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I.O. Komarevtseva, K.V. Balabanova[1], M.O. Ovcharenko, S.A. Usatov, V.M. Komarevtsev, I.V. Frolova, I.I. Zielonyi
State Establishment "Lugansk State Medical University", Rivne
[1]Center for Nursing Development of the MOH of Ukraine, Kyiv

ROUTINE BIOCHEMICAL DIAGNOSIS AND PROGNOSIS OF COVID-19 SEVERITY IN THE CONDITIONS OF THE REGIONAL HOSPITAL

e-mail: kialdmu@ukr.net

The purpose of the study was to evaluate routine laboratory indicators of biochemical blood analysis as markers of potential risk and factors of infection with COVID-19 and the severity of respiratory failure in both women and men. This single-center retrospective study was conducted with the participation of 57 COVID-19-positive individuals hospitalized at the Rivne Regional War Veterans Hospital in October-November 2021. Blood urea levels were higher in respiratory failure group 1 than in respiratory failure group 2 in both women and men. The dynamics of creatinine levels in women and men with COVID-19 in all groups were the same. as well as blood urea levels. The level of total protein in women with COVID-19 decreased with the progression of respiratory failure to stage II, while in men, on the contrary, it increased. Female COVID-19 patients with grade I respiratory failure have higher total protein than male grade I respiratory failure, and female patients with grade 2 respiratory failure have lower total protein levels in women than in men. The dynamics of the level of albumin in women and men with COVID-19 in all groups was the same as the level of total protein. Based on ROC analysis, Kaplan-Meier method; univariate analysis using the log-rank test and the Cox regression model found that blood urea and creatinine levels are biomarkers of the development of respiratory failure (I or II) within 30 days after hospitalization in patients with COVID-19, both women and men.

Key words: COVID-19, biochemical indicators, ROC analysis.

І.О. Комаревцева, К.В. Балабанова, М.О. Овчаренко, С.А. Усатов, В.М. Комаревцев, І.В. Фролова, І.І. Зельоний

РУТИННА БІОХІМІЧНА ДІАГНОСТИКА ТА ПРОГНОЗ ВАЖКОСТІ COVID-19 В УМОВАХ ОБЛАСНОЇ ЛІКАРНІ

Метою дослідження було оцінити рутинні лабораторні показники біохімічного аналізу крові як маркерів потенційного ризику та факторів інфікування COVID-19 та тяжкості дихальної недостатності як у жінок, так і у чоловіків. Це одноцентрове ретроспективне дослідження проводилося за участю 57 COVID-19-позитивних осіб, госпіталізованих до Рівненського обласного госпіталю ветеранів війни у жовтні-листопаді 2021 року. Рівні сечовини крові були вищими в групі дихальної недостатності 1, ніж у групі дихальної недостатності 2, як у жінок, так і у чоловіків. Динаміка рівня креатиніну у жінок і чоловіків із COVID-19 у всіх групах була такою ж, як і рівень сечовини крові. Рівень загального білка у жінок із COVID-19 знижувався з наростанням дихальної недостатності до II стадії, а у чоловіків, навпаки, зростав. Рівень загального білка у хворих на COVID-19 жінок з дихальною недостатністю I ступеня вищий, ніж у чоловіків з дихальною недостатністю I ступеня, а у пацієнтів з дихальною недостатністю 2 ступеня рівень загального білка у жінок нижчий, ніж у чоловіків. Динаміка рівня альбуміну у жінок і чоловіків з COVID-19 в усіх групах була такою ж, як і рівень загального білка. На підставі ROC-аналізу, метода Каплана-Мейєра; однофакторного аналізу з використанням логарифмічного рангового тесту та регресійної моделі Кокса встановлено, що рівні сечовини крові та креатиніну є біомаркерами розвитку дихальної недостатності (I або II) протягом 30 днів після госпіталізації у хворих на COVID-19, як жінок, так і чоловіків.

Ключові слова: COVID-19, біохімічні показники, ROC-аналіз.

The study is a fragment of the research project "Development of new highly economical methods of biomarker diagnostics and prediction of the course and complications of COVID-19 and community-acquired pneumonia in military personnel and civilians", state registration No. 0123U10124; "Development and implementation of innovative technologies for the diagnosis of oncogynecological and oncourological diseases based on liquid biopsy data of extracellular DNA and stem cells", state registration No. 0123U101248.

