15 MEDICAL LABORATORY TESTS important in COVID-19

ORDER OF BIOCHEMISTS, BIOLOGISTS AND CHEMISTS IN THE HEALTHCARE SYSTEM IN ROMANIA (O.B.C.S.S.R.)

05.05.2020

This guide replaces the previous version from 30.04.2020
On page 18- "Cytokine tests, especially IL-6, should be used in order to evaluate where possible patients suspected of hyperinflammation. [30,31]

This version has the following additions compared to the previous one:

On page 4- "The values obtained for medical laboratory tests, including COVID-19 diagnostic tests, must be correlated and should be interpreted in the clinical context for each patient."

Update List
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Contents

1 15 common medical laboratory tests panel important in COVID-19
2 Medical Laboratory tests for COVID-19 recommended by the World Health Organization (WHO)
3 Common medical laboratory tests recommended by I.F.C.C as being important for testing of adult patients with COVID-19
4 Common medical laboratory tests recommended by I.F.C.C whose values decrease compared to the biological reference range in adults with COVID-19
  4.1 White Blood Cell Differential Count: Lymphocytes
  4.2 Complete blood cell count: Platelets
  4.3 Serum albumin
5 Common medical laboratory tests recommended by I.F.C.C whose values increase compared to the biological reference range in adults with COVID-19
  5.1 Complete blood cell count: Leukocytes
  5.2 White Blood Cell Differential Count: Neutrophiles
  5.3 Lactate dehydrogenase (LDH)
  5.4 Alanine aminotransferase (ALT)
  5.5 Aspartate aminotransferase (AST)
  5.6 Total bilirubin
  5.7 Creatinine
  5.8 BUN (blood urea nitrogen)
  5.9 Cardiac troponin
  5.10 D-dimer
  5.11 Fibrinogen
  5.12 aPTT- activated Partial Thromboplastin Time
  5.13 Procalcitonin
  5.14 C-reactive Protein
  5.15 Ferritin
  5.16 Cytokines (IL-6)
6 Conclusions to the O.B.B.C.S.S.R Guide from 05.05.2020

KEY-WORDS: Medical Laboratory (ML) Clinical utility of the test COVID-19 SARS-COV-2
15 medical laboratory tests assessing the prognosis and monitoring the evolution of COVID-19 patients

### Main laboratory abnormalities observed in adult patients with unfavorable COVID-19 progression (Modified 1-30)

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Abnormalities</th>
<th>Potential clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
<td>Increased white blood cell</td>
<td>Bacterial (super)infection</td>
</tr>
<tr>
<td></td>
<td>Increased neutrophil count</td>
<td>Bacterial (super)infection</td>
</tr>
<tr>
<td></td>
<td>Decreased lymphocyte count</td>
<td>Decreased immunological response to the virus</td>
</tr>
<tr>
<td></td>
<td>Decreased platelet count</td>
<td>Consumption (disseminated) coagulopathy</td>
</tr>
<tr>
<td>Blood gases</td>
<td>Estimated modifications</td>
<td>Important in critical care management</td>
</tr>
<tr>
<td>Albumin</td>
<td>Decreased</td>
<td>Impairment of liver function</td>
</tr>
<tr>
<td>LDH</td>
<td>Increased</td>
<td>Pulmonary injury and/or widespread organ damage</td>
</tr>
<tr>
<td>ALT</td>
<td>Increased</td>
<td>Liver injury and/or organ damage</td>
</tr>
<tr>
<td>AST</td>
<td>Increased</td>
<td>Liver injury and/or organ damage</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>Increased</td>
<td>Liver injury</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Increased</td>
<td>Kidney injury</td>
</tr>
<tr>
<td>Urea</td>
<td>Estimated Increase</td>
<td>Kidney injury</td>
</tr>
<tr>
<td>Cardiac troponin</td>
<td>Increased</td>
<td>Cardiac injury</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>Increased</td>
<td>Activation of blood coagulation and/or disseminated coagulopathy</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>Increased</td>
<td>Activation of blood coagulation and/or disseminated coagulopathy</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>Increased</td>
<td>Bacterial (super)infection</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Increased</td>
<td>Severe viral infection/viremia/viral sepsis</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Increased</td>
<td>Severe inflammation</td>
</tr>
<tr>
<td>Cytokines (IL-6)</td>
<td>Increased</td>
<td>Cytokine storm syndrome</td>
</tr>
</tbody>
</table>

15 common medical laboratory tests panel important in COVID-19

O.B.B.C.S.S.R. has proposed to the Ministry of Health the development of a “15 common medical laboratory tests panel relevant in COVID-19” which can be performed in all medical laboratories across Romania.

