Early risk factors for re-detectable positive in the recovered COVID-19 children

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Abstract

Background and Objective: Compared with adult patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), children have a higher proportion of re-detectable positive (RP) in the recovery period.The underlying risk factors remain unknown.We aimed to identify the early risk factors for RP, and to provide a basis for early clinical prediction and risk stratification. Methods: A retrospective analysis was performed on all pediatric cases diagnosed with coronavirus disease 2019 (COVID-19). Results: 14 of 38 (36.8%) pediatric patients were RP.Children have a significantly higher percentage of RP (OR[95%CI] 4.84[2.21-10.59];P=.000).Compared with control group (n=24),RP group (n=14) had more family cluster infections (1.59[1.1-2.3];P=.030),while age ([7.2±4.8] vs [7.6±5.1]),and percentage of male gender (35.7% vs 45.8%), fever (21.4% vs 45.8%),respiratory or digestive symptoms (71.4% vs 50%), asymptom (28.6% vs 33.3%), computed tomography positive findings (85.7% vs 83.3%) and co-infection (7.1% vs 8.3%) were statistically nonsignificant.The laboratory data of RP group had a relatively higher white blood cell count (WBC) (7.5[5.1-9.8] vs 4.8[4.4-7.5];P=.009) and longer plasma prothrombin time (PT) ([12.6±0.7] vs [12.1±0.5];P=.023),while neutrophil percentage and count, lymphocyte percentage and count, hemoglobin, platelets,erythrocyte sedimentation rate,high sensitivity C-reactive protein, interleukin 6,procalcitonin, activated partial thromboplastin time ([37.5±4.6] vs [34.2±5.1]; P=.057),fibrinogen,antithrombin III and D-dimer showed no statistical difference. Conclusions:Family cluster infection,higher WBC and longer PT are the main risk factors for RP in the recovered COVID-19 children.Early activation of coagulation and WBC may be involved in SARS-CoV-2 clearance.

Introduction

As a result of imperfect immune function, children are naturally susceptible to various respiratory viruses and not easy to spontaneously resolve.Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection began in Wuhan city,Hubei province, China.[1-2] more than 2,000 pediatric cases have been reported nationwide in just over two months.[3] Recently, an increasing number of coronavirus disease 2019 (COVID-19) patients were discharged from the hospital and received regular follow-up and observation. Re-detectable positive (RP) of SARS-CoV-2 RNA test in some recovered patients has been reported.[4] Our hospital also reported that 38 of the 262 cured and discharged patients were found to be RP during the convalescence. Among them, patients younger than 14 years old were more common compared with those between the ages of 14 and 60 years.[5] In the long-term follow-up,we also observed that a large proportion of pediatric patients were RP (nasopharyngeal swabs and/or anal swabs) after discharge,even repeated RP and several readmission, asymptoms or with mild symptoms, and chest computed tomography (CT) showed sustained remission of pulmonary lesions.Obviously,RP means that the virus has not been completely cleared.There is still some uncertainty about whether such patients are infectious,but it undoubtedly has a serious impact on the formulation and implementation of prevention and control measures.

Despite great advances in rapid detection, diagnosis and treatment in SARS-CoV-2 infection, little is known about the early risk factors for RP.In particular, data on convalescent children as a special population have

not been reported.We aimed to identify the early risk factors for RP, and to provide a basis for early clinical prediction and risk stratification.

Materials and Methods

Clinical definition and classification

Children are defined as being less than 18 years old. Referring to the guidelines on the diagnosis and treatment of SARS-CoV-2 infected pneumonia (the sixth edition draft) issued by the National Health Commission of China.[6] Fever is recognized when body temperature is higher than or equal to 37.3.Respiratory symptoms include nasal congestion, runny nose, sneezing,sore throat, cough, expectoration, chest pain, dyspnea,etc.Digestive symptoms include nausea, vomiting, abdominal pain, diarrhea, etc.A real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect SARS-CoV-2 RNA positive in nasopharyngeal swab or anal swab samples to confirm diagnosis.All chest CT images were reviewed by two experienced pediatric radiologists. If unilateral or bilateral lung fields have any of the features as follows:(a) ground glass opacities;(b) consolidations with surrounding halo sign;(c) nodules;(d) residual fiber strips;(e) lymphadenopathy.The result is defined as positive CT findings of viral pneumonia.[7-8] Family cluster infection is defined as the occurrence of any of the following criteria in 2 or more family members within a period of less than 1 week: (a) fever;(b) respiratory and or digestive symptoms;(c) positive CT findings of viral pneumonia.