The scientific community is in urgent need for reliable biomarkers related to coronavirus disease 2019 (COVID-19) disease progression, in order to stratify high-risk patients. The rapid disease spread necessitates the immediate categorization of patients into risk groups following diagnosis, to ensure optimal resource allocation [11]. Timely detection of COVID-19 patients at high risk of death and supportive care

can effectively reduce the incidence of persistent critical illness and in-hospital mortality [3, 9, 12]. Extrapulmonary damage from COVID-19 involves acute kidney injury, hepatocellular injury, neurological illnesses, myocardial dysfunction and arrhythmia, and gastrointestinal symptoms leading to severe respiratory failure, kidney injury, myocardial injury, and death [6].

Novel biomarkers are needed to identify patients who will suffer rapid disease progression to severe complications and death. The identification of novel biomarkers is strictly related to the understanding of viral pathogenetic mechanisms, as well as cellular and organ damage. Effective biomarkers would be helpful for screening, clinical management, and prevention of serious complications [11].

Currently, many laboratory markers, such as D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, procalcitonin (PCT), and cytokines, especially interleukin-6, have been proposed to predict and guide the treatment of COVID-19 [4, 10]. However, these markers and some disease scoring systems such as CURB-65 may not be practical for early assessment in terms of risk stratification and prediction to COVID-19 critical illness [7, 9]. Therefore, it is necessary to identify novel, reliable and convenient prognostic biomarkers or predictors of COVID-19 [1, 5, 8].

The purpose of the study was to evaluate routine laboratory parameters of blood biochemistry as markers of potential risk and factors for COVID-19 infection and the severity of respiratory failure in both women and men.

Materials and methods. This single-center retrospective study was carried out on 57 COVID-19-positive individuals hospitalized at Rivne regional hospital for war veterans, between October and November 2021. In accordance with the provisions of the Declaration of Helsinki by the World Medical Association of the last revision (1964-2013) and informed consent for the use of biological material was obtained in all patients prior to inclusion in the study. Research permission was obtained from the Bioethics Committee of the Luhansk State Medical University (01.09.2023). The patients' epidemiological data, laboratory examination, complications, clinical outcomes, computed tomography imaging data, and treatment plan were extracted from medical records. The date of the disease'es onset was the date of the first symptom. All cases of SARS-CoV-2 infection confirmed by a positive result on real-time reverse transcriptase polymerase chain reaction tests of a nasal sample and/or diagnosed by a computed tomography chest scan were included and analyzed. Pneumonia in the recruited patients was defined as a respiratory failure ($spO_2 < 90$ % or $PO_2 < 60$ mmHg at ambient air or specific radiological findings). The primary endpoint of this study was the advent of respiratory failure (I or II) within of hospitalization. The laboratory tests included blood urea, creatinine, total protein, albumin, total bilirubin, direct bilirubin.

Table 1

Main data of recruited patients with COVID-19 (Mean ± SD)

Groups	Women-RF1 (n=11)	Women-RF2 (n=22)	Men-RF1 (n=9)	Men-RF2 (n=15)
Age (years)	61.5±13.5	69.9±9.19	42.1±17.7	58.5±17.3
		p^1=0.043163*	p^3=0.012517*	p^2=0.036590* p^4=0.013243*
Blood urea, mmol/l	9.25±2.73	7.06±1.63	9.56±4.37	6.63±1.57
		p^1=0.007186*	p^3=0.852768	p^2=0.026320* p^4=0.418066
Creatinine, µmol/l	105.4±20.8	83.7±17.5	125.07±19.2	98.6±25.4
		p^1=0.003644*	p^3=0.042254*	p^2=0.013164* p^4=0.041706*
Total protein, g/l	62.3±6.03	57.5±5.86	56.8±4.15	63.07±3.06
		p^1=0.036653*	p^3=0.032582*	p^2=0.000312* p^4=0.001850*
Albumin, g/l	39.8±5.98	35.4±4.02	37.3±4.82	41.9±4.77
		p^1=0.016142*	p^3=0.327763	p^2=0.032847* p^4=0.000067*
Total bilirubin, µmol/l	16.98±2.46	17.3±2.77	17.9±2.09	16.8±2.79
		p^1>0.05	p^3>0.05	p^2>0.05 p^4>0.05
Direct bilirubin, µmol/l	5.05±1.17	5.06±1.16	5.19±1.19	4.94±1.44
		p^1>0.05	p^3>0.05	p^2>0.05 p^4>0.05