The application of the “15 common medical laboratory tests panel relevant in COVID-19” in any hospital from Romania will allow clinicians to:

- identify patients infected with SARS-CoV-2 virus;
- monitor the evolution of COVID-19;

### ATTENTION

- The 15 medical laboratory tests do NOT replace the rRT-PCR diagnostic method for COVID-19!
- The rRT-PCR method is the ONLY COVID-19 diagnostic method recognized by the World Health Organization (WHO).
- The values obtained for medical laboratory tests, including COVID-19 diagnostic tests, must be correlated and should be interpreted in the clinical context for each patient.

The world’s leading organization in the field of Clinical Chemistry and Laboratory Medicine, The International Federation of Clinical Chemistry and Laboratory Medicine (I.F.C.C) is updated in 2020 the “Information Guide on COVID-19” which which synthesizes the international scientific ML information available so far on COVID-19 [1]. In order to bring the results of I.F.C.C's efforts to the global community all this data were summarized in the table taken on page 3 of this GUIDE.

The Order of Biochemists, Biologists and Chemists in the Romanian health system (O.B.B.C.S.S.R.) is an Affiliate Member of the International Federation of Clinical Chemistry and Laboratory Medicine (I.F.C.C) since April 2020 and:

- “Professional, non-governmental, apolitical, non-profit organization with legal personality, which represents the interests of its members” art. 22, para. (1) Law no. 460/2003 [3];
- “Competent authority for the professions of biochemist in the health system, biologist in the health system, chemist in the health system regulated in Romania”, Annex 3, para. 15, Law no. 200/2004 [4].
Medical laboratory tests for COVID-19 recommended by the World Health Organization (WHO)

According to the WHO, a positive case of COVID-19 is a person who has confirmed SARS-CoV-2 virus infection through a medical laboratory test so it is CRUCIAL TO HAVE A QUICK AND CORRECT RESULT provided by the medical laboratory tests. The WHO recommends the rRT-PCR method for SARS-CoV-2 detection [5].

Tests that identify the presence of SARS-CoV-2 virus in the human body are based on a laboratory method which has been put into practice for over 30 years - Polymerase Chain Reaction [6].

Performing each test to identify the presence of SARS-CoV-2 virus in the body takes about 4 hours from the time when the sample reaches the laboratory and sometimes it takes a TAT of 2-4 days if added the time needed for transport and other logistical elements [6].

The biological specimens used to detect SARS-CoV-2 by the rRT-PCR method are:

<table>
<thead>
<tr>
<th>Clinical Specimen</th>
<th>Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchoalveolar lavage fluid</td>
<td>93% (14/15)</td>
</tr>
<tr>
<td>Sputum</td>
<td>72% (72/104)</td>
</tr>
<tr>
<td>Nasal Swab</td>
<td>63% (5/8)</td>
</tr>
<tr>
<td>Fibrobronchoscope brush biopsy</td>
<td>46% (6/13)</td>
</tr>
<tr>
<td>Pharyngeal swabs</td>
<td>32% (126/398)</td>
</tr>
<tr>
<td>Feces</td>
<td>29% (44/153)</td>
</tr>
<tr>
<td>Blood</td>
<td>1% (3/307)</td>
</tr>
<tr>
<td>Urine</td>
<td>0% (0/72)</td>
</tr>
</tbody>
</table>


- COVID-19 positivity rate in case of SARS-COV-2 virus detection using the rRT-PCR method in various biological samples varies from 93% in bronchoalveolar lavage fluid, to a positivity of 62% in sputum, 32% in nasal swab, etc. as shown in the table above [1].
- Based on the practical experience already gained in the fight against the new coronavirus, it appears that one in five tests turned out to be FALSE negative using the rRT-PCR test recommended by the World Health Organization (WHO).
- Scientists around the world are concerned to find other laboratory methods that can guide clinicians in obtaining a fast and correct diagnosis, illness stadialisation and monitoring the evolution of COVID-19.

The best diagnostic solution in COVID-19 could be the combination of the two rRT-PCR and IgM/IgG test methods [7].
Common medical laboratory tests recommended by I.F.C.C as being important for testing of adult patients with COVID-19

The list of laboratory tests recommended by I.F.C.C based on the current scientific literature data together with the major laboratory abnormalities observed in adult COVID-19 patients (I.F.C.C Table- page 3) - are really helpful for clinicians who along with ML specialists can have a quick picture on the status of their utility in paraclinical investigation in COVID-19.

