Section A -Research paper

Association of Inflammatory markers (Ferritin, LDH and D-Dimer) with CO-RADS Scoring (CT imaging) in diagnosis and assessment of COVID-19 severity

- A Retrospective Cross Sectional Study.

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Abstract:

Background:

COVID-19 pandemic outbreak is the major Global Public health issue. Severity spread fromasymptomatic was rapid leads to acute pneumonia, acute respiratory syndrome and even death. Inflammatory markers such as CRP, ferritin, LDH, D-Dimer, IL-6 and procalcitonin were used as prognostic makers in treating patients with COVID-19.For the diagnostic utility, RT PCR and CT findings were used.This study aimed to find the association of routine inflammatory markers, Ferritin, LDH and D-Dimer with CO-RADS scores in diagnosing and assessing the severity of COVID-19.

Methodology:

A retrospective cross sectional study plan was designed. The data was retrieved from the hospital information system from November 2020 to April 2021. There were above 561 patients given samples for the COVID-19 alone with routine biochemical inflammatory markers of Ferritin, LDH, D-Dimer and radiology investigation of chest CT.

Result and Conclusion:

D-dimer, Ferritin and CO-RADS score showed a significant p–value of 0.001 among COVID-19 patients. LDH did not show any significant difference between these two groups.Among the inflammatory markers

D-dimer, ferritin and CO-RADS score are associated with the prediction of COVID-19 clinical severity. The use of the inflammatory biomarkers helps in assessing the disease severity and planning appropriate management.

Key words: Ferritin, LDH, D-Dimer, CORAD Score, COVID-19

Introduction:

COVID-19 pandemic outbreak is the major Global Public health issue currently. COVID-19 infection is caused by a single strand RNA beta coronavirus namely SARS-CoV-2, since it shares a similar RNA sequence of Severe Acute Respiratory Syndrome Corona Virus (SARS- CoV). It was first identified and confirmed in December 2019 at Wuhan China (1). The Clinical symptom and severity of spreading was rapid from asymptomatic to normal person which leads to acute pneumonia, acute respiratory syndrome and even death in infected patients (2,3).

The pathogenesis of the infection triggers the inflammatory and immunological response by releasing cytokines and chemokines (4). As there was increase in the levels of cytokines and chemokines resulting in cytokine storm or multisystem inflammatory syndrome, leading to death. In COVID-19 infection, inflammatory markers play a principal role in pathogenesis of multi-organ dysfunction and mortality in COVID-19 patients. These inflammatory markers such as CRP, ferritin, LDH, D-Dimer, IL-6 and procalcitoninare used as prognostic makers in treating patients with COVID-19(5).

To diagnose SARS-CoV-2, qRT-PCR or CBNATE is used a gold standard method (6). The sensitivity of the test depends upon various factors such as quality of the sample collected, viral load and duration of the infection. This testing method hasn't help clinicians in assessing the severity of the infection (7).

Radiological computed tomography (CT) Chest was used as an alternative investigation, to assess the severity of the disease in patients and report the degree of lung infection and its severity. Dutch Radiological society in March 2020 developed a scoring system based on chest CT and clinical data including clinical finding and laboratory investigation named the COVID-19 Reporting and Data System (CO-RADS). The CO-RADS score categories from 1 to 6 based on the severity of the infection, while 0 is considered to be negative and 6 is severely infected along with RT-PCR positive for SARS-CoV-2 (8).

This study aimed to find the association of routine inflammatory markers, Ferritin, LDH and D-Dimer with CO-RADS scores in diagnosing and assessing the severity of COVID-19.

Methodology:

A retrospective cross sectional study plan was designed. Data was retrieved from the hospital information system for six months from November 2020 to April 2021. Institute research committee and ethics committee approved the study with waiver. There were above 561 patients given samples for the COVID-19 alone with routine biochemical inflammatory markers of Ferritin, LDH, D-Dimer and radiology investigation of chest CT.

With nasopharyngeal swab SARS-CoV-2 was diagnosed by performing qRT-PCR(BIO-RAD). Ferritin and D-Dimer were estimated by immune fluorescence method using Mini-Vidas system (Biomerieux, France). LDH was estimated by the IFCC approved kit method (DiaSys Diagnostic Systems, Germany) using an automated chemistry analyser Sysmex BX 3010. Chest CT was taken by Brivo CT325 CT scan equipment, Wipro GE Healthcare Pvt Ltd.