Notes**:** Age, Creatinine, Total bilirubin, Direct bilirubin – Intergroup by the Mann–Whitney U test Blood urea, Total protein, Albumin data are Means ± SD for Gaussian variables Intergroup by the T-test Students p^1 – significant differences between women-RF1group and women-RF2 group; p^2 – significant differences between men-RF1group and men-RF2; group p^3 – significant differences between women-RF1group and men-RF1; group p^4 – significant differences between women-RF2 group and men-RF2 group

Statistical and graphical analyses were done using STATISTICA 7.0.61.0 (StatSoft, Inc., Tulsa, OK, USA; serial number: AXF003C775430FAN7) and MedCalc Version 20.218 64 bit (MedCalc Software, Ostend, Belgium). Parametric data are presented as a mean±standart deviation (SD). Kolmogorov–Smirnov test was applied to examine the normality of data distribution. To examine group-wise differences, unpaired Student's t-test was used. Frequency calculations were performed using Fisher's exact test. The difference between study groups was tested by a nonparametric Mann–Whitney U test was used. A p-value below 0.05 was considered statistically significant. Receiver operating characteristics (ROC) curve analysis was performed to estimate optimal cut-off values, maximizing sensitivity and specificity according to the Youden index. Analysis of the development of respiratory failure (I or II) during hospitalization was carried out according to the Kaplan-Meier method; univariate analys was undertaken using log rank test and Cox's regression model, respectively. A *p*-value below 0.05 was considered statistically significant.

Results of the study and their discussion. Given that biochemical parameters typically differ between men and women, and that we do not have the same number of extractions in all patients, we decided to divide the data into four groups: women, first extraction, which includes data from all tests who developed grade 1 respiratory failure (women-RF1, n=11); women, final extraction, which includes data from all tests and who developed grade II respiratory failure (women-RF2, n=22); men, initial extraction, same as the first group but with male patients (men-RF1, n=9); and men, final extraction, same as the second group but with male patients (men-RF2, n=15) (Table 1).

