Dr.mahshid ,mehrjerdian

Pathologist

Assistant professor of golestan medical university

Biomarkers

Support tests

- When assessing a patient with COVID-19 infection, biomarkers can be useful to clinicians in starting treatment and close monitoring.
- Though biomarkers may help improve prognosis and outcomes, their significant variability between patients could affect the findings of the studies

- A wide range of different tests is available, with variable sensitivity and specificity, most of which require validation.

- Laboratory tests such as complete blood count, C-reactive protein (CRP), D-dimer, clotting tests, lactic dehydrogenase (LDH), ferritin, and procalcitonin.

C-reactive protein

- CRP is a plasma protein produced by the liver and induced by various inflammatory mediators such as IL-6.
- Despite being non-specific, this acute phase reactant is used clinically as a biomarker for various inflammatory conditions; a rise in CRP levels are associated with an increase in disease severity

- Critically severe patients had significantly higher CRP than severe or non-severe patients
- Higher levels of CRP recorded in the severe group vs non-severe group are suggestive that CRP can be monitored to assess progression of disease
- significant increase in CRP levels prior to changes in CT scores for early periods of severe group

- The results of the first analysis showed that CRP was not significant differentiators of COVID-19 and non-COVID-19 cases. However, in the second analysis, a significantly higher level of CRP was observed in the severe COVID-19 group compared to non-COVID-19 cases, confirming previous reports of the clinical utility of CRP levels as an indicator for severe disease and progressive inflammation
- . CRP levels may indicate lung damage and development of disease

Interleukin-6

- Cytokine release syndrome (CRS) is an over-exaggerated immune response involving an overwhelming release of pro-inflammatory mediators.
- This mechanism underlies several pathological processes including acute respiratory distress syndrome (ARDS)
- .Studies investigating the role of cytokines in SARS and MERS have had also found a link between CRS and disease severity .
- Understanding their role in COVID-19 disease may help facilitate the design of novel immunotherapies

- To investigate the effect of the coronavirus during the acute phase of the disease, plasma cytokines/chemokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β, IL1RA, IL2, IL4, IL5, IL-6, IL-10, IL13, IL15 and IL17A were measured.
- One study showed that macrophages and dendritic cells play crucial roles in an adaptive immune system. These cells contain inflammatory cytokines and chemokines, such as IL-6, IL-8, IL-12, TNF-α, monocyte chemoattractant protein-1, granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor. These inflammatory reactions could cause a systemic inflammation

- Increased expression of IL-2 and IL-6 in serum is expected to predict the severity of COVID-19
- Severity of COVID-19 could be predicted with baseline IL-6 levels
- Significantly higher baseline levels of IL-6 in those requiring ICU compared to those who do not
- Significantly higher levels of IL-6 in sever and critical COVID-19.
- Surveillance may help in early screening of critical illness
- ARDS development in COVID-19 is related to rise in IL-6

White cell count

White blood cells (WBCs), known as leucocytes are a component of blood generated from bone marrow and lymphoid tissue.

They are divided into two major groups, granulocytes and agranulocytes.

Within the granulocyte group are eosinophils, basophils and neutrophils (NC), whereas lymphocytes (LC) and monocytes are present in agranulocytes.

A disproportionate number of these cells may reveal an underlying infection and hence can be measured using blood tests, producing a WCC.

However, the reliability of WCC as a biomarker for COVID-19 remains unproven

- Lymphopenia results from a multifactorial mechanism that includes:
- the cytopathic effect of the virus,
- induction of apoptosis,
- bone marrow suppression by inflammatory cytokines.

- Compared to other biomarkers, there is hesitancy in using WCC alone as it is influenced by many factors such as glucocorticoid treatment which increases it.
- Although WCC encompasses many cell types, NCs and LCs are most clinically relevant biomarkers.
- Multiple studies on COVID-19 have concurred high NLR in severe cases compared to non-severe cases due to high NC and low LC.
- The use of LC independently has been suggested as a potential biomarker of COVID-19 as patients have consistently low LC, with significant lymphopenia reported in critically ill patients .

- Leukocyte and neutrophil counts were significantly lower in COVID-19 compared to non-COVID-19 infections, but contrastingly higher in severe COVID-19 cases relative to non-severe COVID-19.

