The relationship between procalcitonin, D-dimer, ferritin, troponin, and lactate levels with COVID-19

Prokalsitonin, D-dimer, ferritin, troponin ve laktat düzeylerinin COVID-19 ile ilişkisi

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ABSTRACT

Aim: Coronavirus disease-2019 patients may experience an increase in inflammation or clotting disorders. It is believed that some biomarkers can be decisive in decision to hospitalize. Our aim in this study was to investigate the relationship between serum procalcitonin, troponin, D-dimer, ferritin and lactate levels, and Coronavirus disease-2019.

Methods: Patients suspected of suffering from the Coronavirus disease-2019 and whose serum biochemistry and blood gas tests were performed, were included in the study. The relationships between the blood parameters of the patients with negative or positive Polymerase Chain Reaction test results, and those who were hospitalized or non-hospitalized, were examined.

Results: Out of a total of 452 patients, 244 (54%) were male and 208 (46%) were female. There was a statistically significant difference between polymerase chain reaction test positive and test negative groups in terms of procalcitonin, D-dimer, lactate and troponin levels ($p<0.005$). There was no statistically significant difference between hospitalized and non hospitalized patient in terms of ferritin ($p=0.224$). However, there was a significant difference in terms of procalcitonin, D-dimer and troponin levels ($p<0.005$).

Conclusion: Our results revealed that high serum procalcitonin, D-dimer, lactate and troponin levels are associated with the decision to hospitalize Coronavirus disease-2019 patients, whereas ferritin levels played no such role.

Keywords: Coronavirus disease-2019, Procalcitonin, D-dimer, Troponin, Lactate

ÖZ

Amaç: Koronavirüs hastalığı-2019 hastalarında enflamasyon artışı veya pıhtılaşma bozukluğu artışı görülebilmektedir. Bazı biyobelirteçlerin, hastaneye yatış yapılmasında belirleyici olabileceği düşünülmektedir. Bu çalışmadaki amacımız, serum prokalsitonin, troponin, D-dimer, ferritin ve laktat düzeyleri ile Koronavirüs hastalığı-2019 arasındaki ilişkiyi araştırmaktı.

Metod: Koronavirüs hastalığı-2019 şüphesi olan, kan biyokimya ve kan gazı tetkikleri çalışılan hastalar çalışmaya dahil edildi. Polimeraz zincir reaksiyonu test sonuçları negatif veya pozitif olan hastalar ve taburcu veya servise yatışı yapılan hastaların kan sonuçları arasındaki ilişkileri incelendi.

Bulgular: Toplam 452 hastanın, 244 (% 54)'ü erkek ve 208 (%46)'i kadın idi. Polimeraz zincir reaksiyonu testi pozitif olan grup ile negatif olan grup arasında prokalsitonin, D-dimer, laktat ve troponin düzeyleri istatistiksel olarak anlamlı bulundu ($p<0.005$). Yatış yapılan hastalar ile yapılmayan hastalar arasında ferritin seviyesinde istatistiksel olarak bir anlamlılık yoktu ($p=0.224$). Ancak prokalsitonin, D-dimer, troponin seviyelerinde anlamlı bir farklılık çıktı ($p<0.005$).

Sonuç: Elde ettiğimiz sonuçlarla yüksek serum prokalsitonin, D-dimer, laktat ve troponin düzeylerinin Koronavirüs hastalığı-2019 hastalarının hastaneye yatışı ile ilişkisinin olduğunu söyleyebiliriz. Ancak ferritin düzeylerinin hastaneye yatış endikasyonunda bir rolü yoktu.

