The Prognostic Value of Lung Injury and Fibrosis Markers, KL-6, TGF-β1, FGF-2 in COVID-19 Patients

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ABSTRACT

BACKGROUND: Biomarkers of lung injury and interstitial fibrosis give insight about the extent of involvement and prognosis in well-known interstitial lung diseases (ILD). Serum Krebs von den Lungen-6 (KL-6) reflects direct alveolar injury and, transforming growth factor-beta1 (TGF-β1) and fibroblast growth factor-2 (FGF-2) are principal mediators of fibrosis in ILD and in almost all fibrotic diseases. In this sense, we aimed to assess associations of these biomarkers with traditional inflammatory markers and clinical course of COVID-19.

METHODS: Patients with COVID-19 who had confirmed diagnosis with SARS-CoV-2 nucleic acid RT-PCR were enrolled and followed up prospectively with a standardized approach one month after diagnosis. Patients were divided into severe and non-severe groups according to National Institutes of Health criteria. Outcome was assessed for the requirement of intensive care unit (ICU) admission, long term respiratory support and death. Blood samples were collected at enrollment and serum levels of KL-6, TGF-β1, FGF-2 were determined by ELISA. Association between these markers with other prognostic markers and prognosis were analyzed.

RESULTS: Overall 31 severe and 28 non-severe COVID-19 patients were enrolled and were compared with healthy control subjects (n = 30). Serum KL-6 levels in COVID-19 patients were significantly higher (median [IQR]; 11.54 [4.86] vs 8.54 [3.98] ng/mL, $P = .001$] and FGF-2 levels were lower (median [IQR]; 76.84 [98.2] vs 101.62 [210.6] pg/mL) compared to healthy control group. A significant correlation was found between KL-6 values and CRP, fibrinogen, D-dimer and lymphocyte counts. However, we did not find an association between these markers and subsequent severity of COVID-19, mortality and long-term prognosis.

CONCLUSIONS: Serum KL-6 levels were significantly elevated at the diagnosis of COVID-19 and correlated well with the other traditional prognostic inflammatory markers. Serum levels of principal fibrosis mediators, TGF-β1, FGF-2, were not elevated at diagnosis of COVID-19, therefore did not help to anticipate long term prognosis.

KEYWORDS: Coronavirus disease 2019, Krebs von den Lungen-6, transforming growth factor-beta1, fibroblast growth factor-2, biomarker, computed tomography

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Introduction

Since the announcement of COVID-19 as a pandemic, a tremendous progress has been made in all fields of science to understand and to eradicate disease.[1] However, there is still an unmet need for the determination of parameters that would accurately identify patients who will progress into severe disease. Various treatment options have been developed for the different stages of disease, but many of them are effective if they are started early in the disease course.[2] Therefore, stratification of patients at high risk of progressive disease has particular importance s for the effective management of COVID-19.[3] Furthermore, prompt treatment may avoid overloading the health system and facilitate the distribution of limited medical resources to patients requiring aggressive treatment. For this purpose, clinical assessment is indispensable, but laboratory markers, or biomarkers, can provide additional, objective information which can significantly impact critical components of patient care.

Interstitial pneumonia is the most common cause of hospitalizations for COVID-19 and may be complicated by acute respiratory distress syndrome (ARDS) and refractory respiratory failure.[4,5] Moreover, some patients especially those who had severe pneumonia and ARDS might develop progressive pulmonary fibrosis and permanent lung damage leading to death.

Like previous coronavirus infections, that is SARS and MERS, repair process with fibroproliferation, and remodeling