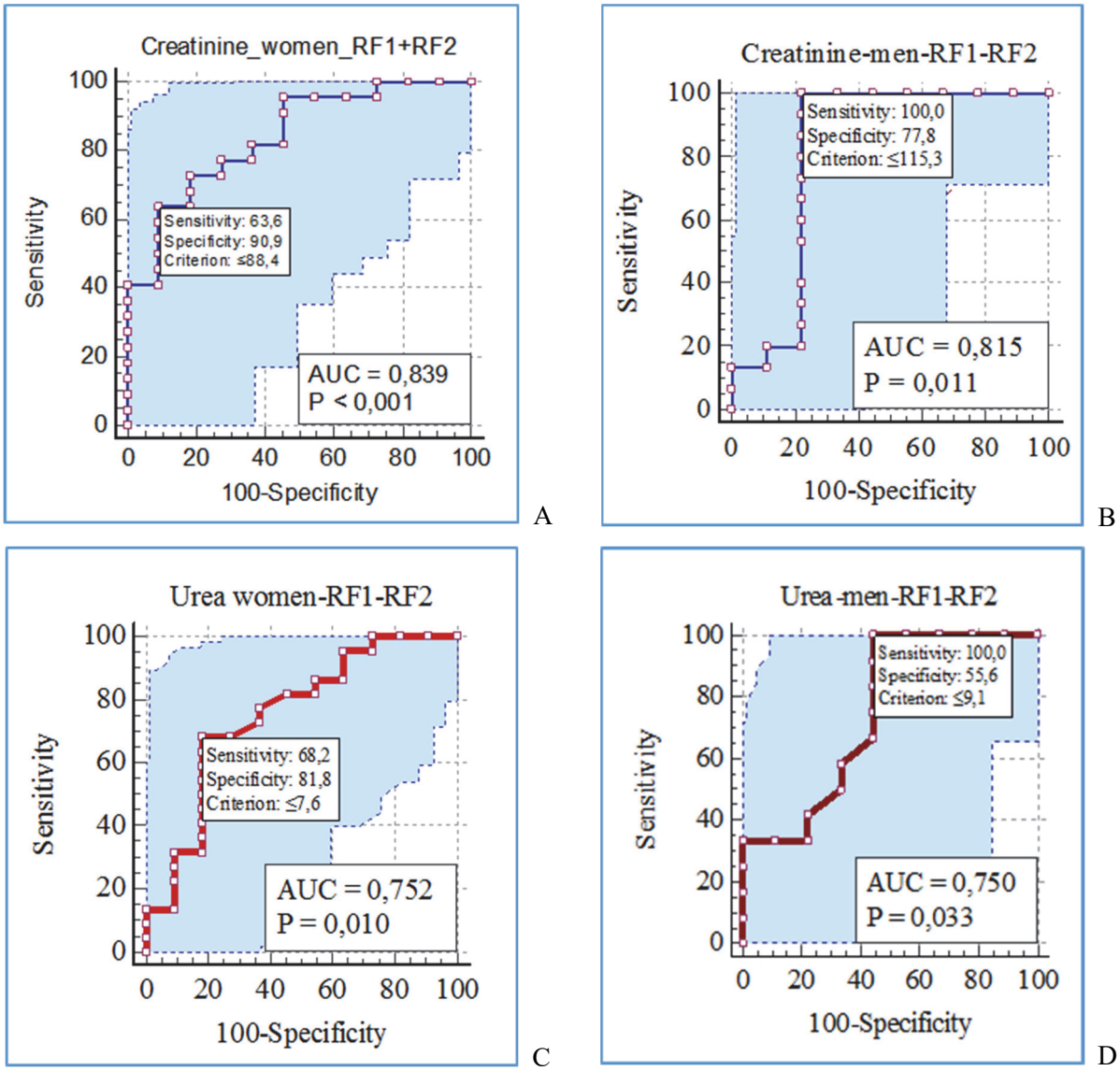

Fig. 1. ROC analysis: receiver operating characteristic (ROC) curves the development of respiratory failure (I or II) within 30 days of hospitalization for Creatinine measured in A – COVID-19-women; B – COVID-19-men; for blood urea measured in C – COVID-19-women; D – COVID-19-men.

Note**:** Here and in the following figures: p<0.001 – calculated by univariate logistic regression analysis.

The mean age of 33 female and 24 male patients was 67.2±11.4 and 52.4±18.9 years old respectively (p=0.000549). The age of women sick with COVID-19 with grade I respiratory failure was 61.5±13.5 and with grade II respiratory failure – 69.9±9.19 (p=0.043163). The age of men sick with COVID-19 with grade I respiratory failure was 42.1±17.7 and with grade II respiratory failure – 58.5±17.3 (p=0.036590). The age of women sick with COVID-19 and with grade I respiratory failure and grade II respiratory failure was higher than that of men.

The levels of blood urea were higher in the RF1 group than those in the RF2 in both women (9.25±2.73 and 7.06±1.63, respectively, p=0.007186) and men (9.56±4.37 and 6.63±1.57, respectively, p=0.026320). There was no statistically significant difference in the levels of blood urea in women and men with COVID-19 in both RF1 and RF2 groups.

The dynamics of creatinine levels in women and men with COVID-19 in all groups was the same as the levels of blood urea: in the RF1 group it was higher than in the RF2 group.