- **Inclusion criteria:**
  - ✔ All patients above 18 years of age with symptoms of COVID-19 who have been tested for RT-PCR.
- **Exclusion criteria:**
  - ✔ All patients who do not have symptoms of COVID-19
  - ✔ Those who had a recent history of travel to the countries with COVID-19.

All the data were expressed in median and 25th - 75th interquartile range. For the comparison of the non-normal distributed data, Mann-Whitney U test was performed.

Result:

Aim of the study was to find association between the inflammatory markers and CORADS score and COVID-19 severity. 561subjects' data was retrievedduring the period of November 2020 to April 2021and grouped them into two groups - COVID 19 negative and positive which had 340 and 221 subjects respectively.

Table 1 depicts that comparison of the inflammatory marker of COVID-19 such as D-Dimer, ferritin and LDH along with radiological scoring i.e. CO-RADS scores. The median value of the D-dimer inflammatory marker among the COVID-19 negative patients is 1410.93 (IQR: 623.64 - 3418.46) and for COVID-19 positive patients is 760.6 (IQR: 469.21 - 1492.9). The median value of the Ferritin inflammatory marker among the COVID-19 negative patients is 194.08 (IQR: 67.86 - 544.71) and for COVID-19 positive patients is 362.26 (IQR: 151.31 - 748.32). D-Dimer and Ferritin showed a significant difference with p–value of 0.001.

The median value of the LDH inflammatory marker among the COVID-19 negative patients is 363 (IQR: 279.75 - 510.5) and for COVID-19 positive patients is 419 (IQR: 286 – 582). There is no significant difference between the groups.

The median value of the CO-RADS score among the COVID-19 negative patients is 1 with an interquartile range of 1 - 3. The median value of the CO-RADS score among the COVID-19 positive patients is 5 with an interquartile range of 5 - 6. They showed significant difference with p–value of 0.001.

Discussion:

The COVID-19 pandemic caused by SARS-CoV-2, clinically ranges from mild influenza-like illness to severe acute respiratory distress syndrome with multisystem involvement and in severe conditions even leads to death (9). The spike glycoprotein of SARS-CoV-2 attaches to the ACE-2 cell surface receptor before moving into the cytoplasm of the cell, where it releases its RNA genome and multiplies to produce more viral particles (10). The cell then breaks down, and the virus spreads to neighbouring cells. Rapid viral multiplication coupled with immunological dysregulation brought on by pyroptosisresults in a large release of inflammatory mediators. This leads to the cytokine storm development and multi-organ damage.

Biomarkers are the quantitative indicators that reflect the underlying pathological processes that take place in the body. Biomarkers are the valuable and cost-effective tools to guide the treatment in COVID-19(11).

D-Dimer assays are frequently used in clinical practice to rule out a diagnosis of venous thromboembolism because elevated levels of the D-dimer suggest an increased risk of irregular blood clotting. D-dimer levels were considerably higher in patients with severe community-acquired pneumonia. Sepsis and coagulation malfunction may result from a viral infection. Therefore, D-dimer had a sign of serious viral infection in addition to venous thromboembolism. The rise in D-dimer was an indirect sign of an inflammatory response because inflammatory cytokines can upset the balance between coagulation and fibrinolysis in the alveoli, which in turn can trigger the fibrinolysis system and raise D-dimer levels. Similar findings were found in our study, which demonstrated that D-dimer is a predictor of clinical severity in COVID-19. (11 - 13)

The storage and binding of iron by ferritin is connected to the immunological and inflammatory response. Poor outcomes of the patients can be predicted by elevated serum ferritin levels in hospitalised individuals especially in patients with influenza like illness (14). In the current study, ferritin is higher in the negative patients on par with the inflammatory response.

Both during hemolysis and the early stages of myocardial infarction, there is an increase in lactate dehydrogenase levels. The liver, striated muscles, heart, kidneys, lungs, brain, and red blood cells are where it is the most active (erythrocytes). Lactate dehydrogenase is released from damaged cells, increasing its content and activity in the blood. In these patients, a high serum LDH activity level is a poor prognostic indicator. LDH is a multi-organ failure marker for a quite number of inflammatory conditions, including MI, infections, cancer, sepsis, and cardio-pulmonary compromise condition.

Limited availability of RT-PCT tests for COVID-19 in some high-prevalence nations, have an impact on the usefulness of the assays.Patients who have positive results on their chest CT scans initially have negative RT-PCR results that eventually turn positive later. It brings out the need to recognise, interpret and communicate the imaging findings pertaining to the lungs.

