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Serum homocysteine level in pediatric patients with COVID-19 and its correlation with the disease severity

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Abstract

Background: Thrombosis and embolism are possible complications in coronavirus disease 2019 (COVID)-19-positive pediatric patients. Although the risk is lesser in children than it is in adults, it does exist during acute infection and multi-inflammatory syndrome in children. Biomarkers such as D-dimer, prothrombin time, and fibrinogen degradation products are ineffective at detecting disease severity. Homocysteine (Hcy) is a prothrombotic factor that has been reported to be higher in adult COVID-19 patients, leading to speculation that it could be used as a biomarker for disease severity.

Purpose: To detect the correlation between serum total homocysteine (tHcy) level and the severity of COVID-19 in pediatrics.

Methods: A cross-sectional study was conducted on 40 children with COVID-19 and 40 healthy control subjects. Serum tHcy was measured by enzyme-linked immunosorbent assay and correlated with the clinical, laboratory, and radiological parameters of the patients.

Results: The median serum tHcy level in COVID-19 patients was 27.5 (interquartile range [IQR]: 23–31.75) µmol/L, while that in the controls was 1.8 (IQR: 1.6–1.875) µmol/L. There was a statistically significant increase in the tHcy level in cases compared to controls ($p < 0.001$). There was a statistically significant positive correlation between serum tHcy and D-dimer, ferritin, alanine transaminase, aspartate transaminase, blood urea nitrogen, and a highly significant positive correlation between tHcy and COVID-19 reporting and data system score, pediatric intensive care unit admission, and the disease severity classification.

Conclusion: Hcy could be a biomarker of importance in predicting the severity of COVID-19 in pediatrics.

KEYWORDS
biomarkers, COVID-19, homocysteine

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CO-RADS, COVID-19 reporting and data system; COVID-19, coronavirus disease 2019; FDPs, fibrinogen degradation products; Hcy, homocysteine; IQR, interquartile range; MTHFR, methylenetetrahydrofolate Reductase; PICU, pediatric intensive care unit; tHcy, total homocysteine.

1 | INTRODUCTION

To stratify high-risk patients of coronavirus disease 2019 (COVID-19), scientists urgently require accurate biomarkers related to COVID-19 illness progression. Vascular injury whether embolism, thrombosis, or infarction affects different organs such as the cardiovascular system, the lungs, and the brain causing significant morbidities and mortality in COVID-19 patients.[1] Importantly, the microvascular changes in COVID-19 are more pronounced than in H1N1-infected lungs, suggesting disease-specific effects not just a common phenomenon of acute respiratory distress syndrome or viral pneumonia. Venous thromboembolism occurs in up to 50% of COVID-19 autopsy series and the reported occurrence of deep-vein thrombosis points to embolic complications in addition to in situ microvascular immunothrombosis.[2] The available biomarkers asD-dimer, prothrombin time, and fibrinogen degradation products (FDPs) are not sensitive enough in detecting the severity of the disease.[1]

Homocysteine (Hcy) is derived from methionine which is an essential amino acid. It is a prothrombotic factor since it is involved in the generation of reactive oxygen species, which causes oxidative stress, endothelial disruption, and irreversible inactivation of protein C and thrombomodulin.[3] Several potential mechanisms were suggested by which Hcy would trigger severe COVID-19 disease or prevent its control.[4,5] High Hcy levels cause thrombus development by increasing inflammatory cytokines that are overproduced in cytokine release syndrome or "cytokine storm." They also cause limit nitric oxide generation and promote endothelial dysfunction. All of these pathologies are hallmarks of severe SARS-Cov2.[6] Additionally, Karst et al.[7] suggested the possible relationship between the hyperhomocysteinemia-causing effect of methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) and severity of COVID-19 disease. Both CT and TT genotypes of MTHFR single-nucleotide polymorphism are highest in Europe, with the highest prevalence in Italy (66.3%) which could explain the high mortality among the Italian COVID-19 patients.

Furthermore, the role of Hcy in different neuropsychiatric disorders like schizophrenia, Alzheimer's disease, dementia, anxiety, and depression[8] may explain the psychiatric symptoms associated with COVID-19.