Anahtar Kelimeler: Koronavirüs hastalığı-2019, Prokalsitonin, D-dimer, Troponin, Laktat

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INTRODUCTION

With reports emanating out of China of viral pneumonia cases with an unknown cause at the end of 2019, various studies were conducted and a new type of coronavirus was soon identified. The Coronavirus disease-2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), quickly spread to 6 continents and more than 200 countries, and became the first ever pandemic caused by a coronavirus [1]. Initially considered to be a respiratory infection, data now suggests that COVID-19 is a systemic (cardiovascular, gastrointestinal, neurological, hematopoietic and immune system) disease. Patients usually present with fever, fatigue, dry cough, loss of appetite, myalgia, dyspnea, loss of smell and taste, nausea, vomiting, diarrhea, nasal discharge and conjunctivitis [2]. It has been found that older people or those with comorbidities have a higher risk of mortality, but fatal complications can also occur in young people without an underlying disease [3]. As of the time this article is written, the number of deaths from COVID-19 has surpassed 2,5 million worldwide, while nearly 28,000 people have lost their lives in Turkey [4] and there remains a great deal that is unknown regarding COVID-19. Some studies have investigated the relationship between blood levels and this coronavirus. For example, lymphopenia, prothrombin time/partial thromboplastin time length and high D-dimer were found to be precursors to severe disease [5]. One current study hypothesized that progressive increase in PCT levels may be positively associated with the severity of COVID-19 and predict a poor prognosis [6].

In light of the high prevalence of COVID-19 disease and limited hospital capacities, determining the profile of patients who needs be hospitalized has become an important goal. Therefore, our study focused on the changes in some blood parameters such as PCT, D-dimer, ferritin and troponin in the follow-up and treatment of patients diagnosed with COVID-19. Our objective was to compare polymerase chain reaction (PCR)-positive and PCR-negative patients and hospitalized versus non-hospitalized patients, based on these parameters.

MATERIAL AND METHODS

A total of 452 patients who came to the emergency medicine (ER) with clinical presentation of COVID-19 between April 1, 2020 and September 1, 2020, had their case studied retrospectively. According to the G-power analysis, it was planned to have at least 350 patients in the study, which received the approval of the Ethics Committee of the Faculty of Medicine of Ataturk University, designated B.30.2.ATA.0.01.00/416, on October 1, 2020. Age, gender, complaints upon presentation, real-time reverse transcription (RT)-PCR test results, PCT, D-dimer, ferritin and troponin levels, as well as hospitalization and non-hospitalization statuses of patients were examined. Laboratory and PCR-test results of patients were obtained from the hospital's database while vital signs and clinical information were gathered from patient files. In our hospital, an RT-PCR test is performed using the ELISA method on an AB Applied Biosystems GeneAmp PCR System 9700 device. D-dimer was tested using the immunoturbidimetric method on a AQT90 Flex device, whereas PCT, ferritin and troponin were tested using the ECLIA method with a Beckman Coluter Unicel Dxl 600 device.Patients who died, patients with incomplete blood parameters and those under 18 years of age, were excluded from the study. The blood levels parameters of the remaining patients who were either hospitalized or non-hospitalized, were thereby compared. Those with a poor clinical picture (breathing difficulty (≥ 30/min, oxygen saturation ≤%93 and/or PaO2/FiO2≤300 mmHg) [7], those who were PCR-positive or negative and had extensive ground-glass opacity showing in thorax computed tomography (CT) that is consistent with viral pneumonia, were accepted as the hospitalized group. High-resolution CT was performed in all patients using a Siemens Somatom Emotion 16 model device. Patients with negative RT-PCR results and normal thorax CTs were accepted as the non-hospitalized group.

Statistical Analysis

The data was provided as mean ± standard deviation (SD), median (range) or number (percentage). For continuous variables, the assumption of normal distribution was checked using the Shapiro-Wilk W-test when the sample size was <50, and

the Kolmogorov-Smirnov test when the sample size was ≥50. In the comparison between the two independent groups, independent samples t-test was conducted when the distribution was normal, and Mann-Whitney u test was used when data was not normally distributed. Categorical variables were compared using the Chi-square test. The receiver operating characteristic (ROC) curve analysis was conducted for PCT, troponin, D-dimer and lactate, to determine whether any of these variables affected the determination to hospitalize. The ROC curve analysis was also utilized to determine the cut-off values, the best of which were calculated using Youden's Index. The data was analyzed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) for Windows. For all values, p<.05 was considered statistically significant.

