Microbes and Infectious Diseases 2022; 3(4): 814-829

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

sIL-2R and sIL-2R/lymphocyte ratio as indicators of severity in COVID-19 pediatric patients

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ARTICLE INFO

Article history:
Received 18 August 2022
Received in revised form 29 August 2022
Accepted 30 August 2022

Keywords:
sIL-2R/lymphocyte ratio
SARS-cov-2
Children
Clinical progression
Cytokines

ABSTRACT

Objectives: To determine the role of sIL-2R and sIL-2R/lymphocyte ratio as indicators of COVID-19 severity and predictors of clinical progression among children and adolescents. **Patients and Methods:** This observational cross-sectional study enrolled 76 pediatric patients [40 (52.6%) males and 36 (47.4%) females] with confirmed COVID-19. Patients were classified into two groups; mild to moderate and severe to critical according to WHO classification of severity and were assessed using COVID-19 severity assessment score and COVID-19 severity index. Soluble IL-2R (sIL-2R) concentrations were measured using a commercial enzyme-linked immunosorbent assay and sIL-2R/lymphocyte ratio was calculated for each patient. **Results**: Receiver-operating characteristic (ROC) curve analysis showed that sIL-2R has a significantly higher discriminative power between patients in both groups (AUC=0.955) as compared to sIL-2R/lymphocyte ratio (AUC=0.711) (*p* value<0.0001). At an associated criterion of >140 ng/l, the sensitivity and specificity of sIL-2R were 81.4.% and 100%, respectively. Soluble IL-2R also showed better performance in predicting the need for supplemental oxygen [threshold>140 ng/l, AUC=0.904 (0.814 to 0.960)], ICU admission [threshold>140 ng/l, AUC=0. 935 (0.854 to 0.979)], and mechanical ventilation [threshold>180 ng/l, AUC=0. 892 (0.799 to 0.951)]. **Conclusion**: Soluble IL-2R can play a potential role as a feasible indicator of COVID-19 severity in children and adolescents, thus informing healthcare providers to direct care to patients who may require intensive or critical care.

Introduction

The coronavirus disease-2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new family of coronavirus [1], resulted in a severe outbreak in China which has rapidly spread to the six continents [2]. The World Health Organization (WHO) has declared COVID-19 as a pandemic on March 11, 2020. Although most cases were mild to moderate, increasing COVID-19 cases led to a significant number of patients developing severe symptoms and death [3]. According to the WHO, as of September 2021, pediatric patients under five years of age represented 1.8 % of reported global cases and 0.1% of reported global deaths while those from 5 to 14 years accounted for 6.3 % and 0.1% of reported global cases and deaths respectively [4]. Unfortunately, 2022 witnessed a dramatic spike in pediatric COVID-19 cases amid the Omicron variant surge [5].

Many studies reported a relationship between serum inflammatory cytokine levels and

DOI: 10.21608/MID.2022.156894.1369
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prognosis of patients with COVID-19 [6–10]. The extremely high concentration of cytokines "cytokine storm" was recorded in plasma of severe cases of COVID 19 patients and was associated with disease severity [6,11]. However, available cytokine markers for predicting the progression of patients with COVID-19 are still limited especially in pediatric population.

Interleukin-2 (IL-2) is critical for the proliferation, differentiation, and function of different T-cell subsets.IL-2 receptor (IL-2R) is composed of 3 subunits: an alpha chain [IL-2Rα (CD 25)], a beta chain [IL-2Rβ (CD122)], and the common gamma chain [IL-2Rγc (CD132)] [12]. The β and γc subunits are important in signal transduction but together they form a low or intermediate affinity dimeric IL-2R. The role of the IL-2Rα is primarily to increase the affinity of the receptor to the cytokine rather than to enhance signal transduction as it has a short intracellular domain [13]. Upon immune activation, the α chain is enzymatically cleaved from the IL-2R and is released from the cell membrane into circulation in the form of soluble IL-2Rα (sIL-2R)[14]. Accordingly, level of sIL-2R has been widely studied as an indicator of immune activation in various pathological conditions[14–17]. Yet, its exact regulatory role in T lymphocyte activation remains controversial [12,13].

It was observed that pediatric research addressing the role of sIL-2R and sIL-2R/lymphocyte ratio in the pathogenesis and outcome of COVID-19 infection, are in paucity. In this study, we examined the role of sIL-2R and sIL-2R/lymphocyte ratio as indicators of COVID-19 severity and predictors of clinical progression among children, and adolescents, thereby guiding appropriate management, and reducing the incidence of complications.

Patients and methods

Study setting and design

This observational cross-sectional, single-center study enrolled 76 pediatric patients with confirmed COVID-19 admitted to Ain Shams University Pediatric Teaching Hospital, Cairo, Egypt, during the period from October 2020 to April 2021. The study was conducted according to the international guidelines of Strengthening the Reporting of Observational Studies in Epidemiology; STROBE [18].

Study population

We included all patients who met the following criteria:

- Confirmed SARS-CoV-2 infection: with at least one positive SARS-CoV-2 real-time reverse transcription polymerase chain reaction (RT–PCR) result for respiratory sample[19].
- Age from 2 months up to <18 years.