In some patients with a positive result rRT-PCR have registered higher or lower values related to the biological reference range compared to those obtained for the negative ones for:
- neutrophils (NEUT);
- C-reactive protein (CRP);
- lactate dehydrogenase (LDH);
- aspartate aminotransferase (AST);
- alanine aminotransferase (ALT);
- serum urea;
- lower lymphocyte count;
- lower serum albumin levels.

The correlated values of ALT, CRP, LDH, Serum Urea, Ferritin, NEUT number had a very good accuracy in detecting SARS-CoV-2 infected patients subsequently confirmed with COVID-19 by the rRT-PCR diagnostic method.

Laboratory test results suggested that [11]:

The values obtained for the number of lymphocytes, C-Reactive Protein and procalcitonin can be used by clinicians to evaluate severe COVID-19 infections.

Note:

This O.B.B.C.S.S.R. GUIDE does NOT contain information on blood gas levels.
**White Blood Cell Differential Count - Lymphocytes**

- **Lymphocyte counts are low in most COVID-19 infected patients** [8].
- Secondary hemophagocytic lymphohistiocytosis (sHLH) is an underestimated hyperinflammatory syndrome characterized by fulminant hypercytokinemia and fatal with multi-organ failure [9].
- **Lymphopenia** (lymphocyte counts under $1.0 \times 10^9/L$ in adults and respectively below $2.5 \times 10^9/L$ in children is also associated with other conditions/factors [10] including:
  - plastic anemia;
  - Hodgkin’s disease and other malignancies;
  - inherited immune system disorders, acquired immunodeficiency syndrome (AIDS) and AIDS immune dysfunction;
  - advanced tuberculosis, renal failure, systemic lupus erythematosus (SLE);
  - congestive heart failure;
  - minor acute viral infections.

**Complete blood cell count - Platelets**

*In patients with COVID-19 thrombocytopenia is very common, being associated to an increased risk of severe COVID-19 disease* [11].

Three hypotheses have been formulated regarding the mechanism of thrombocytopenia [12]:

- **Direct infection of bone marrow cells** by the virus and inhibition of platelet synthesis. Following virus infection, the cytokine storm destroys the progenitor cells of the bone marrow and leads to decreased platelet production. Lung damage indirectly leads to reduced platelet synthesis.
- ** Destruction of platelets by the immune system.**
- **Platelet aggregation in the lungs,** thus causing the appearance of microthrombi and platelet consumption.

Low platelet counts compared to the biological reference range are also associated with other conditions/factors [10] including:

- idiopathic thrombocytopenic purpura, neonatal purpura;
- congestive heart failure, congenital heart disease;
- thrombopoietin deficiency;
- infections;
- lesions involving the bone marrow (e.g., leukemias, carcinomas, myelofibrosis);
- disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura;
- renal insufficiency;
- idiopathic/secondary autoimmune immunological processes;
- alloimmune immunological processes.
Patients with COVID-19 have low serum albumin levels that have been associated with an increased risk of death. Current studies suggest that “albumin therapy could be a potential cure” [12].

Decreased serum albumin levels are also associated with the following conditions/factors [10]:

- acute and chronic inflammation and infections;
- liver pathologies;
- nephrotic syndrome, kidney disease;
- Crohn’s disease, colitis;
- congenital albuminuria;
- burns, severe skin diseases;
- heart failure;
- inadequate intake/decreased absorption;
- thyroid disease;
- increased demand: pregnancy;
- hyperthyroidism;
- hemodilution;
- increased catabolism;
- neoplasia.

Common medical laboratory tests recommended by I.F.C.C whose values decrease compared to the biological reference range in adults with COVID-19.
Common medical laboratory tests recommended by I.F.C.C whose values increase compared to the biological reference range in adults with COVID-19

- Complete blood cell count - Leukocytes
- White Blood Cell Differential Count - Neutrophils
- Lactate dehydrogenase (LDH)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Total bilirubin
- Creatinine
- Cardiac troponin
- D-dimer
- Prothrombin time
- Procalcitonin
- C-reactive protein
- Ferritin
- Cytokines (IL-6)

Most patients with COVID-19, especially those with a severe prognosis, have a significantly increased number of leukocytes [1,13]. Increased white blood cell counts and elevated C-Reactive Protein levels in patients with severe COVID-19 may accompany a bacterial infection.

Leukocytosis occurs in acute infections in which the degree of increase in the number of leukocytes depends on the infection severity, the patient’s resistance, the patient’s age and spinal cord efficiency. Other causes of leukocytosis include the following [10]:

- leukemia, myeloproliferative disorders;
- trauma or tissue damage (e.g. surgery);
- neoplasms, bronchogenic carcinoma;
- toxins, uremia, coma, eclampsia, thyroiditis burst;
- acute hemolysis;
- acute hemorrhage after splenectomy;
- polycythemia vera (Vasquez disease);
- tissue necrosis.