The present study aims to assess the serum level of Hcy and to correlate it with clinical, laboratory, and radiological findings in COVID-19 pediatric patients, evaluating its potential role as a prognostic marker of COVID-19 infection outcomes.

2 | METHODOLOGY

A case-control study was conducted in the Children's Hospital, Ain Shams University, Cairo, Egypt. It included 40 cases (children with COVID-19 diagnosed with positive reverse transcription-polymerase chain reaction[RT-PCR] with clinical, laboratory, and radiological evidence of COVID-19 infection), and 40 controls (healthy pediatric subjects with COVID-19 negative RT-PCR and no clinical, laboratory, and radiological evidence of COVID-19 infection, age, and sex-matched with the cases).

The study was conducted following the ethical principles of the Local Ethics Committee, Children's Hospital, Ain Shams University.

Exclusion criteria:Patients with a previous history of cardiovascular diseases, thromboembolism, or hypertension.

All studied COVID-19 patients were subjected to:

- Full history taking (age, sex, symptoms, disease comorbidities, duration of symptoms, and infected contact).
- Clinical examination (vital data, anthropometric measurements, skin lesions, and chest examination), severity of COVID-19 infection were classified into mild, moderate, severe, and critically ill.[9]
- Laboratory investigations: Complete blood count was performed on the Sysmex XN-1000 (Sysmex Corporation),D-dimer was performed on the STA Compact Max (Stago), and C-reactive protein (CRP), lactate dehydrogenase, ferritin, liver, and renal function tests were performed on the automated Beckman Coulter AU480 analyzer (Beckman Coulter).
- Computerized tomography (CT) scan: The radiological severity was assessed by the COVID-19 reporting and data system (CO-RADS) classification which has grades from 1 to 6.[10]
- Measurement of total homocysteine (tHcy) level by quantitative Enzyme-Linked Immunosorbent Assay Kit supplied by (SunRed Biotechnology Laboratory) in the serum of patients and controls.[11] Each standard and sample was dispensed into a selected well coated with an Hcy antibody. Enzyme conjugate was added and incubated. After washing, chromogens A and B were added to each well and incubated. Stop Solution was applied to each well and the absorbance was determined at 450 nm by a spectrophotometer. A standard curve was constructed from which the concentration of tHcy in each sample was determined.

3 | RESULTS

This current study was conducted in the period from October 2020 to March 2021 on a total number of 80 subjects; 40 cases with COVID-19; and 40 age and sex-matched healthy subjects as a control group. Patients were 15 (37.5%) males and 25 (62.5%) females whose ages ranged from 2 months to 16 years with 5.425 ± 4.81 years as a mean ± SD value. Control subjects were 15 (37.5%) males and 25 (62.5%) females and their ages ranged from 2 months to 16 years with 6.78 ± 4.43 years as a mean ± SD value.

Only six (15%) patients had evidence of contact with COVID-19 positive patients. There were comorbidities in 15 (37.5%) patients. The comorbidities were: osteosarcoma, biliary atresia, nephrotic syndrome, polytrauma, systemic lupus erythematosus, malignancy, neurodegenerative disease, renal tubular acidosis, hemophagocytic lymphohistiocytosis (HLH), systemic juvenile idiopathic arthritis, and acute disseminated encephalomyelitis.

The mean duration of hospital admission was 12.51 ± 5.53 days, where 11(27.5%) patients were admitted to the pediatric intensive

care unit (PICU). The disease was mild in 13 (32.5%), moderate in 9 (22.5%), severe in 7 (17.5%), and critically ill in 11 (27.5%) patients.

As regards the symptoms of the studied patients; fever(100%), abdominal pain (45%), cough (37.5%), rash (35%), nausea and vomiting (32.5%), dyspnea (30%), runny nose (25%), fatigue (25%), diarrhea (22.5%), conjunctivitis (22.5%), sore throat (20%), muscle ache (17.5%), joint pain (12.5%), seizures (12.5%), headache (10%), and finally 2.5% of patient lost the ability to smell and taste.