Exclusion criteria included patients with other clinical conditions known to be associated with elevated sIL-2R levels like lymphoma, autoimmune diseases, lymphoproliferative syndrome, and hemophagocytic syndromes [12,13].

Patients were classified into two groups:

- Group I: included patients with mild to moderate manifestations.
- Group II: included patients with severe to critical manifestations.

In order to assign patients to the appropriate group, we followed the WHO classification of COVID-19 disease severity [20] .

Ethical considerations

The study was reviewed and approved by the Research Ethical Committee, Faculty of Medicine, Ain Shams University, Children's Hospital, Cairo, Egypt (Approval number: 000017585). Informed consents were obtained from the patients' caregivers prior to their inclusion in the study which followed the ethical principles of Declaration of Helsinki developed by the World Medical Association [21].

Patients included in the study were managed according to the Egyptian national guidelines for clinical management and treatment of COVID-19 [22].

Data collection

We collected relevant clinical and demographic data (age, sex, residence, socioeconomic status using El-Gilani score [23], history of any chronic illnesses), history of exposure to confirmed COVID-19 patient, time of disease onset, duration of symptoms before presentation, presenting symptoms and signs suggestive of COVID-19 infection which included fever (temperature ≥38 °C), cough, dyspnea, bony aches, sore throat, loss of taste or smell, vomiting, diarrhea or abdominal pain, extreme fatigue and/or irritability [24,25].

During the course of hospital stay, the following data were recorded: the duration of hospital stay (defined as time between onset of admission till discharge), duration of illness (defined as time of

disease onset till outcome), lines of treatment, associated comorbidities, and the outcome data; either discharge, need for intensive care unit (ICU) admission, mechanical ventilation or death.

Radiologic investigations

Chest X-rays (CXR), and computed tomography (CT) of the chest were performed and the findings were independently interpreted by two experienced radiologists according to the radiological society of North America guidelines [26].

Routine laboratory tests for COVID-19 patients

The results of the following laboratory tests (performed for all COVID-19 patients as part of clinical assessment and management according to standard protocols [27,28] were collected from patients' records.

- Complete blood picture.
- International normalized ratio, full liver and renal function tests, cardiac enzymes and serum glucose levels.
- Inflammatory markers including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), serum ferritin, fibrinogen and D dimer.

Soluble IL-2R concentration and sIL-2R/lymphocyte ratio

Serum samples were collected from participants upon confirmation of COVID-19 diagnosis. Soluble IL-2R concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Bioassay Technology Laboratory, Shanghai, China) following the manufacturer's instructions [29]. The assay is a quantitative double antibody sandwich ELISA designed to measure human sIL-2R in serum, plasma, cell culture supernates, cell lysates and tissue homogenates. Optical densities at 450 were read within 10 min of adding the stop solution. The detection range is from (5ng/L-1000ng/L).

Accordingly, sIL-2R/lymphocyte ratio was calculated for each patient.

Severity assessment

All the study participants were assessed using the following severity scores:

- COVID-19 severity assessment score (COSA): This score predicts the likelihood of severe disease courses and adverse clinical outcomes for SARS-CoV-2 positive patients. It relies mainly on routine laboratory parameters (hemoglobin < 100 g/L, CRP > 25 mg/L, leucocyte counts > 10 G/L, glucose > 10 mmol/L, estimated glomerular filtration rate < 75 mL/min and sodium > 144 mmol/L), in addition to male sex as a categorial variable. The scoring system ranges from 0 to 10 with higher scores indicating higher risk for severe COVID-19 [30].
- COVID-19 severity index: This score assesses COVID 19 severity using a set of selected clinical and laboratory parameters. The variables included age, male sex, respiratory rate, oxygen saturation, heart failure, diabetes, systolic blood pressure, temperature, pulse rate, D dimer, dyspnea, lymphocytes, and platelets counts. Patients are divided into four risk categories based on their score; Low 0-2; Moderate 3-5; High 6-7; Critical 8 or more [31].

Sample size calculation

Using PASS11 program for sample size calculation, assuming the area under the ROC curve (AUC) of 0.70 for sIL-2R/lymphocyte ratio, for differentiation between 2 study groups (mild to moderate versus severe to critical), a sample size of at least 31 patients in each group, achieved a study power of 80 % to detect significance for the comparisons between both groups, with Alpha error at 0.05, Beta error of 0.2.