Patients infected with the new coronavirus (SARS-CoV-2) tend to have a higher neutrophil/lymphocyte ratio in favour of neutrophils (NLR) [14]. NLR is an independent risk factor for hospital mortality in COVID-19 patients, especially in men. NLR evaluation can help identify individuals at high risk for COVID-19 [14].

Patients with new coronavirus infection (COVID-19) have higher blood levels of neutrophil extracellular traps (NETs) that have the potential to spread inflammation and microvascular thrombosis- including in the lungs of patients with acute respiratory distress syndrome [15].

Neutrophilia (increase in absolute number and relative percentage of neutrophils) > 8.0 × 10⁹/L is also associated with the following conditions/factors including [10]:

- acute, localized and general bacterial infections, fungal and spirochete and some parasitic infections and rickettsia;
- some viral infections (in early stages) and some parasitic infections (usually neutrophilia is mild and is present only in the initial phase);
- inflammations;
- acute hemorrhage, hemolytic anemia, hemolytic reaction, transfusion, major surgical procedures, postsplenectomy;
- chronic inflammatory diseases;
- metabolic diseases;
- eclampsia, thyrotoxicosis;
- tissue necrosis;
- neonatal sepsis;
- malignant tumors, especially carcinomas (gastrointestinal, pulmonary);
- myeloproliferative diseases [10].
Patients with COVID-19 with elevated lactate dehydrogenase (LDH) and a high number of neutrophils at admission indicated higher exposure to the new coronavirus. Increased initial lactate dehydrogenase (LDH) levels compared to the biological reference interval and the high number of neutrophils at admission of elderly COVID-19 patients were independently correlated with a poor clinical prognosis [16].

The level of lactate dehydrogenase (LDH) also has diagnostic utility in:
- cardiac pathology (IM staging, acute myocarditis);
- pulmonary embolism;
- liver pathology (hepatitis, cirrhosis);
- differential diagnosis of hemolytic anemia;
- oncological pathology (e.g. monitoring of tumor activity in lung cancers, advanced neoplasms, oncological hematology);
- collagen diseases.

A 5-fold increase compared to the biological reference range for the ALT values is associated to a 7-fold increase for the risk of mortality in COVID-19 patients.

In most patients who had elevated ALT serum levels during COVID-19, it was observed that after recovery ALT values returned to the biological reference range [17].

High ALT levels compared to the biological reference range are also associated with other conditions/factors [10], including:
- hepatic steatosis (increases 2-3 times higher than normal biological reference values);
- infectious mononucleosis, acute lymphoblastic leukemia (children);
- pancreatitis (mild growth) and acute pancreatitis (moderate growth);
- myocardial infarction (less significant than AST);
- heart failure;
- polymyositis;
- severe burns, striated muscle trauma, severe shock.
Aspartate aminotransferase (AST)

High levels of AST compared to the biological reference range were observed both in patients with non-severe COVID-19 disease as well as in a double number of patients with severe disease [18].

Patients diagnosed with COVID-19 investigated by CT scan in the subclinical phase had a significantly lower level of AST than the patients diagnosed after the onset of symptoms [19].

Elevated AST levels compared to the biological reference range are also associated with other conditions/factors [10] including:

- myocardial infarction (4 to 10 times higher);
- acute hepatitis and chronic hepatitis (ALT > AST);
- active cirrhosis (drug/alcohol induced: AST > ALT);
- infectious mononucleosis;
- necrosis and liver metastases;
- primary or metastatic carcinoma;
- alcoholic hepatitis;
- Reye’s syndrome;
- acute viral hepatitis and ethanolic hepatitis.

Total bilirubin

Elevated bilirubin levels compared to the reference biological range are significantly associated with the severity of COVID-19 disease [19].

Increases in total bilirubin, accompanied by jaundice, may be caused by the damage in liver physiology or hemoglobin metabolism, with various etiologies [10]:

- hepatocellular jaundice caused by lesions or diseases of the liver parenchymal cells associated with certain conditions (viral hepatitis, cirrhosis, infectious mononucleosis) or as a reaction to the administration of certain drugs (e.g. chlorpromazine);
- obstructive jaundice, caused by obstruction of the bile ducts or liver ducts in patients with lithiasis or neoplasms, which has the effect of increasing the serum level of conjugated bilirubin.
Serum creatinine (SCr) value at admission is a predictor of death for hospitalized COVID-19 patients [20].

In the management of COVID-19, more frequent measurements of serum creatinine (SCr) are recommended to improve the early detection of renal lesions in patients with COVID-19.