General examination revealed that 22 (55%) of patients were toxic and 2 (5%) of them were in shock. Tachycardia for age was recorded in 21 cases (52.5%). Hypoxia was recorded in seven (17.5%) cases. Hypotension was recorded in nine cases (22.5%) and hypertension in two cases (5.0%). Three cases had cyanosis. There were no thromboembolic symptoms in any of our subjects.

According to the weight-for-age *z*-score, five (12.5%) cases were mildly underweight (−1 to −2) and five (12.5%) were moderately underweight (−2 to −3).

Laboratory tests results are demonstrated in Table 1.

According to CO-RADS classification, CT score was 1 in 13 (32.5%) patients, 3 in 1 (2.5%) patient, 5 in 1 (2.5%) patient, and it was 6 in 25 (62.5%) patients.

Echocardiographic (ECHO) findings, cardiomegaly was found in one patient (2.5%), carditis in six (15%), dilated cardiomyopathy in one patient (2.5%), dilated left ventricle in two (5%), aortic stenosis in one patient (2.5%), and mitral and aortic regurge in two patients (2.5%) (5%).

As regards serum Hcy, the COVID-19 group had statistically significantly higher levels (range: 7–64 µmol/L and median [interquartile range, IQR]: 27.5 [23–31.75] µmol/L) in comparison to the control group (range: 1.00– 2.20 µmol/L and median [IQR]: 1.8 [1.6–1.875] µmol/L) ($p < 0.001$) (Table 2).

Serum Hcy levels were significantly higher in PICU admitted COVID-19 cases compared to the ward admitted cases, also they were higher in cases withD-dimer ≥1 mcg/ml compared to cases withD-dimer lower than 1 mcg/ml, and serum Hcy concentration increased as the grade of severity of COVID-19 increased (Table 3).

There was a highly significant positive correlation between serum Hcy and CO-RADS score, and the disease severity classification, as well as a statistically significant positive correlation between it and D-dimer, ferritin, alanine transaminase (ALT), aspartate transaminase (AST), and blood urea nitrogen (BUN) (Table 4).

TABLE 1 Distribution of laboratory investigation among the studied children with COVID-19

	Mean	SD	Abnormalities	*N*	%
TLC ($\times 10^3$/µl)	11.91	6.81	Leucocytosis	14	35.5
			Leucopenia	3	7.5
Lymphocytes ($\times 10^3$/µl)	2.72	1.47	Lymphopenia	6	15
Neutrophils ($\times 10^3$/µl)	8.32	5.65	Neutrophilia	16	40
Hemoglobin (g/dl)	10.23	1.56	Anemia	36	90
			Polycythemia	1	2.5
Platelets ($\times 10^3$/µl)	318.00	158.16	Thrombocytosis	7	17.5
			Thrombocytopenia	3	7.5
ESR (mm/h)	58.00	36.27	Increased ESR	34	85
CRP (mg/L)	112.64	99.97	Increased CRP	34	85
LDH (IU/L)	371.33	145.85	Increased LDH	22	55
D-dimer (mcg/ml)	2.76	2.76	IncreasedD-dimer	30	75
Ferritin (µ/L)	1013.33	1081.87	Increased ferritin	33	82.5
PT (s)	13.11	2.57	Increased PT	2	5
PTT (s)	39.38	4.64	Increased PTT	2	5
INR	1.10	0.21	Increased INR	4	10
ALT (IU/L)	52.30	45.56	Increased ALT	20	50
AST (IU/L)	64.95	53.62	Increased AST	20	50
BUN (mg/dl)	19.70	5.18	Increased BUN	11	27.5
Creatinine (mg/dl)	0.50	0.21	Increased Creatinine	9	22.5

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PT, prothrombin time, PTT, partial thromboplastin time; TLC, total leukocytic count.