Statistical methods

Data were analyzed using IBM© SPSS© Statistics version 26 (IBM© Corp., Armonk, NY) and MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Non-normally distributed continuous variables are presented as median and interquartile range and differences are compared with the Mann-Whitney test (for two-group comparison) or the Jonckheere-Terpstra trend test (for multiple-group comparison). The Conover test was used for post hoc comparison if needed. The critical level of significance for post hoc comparisons is set at $p < 0.0083$ (Bonferroni method). Categorical variables are presented as counts and percentages and between-group differences are compared using the Pearson chi-squared test or Fisher's exact test. Ordinal data are compared with the chi-squared test for trend. Correlations between numerical variables are tested non-parametrically using the Spearman rank correlation (Spearman's rho). The correlation coefficient (Spearman's rho) is interpreted as

follows: <0.2 = very weak, 0.2 to 0.39 = weak, 0.4 to 0.59 = moderate, 0.6 to 0.79 = strong, ≥0.8 = very strong. Receiver-operating characteristic (ROC) curve analysis is used to examine the predictive value of the biomarkers. The AUC is interpreted as follows: AUC < 0.6 = fail, 0.6 to 0.69 = poor, 0.7 to 0.79 = fair, 0.8 to 0.89 = good, ≥ 0.9 = excellent. Multivariable stepwise binary logistic regression analysis was used to examine the predictors of severe to critical COVID-19. Inter-method agreement is examined using weighted Cohen's kappa (κ). Cohen's kappa is interpreted as follows: <0.2 = poor agreement, 0.21 to 0.40 = fair agreement, 0.41 to 0.6 = moderate agreement, 0.61 to 0.8 = good agreement, >0.8 = very good agreement. *p*-values <0.05 are considered statistically significant.

Results

During the study period, a total of 76 pediatric patients during the early phase of infection (i.e., <7 days after symptom onset) were eligible for inclusion. **Tables (1&2)** present the characteristics of the whole study population and the comparison between both study groups as regards all studied parameters.

Demographic and epidemiologic parameters

The patients' ages ranged from 2 months to 16 years with a median of 6 years. They were 40 (52.6%) males and 36 (47.4%) females of different disease severities, where 33 (43.4%) patients belonged to group I [mild (11 patients) to moderate cases (22 patients)] and 43 (56.6%) patients belonged to group II [severe (26 patients) to critical cases (17 patients)].

Most of the children in group I were in the scholar age group (60.6 %), while (51.2%) of group 2 patients were in the infantile and preschooler age groups (p=0.124). No statistically significant difference was found between both groups as regards demographic or epidemiologic data.

Clinical and laboratory parameters

The majority of the patients presented with fever (96.1%), while cough, dyspnea and lower respiratory symptoms were significantly reported among the patients of group II (p=0.022, p=0.012, p=0.011, respectively).

Among the laboratory parameters, we observed a significant difference in total leucocytic count between both groups (p=0.022). Regarding differential leucocytic count, lymphopenia was statistically significant among patients in group II (p=0.046).

Serum sIL-2R and sIL-2R/lymphocyte ratio as predictors of severity

Both sIL-2R and sIL-2R/lymphocyte ratio showed statistically significant higher levels in group II as compared to group I (p<0.001 and p=0.002, respectively) **(Table 1).**

As illustrated in both **figure (1)** and **table (3)**, ROC curve analysis showed that sIL-2R has a significantly higher discriminative power between patients in both groups (AUC=0.955) as compared to sIL-2R/ lymphocyte (AUC=0.711) (p value<0.0001). At an associated criterion of >140 ng/l, the sensitivity and specificity of sIL-2R were 81.4.% and 100%, respectively.

Soluble IL-2R also showed better performance in predicting the need for supplemental oxygen [threshold>140 ng/l, AUC=0.904 (0.814 to 0.960)], ICU admission [threshold>140 ng/l, AUC=0. 935 (0.854 to 0.979)], and mechanical ventilation [threshold>180 ng/l, AUC=0. 892 (0.799 to 0.951)]. In predicting mortality, both sIL-2R and its ratio to lymphocytes showed comparably poor performance [threshold>110 ng/l, AUC=0. 542 (0.424 to 0.657) versus threshold>31.81, AUC=0. 620 (0.501 to 0.729)] **(Figure 2).**

As mentioned previously, we relied on the WHO criteria for patients' classification who were also assessed using COSA and COVID-19 severity index. Both scores showed high statistically significant difference between both groups (p <0.001) **(Table 2).** However, we noted fair statistical agreement between COSA, COVD-19 severity index and WHO classification **(Table 5).**

Soluble IL-2R levels showed positive correlation with WHO classification of severity (r=0.893, p<0.001), COSA score (r=0.559, p<0.001) and COVID-19 severity index (r=0.428, p<0.001) **(Table 4)**

Table 1. Demographic, laboratory and general clinical characteristics of the study population