Mortality in patients with COVID-19 was significantly associated with elevated levels of proteinuria, hematuria, urea-derived blood nitrogen (BUN), serum creatinine, uric acid, D-dimers.

Patients with COVID-19 have renal impairment since admission with elevated serum levels above the reference values [20], for:

- BUN (blood urea nitrogen);
- serum creatinine;
- uric acid;
- creatinine kinase (CK);
- lactate dehydrogenase (LDH)- in some COVID-19 patients;
- proteinuria;
- haematuria.

Elevated serum creatinine levels compared to the biological reference range are also associated with the following conditions/factors [10]:

- renal failure and acute or chronic renal disease of glomerular or tubulo-interstitial cause;
- chronic nephritis;
- urinary tract obstruction;
- muscle disorders (gigantism, acromegaly, myasthenia gravis, muscular dystrophy, poliomyelitis);
- decreased renal perfusion (prerenal azotemia) from congestive heart failure, shock, dehydration;
- rhabdomyolysis;
- hyperthyroidism.

Kidney failure causes a 5-fold increase in the risk of death for COVID-19 patients.
**Cardiac troponin**

In patients confirmed with COVID-19, elevated levels of highly sensitive troponin I (hs-cTnI) were observed during hospitalization, and more than 50% of those who died had a significantly higher concentration of hs-cTnI compared to the biological interval of reference [21].

As measuring troponin levels is useful in the diagnosis of myocardial infarction, the change in cTn concentration in patients confirmed with SARS-CoV-2 infection should be interpreted in the clinical context.

Elevated for another cardiac marker, NT-proBNP (>88.64 pg/mL), may be an independent predictor of mortality in COVID-19 confirmed patients.

**ACC experts recommend that cardiologists pay special attention to assessing a possible exacerbation of heart failure in a COVID-19 positive patient and emphasize the priority of clinical examination** [21].

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**BUN (Blood urea nitrogen)**

Elevated serum urea levels (azotemia) compared to the biological reference range are also associated with the following conditions / factors [10]:

- renal failure caused by/associated with congestive heart failure, acute myocardial infarction;
- shock, stress, high salt intake and dehydration;
- chronic kidney disease (e.g. glomerulonephritis, pyelonephritis);
- urinary tract obstruction;
- hemorrhages of the gastrointestinal tract;
- diabetic ketoacidosis;
- excessive protein intake or increased protein catabolism (neoplasms, prolonged fever, stress, burns, myocardial infarction or cancer);
- use of anabolic steroids.

The differentiation between prerenal and postrenal azotemia is based on the urea/creatinine ratio [10].

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Common medical laboratory tests recommended by I.F.C.C whose values increase compared to the biological reference range in adults with COVID-19
D-dimers are the most important prognostic element in monitoring patients with severe forms of COVID-19.

Elevated levels of D-dimers compared to the biological reference range observed at admission of patients with COVID-19 and their marked increase, up to 3-4 fold over the initial value, were associated with increased mortality, which probably reflects the activation of coagulation in infection/sepsis, cytokine storm and imminent organ failure [22,23,24].

The continuous increase in the value of D-dimers compared to the biological reference range indicates a progressive severity of infection in COVID-19 [22,23,24].

The decrease in the value of D-dimers compared to the biological reference range is a good prognostic element in COVID-19 [22,23,24].

Some patients with severe COVID-19 infection may develop coagulopathy (according to the criteria of ISTH- The International Society of Thrombosis and Haemostasis) with fulminant activation of coagulation and consumption of coagulation factors leading to a marked increase in D-dimers.

D-dimers result from the degradation of stabilized fibrin (cross-polymerized) under the action of plasmin, which proves a fibrinolysis secondary to coagulation.

This test has a higher diagnostic value than PDF (fibrin degradation products), especially for Disseminated Intravascular Coagulation (DIC) and thrombotic manifestations, including those caused by COVID-19.

The determination of D-dimers is also indicated in:

- deep vein thrombosis of the lower limbs and pulmonary thromboembolism;
- plasma D-dimer levels increase progressively during pregnancy and have poor predictive value in ruling out the diagnosis of deep vein thrombosis after 20 weeks of gestation. During labor, the D-dimers increase to a high extent, after which they decrease rapidly at 3 days postpartum and slowly return to normal values after about 30 days. From this moment they begin to regain their usefulness;
- D-dimers have predictive value on the risk of recurrence of thrombosis;
- D-dimers are a sensitive marker for Disseminated Intravascular Coagulation (DIC), being recommended for confirming / refuting a diagnostic suspicion, estimating the potential risk in patients with existing DIC, as well as for monitoring the initiated therapy;
- monitoring the degree of clot lysis during thrombolytic therapy;
- choosing a more sustained therapy in patients with increased initial levels of D-dimers;
- subclinical thrombophilia, being useful in the investigation of patients with unexplained primary or secondary infertility and thrombophilia.