Significantly higher NLR in severe patients. Monitoring may aid in early screening of critical illness.

Non-ICU vs ICU patients had drastically lower WCC, checking low LC and high NC may help in early detection of disease progression.

- Surveillance of NC/LC may reflect severity of lung abnormalities.

- **Several reports have suggested lymphopenia as a strong indicator of COVID-19 infection.**

- lymphocyte counts were found significantly lower in the severe group, which is in line with previous reports which observed progressive lymphopenia as a strong indicator of disease severity.

- Complete blood count – lymphopenia, eosinopenia, and neutrophil/lymphocyte ratio ≥ 3.13 are related to greater severity and worse prognosis.

Dysplastic changes in peripheral blood :

- . A) reactive lymphocyte
- B) Pseudo-Pelger-Huet anomaly of neutrophils.(Hyposegmented)
- C) Lobulation anomaly of the neutrophils.
- D) giant platelet.

Lactate dehydrogenase

- In glucose metabolism, the enzyme LDH converts pyruvate to lactate.
- LDH secretion is triggered by necrosis of the cell membrane, hinting to viral infection or lung damage, such as the pneumonia induced by SARS-CoV-2
- There is convincing evidence linking LDH levels to the development of COVID-19 disease

- Higher LDH levels reported in severe patient's vs non sever
- LDH measured in COVID-19 positive vs negative patients and higher levels apparent in positive groups

D-dimer

- D-dimer originate from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis . Early studies have associated COVID-19 with haemostatic abnormalities with one study observing elevated levels of D-dimer, the measure of coagulation, in non-survivors compared to survivors

- Abnormal coagulation results with markedly elevated D-dimer are common in deaths with COVID-19
- D-dimer levels >1 µg/mL can help clinicians in identifying patients with poor prognosis at earlier stage
- D-dimer levels much higher in those requiring ICU admission and invasive ventilation however statistical analysis not performed
- Compared to non-ICU patients, ICU patients had significantly higher levels of D-dimer
- D-dimer on admission of >2.0 µg/mL could effectively predict in-hospital mortality in patients with COVID-19 and could be an early and helpful marker to improve management

Platelet count

- COVID-19 infection leads to severe haematological changes leading to thrombocytopenia.

- Furthermore, extreme thrombocytopenia is often observed in severe and critical conditions of illness due to the development of disseminated intravascular coagulopathy (DIC), reflected excessive coagulation activity marked by significantly higher levels of D-dimers found in our analysis among patients of severe COVID-19 infection.
- The mechanism behind thrombocytopenia in both early and late stages of COVID-19 infection is likely multifactorial, as a result of both indirect and direct mechanisms that interfere with thrombocyte production, activation, or consumption.
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- Possible direct mechanisms of thrombocytopenia previously characterized in the SARS-CoV is viral bone marrow invasion and infection of progenitor cells, therefore interfering with thrombocyte production from megakaryocytes by inhibition of growth and by inducing apoptosis.
- Other potential mechanisms of thrombocytopenia include excess activation of thrombocytes by virus-triggered immune complexes, and increased consumption due to excessive thrombosis that occur during lung damage.

- In conclusion, thrombocyte count was significantly lower in COVID-19 compared to non-COVID-19 infections, and persistently low in severe disease, implying that thrombocyte measurement is a key laboratory parameter for both diagnosis and prognosis..

- blood parameter can aid both diagnosis and monitoring of progression and should be assessed continually during care of COVID-19 infections.

- thrombocytopenia has been implicated as a marker for severe SARS-CoV-2 infection.
- thrombocyte counts can also be used as a marker to differentiate between COVID-19 and non-COVID-19 infections, regardless of severity.
- In areas with concurrently high dengue infection rates such as South East Asia, the similarities between initial clinical and laboratory presentations of SARS-CoV-2 and dengue infection should be carefully considered during the current pandemic.
- A report from Singapore[49] have shown covert COVID-19 infection in patients originally suspected of dengue infection, with consistent thrombocytopenia observed in the two reported cases. Moreover, false positive dengue serology occurred in the two COVID-19 cases, further complicating the distinction and posing a risk to the intense surveillance required during the current pandemic.