take place after acute lung injury in COVID-19. Repair process includes regeneration of local stem cells and accumulation of connective tissue for replacing damaged areas.[6] Connective tissue accumulation occurs via alveolar fibroblastic invasion, the transformation of fibroblasts to myofibroblasts, and excessive extracellular matrix (ECM) production. Fibroblasts are located in the alveolar interstitium and synthesize collagen, fibronectin, and basic ECM material resulting in progressive pulmonary fibrosis. Fibrosis mediators, transforming growth factor-beta1 (TGF-β1), fibroblast growth factor-2 (FGF-2), and a mucinous high-molecular-weight glycoprotein Krebs von den Lungen-6 (KL-6), plays central role in post pneumonia fibrosis via induction of proliferation and differentiation of fibroblasts into myofibroblasts.[7,8] Myofibroblasts produce ECM more intensively but also more irregularly than fibroblasts and remain for a longer time in the wound area, hence pulmonary fibrosis develops as a consequence of long standing, progressive, dysregulated immune process.[9] Since, post pneumonia lung damage and fibrosis generally ensue after severe or long lasting infection, determination of biomarkers that may predict severe lung involvement and prolonged recovery are imperative for the commencement of anti-inflammatory and anti-fibrotic treatments promptly, before the development of progressive, irreversible and untreatable complications. Some studies suggested that in patients with severe COVID-19, elevated levels of serum markers such as ferritin, soluble interleukin-2-receptor (sIL2-R), and lactate dehydrogenase (LDH) might be beneficial for the prediction of progressive disease.[10] Unfortunately, at present, there is still no optimal biomarker for anticipation of severe COVID-19.

The present study aimed to evaluate utility of fibrotic and inflammatory markers such as KL-6, FGF-2, and TGF-β1 for anticipation of severity and prognosis of COVID-19 patients.

Materials and Methods

Fifty-nine COVID patients and thirty healthy controls were recorded in this cross-sectional, observational study. Patients were divided into severe (n = 31) and non-severe groups (n = 28) according to National Institutes of Health criteria. The total sample size calculated by G*Power3.0.10 revealed the minimum number of patients is equal with power = 0.8 (1-Type 2 error) and α = .05 (Type 1 error) as 87 for 3 groups (severe group, non-severe group, healthy group) when it was assumed that the effect size was 0.34 according to a previous study.[11]

According to National Institutes of Health (NIH) severity criteria: patients with mild/moderate symptoms, did not have shortness of breath and SpO_2 value of ⩾%94 at ambient air at sea level were constituted non-severe group whereas those with respiration rate of >30 bpm, SpO_2 <94% at ambient air, FiO_2 <300 mmHg or lung infiltrates involving >50% of lung volume, respiratory support requirement, septic shock and/or multiple organ dysfunction formed the severe COVID-19 group. Outcome was assessed for the requirement of intensive care unit (ICU) admission, long term respiratory support and death.

Patients who diagnosed COVID-19 with positive nucleic acid reverse transcription-polymerase chain reaction (rt-PCR) of sputum and nasopharyngeal swabs between April 2019 and March 2022 were enrolled and followed up 1 month after hospital discharge or until death. All patients underwent a standardized approach including blood samples, computed tomography (CT), and pertinent microbiological examination. Patients with (a) infections caused by other pathogens such as bacteria, fungi, and other respiratory viruses (b) previous history of lung disease (c) neoplastic conditions (d) pregnancy (e) age of <18 were excluded from the study. Thirty healthy volunteers constituted the control group age, sex, smoking habits, comorbidities, oxygen saturation (SpO2), laboratory data, chest CT findings, and follow up data were collected from patient files and electronic medical records (EMRs). Information regarding co-morbidities such as diabetes mellitus, cardiovascular disease, hypertension, chronic renal disease, chronic liver disease, were retrieved from EMRs or patient interviews.

One serum sample were screened one time for both controls and patients' group before admission. Serum samples were obtained from the antecubital vein of patients at the time of confirmation of COVID-19 diagnosis before the commencement of any medications. Collected samples were centrifuged at 3000 rpm for 10 minutes and stored at −80°C until the time of analyze. Serum KL-6, TGF-β1, and FGF-2 levels were investigated by using commercial Enzyme-linked immunosorbent assay (ELISA) test kits (Bioassay Technology, Shanghai).

Patients were undergoing screening with high-resolution chest CT which were performed with 192-channel CT scanner (Somatom Force; Siemens Healthineers; Germany) in the supine position with breath-holding at the time of diagnosis and on follow up as per indication. Scanning parameters were as follows: slice thickness in range 1 and 5 mm, peak kilovoltage of 120 to 140 in range, and a minimum tube current of 45 mA. Images were transferred to a dedicated workstation (syngo.via VB10, Siemens, Erlangen, Germany) and examined by an experienced pulmonary radiologist (G.E) for the distribution, number and extent of lesions, the percentage of involved parenchymal area, ground-glass opacities, reticulation, traction bronchiectasis, and honeycombing.