RESULTS

The mean age of patients was 54 ± 20 (range: 18-96). The number of male patients was 244 (54%), while the number of female patients was 208 (46%). According to the results of combined throat / nasal swab test performed in the ER, 67 (14.8%) patients were PCR-positive and 385 (85.2%) patients were PCR-negative.

Complaints of the patients with suspicious contact admitted to the ER were muscle pain (myalgia) (48.2%), fatigue (19.7%), fever (18%). The others were asymptomatic (13.7%) (Table 1).

Table 1. Demographic and clinic data of COVID-19 patients.

Gender (n,%)	Female	208 (46)
	Male	244 (54)
Presenting Complaint (n,%)	Myalgia	218 (48.2)
	Fatigue	89 (19.7)
	Asymptomatic	62 (13.7)
	Fever	83 (18.4)
RT-PCR (n,%)	Positive	67 (14.8)
	Negative	385 (85.2)
Hospitalization (n,%)	Hospitalized	108 (23.9)
		344 (76.1)

All blood parameters of the PCR-positive group were lower than those of the PCR-negative group. There was no statistically significant difference between the two groups in terms of serum ferritin levels (p=0.713). However, there was a statistically significant difference between PCT, D-dimer, troponin and lactate levels of two groups

(p values were 0.001, 0.001, 0.001 and 0.001, respectively) (Table 2).

Table 2. Evaluation of blood parameters according to RT-PCR result

	RT-PCR positive (n:67)	RT-PCR negative (n:385)	P value
Age	50±20	55±20	0.032*
Ferritin	1188.8±1658	1223.6±1916.4	0.713*
PCT	3.33±13.7	5.45±43.43	0.001*
D-dimer	1222.8±3773.6	1923.9±6368.3	0.001*
Troponin	20.8±90.6	200.1±1844.3	0.001*
Lactate	1.6±1.0	2.1±1.6	0.001*

P*: Mann-Whitney U test, PCT: Procalcitonin

The number of patients hospitalized after ER admission was 108 (23.9%), while the number of discharged outpatients was 344 (76.1%) (Table 1). The mean age of hospitalized patients was higher than the mean age of non-hospitalized patient, however, this finding was determined not to be statistically significant (p=0.009). The blood parameters of hospitalized and non-hospitalized patient were also compared. There was no statistically significant difference in ferritin levels between these two groups (p=0.224), however there was a statistically significant difference between PCT, D-dimer, troponin and lactate levels of the two groups (p values were 0.001, 0.001, 0.004 and 0.001, respectively) (Table 3).

Table 3. Evaluation of blood parameters by hospitalization

	Hospitalized Patients (n:108)	Non-hospitalized Patients (n:344)	P value
Age	59 ± 20	53 ± 20	0.009**
Ferritin (ng / ml)	1602.5 ± 2268	1090.5 ± 1714.4	0.224**
PCT (ng/ml)	15.5 ± 79.6	1.6 ± 5.3	0.001**
D-dimer (ng/ml)	4468.8 ± 11575.3	937.1 ± 1188.1	0.001*
Troponin (ng/m)	589.1 ± 3374.1	35.1 ± 149.4	0.004**
Lactate (mmol/L)	2.7 ± 2.5	1.9 ± 1.1	0.001**