Variables	All Patients	Mild to moderate COVID-19 (No. = 33)	Severe to critical COVID-19 (No. = 43)	*p*-value*
Demographic and epidemiologic parameters				
Age (in years)	6 (2-10)	7 (3 – 10)	4 (1 – 8)	0.069
Sex				
Males	36 (47.4%)	13 (39.4%)	23 (53.5%)	0.223
Females	40 (52.6%)	20 (60.6%)	20 (46.5%)	
Residence				
Urban	53 (69.7%)	23 (69.7%)	30 (69.8%)	0.995
Rural	23 (30.3%)	10 (30.3%)	13 (30.2%)	
Socioeconomic level				
Low	25 (32.9%)	10 (30.3%)	15 (34.9%)	0.812†
Middle	48 (63.2%)	22 (66.7%)	26 (60.5%)	
High	3 (3.9%)	1 (3.0%)	2 (4.7%)	
Source of infection				
Community	60 (78.9%)	24 (72.7%)	36 (83.7%)	0.244
Healthcare associated	16 (21.1%)	9 (27.3%)	7 (16.3%)	
History of exposure to confirmed case	25 (32.9%)	13 (39.4%)	12 (27.9%)	0.291
Clinical parameters				
BMI kg/m2	15.8 (13.3-19.1)	16.6 (14.8 -19.1)	15.5 (12.5 -19.1)	0.405
Comorbid medical conditions	31 (40.8%)	14 (42.4%)	17 (39.5%)	0.799
URT symptoms	69 (90.8%)	28 (84.8%)	41 (95.3%)	0.229‡
Fever	73 (96.1%)	32 (97.0%)	41 (95.3%)	1.000‡
Cough	50 (65.8%)	17 (51.5%)	33 (76.7%)	**0.022**
LRT symptoms	51 (67.1%)	17(51.5%)	34 (79.1%)	**0.011**
Wheezes	44 (57.9%)	16 (48.5%)	28 (65.1%)	0.146
Dyspnea	53 (69.7%)	18 (54.5%)	35 (81.4%)	**0.012**
RD	52 (68.4%)	19 (57.6%)	33 (76.7%)	0.075
RD grade				
No RD	21 (27.6%)	13 (39.4%)	8 (18.6%)	**0.003†**
Mild RD	7 (9.2%)	6 (18.2%)	1 (2.3%)	
Moderate RD	40 (52.6%)	13 (39.4%)	27 (62.8%)	
Severe RD	8 (10.5%)	1 (3.0%)	7 (16.3%)	
RR (bpm)	30 (25-40)	30 (25-35)	35 (23-45)	0.490
GIT symptoms	41 (53.9%)	18 (54.5%)	23 (53.5%)	0.927
Laboratory parameters				
sIL-2R (U/ml)	140 (120-180)	110.0 (80.0-125.0)	165.0 (150.0-240.0)	**<0.001**
sIL-2R/lymphocyte ratio	63.0 (28.2-98.1)	35.5 (23.7-69.4)	82.1 (43.5-188.7)	**0.002**
WBC (k/mm^3)	10.7 (7.2-17.5)	14.4 (8.8 – 21.1)	9.6 (6.7-14.9)	**0.039**
Abnormal WBC	37 (48.7)	21 (63.6%)	16 (37.2%)	**0.022**
Leucopenia	7 (9.2%)	3 (9.1%)	4 (9.3%)	1.000‡
Leucocytosis	30 (39.5%)	18 (54.5%)	12 (27.9%)	**0.019**
Neutrophils (k/mm^3)	6.9 (4.2-12.3)	9.0 (5.3-12.5)	6.1 (3.0 -11.5)	0.081
Abnormal neutrophil count	36 (47.3)	23 (69.7%)	13 (30.2%)	**0.001**
Neutropenia	3 (3.9%)	3 (9.1%)	0 (0.0%)	0.078‡
Neutrophilia	33 (43.4%)	20 (60.6%)	13 (30.2%)	**0.008**
Lymphocytes (k/mm^3)	2.6 (1.4-4.6)	3.1 (1.4-5.7)	2.6 (1.6-4.5)	0.463
Abnormal lymphocytic count	38 (50%)	17 (51.5%)	21 (48.8%)	0.817
Lymphopenia	28 (36.8%)	8 (24.2%)	20 (46.5%)	**0.046**
Lymphocytosis	10 (13.2%)	9 (27.3%)	1 (2.3%)	**0.002‡**

Monocytes (k/mm^3)	0.4 (0.2-0.8)	0.5 (0.3-0.9)	0.4 (0.2-0.7)	0.069
Hb (g/dl)	10.1 (8.9-11.5)	10.0 (8.7-11.3)	10.7 (9.3-12.1)	0.337
Platelets (k/mm^3)	249 (164-354)	228 (132-327)	271 (176-358)	0.209
Albumin (g/dl)	3.2 (2.9-3.8)	3.1 (2.7-3.6)	3.3 (2.9-3.8)	0.280
AST (IU/l)	30 (22-50)	32 (23-77)	25 (21-44)	0.155
High AST	30 (39.5%)	14 (42.4%)	16 (37.2%)	0.645
ALT (IU/l)	18 (12-34)	19 (12-46)	16 (12-24)	0.164
High ALT	14 (18.4%)	8 (24.2%)	6 (14.0%)	0.251
Total bilirubin (mg/dl)	0.4 (0.2-0.5)	0.4 (0.2-0.6)	0.4 (0.2-0.5)	0.575
High total bilirubin	5 (6.6%)	3 (9.1%)	2 (4.7%)	0.647‡
CRP (mg/l)	45.9 (6.6-156.5)	47.9 (12.0-225.1)	37.9 (6.0-115.5)	0.103
High CRP	65 (85.5%)	29 (87.9%)	36 (83.7%)	0.747‡
ESR (mm/h)	0 (0-33)	0 (0-35)	0 (0-25)	0.577
D-Dimer (µg/ml FEU)	1.77 (0.70-3.56)	2.17 (0.74-3.70)	1.47 (0.62-3.41)	0.540
LDH (IU/l)	363 (294-493)	369 (306-494)	355 (285-474)	0.267
Ferritin (ng/ml)	391.1 (174.0-841.1)	396.0 (176.0-779.2)	386.1 (172-960)	0.564
Fibrinogen (g/l)	0 (0-0)	0 (0-0))	0 (0-0)	0.695
Total CK (IU/l)	23 (0-75)	24.0 (0.0-72.0)	21.0 (0-0.76)	0.584
Troponin (ng/ml)	0 (0-0)	0 (0-0)	0 (0-0.01)	0.852