Ruling out the diagnosis of deep vein thrombosis after 20 weeks of gestation. During labor, the D-dimers increase to a high extent, after which they decrease rapidly at 3 days postpartum and slowly return to normal values after about 30 days. From this moment they begin to regain their usefulness.
Fibrinogen

Fibrinogen is a laboratory test used to monitor COVID-19 patients. At the onset of infection, elevated fibrinogen values were found compared to the biological reference range.

During the disease, especially in patients who develop severe DIC-type coagulopathies associated with COVID-19, the value of fibrinogen can decrease dramatically, even below 100 mg/dl [22,23,24].

This correlates with the growth in parallel for the levels of another inflammation marker (CRP-C Reactive Protein) [22,23,24].

Elevated values of fibrinogen compared to reference values may be found in [10]:

- acute rheumatoid arthritis;
- chronic inflammation;
- Hodgkin’s disease;
- neoplasms;
- liver disease;
- estrogen treatment;
- compensated intravascular coagulation;
- hypertension;
- diabetes, obesity;
- nephrotic syndrome;
- multiple myeloma.

aPTT- activated Partial Thromboplastin Time

Patients who develop severe coagulopathies such as Disseminated Intravascular Coagulation (DIC) associated with COVID-19 disease are also monitored by determining aPTT (activated Partial Thromboplastin Time), a common laboratory test, scientifically proven to be useful in monitoring patients with COVID-19 who develop severe coagulopathies [22,23,24].

Unlike the classic pattern of DIC caused by sepsis or trauma, the degree of increase in aPTT in COVID-19 is often lower than that of PT (especially due to increased factor VIII levels).

Elevated aPTT values indicate deficiency of the factors involved and allow the detection of hemophilia, generally suggesting a hemorrhagic disease.

aPTT is prolonged [10] in all intrinsic congenital deficiencies (including haemophilia A and B, von Willebrand’s disease), treatment with heparin and thrombin inhibitors, treatment with oral anticoagulants, vitamin K deficiency, malnutrition, hypofibrinogenemia, hepatitis, DIC, presence of fibrin degradation products, presence of circulating factor-specific or nonspecific-primary or secondary lupus anticoagulants in Systemic Lupus Erythematosus (SLE) and other autoimmune diseases, malignancies, tuberculosis, multiple glomerulonephritis, after transfusion in response to certain medications.
Monitoring of COVID-19 patients who develop coagulopathies associated with the disease is also performed by determining the PT-prothrombin time/Quick time. \[22,23,24\]

Some patients with severe COVID-19 infection may develop coagulopathy (according to the criteria of ISTH-The International Society of Thrombosis and Haemostasis) with fulminant activation of coagulation and consumption of coagulation factors which is also reflected by prolonged prothrombin time (PT) \[22,23,24\].

Elevated values of PT-prothrombin time/Quick time- are also present \[10\] in:

- treatment with oral anticoagulants;
- congenital or acquired deficiency of factors II, V, VII, X, vitamin K deficiency in newborns, alterations of the liver parenchyma (acute hepatitis, chronic hepatitis, liver cirrhosis);
- biliary obstruction;
- intestinal fat absorption disorders;
- (celiac disease, chronic diarrhea), hereditary hypoprothrombinemia;
- hyperfibrinogenemia, hypofibrinogenemia, dysfibrinogenemia;
- the presence of circulating anticoagulants, DIC syndrome, hemorrhagic diathesis in newborns;
- malnutrition.

Prothrombin Time (PT)

Elevated procalcitonin values compared to the biological reference range are associated with an almost 5-fold higher risk of severe SARS-CoV-2 infection \[25\].

Periodic measurement of procalcitonin may play an important role in predicting the course of COVID-19 to a more severe form of the disease \[25\].

The synthesis of this biomarker is inhibited by interferon (INF)-gamma, whose concentration increases during viral infections which could explain why procalcitonin value would remain in the reference range in several patients with uncomplicated SARS-CoV-2 infection.

PCT shows elevated concentrations in severe bacterial, fungal, and parasitic infections as well as in sepsis.

Concentrations > 2 ng/mL indicate an increased risk of severe sepsis and/or septic shock.
An increased level of serum C-Reactive Protein (CRP) was observed in most patients with COVID-19. In patients with COVID-19, the CRP value at admission correlated with the severity of the disease and proved to be a good predictor of adverse outcomes [1,26]. In the early stages of COVID-19, CRP levels were positively correlated with lung lesions and could reflect the severity of the disease [27].