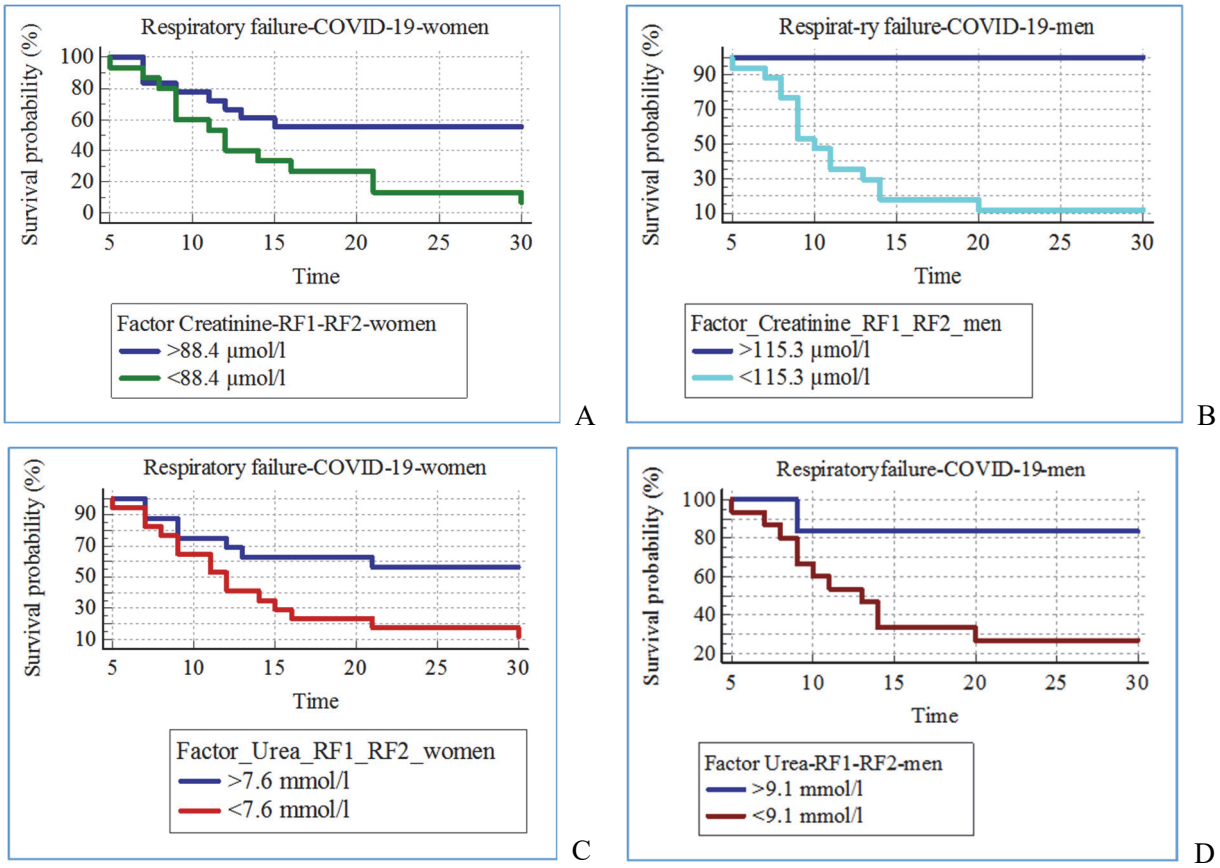

Fig. 2. Kaplan–Meier curves of the development of respiratory failure (I or II) within 30 days of hospitalization of A – COVID-19-women (predictor – Creatinine); B – COVID-19-men (predictor – Creatinine); C – COVID-19-women (predictor – blood urea); D – COVID-19-men (predictor – blood urea) with different cut-off values of the of indexes investigated. p value by Long-rank test.

Note: Here and in the following figures: p<0.0001 – calculated by univariate logistic regression analysis.

The levels of total protein in women with COVID-19 decreased with the growth of respiratory failure to stage II (62.3±6.03 and 57.5±5.86, respectively, p=0.036653), while in men, on the contrary, they increased (56.8±4.15 and 63.07±3.06, respectively, p=0.000312). The levels of total protein in women sick with COVID-19 with grade I respiratory failure are higher than in men sick with grade I respiratory failure, while in patients with grade 2 respiratory failure the levels of total protein in women are lower than in men.

The dynamics of albumin levels in women and men with COVID-19 in all groups was the same as the levels of total protein.

There was no statistically significant difference in the levels of total bilirubin and direct bilirubin between all groups.

Analysis of the ROC curve in patients with COVID-19 is shown in Fig. 1.

Analysis of the ROC curve in patients with COVID-19 showed Creatinine and blood urea as a predictor of the development of respiratory failure (I or II) within 30 days of hospitalization. The area under ROC curve of Creatinine was greatest in all groups patients with COVID-19 (women – 0.839, p< 0.001,

optimal cut-off values of Creatinine – ≤88.4 μmol/l; men – 0.815, p=0.011, optimal cut-off values of Creatinine – ≤115.3 μmol/l); the area under ROC curve of blood urea was also reliably high in both women (women – 0.752, p=0.010, optimal cut-off values of blood urea – ≤7.6 mmol/l) and men (men – 0.750, p=0.033, optimal cut-off values of blood urea – ≤9.1 mmol/l), which reflects the high predictive efficacy of these indices for assessing the development of of respiratory failure (I or II) within 30 days of hospitalization.

ROC analysis did not confirm the significant diagnostic accuracy of .Age, total protein, albumin, total bilirubin, direct bilirubin.

Kaplan-Meier survival curves after classifying patients based on Youden cut-off values obtained using ROC curves showed significantly earlier development of respiratory failure (I or II) within 30 days of hospitalization as a function of Creatinine levels (Fig.2): COVID-19 patients (women: HR = 0.31; 95 % CI 0.13 to 0.76, p = 0.0108; men: HR = 0.18; 95 % CI 0.061 to 0.53, p = 0.0020), blood urea levels (women: HR = 0.33; 95 % CI 0.14 to 0.79, p = 0.0134 men: HR = 0.28; 95 % CI 0.083 to 0.94, p =0.0401).

Next, we performed a Cox proportional hazards regression analyses of predictors for assessing the development of respiratory failure (I or II) within 30 days of hospitalization in all groups patients with COVID-19 are presented in Table 2.