Categorical variables are presented as counts and percentages. continuous variables are presented as median and interquartile range
*. Mann-Whitney test for continuous variables or Pearson chi-squared test for categorical variables unless otherwise indicated
†. Chi-squared test for trend
‡. Fisher's exact test
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; ESR. erythrocyte sedimentation rate; Hb, hemoglobin; LDH, lactate dehydrogenase; WBC, white blood cells

Table 2. COVID-19 severity, progression, and outcome of the whole study population.

Variables	**All Patients**	**Mild to moderate COVID-19 (No. = 33)**	**Severe to critical COVID-19 (No. = 43)**	***p*-value***
Severity scores				
COSA Score	5 (4-6)	4 (2-4)	5 (5-6)	**<0.001**
COSA Score interpretation				**<0.001†**
Low risk	19 (25.0%)	16 (48.5%)	3 (7%)	
Moderate risk	36 (47.4%)	17 (51.5%)	19 (44.2%)	
High risk	21 (27.6%)	0 (0%)	21 (48.8%)	
Very high risk	0 (0.0%)	0 (0%)	0 (0%)	
COVID-19 Severity Index	7 (5-11)	6 (4-7)	8 (6-13)	**<0.001**
COVID-19 Severity Index interpretation				**<0.001**
Low clinical risk	4 (5.3%)	3 (9.1%)	1 (2.3%)	
Moderate clinical risk	19 (25.0%)	13 (39.4%)	6 (14.0%)	
High clinical risk	21 (27.6%)	11 (33.3 %)	10 (23.3%)	
Very high clinical risk	32 (42.1%)	6 (18.2 %)	26 (60.5%)	
Progression and outcome				
ICU admission	44 (57.9%)	1 (3.0%)	43 (100%)	**<0.001**
Mechanical ventilation	10 (13.2%)	0 (0.0%)	10 (23.3%)	**0.004‡**
Supplemental O_2	41 (53.9%)	1 (3.0%)	40 (93.0%)	**<0.001**
Mortality	7 (9.2%)	1 (3.0%)	6 (14.0%)	0.131‡
Disease onset to outcome (days)	12 (9-16)	12 (9-16)	12 (9-17)	0.769
Admission to outcome (days)	10 (7-16)	10 (9-16)	10 (7-15)	0.748

Categorical variables are presented as counts and percentages. continuous variables are presented as median and interquartile range
*. Mann-Whitney test for continuous variables or Pearson chi-squared test for categorical variables unless otherwise indicated
†. Chi-squared test for trend
‡. Fisher's exact test
COSA, COVID-19 severity assessment score; ICU, intensive care unit

Table 3. Performance of sIL-2R or sIL-2R/lymphocyte ratio in discrimination between patients with mild to moderate COVID-19 (group I) and those with severe to critical COVID-19 (group II).

ROC curve parameters	**Markers**	
	sIL-2R	sIL-2R/lymphocyte ratio
AUC	0.955	0.711
Standard Error	0.019	0.059
95% Confidence interval	0.882 to 0.989	0.596 to 0.810
z statistic	23.493	3.570
Significance level P (Area=0.5)	<0.0001	0.0004
Youden index J	0.814	0.359
Associated criterion	>140	>37.5
Sensitivity (%)	81.4	81.4
Specificity (%)	100	54.6
ΔAUC	0.244	
SE	0.056	
95% CI	0.135 to 0.353	
Z	4.38	
P-value	**<0.0001**	

AUC, area under the ROC curve; CI, confidence interval; SE, standard Error; Youden index J = (Sensitivity + Specificity) – 1; Z = Z-statistic

Table 4. Correlations of sIL-2R and sIL-2R/lymphocyte ratio with demographic, clinical and other laboratory variables.