Determining C-reactive protein concentrations in dynamics helps in monitoring the response to antimicrobial treatment and its determining serum concentration levels is useful [10] in:

- chronic inflammatory diseases and cancers (moderate CRP values);
- inflammatory rheumatism (values between 10-100 mg/dL);
- surgery, PCR being a marker of post-surgical intervention monitoring;
- in uncomplicated cases the CRP concentration reaches a peak in the first 48-72 hours post-surgical intervention and returns to normal until the 7th day after;
- in case of post-surgical complications (inflammation or septic condition) the CRP level remains increased;
- in acute bacterial infections, where it increases rapidly, in 4-6 hours, to values >100 mg/dL and decreases in a few days with the remission of the bacterial infection.

In patients with COVID-19, the CRP value at admission correlated with the severity of the disease and proved to be a good predictor of adverse outcomes [1,26]. In the early stages of COVID-19, CRP levels were positively correlated with lung lesions and could reflect the severity of the disease [27].

Ferritin

Hyperferritinemia has been associated with an increased severity of COVID-19 disease because at elevated ferritin levels compared to the biological reference range the so-called “Cytokine storm” is triggered, which can be fatal to half of COVID-19 patients, especially in the elderly [28].

The increase in ferritin (values > 400 ng/mL) occurs in case of excessive exogenous iron intake and in the following conditions/situations [10]:

- iron overload syndrome (hemochromatosis or hemosiderosis);
- oral/parenteral administration of iron;
- inflammatory diseases;
- acute/chronic liver diseases associated with alcoholism;
- malignant pathology (lymphoblastic leukemias, Hodgkin's disease, malignant lymphomas, neuroblastoma, breast/renal cell carcinoma, pancreatic/lung cancer);
- hyperthyroidism;
- hemolytic anemia, megaloblastic anemia, thalassemia, sideroblastic anemia;
- the final stage of kidney disease;
- late cutaneous porphyria;
- infectious diseases;
- inflammatory diseases;
- acute myocardial infarction;
- X-ray therapy.
The immune response plays an important role in the evolution of COVID-19 disease and in the occurrence of severe forms.

Excessive amounts of proinflammatory cytokines and chemokines: interferon, TNF alpha, interleukins 1beta, 6, 12, 18, 33, and others are released by immune cells that respond to SARS-CoV-2 infection by activating a violent immune response against the lungs (producing ARDS) and other organs (leading to multiple organ failure), which rapidly has lethal consequences [29].

Cytokine tests, especially IL-6, should be used where possible for the assessment of patients suspected of hyperinflammation [30,31].

Acute Respiratory Distress Syndrome (ARDS) is, according to scientific studies data, a leading cause of mortality in COVID-19 [29].

It was observed in the SARS-CoV-2 virus infection that the number of CD4+ and CD8+ T lymphocytes is low in the peripheral blood (as they are destroyed in the process of removing the virus), but there are high concentrations of activation markers.

Cases have been reported in which SARS-CoV-2 virus infection was detected even after the patient has been considered cured.

This indicates a difficulty for the immune system to effectively eliminate the virus, in which case a potential vaccine would have limited efficacy.
Parameters/analytes (COMMON MEDICAL TESTS) showing a SEVERE/UNFAVOURABLE PROGNOSIS in COVID-19

<table>
<thead>
<tr>
<th>Lt. no.</th>
<th>Analyte/parameter (medical test)</th>
<th>Value compared to the biological reference range</th>
<th>Clinical significance in COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neutrophils/lymphocytes ratio (NLR)</td>
<td>Low lymphocyte count Increased number of neutrophils</td>
<td>Increased value Independent risk factor inpatient mortality rate, especially in men</td>
</tr>
<tr>
<td>2</td>
<td>Serum albumin</td>
<td>Low value</td>
<td>Associated with an increased risk of death</td>
</tr>
<tr>
<td>3</td>
<td>Leukocytes</td>
<td>Increased number</td>
<td>Severe prognosis</td>
</tr>
<tr>
<td>4</td>
<td>Lactate dehydrogenase</td>
<td>Increased value</td>
<td>Correlated with a poor clinical prognosis</td>
</tr>
<tr>
<td>5</td>
<td>Alanine aminotransferase</td>
<td>Increased value</td>
<td>A 5-fold increase compared to the biological reference range is associated with a 7-fold increase for the risk of mortality</td>
</tr>
<tr>
<td>6</td>
<td>Serum creatinine</td>
<td>Increased value</td>
<td>Serum creatinine (SCr) value at admission is a predictor for COVID-19 inpatient death</td>
</tr>
<tr>
<td>7</td>
<td>Highly sensitive I troponinin (hs-cTnI)</td>
<td>Increased value</td>
<td>Prediction of mortality in confirmed COVID-19 patients</td>
</tr>
<tr>
<td>8</td>
<td>Type B natriuretic peptide (NT-proBNP)</td>
<td>Increased value</td>
<td>Prediction of mortality in confirmed COVID-19 patients</td>
</tr>
<tr>
<td>9</td>
<td>D-dimeri</td>
<td>Increased value</td>
<td>Associated with increased mortality</td>
</tr>
<tr>
<td>10</td>
<td>C-Reactive Protein (CRP)</td>
<td>Increased value</td>
<td>Positively correlated with lung lesions and could reflect the severity of the disease</td>
</tr>
<tr>
<td>11</td>
<td>Ferritin</td>
<td>Increased value</td>
<td>Increased severity of COVID-19 disease that can be fatal</td>
</tr>
<tr>
<td>12</td>
<td>Interferon, TNF alpha, Interleukins 1 beta, 6, 12, 18, 33</td>
<td>Increased value</td>
<td>Activates a violent immune response that is rapidly evolving towards death</td>
</tr>
</tbody>
</table>