The study was approved by the Local Ethics Committee (Approval 2020-05-07T21_15_36) and written informed consent was obtained from the participants at inclusion.

Statistical Analysis

For statistical analyses, NCSS (Number Cruncher Statistical System) (Kaysville, Utah, USA) software was used. In the evaluation of the data, descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used. In the comparison of quantitative data, for normally distributed parameters, Student's *t*-test, and for not normally distributed parameters Mann-Whitney *U* test was utilized. In the evaluation of the relationship between variables, Spearman's correlation analysis was used. For the comparison of qualitative data,

Table 1. Demographic and clinical features of COVID-19 patients and healthy control subjects.

	COVID-19 PATIENTS (N = 59)	HEALTHY CONTROLS (N = 30)	*P*
Age, year (mean ± SD)	59.8 ± 13.5	54.3 ± 13.9	.080
Gender, male, n (%)	32 (54.2)	16 (53.3)	.936
Smoking, (ever), n (%)	8 (13.6)	11 (36.7)	.012
Comorbidities			
Hypertension, n (%)	23 39	10 (33.3)	.602
Diabetes mellitus, n (%)	16 (27.1)	5 (16.7)	.272
Cardiovascular diseases, n (%)	9 (15.5)	2 (6.7)	.319
Chronic kidney disease, n (%)	3 (5.1)	2 (6.7)	1.000
Cancer, n (%)	5 (8.5)	1(3.3)	.659
Number of co-morbidities, n (%)			
1	17 (53.1)	16 (84.2)	.043
2	13 (40.6)	2 (10.5)	
⩾3	2 (6.3)	1 (5.3)	
KL-6, ng/mL (median, IQR)	11.54 (4.86)	8.54 (3.98)	.001
FGF-2, pg/mL (median, IQR)	76.84 (98.2)	101.62 (210.6)	.013
TGF-β1, pg/mL (median, IQR)	291.2 (359.2)	291.2 (919.5)	.189

Abbreviations: FGF-2, fibroblast growth factor-2; IQR, inter quartile range; KL-6, Krebs von den Lungen-6; SD, standard deviation; TGF-β, transforming growth factor beta.

Pearson's chi-square, Fisher-Freeman-Halton, and Fisher's Exact tests were used. In multivariate evaluations, logistic regression analysis was employed using the forced entry method with including age, sex, and parameters with a *P*-value of <.01 in univariate analyzes.

Results

Study comprised 31 severe, 28 non-severe COVID-19 patients and 30 healthy control subjects. There was no significant difference between groups with respect to age and sex ($P > .05$) (Table 1). In the patient group, (n = 13) 40.6 % had at least two co-morbid condition was higher compared to control group (n = 2) 10.5%. There was no significant difference between groups for the frequency of hypertension, diabetes mellitus, cardiovascular diseases, chronic renal failure and cancer. KL-6 values were found to be significantly higher in COVID-19 patients than control group (median [IQR]; 11.54 [4.86] vs 8.54 [3.98], $P = .001$) whereas, FGF-2 values were found to be lower compared to healthy controls (median; IQR 76.84 [98.2] vs 101.62 [210.6], $P = .013$). TGF-β1 values were found similar between patients and healthy controls.

We did not find an association between the severity of disease and age, sex, smoking history and presence of comorbidities (Table 2). Radiographic extent of lung involvement is more prominent in severe patients on chest CT. In severe patients LDH, CRP, procalcitonin, fibrinogen, and ferritin values were found to be significantly higher than those with non-severe disease (Table 3, $P < 0.01$). However, there were no difference between severity groups for the serum levels of KL-6, FGF-2, and TGF-β1 (Table 3). In severe group 19 of 31 patients (61.3%) required ICU admission, 6 intubated (19.4%), and 4 of them eventually died (13.3%) despite the employment of corticosteroids and tocilizumab, whereas in non-severe group a single patient needed ICU care and deceased afterward (Table 4).

Serum KL-6 levels correlated with CRP (r = .362; $P = .006$), fibrinogen (r = .291; $P = .028$), D-dimer (r = .403; $P = .002$), and lymphocyte counts (r = −.305; $P = .021$, Table 5). However, no significant correlation was found between KL-6 measurements and WBC, platelet, ALT, AST, LDH, creatine, troponin, ferritin, procalcitonin, SPO_2, duration of hospitalization, and radiographic severity of disease. FGF-2 and TGF-β1 not correlated with the any of the prognostic parameters. No significant association was found between the KL-6, TGF-β1, and FGF-2 serum levels and final outcome of COVID-19 patients.

