complement depends on the % of TBSA and the duration of the burn but the decrease in C4 complement value, i.e., its activation, is not significant between individual groups with different % of TBSA. How long the burn lasts affects the activation of serum C4 complement. In each group individually, serum C4 complement was lower on the seventh day when compared to the first day after the burn, but this reduction was not significant. Greater burn areas have the consequence of the higher complement consumption and the significantly lower serum C3 complement values, but do not significantly lower the serum C4 complement.

The results of our research are similar to experimental works of Gelfand et al (2). They found massive activation of an alternative complement pathway but not the classical activation pathway in a mouse experiment, %TBSA 25%-60%. The authors found de novo immunoelectrophoretic conversion of C3 complement 15 minutes to 2 hours after the burn. The activation of the alternative complement pathway was associated with increased aggregation of neutrophils in plasma and lungs, but also with increased permeability of blood vessel walls in the lungs and the formation of pulmonary edema. Decomplementation with one factor of cobra venom, or in the case of a genetic defect of the C5 component of complement, reduced complement activation by an alternative route, reduced aggregation of neutrophils in pulmonary blood vessels, reduced pulmonary edema, and reduced mortality from burns in the first 24 hours after the burn. The same authors followed the activation of the classical and alternative complement activation pathways, measuring the hemolytic activity of CH50 complement in 8 burnt patients with %TBSA 30%-90%. In the first 24 hours after the burn, they found primary activation of an alternative complement activation pathway in seven patients who developed bacteremia, pneumonia, and acute respiratory distress syndrome (ARDS). The classical complement activation pathway was not significantly activated. In the serum of burnt patients, the activating products, i.e., de newly created proteins, which activate an alternative pathway of complement activation, the mentioned authors, failed to prove. The authors hypothesize that these are the consequences of rapid in vivo clearance of de newly created substances that activate an alternative pathway of complement activation in humans.

The degree of complement reduction may be one of the indicators of the survival of the burnt patient (3). In burn patients a massive inflammatory response is induced that negatively affects the healing process of the burn wound and additionally exerts systemic effects. An important factor here is the complement system (4).

Bjornson et al. (5) experimentally examined the opsonic activity of C3 complement against E. coli in the serum of five patients with burns, with %TBSA of 40%-80%, for three weeks after the burn. Opsonic activity and serum C3 levels were reduced in all patients during the first seven days after the burn. The opsonic activity of the C3 complement remained significantly reduced for three weeks after the burn injury. By adding the serum of healthy persons, and the serum of burnt patients, the opsonic activity of the C3 complement returned to normal limits, but the ability to activate the C3 complement did not return. The authors believe that the reduced opsonization is a consequence of serum protein deficiency in patients with burns, and the decrease in C3 conversion may also be caused by some inhibitor present in the circulation. Mc Cabe (6) found in his studies that reduced complement values
are important predictors of immune suppression and concluded that immunosuppression is stronger if the %TBSA is greater.

Some authors attribute a direct role in the lethal outcome of an extensively burnt patient to the activation of the alternative complement pathway. Gelfand et al. (7) believe that, in extensive burns, shock does not occur as a result of protein loss over burnt surfaces, but due to increased aggregation of neutrophils in plasma and lungs, increased permeability of blood vessel walls in the lungs, formation of pulmonary edema and lethal outcome, and all changes as a consequence of complement activation by an alternative pathway. Kang et al. (3) found, in patients with extensive burns with a lethal outcome, simultaneous activation of both the classical and alternative complement activation pathways. The function of the complement system may be determined by a delicate balance between positive and negative regulation. Bain et al. have shown that increased alternative complement pathway function is associated with improved survival during critical illness, possibly due to improved host immune capacity (8). The immune response of the organism after a burn is very complex and is caused by several factors. The serum complement is one of these crucial factors. Our earlier research shows that the behavior of T lymphocytes in patients with different %TBSA is similar to the behavior of serum complement in this research. During the burns, several changes in the T-lymphocyte population were observed. The suppression of the immune response is greater the more severe the burn is (9).