SOURCE: Scientific papers available and accessed until May 4-th 2020
### Parameters/analytes (COMMON MEDICAL TESTS) showing SEVERITY in COVID-19

<table>
<thead>
<tr>
<th>It. no.</th>
<th>Analyte/parameter (medical test)</th>
<th>Value compared to the biological reference range</th>
<th>Clinical significance in COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Platelets</td>
<td>Low number</td>
<td>Increased risk of severe disease</td>
</tr>
<tr>
<td>2</td>
<td>Bilirubin</td>
<td>Increased value</td>
<td>Significantly associated with disease severity</td>
</tr>
<tr>
<td>3</td>
<td>Fibrinogen</td>
<td>Value increased at onset and decreases in DIC</td>
<td>Develops severe DIC-type coagulopathies</td>
</tr>
<tr>
<td>4</td>
<td>Prothrombin time (PT)</td>
<td>Value extension</td>
<td>Develops severe DIC-type coagulopathies</td>
</tr>
<tr>
<td>5</td>
<td>Procalcitonin</td>
<td>Increased value</td>
<td>Associated with an almost 5-fold higher risk of severe infection with SARS-CoV-2</td>
</tr>
<tr>
<td>6</td>
<td>C-Reactive Protein (CRP)</td>
<td>Increased value</td>
<td>Good predictor of adverse outcomes</td>
</tr>
<tr>
<td>7</td>
<td>Ferritin</td>
<td>Increased value</td>
<td>Associated with increased disease severity</td>
</tr>
<tr>
<td>8</td>
<td>Urea nitrogen (BUN)</td>
<td>Increased value</td>
<td>Significantly associated with mortality</td>
</tr>
</tbody>
</table>

### SOURCE:
Scientific papers available and accessed until May 4-th 2020

### Conclusions to the O.B.B.C.S.S.R GUIDE from 05.05.2020

Dr. Constanța Popa
COMMON TESTS FOR BIOCHEMISTRY, HEMATOLOGY-HEMOSTASIS, IMMUNOLOGY published by I.F.C.C in March 2020 [1], which provide to clinicians an extremely useful information on:

- staging
- prognosis
- monitoring
- detection of COVID-19

based on the increased or decreased ML test values compared to the biological reference range and are performed by specialists licensed by the Ministry of Health, explicitly nominated by paragraph (3), art. 5, Order of the Minister of Health 1301/2007:

"In the medical analysis laboratory, doctors with the specialty of laboratory medicine-clinical laboratory, microbiology, laboratory medicine, other personnel with higher education licensed to work in the medical field".
REFERENCES

1. I.F.C.C Information Guide on COVID-19 - Monday 4 May updates Published: Thursday, March 26, 2020
2. www.ifcc.org
3. Law no.460/2003
4. Law no. 200/2004
8. www.jwatch.org Coagulopathy Associated with COVID-19 April 6, 2020
9. www.doi.org/10.1016/S0140-6736(20)30628-0
12. BMJ 2020; 368 www.doi.org/10.1136/bmj.m810
15. neurosciencenews.com/tests-blood-covid-19-16241/
16. www.ncbi.nlm.nih.gov/pmc/articles/PMC3816785/
17. www.ncbi.nlm.nih.gov/pmc/articles/PMC4305785/
18. www.journal-of-hepatology.eu/article/S0168-8278(20)30218-X/fulltext
20. www.raportuldegarda.ro
29. MH Order No.1301 July 20-07
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