Variable		**Marker**	
		sIL-2R	sIL-2R/lymphocyte ratio
Age	Spearman's rho	-0.008	0.274*
	P-value	0.947	0.017
BMI	Spearman's rho	0.034	0.117
	P-value	0.774	0.316
Onset to outcome time	Spearman's rho	0.046	0.086
	P-value	0.690	0.460
Admission to outcome time	Spearman's rho	-0.048	0.069
	P-value	0.681	0.555
WHO classification	Spearman's rho	0.893**	0.494**
	P-value	<0.001	<0.001
COSA score	Spearman's rho	0.559**	0.271*
	P-value	<0.001	0.018
COSA risk category	Spearman's rho	0.519**	0.283*

	P-value	<0.001	0.013
COVID-19 Severity Index	Spearman's rho	0.428**	0.233*
	P-value	<0.001	0.043
COVID-19 Severity Index risk category	Spearman's rho	0.387**	0.218
	P-value	0.001	0.059
Respiratory distress grade	Spearman's rho	0.164	-0.041
	P-value	0.158	0.722
Respiratory rate	Spearman's rho	-0.076	-0.147
	P-value	0.514	0.204
SpO2	Spearman's rho	-0.717**	-0.358**
	P-value	<0.001	0.001
WBC	Spearman's rho	-0.221	-0.504**
	P-value	0.056	<0.001
Neutrophils	Spearman's rho	-0.122	-0.200
	P-value	0.295	0.084
Lymphocytes	Spearman's rho	-0.166	-0.799**
	P-value	0.151	<0.001
Monocytes	Spearman's rho	-0.236*	-0.479**
	P-value	0.040	<0.001
Hb	Spearman's rho	0.037	0.039
	P-value	0.748	0.741
Platelets	Spearman's rho	0.113	-0.213
	P-value	0.331	0.064
AST	Spearman's rho	-0.127	0.024
	P-value	0.274	0.836
ALT	Spearman's rho	-0.113	0.083
	P-value	0.333	0.476
Total bilirubin	Spearman's rho	0.080	0.131
	P-value	0.494	0.258
Albumin	Spearman's rho	0.167	-0.046
	P-value	0.148	0.691
CRP	Spearman's rho	-0.030	0.113
	P-value	0.795	0.329
ESR	Spearman's rho	0.011	0.052
	P-value	0.926	0.655
D-Dimer	Spearman's rho	0.043	0.160

	P-value	0.710	0.167
LDH	Spearman's rho	-0.168	-0.193
	P-value	0.148	0.095
Ferritin	Spearman's rho	-0.016	0.117
	P-value	0.892	0.315
Fibrinogen	Spearman's rho	-0.092	-0.116
	P-value	0.431	0.320
Total CK	Spearman's rho	-0.006	0.076
	P-value	0.961	0.516
Troponin	Spearman's rho	0.009	0.186
	P-value	0.936	0.107

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK, creatine kinase; COSA, COVID-19 severity assessment score; CRP, C-reactive protein; ESR. erythrocyte sedimentation rate; Hb, hemoglobin; LDH, lactate dehydrogenase; WBC, white blood cells; WHO, World Health Organization

Table 5. Inter method agreement between WHO classification, COSA classification and COVID-19 Severity Index.

Agreement between WHO classification and COSA classification					
	WHO classification				
COSA classification	**Mild**	**Moderate**	**Severe**	**Critical**	**Total**
Low risk for severe COVID-19	7	9	3	0	19 (25.0%)
Moderate risk for severe COVID-19	4	13	13	6	36 (47.4%)
High risk for severe COVID-19	0	0	10	11	21 (27.6%)
Very high risk for severe COVID-19	0	0	0	0	0 (0.0%)
Total	11 (14.5%)	22 (28.9%)	26 (34.2%)	17 (22.4%)	76
Agreement statistics					
Weighted Kappa					0.33
Standard error					0.06
95% CI					0.22 to 0.44
Agreement between WHO classification and COVID-19 severity index					
	WHO classification				
COVID-19 severity index	Mild	Moderate	Severe	Critical	Total
Low clinical risk	2	1	1	0	4 (5.3%)
Moderate clinical risk	2	11	6	0	19 (25.0%)
High clinical risk	6	5	6	4	21 (27.6%)

Very high clinical risk	1	5	13	13	32 (42.1%)
Total	11 (14.50%)	22 (28.90%)	26 (34.20%)	17 (22.40%)	76
Agreement statistics					
Weighted Kappa					0.31
Standard error					0.07
95% CI					0.17 to 0.46
Agreement between COSA classification and COVID-19 severity index					
	COSA classification				
COVID-19 severity index	Low risk for severe COVID-19	Moderate risk for severe COVID-19	High risk for severe COVID-19	Very high risk for severe COVID-19	Total
Low clinical risk	2	2	0	0	4 (5.3%)
Moderate clinical risk	9	10	0	0	19 (25.0%)
High clinical risk	5	13	3	0	21 (27.6%)
Very high clinical risk	3	11	18	0	32 (42.1%)
Total	19 (25.0%)	36 (47.4%)	21 (27.6%)	0 (0.0%)	76
Agreement statistics					
Weighted Kappa					0.31
Standard error					0.07
95% CI					0.17 to 0.46
CI, confidence interval; COSA, COVID-19 severity assessment score; WHO, World Health Organization					

Figure 1. ROC curve illustrating performance of sIL-2R (AUC=0.955), lymphocyte count (AUC=0.549), and sIL-2R/lymphocyte ratio (AUC=0.711) in discrimination between patients with severe to critical and mild to moderate COVID-19.