procalcitonin

- The results of the first analysis showed that was not significant differentiators of COVID-19 and non-COVID-19 cases.
- In contrast to a previous meta-analysis, procalcitonin level between severe and non-severe groups were not significantly different in this analysis, which pooled the procalcitonin results of 14 studies.
- The changes in procalcitonin level among severe COVID patients remain unclear, because although half of the 14 analyzed studies showed an insignificant difference between procalcitonin levels between the two groups, the remaining seven analyzed studies reported significantly higher PCT values in the severe group.
- These reports postulate that raised PCT indicates the presence of secondary bacterial infection.

Cardiac troponin

- There is growing evidence of higher mortality rates among those with underlying cardiovascular disease due to COVID-19 infection
- . Some have investigated the use of high-sensitivity cardiac troponin I (hs-TnI) as a marker of disease progression and mortality

- Early recognition of myocardial injury indicated by elevated hs-TnI aids in appropriate triage to a critical care area and informs the use of inotropes and vasopressors.
- However, elevated levels are common in hospitalised patients and are likely to be due to non-ischaemic causes of myocardial injury.
- This may lead to inappropriate use of cardiology consultation and downstream testing and increased risk to cardiac physiology staff.

- Significantly higher levels of hs-TnI in patients who require mechanical ventilation compared to those who do not

Renal markers

- There is also evidence that chronic kidney disease is associated with severe forms of COVID-19 infection
- Serum values of urea, creatine and cystatin-C significantly increase in severe COVID-1
- Raised creatinine levels associated with poor outcome in COVID-19 infection

- Interestingly, another study showed a potential role for urinalysis over serum markers of kidney function .
- Here, abnormalities in the routine urine test on admission correlated strongly with disease severity.
- They go on to suggest that urinalysis may reveal kidney impairment more readily than evaluation of serum renal biomarkers.
- However, these tests were only carried out on admission and so patients in earlier stages of the infection had changes in serum levels obscured by compensatory kidney function.
- Hence renal abnormalities on admission may indicate higher risks of deterioration, ensuring appropriate triaging.

Liver injury

- Primarily mild elevation of AST and ALT (1 - 2 times upper limit of normal
- AST > ALT > bilirubin > alkaline phosphatase (ALP)
- Gamma glutamyl transferase (GGT) has been reported to be elevated in up to 54% of patients with COVID-19 during hospitalization

- Microscopic finding:
- Steaosis
- Acute hepatitis
- cholestasis

- Bronchoalveolar lavage (BAL):
- Abundant activated plasma cells, as per a single case report

- Alveolar macrophages may feature nuclear clearing or intranuclear cytopathic inclusions

ICU /nonICU

- Compared to non-ICU patients, ICU patients had higher plasma levels of :
- IL-6,
- D-dimer
- Lymphopenia
- Higher WCC
- specifically higher NC, lower LC and higher NLR in ICU vs non-ICU patients.

Sever /non sever

- Significantly higher levels of CRP, IL-6, LDH and NC but low LC in severe COVID-19,vs non-severe group. Surveillance may help in early screening of critical illness

Prognostic

- ROC analysis showed strong association found between CRP levels and progression of disease.
- Increased CRP and decreased albumin strongly correlated with disease progression
- D-dimer levels >1 μg/mL can help clinicians in identifying patients with poor prognosis at earlier stage

- LDH, NC, CRP and platelet count were higher in refractory vs general patients.

- Refractory patients had more cases of lung abnormalities, suggesting these biomarkers correlate with development of disease

Critically severe patients

- Critically severe patients had significantly higher CRP and WCC than severe or non-severe patients.

- Greater CRP values correspond with the critical group, as groups were determined based on the diameter of largest lung lesion - CRP levels may indicate lung damage and development of disease

mortality

- Abnormal coagulation results with markedly elevated D-dimer are common in deaths with COVID-19

- Cardiac troponin, Myoglobin, CRP and IL-6 significantly increased in cases with mortality
- Prior to the death of critical patients: NC, D-dimer, blood urea, and creatinine levels rose throughout until death, whilst the LC carried on falling.