P*: Mann-Whitney U test, P**: Independent t-test, PCT: Procalcitonin

The area under the ROC curve (AUC) and statistic values of each study variable with a 95% confidence interval are specified in Table 4, to show which ones indicate the risk of hospitalization in COVID-19 patients. Based on the findings, a PCT higher than 0.16 ng/ml, D-dimer higher than 1430 ng/ml, troponin higher than 26.6 ng/m and lactate higher than 2.75 mmol/L, are associated

with a higher risk of hospitalization in COVID-19 patients (Table 4). Figure 1 presents the sensitivity and specificity of the PCT, D-dimer, troponin and lactate in predicting hospitalization of COVID-19 patients in the ROC curve analyze (Figure 1).
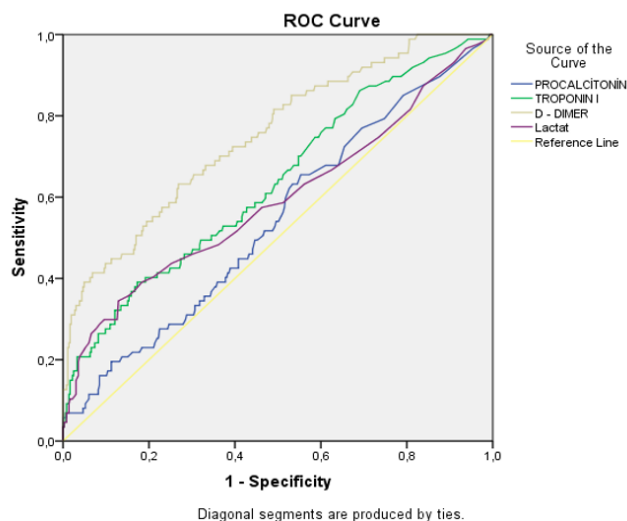

Figure 1. Presents the sensitivity and specificity of the PCT, D-dimer, troponin and lactate in predicting hospitalization of COVID-19 patients.

DISCUSSION

In this study, we examined whether there was any relationship between the levels of some biomarkers, hospitalization indication and RT-PCR test results in COVID-19 patients. We have found a relationship between high serum PCT, D-dimer, lactate and troponin levels and hospitalization of these patients.

As a result of the increase in the number of COVID-19 patients in recent months, the issue of which patients will be hospitalized becomes more important. Even though the RT-PCR test, which is still required for COVID-19 diagnosis, comes back negative, the diagnosis should be supported with laboratory and radiological findings [8]. RT-PCR positive patients are considered confirmed cases. In the early period, PCR negative patients should also undergo thorax CT as the radiological imaging method. However, thorax CT follow-up is not recommended for evaluating the response to treatment [9]. PCR test results are still the main focus for diagnosis, treatment and hospitalization, however given the low cost and rapid results, blood parameters can be used, without waiting for PCR test results.

The most frequent laboratory findings in seriously ill hospitalized patients are lymphopenia, hypoalbuminemia, and increased ferritin and D-dimer (10). Leukocyte levels and lymphocyte counts, which are indicators for inflammation, are normal in the early stage of the disease when there are non-specific symptoms [11]. However, clinical signs of the disease start to become apparent 7 to 14 days after symptom onset and this is the systematic increase of inflammatory markers and cytokines, which is also known as the cytokine storm [12]. It is believed that the severity of COVID-19, even its fatal outcomes, is a consequence of the cytokine storm syndrome [13].

Ferritin is an important mediator of immune system disorders. Hyperferritinemia contributes to cytokine storm through direct immunosuppressive and proinflammatory effects [14]. A study on patients who died of COVID-19 found that ferritin levels were high after hospitalization as well as during their hospital stay [15]. In their study, Wu et al. showed high serum ferritin to be associated with ARDS development; no significant association was found with survival [16]. Although ferritin levels were high in the hospitalized patient group in our study, no statistical significance was found between hospitalized and non-hospitalized patient groups.

Coagulation disorders appear to be more common in severe cases of COVID-19. D-dimer levels are associated with negative outcomes among community-acquired pneumonia patients, which may also reflect the severity of COVID-19 [17]. In a descriptive study by Chen et al, high D-dimer levels were measured in 36% of 99 patients [18]. Another study on 41 patients in China, showed that D-dimer levels were higher at the time of admission among patients requiring intensive care. The median D-dimer for intensive care patients was 2.4 mg/L and 0.5 mg/L for non-intensive care patients (p = 0.004) [19]. Our study reached conclusions similar to those in the literature: D-dimer levels were high in both PCR-negative and hospitalized groups.