TABLE 2 Comparison between cases and controls as regards serum tHcy level

tHcy level	COVID-19 cases ($n = 40$)	Control group ($n = 40$)	Test of significance	p
Range	(7–64)	(1.00–2.20)	$U = 0.00$	<0.001*
Median (IQR)	27.5 (23–31.75)	1.8 (1.6–1.875)		

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; p, the p-value for comparing the two studied groups; tHcy, total homocysteine; U, Mann–Whitney test.

*Statistically significant at $p \leq 0.05$.

TABLE 3 Statistical comparison between serum total homocysteine (tHcy) level and other variables of studied children with COVID-19

	tHcy level Median (IQR)	Range	Test value	p	Significance
Age, y					
<1	27 (24.5–30.5)	11–56	0.065[a]	0.968	NS
1–5	27 (18–32)	10–52			
>5	28 (23–36)	7–64			
Sex					
Female	28 (23–31)	7–52	−0.644[b]	0.520	NS
Male	27 (23–52)	12–64			
Duration of admission, d					
<5	10 (10–10)	10–10	−1.562[b]	0.118	NS
≥5	28 (23–32)	7–64			
D-dimer, mcg/ml					
<1	21 (11–27)	7–28	−3.226[b]	0.001	HS
≥1	30 (24–41)	12–64			
Cardiac affection					
No	27 (21–31)	7–64	−1.047[b]	0.295	NS
Yes	28 (24–36)	21–52			
PICU admission					
No	26 (21–30)	7–56	−2.762[b]	0.006	HS
Yes	34 (28–52)	21–64			
Comorbidities					
No	28 (24–34)	7–56	−1.568[b]	0.117	NS
Yes	25 (21–30)	10–64			
Clinical severity					
Mild	19.5 (11–26)	7–28	20.682[a]	0.000	HS
Moderate	27 (25–30)	23–32			
Severe	31 (28–52)	24–56			
Critically ill	35 (28–52)	21–64			
Outcome					
Died ($n = 3$)	30 (21–31)	21–31	−0.129[b]	0.898	NS
Improved ($n = 37$)	27 (23–32)	7–64			

Note: $p > 0.05$: nonsignificant (NS); $p < 0.05$: significant; and $p < 0.01$: highly significant (HS).

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; PICU, pediatric intensive care unit.

[a]Kruskal–Wallis test.

[b]Mann–Whitney test.

TABLE 4 Correlation between serum levels of total homocysteine (tHcy) with other studied parameters

Studied parameters	Serum levels of tHcy Spearman's correlation	*p*
Oxygen saturation (%) (room air)	−0.223	0.167
Blood systolic pressure (mmHg)	−0.300	0.060
Blood diastolic pressure (mmHg)	−0.123	0.448
Lymphocytes (×10^3/μl)	−0.029	0.861
Neutrophils (×10^3/μl)	0.077	0.638
Platelets (×10^3/μl)	−0.123	0.448
CRP (mg/dl)	0.202	0.211
ESR (mm/h)	0.057	0.726
LDH (IU/L)	0.206	0.201
D-dimer (mcg/ml)	0.385	0.014*
Ferritin (μg/L)	0.340	0.032*
PT (s)	0.018	0.911
PTT (s)	0.216	0.180
INR	0.106	0.513
ALT (IU/L)	0.357	0.024*
AST (IU/L)	0.339	0.033*
BUN (mg/dl)	0.391	0.013*
Creatinine (mg/dl)	0.219	0.174
CO-RADS score	0.649	0.000**
Disease severity classification	0.714	0.000**

Note: Spearman's rank correlation coefficient analysis, $p > 0.05$ insignificant.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PT, prothrombin time, PTT, partial thromboplastin time; TLC, total leukocytic count.

*$p \leq 0.05$ significant; **$p \leq 0.001$ highly significant.

Three of our patients died, two of them were females below 6 months of age and the other was male 14 years old. Two had comorbidities (osteosarcoma, and HLH), and one had evidence of carditis by ECHO. Two of them were admitted to PICU. All cases had CO-RAD 6 with ground-glass opacities and consolidation. Their serum Hcy ranged from 21 to 31 μmol/L, and there was no statistically significant difference between the level of Hcy in the dead and survived subjects with COVID-19 (Table 5).