Discussion

Early diagnosis of severe disease is crucial for controlling the disease and improving the prognosis of patients with limited medical resources. COVID-19 infection is associated with a potential lung fibrotic process caused by alveolar damage. KL-6, TGF-β1, and FGF-2 are fibrotic markers that involved in lung diseases with alveolar parenchymal damage and

Table 2. Demographic and clinical features of severe and non-severe COVID-19 patient groups.

	SEVERE GROUP (N=31)	NON-SEVERE GROUP (N=28)	*P*
Age, (mean ± SD)	59.7 ± 14.7	59.8 ± 12.2	.96
Gender, male, n (%)	17 (54.8)	15 (53.6)	.92
Smoking (ever), n (%)	5 (16.1)	3 (10.7)	.71
Number of co-morbidities, n (%)			
1	8 (50.0)	9 (56.3)	.309
2	8 (50.0)	5 (31.3)	
⩾3	0 (0)	2 (12.5)	
Duration of symptoms, days, mean (min-max)	5.16 (1-10)	5.54 (0-15)	.401
Abnormal chest X-ray	29 (93.5)	19 (67.9)	.011
Computed tomography findings			
Bilateral lesions, n (%)	28 (96.6)	23 (88.5)	.74
Multifocal lesions, n (%)	27 (93.1)	23 (88.5)	.66
Extent of lesions involving >%50 of the lung area, n (%)	25 (96.2)	16 (55.2)	.001

Abbreviations: SD, standard deviation.
The data are presented as median unless indicated.

fibrosis. Therefore, the use of these biomarkers may offer an opportunity for the early intervention to predict severe respiratory disease.

There are previous studies on the relation between the severity of lung damage and serum KL-6 however, to our knowledge, this is the first study investigating three fibrotic markers in combination and their correlation with the degree of involvement detected by CT. In the present study, the association between serum KL-6 levels and COVID-19 pneumonia has been corroborated. This relation has also been supported by the degree of involvement in CT, the need for mechanical ventilation, and other infection severity indicators.

KL-6 is MUC-1 mucin found in regenerative type II alveolar epithelial cells and is regarded as a biological marker of pulmonary epithelial injury.[12] Pulmonary infections might induce proliferation of type II alveolar epithelial cells and production of KL-6 which enters blood circulation due to increased vascular permeability. In 2004, Sato et al[13] reported elevated levels of KL-6 in ARDS and its association with oxygenation and survival. Subsequently, increased serum KL-6 levels reported in interstitial lung disease (ILD) patients and its association with mortality.[14] Similarly, COVID-19 pneumonia might induce proliferation of type II alveolar epithelial cells and production of KL-6.[15] In the present study, serum KL-6 levels were significantly higher than healthy control subjects. Recently, Frix et al[16] showed raised serum KL-6 levels in COVID-19 patients compared to healthy subjects, but not as much of ILD patients. However, they did not find an association between KL-6, dyspnea, ICU admission and conventional inflammatory markers such as CRP.[16] Yamaya et al[17] reported correlation with severity of pneumonia and serum KL-6 levels with a favorable sensitivity and specificity. Additionally, Xue et al[11] proposed in their study that measurement of KL-6 on admission might predict development of pulmonary fibrosis in the follow up of COVID-19 patients. On the other hand, a similar study from United Kingdom did not confirm utility of KL-6 measurement on admission for subsequent severity, ICU need and long term clinically significant dyspnea.[18] In our study, although we found elevated levels in severe patients compared to non-severe subjects it did not reach statistical significance. We did not find an association between mortality and long-term pulmonary complications probably small number of these outcomes.

TGF-β1 is a member of a larger family of polypeptide growth factors that have key functions in growth, development, and tissue remodeling. Recent evidence has indicated that TGF-β1 is an isoform of TGF-β1, which induces connective tissue synthesis and supports fibroblast proliferation in an autocrine or paracrine manner in various cell types and disease models.[19] TGF-β1 is probably the strongest profibrotic mediator and plays a critical part in the activation of fibroblasts. TGF -β1 is remarkably expressed in epithelial cells of patients with idiopathic pulmonary fibrosis or lung tissues of animals with sustained pulmonary fibrosis.[20] Yet, in the present study, no significant difference was found between groups with respect to the TGF-β1 level.