CONCLUSIONS

Our research has shown that complement activation occurs on the first day after the burn trauma. Patients with a higher %TBSA already on the first day after the burn had lower complement values compared to patients with a lower %TBSA. On the 1st day after burn trauma, we found a negative correlation between %TBSA and serum C3 complement. Complement C3 was significantly lower on 7th day compared to day 1 after the burn in all burnt patients with different %TBSA, but not serum C4 complement. Full-thickness burns resulted in a significant decrease in serum C3 complement compared to partial-thickness burns. The larger burn area has the consequence on the higher complement consumption and the significantly lower serum C3 complement values, but not significantly lower the serum C4 complement. Serum C3 complement is a significant predictor of burn severity on the 1st day. Predictory significance of serum C4 complement is not significant on day 1.

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Sažetak

SERUMSKI C3 I SERUMSKI C4 KOMPLEMENT KOD PACIJENATA SA OPEKOTINAMA I KORELACIJA SA TEŽINOM OPEKOTINE

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Uvod: Opekotina, ovisno o stepenu težine, indukuje značajan patofiziološki odgovor organizma. Serumski complement učestvuje u imunološkom odgovoru opečenog organizma u sklopu opšteg imunološkog odgovora indukovanog opekotinskom traumom.

Ciljevi istraživanja: Ciljevi istraživanja su ispitati kako težina opekotine utiče na vrednosti serumskog C3 i C4 komplementa, da li težina opekotine koreli sa vrednostima serumskog C3 i C4 komplementa, i proceniti prediktivnu važnost serumskog C3 i C4 komplementa za procenu težine opekotine.
Pacijenti i metod rada: Prospektivno ispitivanje je sprovedeno kod 90 pacijenata. Prema stepenu %TBSA, pacijente smo klasifikovali u 3 grupe: pacijente sa %TBSA < 15% (30 pacijenata), pacijente sa %TBSA od 15%-25% (30 pacijenata) i pacijente sa %TBSA 25%-40% (30 pacijenata). Partial-thickness burns, imalo je 39 pacijenata, a full-thickness burns 51 pacijent. Kod svih pacijenata određivali smo serumski C3 i C4 komplement prvog i sedmog dana nakon opekotine.

Rezultati i straživanja: Serumski C3 komplement signifikantno je niži kod grupe sa %TBSA 25%-40% i grupe sa %TBSA 15%-25% prvog i sedmog dana u odnosu na pacijente sa %TBSA < 15%, p < 0.005. Serumski C4 komplement nije se signifikantno razlikovao između ispitivanih grupa prvog i sedmog dana. Serumski C3 komplement signifikatno je niži u grupi pacijenata sa full-thickness burns u poređenju sa grupom pacijenata sa partial-thickness burns, p < 0.0005. Serumski C4 komplement bio je niži u grupi pacijenata sa full-thickness burns u poređenju sa pacijentima sa partial-thickness burns, ali to sniženje nije signifikantno. Već prvog dana nakon opekotinske traume, %TBSA je u negativnoj korelaciji sa serumskim C3 komplementom, ali ne i sa serumskim C4 komplementom.

Zaključak: Naše istraživanje pokazalo je da se serumski C3 komplement aktivira već prvog dana na opekotine. Pacijenti sa većim %TBSA već prvog dana imali su niže vrednosti komplementa u odnosu na pacijente sa nižim %TBSA. Prvog dana opekotine prisutna je negativna korelacija serumskog C3 komplementa i %TBSA. Serumski C3 komplement signifikatno je niži sedmog dana u poređenju sa prvim danom opekotine kod svih pacijenata sa različitim %TBSA. Full-thickness burns pacijenti imaju signifikantno niži serumski C3 komplement u poređenju sa pacijentima koji su imali partial-thickness burns. Veći stepen %TBSA i dubina opekotine signifikantno smatraju serumski C3 komplement, ali ne i serumski C4 komplement. Serumski C3 komplement je značajni prediktor težine opekotine u većim stepenima %TBSA, ali to sniženje nije signifikantno. Serumski C4 komplement je značajni prediktor težine opekotine u većim stepenima %TBSA, ali to sniženje nije signifikantno. Serumski C3 komplement je značajni prediktor težine opekotine već prvog dana nakon opekotinske traume, ali ne i serumski C4 komplement. Serumski C3 komplement je značajni prediktor težine opekotine u većim stepenima %TBSA, ali to sniženje nije signifikantno. Serumski C4 komplement je značajni prediktor težine opekotine u većim stepenima %TBSA, ali to sniženje nije signifikantno.

Ključne reči: opekotine, %TBSA, dubina opekotine, serumski C3 komplement, serumski C4 komplement, prediktorni značaj serumskog komplementa.

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