4 | DISCUSSION

Since the outbreak of the COVID-19 pandemic, the medical and scientific communities have struggled to manage patients and develop accurate biomarkers connected to illness progression to quickly stratify patients for their risk of critical symptoms.[12] There are no clear prognostic criteria of thromboembolic illness other than D-dimer and FDPs. Previous research has linked elevated Hcy levels to thrombosis through a variety of pathways, including increased tissue factor expression, weakened anticoagulant processes, increased platelet reactivity, decreased fibrinolytic capability, and endothelial dysfunction.[13] Hcy levels are employed as thromboembolic risk predictors in cardiovascular patients; however, they have not yet been applied to COVID-19 risk categorization.

When comparing COVID-19 cases to healthy controls, the present study found a statistically significant increase in Hcy levels ($p < 0.001$). We also found a significant correlation between serum Hcy levels and illness severity and PICU admissions. Hcy has been suggested as a potential biomarker of COVID-19 infection severity in several studies performed on adults.[1,14] Hcy and troponin are predictors of severe COVID-19 disease progression ($p < 0.05$) according to Ponti et al.[1] In contrast, Kryukov et al.[15] reported that there was no statistically significant variation in Hcy levels between individuals with varying degrees of coronavirus infection severity.

The study by Ponti et al.[5] COVID-19 patients reported that plasma Hcy levels were significantly higher in non-survivors in comparison to survivors showing a strong correlation between MTHFR 677 T allele, elevated Hcy, and mortality in COVID-19. However, in our study, there was no statistically significant difference between survivors and non-survivors ($p > 0.05$). The small number of nonsurvivors in the current study, as well as the existence of additional significant comorbidities, may explain this.

D-dimer could predict mortality in COVID-19 patients, especially in levels above 2 μg/ml (fourfold increase).[16,17] D-dimer levels were elevated in 30 (75%) of the cases in this study, which is consistent with Huang et al.'s[18] finding that D-dimer values were nearly fivefold higher in those with severe disease than in those with mild disease ($p = 0.004$). In COVID-19 patients, we found a positive correlation between serum Hcy and D-dimer levels. Furthermore, compared to patients with D-dimer levels below 1 μg/ml, there was a statistically significant rise in the level of Hcy in the group of patients with D-dimer levels greater than 1 μg/ml.

CT abnormalities were seen in 63% of cases in a review study by de Souza et al.[19] (on 1117 children), which is similar to our findings (67.5%). While Qi et al.[20] conducted a meta-analysis, they found ground-glass opacities of 32.9% of the cases (far lower than our study's 65%). The CO-RADS score was found to be effective in diagnosing COVID-19 patients, even those who had a negative RT-PCR.[21] The current study reported a link between serum Hcy levels and the chest CT grading measured by the CO-RADS score. The findings of Yang et al.'s[22] study on adult COVID-19 patients supported our findings, as it showed that Hcy levels were substantially linked with imaging progression on chest CT in COVID-19 patients ($p = 0.009$). Todua et al.[23] showed a strong association between serum Hcy, D-dimer, and CT pulmonary angiography in predicting pulmonary embolism, making Hcy a useful marker in COVID-19 diagnosis and prognosis.

In our study, ferritin, which is an acute-phase reactant, had a significant correlation with Hcy. Many research studies have shown

TABLE 5 Nonsurvivors with COVID-19 as regards serum tHcy level

tHcy level	Outcome Nonsurvivors No. = 3	Survivors No. = 37	Test value‡	*p*	Significance
Median (IQR)	30 (21–31)	27 (23–32)	−0.129	0.898	NS
Range	21–31	7–64			

Note: $p > 0.05$: nonsignificant (NS); $p < 0.05$: significant; $p < 0.01$: highly significant. ‡Mann–Whitney test.
Abbreviations: COVID-19, coronavirus disease 2019; IQR, Interquartile range; tHcy, total homocysteine.

that elevated ferritin is a predictor of poor prognosis in COVID-19 patients, such as Cheng et al.'s study,[24] which found a higher level of ferritin in patients with severe COVID-19, nonsurvivors, those with comorbidities, acute liver injury, COVID-19 patients admitted to intensive care units, and those who required mechanical ventilation.