Fibroblasts are located in the alveolar interstitium. Following alveolar injury, fibroblast migration to the site of the injury is stimulated by fibroblast growth factor (FGF).[21] TGF-β promotes

Table 3. Laboratory features of severe and non-severe COVID-19 patient groups.

	SEVERE GROUP (N=31)	NON-SEVERE GROUP (N=28)	*P*
WBC (×10³/mm³; median, Q25-Q75)	6.0 (4.2-9.5)	5.3 (4.9-7.3)	.554
Lymphocytes (×10³/mm³; median, Q25-Q75)	0.9 (0.6-1.3)	1.2 (0.8-1.6)	.133
Hemoglobin (g/dL; median, Q25-Q75)	12.4 (11.1-13.7)	12.8 (11.5-13.7)	.350
Platelets (×10³/mm³; median, Q25-Q75)	188 (148-256)	196.5 (151-252)	.665
AST (U/L; median, Q25-Q75)	39 (26-63)	28 (23-39)	.050
ALT (U/L; median, Q25-Q75)	27 (18-46)	28 (21-41.5)	.933
Creatinine (mg/dL; median, Q25-Q75)	0.8 (0.6-1.2)	0.9 (0.7-1.0)	.574
LDH (IU/mL;median, Q25-Q75)	426 (258-516)	252 (201-325)	.002
Troponin (ng/mL; median, Q25-Q75)	8 (5-23)	7.5 (5-12.5)	.506
D-dimer (ng/mL; median, Q25-Q75)	0.7 (0.5-1.4)	0.5 (0.4-1.0)	.058
CRP (mg/L; median, Q25-Q75)	99.2 (71.5-173)	29.2 (11.9-79.8)	.001
Procalcitonin (ng/mL; median, Q25-Q75)	0.1 (0.0-0.4)	0.0 (0.0-0.1)	.015
Fibrinogen, mg/dL (mean ± SD)	569.13 (±157.09)	451.61 (±122.61)	.002
Ferritin (µg/L; median, Q25-Q75)	426 (241-742)	198 (81-359)	.001
KL-6 (ng/mL; median, Q25-Q75)	12.3 (6.6)	11.2 (5.0)	.326
FGF-2 (pg/mL; median, Q25-Q75)	73.9 (71.5)	76.8 (102.4)	.287
TGF-β (pg/mL; median, Q25-Q75)	294.1 (367.8)	286.9 (325)	.538

Abbreviations: ALT, alanine amino transferase; aPTT, activated partial thromboplastin tim;, AST, aspartate amino transferase; BUN, blood urea nitrogen; CK, creatine kinase; CRP, C-reactive protein; FGF-2, fibroblast growth factor-2; IQR, inter quartile range; KL-6, Krebs von-6; LDH, lactate dehydrogenase; SD, standard deviation; TGF-β, transforming growth factor beta; WBC, white blood cells.
The data are presented as median, IOR unless indicated.

Table 4. Treatment characteristics and outcome of COVID-19 patients.

	SEVERE GROUP (N=31)	NON-SEVERE GROUP (N=28)	*P*
Glucocorticoid use	28 (90.3)	7 (25.0)	.001
Tocilizumab use	8 (25.8)	0	.005
LMWH use	31 (100)	26 (92.9)	.22
ICU admission, n (%)	19 (61.3)	1 (3.6)	.001
Intubation	6 (19.4)	1 (3.6)	.106
Death, n (%)	4 (13.3)	1 (3.6)	.354

Abbreviations: ICU, intensive care unit; LMWH, low molecular weight heparin.