COVID-19 Reporting and Data System (CO-RADS), was released in mid-March 2020 by Dutch Radiological society,encourages the use of precise, detailed language to lessen report ambiguity,

differentiatemoderate-to-severeof the disease. With the help of the CO-RADS evaluation, a patient's non-enhanced chest CT scan can be divided into categories based on the percentage of lung involvement.

The classification's ability to distinguish between radiological abnormalities associated with a low and high probability of COVID-19, tested against both a clinical diagnosis and positive results for RT-PCR testing, is another significant strength of the system. The CO-RADS was categories between 1 to 6 represent the progression of COVID-19 risk, from very low risk (CO-RADS 1) to infection that has been established with a positive RT-PCR experiment (CO-RADS 6).

Cases that either had a normal chest CT scan or one that had abnormalities that could clearly be ascribed to non-infectious diseases fall under the CO-RADS 1 category. Emphysema, perifissural nodules, lung tumours, or fibrosis are examples of findings that would support this judgement. The presence of interlobular interstitial thickening with pleural effusion should be classified under this category if it represents interstitial pulmonary edema.(Figure 1) Cases with radiological findings of infectious diseases that are not compatible with COVID-19 but are typical of other lung infections, such as bronchitis, bronchiolitis, bronchopneumonia, centrilobular ground-glass opacities, lobar pneumonia, or pulmonary abscesses, fall under the CO-RADS 2 category(Figure 2)(8)(9)

The CO-RADS 3 category contains radiological findings related to COVID-19 lung involvement which are also found in other viral pneumonias and non-infectious lung diseases. Peri-hilar ground-glass, homogeneous, and extensive ground-glass opacities, ground-glass opacities linked to interlobular interstitial thickening, and patterns of organising pneumonia if other COVID-19-typical findings are imaging findings included in this category.(Figure 3)

The CO-RADS 4 category includes those results, while typical for COVID-19, have some overlap with other viral pneumonias. This includes results that are similar to those in CO-RADS 5 but have an atypical distribution, such as an absence of contact with the visceral pleura, unilaterality, peribronchovascular predominance, or when superimposed on severe and widespread pre-existing pulmonary changes.(Figure 4)

CO-RADS 5 category implies mandatory features that include ground-glass opacities, with or without consolidation, located near visceral pleural surfaces (including the fissures), and multifocal bilateral distribution. Subpleural sparing is allowed.There are three confirmatory patterns, and they usually appear at various points throughout the disease's progression. Early on, this pattern exhibits numerous ground-glass areas that can be rounded or half-rounded in form with an unsharp demarcation or numerous and sharply limited ground-glass areas delineating the boundaries of numerous adjacent secondary pulmonary lobules. A "crazy paving" pattern is created later in the disease's progression by the obvious intra-lobular interstitial thickening linked to the ground-glass opacities. The pattern then represents an organising pneumonia pattern, including the reversed halo sign, ground-glass consolidation connected to extensive subpleural consolidations with air bronchogram and curvilinear subpleural bands (Figure 5) (8)(9)This is seen in the COVID-19 positive patients only in our study.

Thus the study showed significant difference between the inflammatory markers such as D-dimer, Ferritin and CO-RADS among the COVID-19 positive and negative patientsrespectively. The inflammatory markers like D-dimer, Ferritin and CO-RADS score were high in the COVID-19 negative patients than in the positive patients because patients may be asymptomatic or may be in the incubation period despite the negative results of the RT-PCR tests. These high inflammatory markers may be the indirect manifestation of the inflammatory reaction, as the inflammatory cytokines cause the imbalance of coagulation and fibrinolysis in the alveoli that activates the fibrinolysis system and increase the level of D-dimer, ferritin and CO-RADS score. CO-RADS 1 is also significantin the COVID-19 negative patients because they may be asymptomatic. (13)

Conclusion:

The study highlights the significant association between theinflammatory markers, Ferritin, LDH, D-Dimer and CO-RADS score within COVID-19 patients. Among the inflammatory markers D-dimer, ferritin and CO-RADS score are associated with the prediction of COVID-19 clinical severity. This is during the pre-vaccination time which reflected the disease severity and the role ofinflammatory markers in the COVID-19 negative patients also.

Limitation:

- It is a retrospective study design which projects the judicious use of the inflammatory biomarkers in the prediction of the clinical severity of COVID-19.
- It does not reveal the various drug interventions on the outcome.