Anemia was present in 90% of the patients in our study, and 25% of the patients were underweight. In a similar study, Kulkarni et al.[25] reported malnutrition and anemia in 23% of the COVID patients investigated. We did not find a significant correlation between Hcy and hemoglobin levels. Sirdah et al.,[26] on the other hand, found a link between homocysteine and iron deficiency anemia. We also did not find a significant correlation between body weight and Hcy. Comparably, Xiang et al.[27] found a negative association between being underweight and the risk of developing of hyperhomocysteinemia.

COVID-19 liver injury could be caused by direct viral invasion via angiotensin-converting enzyme 2 receptors in the liver, or it could be immune-mediated or drug-induced.[28] Previous studies found increased liver enzymes in 25% of children with COVID-19, which reached 50% in severe and critically ill individuals.[29] Omran et al.[30] reported that elevated liver enzymes are predictors of the severity of COVID-19. In the current study, ALT and AST had a positive correlation with Hcy levels, which is consistent with the findings of Lv et al.[31]

Although Hcy levels rise in patients with renal failure, we could not detect a link between Hcy and serum creatinine. However, there was a strong link between BUN and Hcy. BUN and D-dimer are also predictors of death in COVID-19 with a sensitivity (85%) and specificity (91%) according to Cheng et al.,[24] and the BUN/creatinine ratio is a prognostic factor of COVID-19 severity and mortality according to Ok et al.[32]

CRP was elevated in 85% of our cases, in contrast to the Qi et al.[20] study (14%). CRP and Hcy have a positive correlation, according to Berbert[4] but there was no significant correlation between CRP and Hcy in our study. Caldeira-Arajo et al.[33] reported that the level of Hcy in pediatric patients was altered by age; however, this was not confirmed in our study. And, despite the fact that the link between Hcy and cardiac affection is widely established,[34] we were unable to prove this in our research.

Hyperhomocysteinemia can be prevented by eating a diet rich in folate, vitamin B2, B6, B12, betaine, choline, limiting tea, coffee, high-protein meals, serine, and cystine. Kumrungsee et al.[35] found that Vitamin B6 plays a role in reducing the severity of COVID-19 by acting as an anti-inflammatory and antioxidant, particularly in the lungs, preventing pneumonia and diabetes in COVID-19 patients. If the level of Hcy is greater than 8 μmol/L, Karst et al.[7] recommended adding 5-methylenetetrahydrofolate, vitamin B2, B6, and B12 to the diet of COVID-19 patients. Dietary intervention to lower Hcy levels in the blood is a promising topic for future research to prevent COVID-19 thromboembolic consequences.[36]

The current study included patients with multiple comorbidities, hospitalized with COVID-19 infection. The results cannot, therefore, be generalized to all COVID-19 patients. If confirmed by larger studies, serum Hcy levels and MTHFR gene sequencing may become routine markers for clinical management of COVID-19 infection, as well as an important clinical target that should be normalized through vitamin and nutrient supplementation (folic acid and vitamin B12).

5 | CONCLUSION

A significantly higher level of serum Hcy in patients with COVID-19 may be used as a biomarker of disease severity being correlated with other inflammatory markers as D-dimer, ferritin, ALT, AST, BUN, CO-RAD score, PICU admission, and clinical severity grading. Larger studies investigating MTHER gene sequencing and the role of vitamins and nutrient supplementation may be a promising area of research for clinical severity assessment of COVID-19 infection.

AUTHOR CONTRIBUTIONS

Eman M. Fouda: Conceptualization (equal); methodology (equal); supervision (equal); and writing – review and editing (equal). **Nancy S. Wahba**: Investigation (equal); methodology (equal); and writing – review and editing (equal). **Asmaa I. M. Elsharawy**: Data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); software (equal); and writing – original draft (equal). **Sally R. Ishak**: Conceptualization (equal); data curation (equal); methodology (equal); writing – original draft (equal); and writing – review and editing (equal).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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