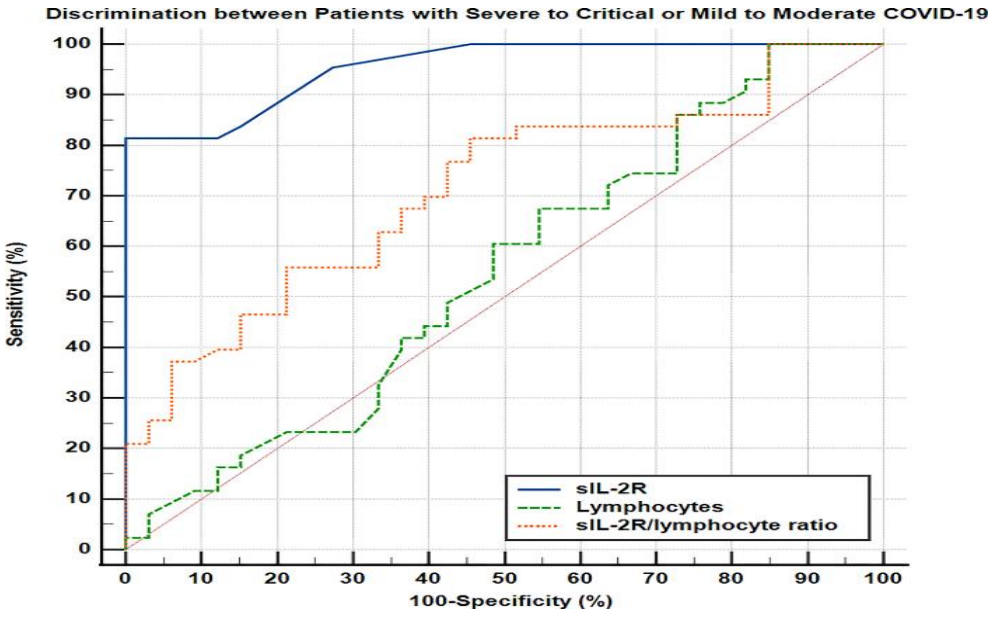
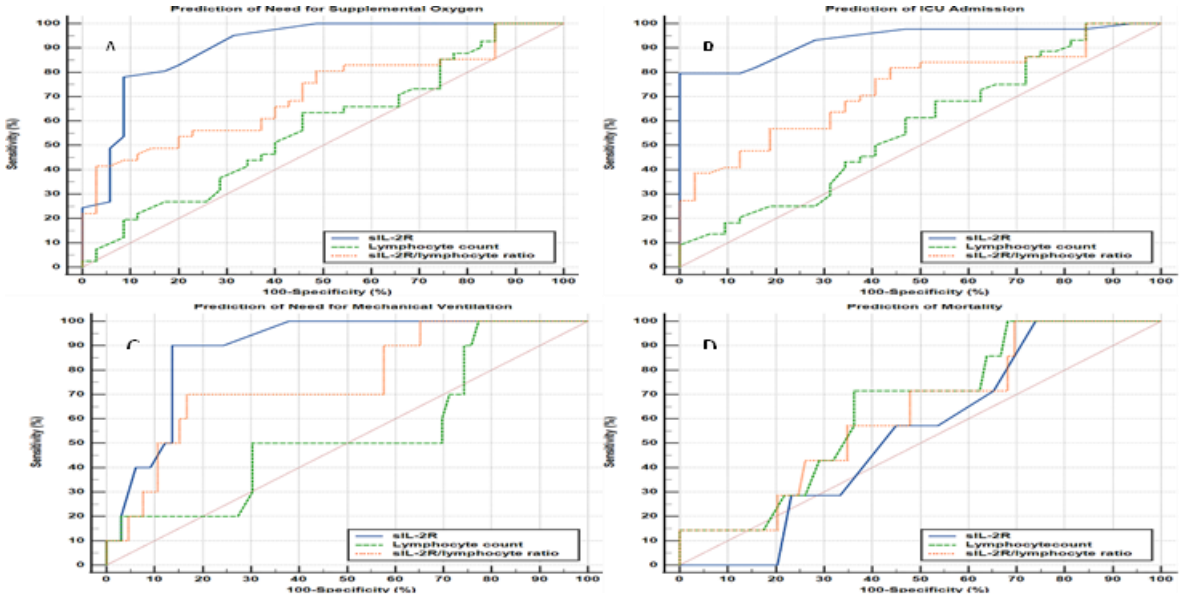


Figure 2. ROC curves illustrating performance of sIL-2R, lymphocyte count, and sIL-2R/lymphocyte ratio in predicting clinical outcome in COVID-19 pediatric patients
A) Need for supplemental oxygen (sIL-2R: AUC=0.904, lymphocyte count: AUC=0.572, and sIL-2R/lymphocyte: AUC=0.709)
B) ICU admission (sIL-2R: AUC=0.935, lymphocyte count: AUC=0.576, and sIL-2R/lymphocyte: AUC=0.733)
C) Need for mechanical ventilation (sIL-2R: AUC=0.892, lymphocyte count: AUC=0.542, and sIL-2R/lymphocyte: AUC=0.755)
D) Mortality (sIL-2R: AUC=0.542, lymphocyte count: AUC=0.646, and sIL-2R/lymphocyte: AUC=0.620).