Table 2

Unadjusted and adjusted hazard ratios for respective univariate Cox proportional hazard models for assessing the development of respiratory failure (I or II) within 30 days of hospitalization

Factor	Univariable		
	HR (95 % CI)	Harrell's C-index	p-Value
Creatinine-COVID-19-women	0.98 (0.96–0.99)	0.609	p=0.0113
Creatinine-COVID-19-men	0.98 (0.97–1.00)	0.665	p=0.0170
Blood urea-COVID-19-women	0.78 (0.63–0.97)	0.621	p=0.0274
Blood urea-COVID-19-men	0.6 (0.38–0.95)	0.721	p=0.0305

Note: Here and in the following figures: p<0.0001 – calculated by univariate logistic regression analysis, hazard ratios (HR)

Harrell's C-index, also known as the concordance index, is a goodness of fit measure for models which produce risk scores. All parameters that were significant at a p value less than 0.10 could predict the development of respiratory failure (I or II) within 30 days of hospitalization in patients with COVID-19: for Creatinine (women – C-index=0.609, men C-index=0.665), for blood urea (women – C-index=0.621, men C-index=0.721) increases the risk of a poor prognosis of the development respiratory failure (I or II) within 30 days of hospitalization.

The epidemic data from China, Italy, Japan, Singapore, Canada, and South Korea showed an age-dependent disparity in susceptibility to COVID-19. Age dependence in susceptibility to COVID-19 was markedly lower in younger age groups in all regions [12]. Males are predisposed to SARS-CoV-2 infection. Initial pandemic reports from China indicated that men accounted for ~ 60 % of COVID-19 patients. A meta-analysis of 59 studies comprising 36,470 patients showed that men had a higher risk of infection, disease severity, intensive care unit (ICU) admission, and death than women [9]. Our study established a gender difference in age in COVID-19 patients with women being greater than men both in patients with stage I and stage II respiratory failure.

Meta-analyses of multiple studies have shown significant correlations between several laboratory factors and the severity and mortality of COVID-19. These laboratory parameters included the following: (1) changes in blood cell counts, including increased leukocyte and neutrophil counts, neutrophil-to-lymphocyte ratio, and decreased lymphocyte and eosinophil counts; (2) increase in the level of biochemical parameters: lactate dehydrogenase, CRP, procalcitonin, aspartate amino transferase, alanine aminotransferase, and blood urea nitrogen; and (3) changes in coagulation indices: decreased platelet counts, increased D-dimer, fibrinogen, change in prothrombin time (PT), and activated partial-thromboplastin time (APTT). These changes may be associated with an aggravated disease course of COVID-19 [3, 12]. Our study supplemented the specified list of laboratory parameters in patients with COVID-19 with the levels of blood urea, creatinine, total protein, albumin, total bilirubin and direct bilirubin, as a potential predictive factor for the development of respiratory failure. Of all these indices, only creatinine and urea, based on the ROC analysis and Kaplan-Meier survival curves, gave us the possibility of predicting the development of severe respiratory syndrome in the form of respiratory failure.

Izcovich A. et al. [7] reported bilirubin as prognostic factor however we found low certainty evidence on those variables in our primary analysis. These authors, like us, found high levels of creatinine

and urea in patients with COVID-19, but, unlike our results, did not find their prognostic significance. They did not study gender differences in laboratory parameters, unlike us. Therefore, it is difficult to compare the results obtained by these authors, for example, the increased level of albumin in the general group of their patients with COVID-19. According to our data, hyperalbinuria was observed in male patients with increasing respiratory failure, which was not the case in women. Sulmaz Ghahramani et al., on the contrary, showed in their study low albumin levels in patients with COVID-19, as we observed in the group of women with COVID-19.

Given the gender-specific complications and outcomes of COVID-19 [2], our study, which established the dynamics and prognostic nature of routine laboratory parameters taking into account gender distribution, is relevant for practical healthcare, especially in small hospitals.

Conclusions

1. With the increase in respiratory failure in patients with COVID-19, the levels of total and direct bilirubin, both in women and men, did not change; the levels of blood urea and creatinine – decreased in both women and men (although in respiratory failure I the level of creatinine in men was significantly higher than in women); the levels of total protein and albumin in women decreased, while in men, on the contrary, they increased.

2. The levels of blood urea and creatinine is a biomarker of respiratory failure's development (I or II) within 30 days of hospitalization in patients with COVID-19, both women and men.

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