PCT is released by thyroid parafollicular C cells, a 116-amino acid peptide precursor to the hormone calcitonin [20]. PCT has been widely researched

for its use as a biomarker in bacterial infections [21]. There have been some studies that proved PCT levels are positively correlated with the severity of COVID-19. In a study of Lippi et al. where they measured the PCT levels of 38 patients, 32 were discharged and 6 died in intensive care: PCT levels were low in non-hospitalized patients [22]. Our study found PCT levels to be high among hospitalized patients. Statistically significant results were obtained in PCR-positive and the hospitalized group.

Troponin is a marker of myocardial damage, mainly myocardial infarction or myocarditis. High troponin levels are quite common among COVID-19 patients and are associated with fatal outcomes. Along with the fact that it does not have a definitively proven mechanism, it is believed that it may be through viral myocarditis, cytokine-induced myocardial damage and microangiopathy [23]. High troponin levels were found to be common among hospitalized COVID-19 patients and the duration of stay at the hospital was also longer in patients with high troponin levels [24]. Our study found that the troponin level was higher among PCR-positive patients, compared to PCR-negative patients. We assume that this finding is independent of PCR tests and may be related to the patient's clinical status. The troponin levels of hospitalized patients were quite high compared to the non-hospitalized group.

Serum lactate is a byproduct of anaerobic metabolism. Lactate levels can show the severity of tissue hypoperfusion and hypoxia [25]. In tissue hypoxia, where there is no oxygen, pyruvate molecules cannot enter the mitochondria and are converted to lactate. Lactate accumulation is a result of increased production and/or decreased removal. In one study, higher lactate levels suggested that anaerobic glycolysis began early due to insufficient oxygen supply [26], therefore higher lactate levels may be considered an indicator for insufficient oxygen supply.

When we searched the literature, we found that there were few studies investigating the relationship between COVID-19 and lactate levels, it was therefore our hope that ours would guide future studies. We found lactate levels in the PCR-negative group were significantly higher than in the PCR-positive group and seem to be a rapid and reliable indicator for lung damage, and could even be used to better adapt the treatment of COVID-19 patients. Although there are an increasing number of studies being performed on this coronavirus, we hope the results of our study can be of use as it is the first to compare this quantity of blood parameters according to PCR test results, as well between hospitalized and non-hospitalized patients.

Limitations of the study

Our study has some limitations, One of which was that the hospital where the study was conducted is one of two large hospitals in the city and the other institution being used as the designated pandemic-response hospital. This situation resulted in a relatively low number of COVID-19 patients presenting. Another limitation is that the epidemiologist made the determination of whether a patient should be hospitalized or not, and that the hospitalization criteria depend on multiple factors. The presence of various diseases that might affect the patients' parameters and the fact that we did not evaluate other blood parameters, are both limitations. Finally, the retrospective design of this study may be considered an additional limitation.

In conclusion, patients can be hospitalized according to some blood parameters and RT-PCR results. In our study, we found that besides the clinic, serum PCT, D-dimer, lactate and troponin levels were higher in hospitalized patients. We are of the opinion that extensive studies are needed to investigate the relationship between these blood parameters and the severity of COVID-19.

REFERENCES

1. Dikmen AU, Kına MH, Özkan S, İlhan MN. Epidemiology of COVID-19: What We Learn From Pandemic. Journal Biotechnol and Strategic Health Res. 2020;1(Özel Sayı):29-36. doi: 10.34084/bshr.715153.

2. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical Characteristics of COVID-19 in New York City. N Engl J Med. 2020;382(24):2372-4 doi: 10.1056/NEJMc2010419.

3. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-7. doi: 10.1111/jth.14768.

4. WHO Coronavirus Disease (COVID-19) Dashboard Data last updated: 2021/02/21, 04:21 pmCEST

5. Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGH, Tan GB, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020;95(6):E131-4 doi: 10.1002/ajh.25774.

6. Zhang J, Dong X, Cao Y, Yuan Y, Yang Y, Yan Y, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020;75(7):1730-41 doi:10.1111/all.14238.

7. Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: a meta-analysis. J Med Virol. 2020;92(11):2283-5 doi: 10.1002/jmv.25948.

8. Bryant JE, Azman AS, Ferrari MJ, Arnold BF, Boni MF, Boum Y, et al. Serology for SARSCoV-2: Apprehensions, opportunities, Sci Immunol. 2020;5(47):eabc6347 doi: 10.1126/sciimmunol.abc6347.

9. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020;296(2):E32-40. doi: 10.1148/radiol.2020200642.

10. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features in severe and moderate coronavirus disease 2019. J. Clin. Invest. 2020;130(5):2620–9. doi: 10.1172/JCI137244.

11. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M et al. Hematological findings and complications of COVID-19. American J Hematol. 2020;95(7): 834-47. doi: 10.1002/ajh.25829.

12. Li T, Lu H, Zhang W. Clinical observation and management of COVID19 patients. Emerg Microbes Infect. 2020;9(1):687-90. doi: 10.1080/22221751.2020.1741327.

13. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.

14. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164–74. PMID: 24778671

15. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62. doi: 10.1016/S0140-6736(20)30566-3.

16. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934-43. doi: 10.1001/jamainternmed.2020.0994.

17. Snijders D, Schoorl M, Schoorl M, Bartels PC, van der Werf TS, Boersma WG. D-dimer levels in assessing severity and clinical outcome in patients with community-acquired pneumonia. A secondary analysis of a randomised clinical trial. Eur J Intern Med. 2012;23(5):436-41. doi: 10.1016/j.ejim.2011.10.019.

18. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223): 507-13. doi: 10.1016/S0140-6736(20)30211-7.

19. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.

20. Lippi G, Cervellin G. Procalcitonin for diagnosing and monitoring bacterial infections: for or against?. Clin Chem Lab Med. 2018;56(8):1193-5. doi:10.1515/cclm-2018-0312.

21. Creamer AW, Kent AE and Albur M. Procalcitonin in respiratory disease: use as a biomarker for diagnosis and guiding antibiotic therapy. Breathe. 2019;15(4):296–304. doi: 10.1183/20734735.0258-2019.

22. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chim Acta. 2020;505:190-1. doi:10.1515/ cclm-2020-0198.

23. Tersalvi G, Vicenzi M, Calabretta D, Biasco L, Pedrazzini G, Winterton D. Elevated troponin in patients with Coronavirus Disease 2019 (COVID-19): possible mechanisms. J Card Fail. 2020;26(6):470-5. doi: 10.1016/j.cardfail.2020.04.009.

24. Vrsalovic M, Presecki AV. Cardiac troponins predict mortality in patients with COVID-19: A meta-analysis of adjusted risk estimates. J Infect. 2020;81(3):e99-100. doi: 10.1016/j.jinf.2020.05.022.

25. Fu YQ, Bai K, Liu CJ. The impact of admission serum lactate on children with moderate to severe traumatic brain injury. PloS One. 2019;14(9):e0222591 doi: 10.1371/journal.pone.0222591.

26. Kubat E, Çalışkan A, Ünal EU, Usanmaz SE, Türkcan BS, Sarıtaş, A et al. Effect of Selective Antegrade Cerebral Perfusion with Moderately Hypothermic Lower Body Circulatory Arrest on Biomarkers Related to Endothelial Function. Acta Med. Alanya 2020;4(1):48-55. doi: 10.30565/medalanya.642337

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