Discussion

This study was carried out during the first year of COVID-19, in a tertiary teaching hospital in Cairo, Egypt; a lower middle-income country in the Eastern Mediterranean region. During this period, the Egyptian authorities adopted a policy of in hospital isolation and treatment of all COVID-19 confirmed patients-even those classified as mild cases. This explains that 14.5% and 28.9% of the patients enrolled in this study suffered mild or moderate symptoms, respectively. The severe and critical cases together accounted for 56.6% of cases. This could be attributed to the fact that 40.8% of the patients had comorbid illnesses and a considerably high percentage (21%) of them acquired the infection during their hospitalization for other medical conditions.

A plethora of articles addressed the need for a laboratory biomarker that can determine and/or predict the severity and outcome of COVID-19 patients. Many proinflammatory and inflammatory markers, coagulation and biochemical parameters were investigated in literature with sometimes contradicting results [32–36]. **Gatselis et al.** [37] proposed utilizing sIL-2R as a more specific marker of disease severity and predictor of mortality considering its established role in other diseases characterized by immune dysregulation [12,15,38]. In the aforementioned study, sIL-2R levels were significantly higher in adult patients with severe COVID-19, compared with those with moderate disease. It was also found to be the strongest laboratory predictive factor for mechanical intubation and death [37]. **Hou et al.** [39] observed that the ratio of sIL-2R to lymphocytes surpassed CRP and ferritin in a multivariate log regression analysis in discriminating critical from both mild and severe illnesses in adult COVID-19 patients in China.

In our work, which included pediatric patients, despite that both sIL-2R and sIL-2R/ lymphocyte ratio were highly significant in severe and critically ill patients as compared to mild and moderate patients, sIL-2R has a significantly higher discriminative power between patients in both groups as compared to its ratio to lymphocytes. It also showed better performance in predicting the clinical progression including the need for supplemental oxygen, ICU admission and mechanical ventilation. It also showed strong correlation with WHO classification of severity, COSA score and COVID-19 severity index. In another study, sIL-2R corelated positively with the

severity of COVID-19 pneumonia and patient mortality but not mechanical ventilation[40]. Similar finding was reported by **Liu et al.** [33], where disease severity and in-hospital mortality were associated with elevated sIL-2R levels. In the current study, sIL-2R, lymphocytic count and sIL-2R /lymphocyte ratio all showed poor performance in predicting mortality. In this concern, it is worth noting that there was in fact no statistically significant difference between both groups of study participants as regards mortality and one of the patients in the first group died of preexisting medical condition.

We should also be aware of the variabilities among studies in classifying patients according to their outcome and disease severity especially in the pediatric age group [41]. In this study we initially applied the WHO classification of COVID-19 disease severity [20] to classify the patients. And we also assessed the severity using COSA and COVID-19 severity index. Despite that they were both able to discriminate between both groups, yet there was only statistically fair agreement between each score and the other. Our finding thus highlights an urgent need for developing a unified assessment score for this age group. For instance, in a systematic review and meta-analysis performed by **Shi et al.** [41], they questioned the value of recognizing male sex (a parameter in both COSA and COVID-19 severity index) as a risk factor for poor prognosis in COVID-19 considering that boys have an established higher prevalence of childhood diseases as compared to girls. In this study, though male patients suffered more severe or critical illness than females, yet it didn't reach statistical significance. Other studies showed contradicting results in this context [42–45]

According to accumulating data from literature, lymphopenia is characteristic in patients with severe and critical COVID-19 despite of the high levels of IL-2 levels (as part of the "cytokine storm") in both adults and pediatric patients [33,35,46–48]. In this study, and in line with previously published data [7,39,49], we observed negative correlation between sIL-2R levels and lymphocytic count. Despite this negative correlation is non-significant, it might support the assumption that sIL-2 may have a role in the mechanisms of lymphopenia in COVID-19 by acting as a decoy and inhibiting IL-2 signaling and hence its proliferative function on T lymphocytes [50]. However, this point needs further elucidation.

This study is limited by the small sample size which did not allow for investigating the role of underlying comorbidities. Despite all patients were included during the early phase of infection, it was not applicable to measure sIL-2R at the same date from the onset of infection for all patients. Also, sequential measurement of sIL-2R and lymphocytic count during the course of illness would have provided better insight on their role in the pathogenesis of the disease.

Conclusion

Soluble IL-2R can play a potential role as a feasible indicator of COVID-19 severity among children and adolescents, thus informing clinical providers to direct care to patients who may require intensive or critical care. The role of sIL-2R in lymphopenia in COVID-19 patients still needs further investigations.

Conflict of interest : None.

Funding : None.

Acknowledgement

The authors deeply appreciate the help of all study participants, their guardians and ASUH administration to complete this work.

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