migration, proliferation, activation and differentiation of fibroblasts into myofibroblasts and is the key player in nearly all fibrotic processes.[22] Previous studies reported that in both patients with IPF and pulmonary fibrosis model, in rats injected bleomycin, TGF-β1 and FGF-2 increased.[23,24] Hence, targeting these pathways with tyrosine kinase inhibitors have become a treatment strategy in idiopathic pulmonary fibrosis (IPF) and systemic sclerosis. Similarly, early use of these drugs recommended by some authors to halt progression to post-COVID-lung fibrosis in high-risk individuals.[25] Indeed, excessive expression of TGF-β has been demonstrated in the lung tissues of deceased COVID-19 patients.[26] TGF-b1 is not only profibrotic but also a pro-inflammatory cytokine and always releases in ARDS patients. A large number of papers declared increased levels of TGFb1 in both serum or BALF in either COVID-19 patients or experimental models of COVID.[27-30] However, in a small study consisting of 22 COVID-19 patients no difference was noted with healthy subjects for the serum levels of FGF-2 and TGF.[31] In our

Table 5. Correlations between investigated biomarkers and pertinent laboratory parameters, (n = 59).

PARAMETERS	KL-6		TGF-B		FGF-2	
	R	*P*	R	*P*	R	*P*
CRP	.362	**.006**	−.138	.267	−.105	.430
WBC	.132	.327	−.033	.803	.073	.582
Lymphocytes	−.305	**.021**	.032	.807	−.012	.928
Platelets	.247	.064	−.053	.688	.029	.828
ALT	−.062	.649	−.085	.521	−.091	.494
AST	.066	.626	−.092	.486	−.115	.385
LDH	.141	.294	−.253	.053	−.127	.336
Creatinine	.063	.641	−.071	.594	.003	.983
Troponin	.217	.108	.014	.916	.027	.840
Ferritin	.116	.393	−.083	.536	.018	.896
D−dimer	.403	**.002**	.116	.380	.072	.587
Fibrinogen	.291	**.028**	.017	.897	.019	.885
Procalcitonin	.049	.720	.027	.840	.030	.820
SPO2	−.187	.213	.106	.474	.092	.532
Duration of hospitalization	.129	.342	−.109	.415	−.130	.330

ALT, alanine amino transferase; AST, aspartate amino transferase; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; SPO_2, oxygen saturation; WBC, white blood cells.

study, we also did not find a significant increase in TGF-β and FGF-2 levels and even lower levels of the latter mediator in COVID-19 patients than healthy control. Colarusso et al[32] demonstrated significantly increased serum TGF-β values of patients who developed pulmonary fibrosis after the COVID-19 compared to healthy subjects. In the same study TGF-β values correlated with the severity of pulmonary disease. In the light of these observations, we suggest that major fibrotic mediators do not increase at the initial phase of COVID-19 but take place later, especially in patients who have prolonged disease or prominent lung damage.

Limitations

We have several limitations in our study. As mentioned, serial measurements of mediators in different stages of disease might better show their role in long term prognosis and give insights about timing of potential effective treatments. Second, although number of severe and non-severe patients balanced, inclusion of more patients in both groups might reduce risk of type 2 statistical error. There are several fibrosis mediators like interleukin-1 family cytokines which we did not evaluate them in our study. We did not take a follow up lung CT routinely for ethical reasons which might better show potential ongoing subclinical lung inflammation and fibrosis in the long term. Nevertheless, simultaneous assessment of the most intimate mediators of fibrosis and a reliable lung injury biomarker let us draw some suggestions for future studies.

Conclusion

In conclusion, in the present study, we confirmed that serum KL-6 levels increase at the initial stage of COVID-19 but not superior to clinical parameters to ascertain severity and outcome of disease. Primary fibrotic mediators did not seem to increase early in the disease course, but rather pulmonary involvement became critical and recovery is prolonged. Well-designed prospective longitudinal studies are needed to demonstrate at what stage of disease fibrosis mediators take place for the proper use of anti-fibrotic therapies.

Declarations

Ethics approval and consent to participate

This study was approved by Gazi University ethics committee, conducted in accordance with the 1975 Helsinki Declaration. Written informed consent was obtained from the participants.

Consent for publication

This study was approved by Gazi University ethics committee.

Author contributions

HK; Conceptualization, Investigation, Writing—original draft. AAG; Investigation, Visualization. HSÖ; Methodology, Investigation, Visualization. PAY; Methodology, Resources. GE; Methodology, Resources. GB; Investigation, Methodology, Resources. TDB; Investigation, Visualization. ÖG; Methodology, Investigation, Resources. DY; Methodology, Investigation, Formal analysis. HK; Conceptualization. MAÖ; Conceptualization, Supervision. AT; Conceptualization, Writing—original draft, Writing—review & editing.

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