Reference:

1. Pal M, Berhanu G, Desalegn C, Kandi V. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. Cureus. 2020;12(3):e7423. Published 2020 Mar 26. doi:10.7759/cureus.7423.

2. Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr. 2020;87(4):281-286. doi:10.1007/s12098-020-03263-6

3. Johnson KD, Harris C, Cain JK, Hummer C, Goyal H, Perisetti A. Pulmonary and Extra-Pulmonary Clinical Manifestations of COVID-19. Front Med (Lausanne). 2020;7:526. Published 2020 Aug 13. doi:10.3389/fmed.2020.00526

4. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017;9(6):7204-7218. Published 2017 Dec 14. doi:10.18632/oncotarget.23208

5. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. Ther Adv Respir Dis. 2020;14:1753466620937175. doi:10.1177/1753466620937175

6. Dramé M, TabueTeguo M, Proye E, et al. Should RT-PCR be considered a gold standard in the diagnosis of COVID-19?. J Med Virol. 2020;92(11):2312-2313. doi:10.1002/jmv.25996

7. Prokop M, van Everdingen W, van Rees Vellinga T, Quarles van Ufford J, Stöger L, Beenen L, et al. CO-RADS – A categorical CT assessment scheme for patients with suspected COVID-19: definition and evaluation. Radiology. 2020 Apr 27;201473

8. Penha, D., Pinto, E. G., Matos, F., Hochhegger, B., Monaghan, C., Taborda-Barata, L., Irion, K., &Marchiori, E. (2021). CO-RADS: Coronavirus Classification Review. Journal of clinical imaging science, 11, 9.

9. Prokop M, van Everdingen W, van Rees Vellinga T, Quarles van Ufford J, Stöger L, Beenen L, et al. CO-RADS – A categorical CT assessment scheme for patients with suspected COVID-19: definition and evaluation. Radiology. 2020 Apr 27;201473.

10. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus Biology and replication: Implications for SARS-COV-2 [Internet]. Nature News. Nature Publishing Group; 2020 [cited 2023Feb5]. Available from: https://www.nature.com/articles/s41579-020-00468-6

11. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, Deng G. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis. 2020 Jul;96:467-474.

12. Samprathi M, Jayashree M. Biomarkers in COVID-19: An Up-To-Date Review. Frontiers in Pediatrics. 2021;8.

13. Karimi A, Shobeiri P, Kulasinghe A, Rezaei N. Novel Systemic Inflammation Markers to Predict COVID-19 Prognosis. Frontiers in Immunology. 2021;12.

14. Moreira AC, Mesquita G, Gomes MS. Ferritin: An Inflammatory Player Keeping Iron at the Core of Pathogen-Host Interactions. Microorganisms. 2020;8(4):589.

15. Marimuthu, Aishwarya K1,;Anandhan, Monisha2; Sundararajan, Lakshmikanthan2; Chandrasekaran, Jagadeesh1; Ramakrishnan, Balasubramaniam3. Utility of various inflammatory markers in predicting outcomes of hospitalized patients with COVID-19 pneumonia: A single-center experience. Lung India: September–October 2021 - Volume 38 - Issue 5 - p 448-453 doi: 10.4103/lungindia.lungindia_935_20

Table 1: Comparison of Inflammatory markers (D-Dimer, Ferritin and LDH) and CO-RADS between COVID-19 Negative and Positive group patients.

Parameters	COVID – 19		p' Value
	Negative (N = 340)	Positive (N = 221)	
	Median (25th - 75th)	Median (25th - 75th)	
D-Dimer (ng/ml)	1410.93 (623.64 - 3418.46)	760.6 (469.21 - 1492.9)	0.001
Ferritin (ng/ml)	194.08 (67.86 - 544.71)	362.26 (151.31 - 748.32)	0.001
LDH (U/L)	363 (279.75 - 510.5)	419 (286 - 582)	0.170
CO-RADS	1 (1 - 3)	5 (5 - 6)	0.001

Note: Mann-whitney U test - p Value < 0.05 is significant

Figures:

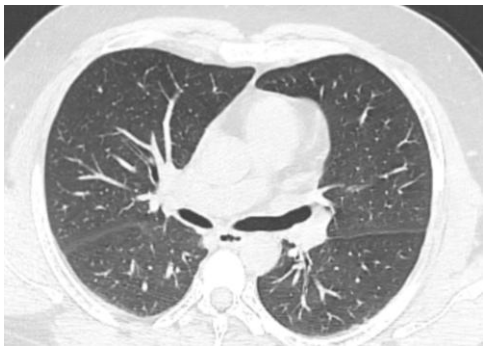

Figure 1: Normal Lung parenchyma -CORADS 1

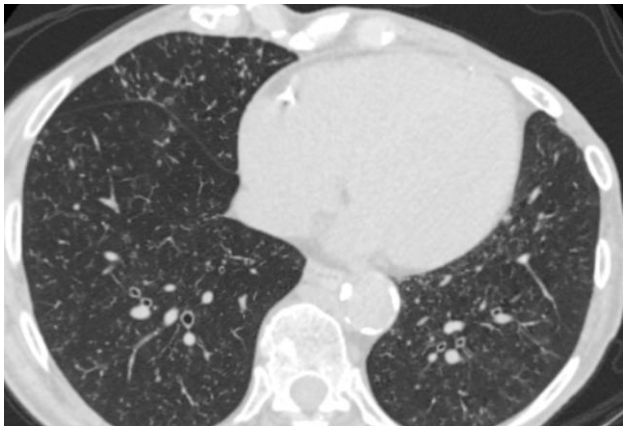

Figure 2: HRCT Lung showing multiple tree in bud and centrilobular micronodules scattered in bilateral lung fields – suggesting infectious etiology other than COVID.

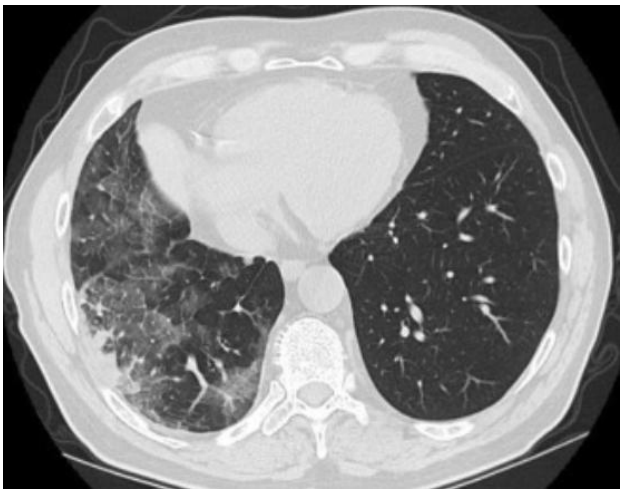

Figure 3: Asymmetrical unilateral groundglass opacities in a case of pneumonia suggesting CORADS 3.

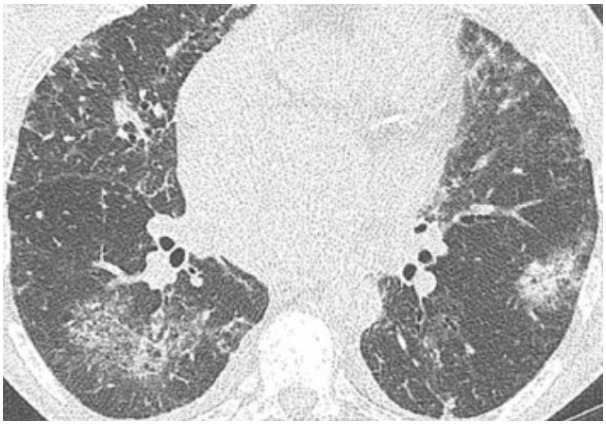

Figure 4: Ground glass changes noted in an atypical peri-bronchovascular pattern consistent with CORADS 4

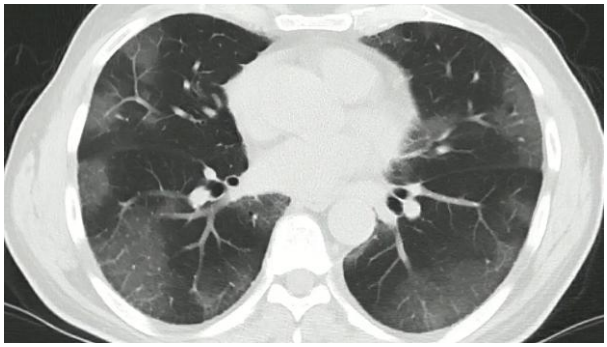

Figure 5: Typical Image findings of COVID including peripheral wedge-shaped ground glass opacities in subpleural and peripheral distribution.