Discharge criteria: All clinical symptoms of the COVID-19 children disappeared, absorption of lung lesions improved, and two consecutive nucleic acid tests of both nasopharyngeal swab and anal swab were negative at least 24 hours apart.

Follow-up procedure after discharge: All discharged COVID-19 children were isolated and observed at home for 2 weeks, and community health workers visited house twice a week to collect nasopharyngeal swabs for SARS-CoV-2 RNA detection.They were also followed-up once every two weeks for at least two additional weeks after isolation. Among them, the RP pediatric patients were re-admitted to hospital for further medical observation and close contacts were also followed-up.The rest of the recovered non-RP pediatric patients were closely followed-up outside the hospital.

Data collection

We conducted a retrospective study.The medical records of all COVID-19 children were reviewed.Clinical and laboratory data of the first two days after admission were collected.Based on the SARS-CoV-2 RNA test results during follow-up, the RP group was defined as at least one positive test result. The control group was defined as all tests were negative and no suspicious clinical symptoms appeared, and the differences between the two groups were compared.

Inclusion criteria: all confirmed pediatric cases.

Exclusion criteria: lost follow-up cases.

Statistical Analysis

All analyses were conducted by using of IBM Statistical Product and Service Solutions software Version 24 (SPSS Inc,Chicago,IL).Continuous variables were summarized as the median with interquartile ranges (IQRs) or mean with standard deviations (SDs), median [IQR] or [mean±SD], depending on whether their distributions were normal or not.Comparisons of categorical variables were performed using the Pearson Chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for statistically significant variables.The parametric tests (independent sample Student t-test) or non-parametric tests (Mann-Whitney U test) were used to analyse variables.$P < .05$ was considered as statistically significant in all tests if applied.

Results

From Jan 22nd to Mar 10th, a total of 39 confirmed pediatric patients infected with SARS-CoV-2 were admitted with 1 (2.6%) case excluded due to lost follow-up,and 14 of 38 (36.8%) were RP. Referring to data

reported by our hospital,[5] 24 of 223 (10.8%) adult patients were RP.Children have a significantly higher percentage of RP (OR[95%CI] 4.84[2.21-10.59];P=.000).All included pediatric cases are divided into control group (n=24) and RP group (n=14).

Compared with control group,RP group had more family cluster infections (OR[95%CI] 1.59[1.1-2.3];P=.030),while age ([7.2+-4.8] vs [7.6+-5.1]),and percentage of male gender (35.7% vs 45.8%), fever (21.4% vs 45.8%),respiratory or digestive symptoms (71.4% vs 50%), asymptom (28.6% vs 33.3%), CT positive findings (85.7% vs 83.3%) and co-infection (7.1% vs 8.3%) were statistically nonsignificant (Table 1). The laboratory data of RP group had a relatively higher white blood cell count (WBC) (7.5[5.1-9.8] vs 4.8[4.4-7.5];P=.009) and longer plasma prothrombin time (PT) ([12.6+-0.7] vs [12.1+-0.5];P=.023), while neutrophil percentage (48.7[12.3-55.7] vs 37.5[31.1-44.4]) and count (2.9[1.7-4.0] vs 2.1[1.4-2.9]), lymphocyte percentage (7.5[5.1-9.8] vs 50.8[44.2-56.2]) and count (2.5[1.9-3.6] vs 3.0[2.2-5.7]),hemoglobin (129.0[121.0-142.0] vs 124.5[123.0-138.5]), platelets ([268.6+-82.4] vs [292.7+-64.0]), erythrocyte sedimentation rate (ESR) (13.0[7.0-20.0] vs 11.4[7.8-15.0]),high sensitivity C-reactive protein (hs-CRP) (1.2[0.6-2.7] vs 0.6[0.4-2.9]), interleukin 6 (IL-6) (3.0[1.9-6.3] vs 2.9[1.9-4.1]),procalcitonin (PCT) (0.04[0.02-0.08] vs 0.05[0.03-0.06]), activated partial thromboplastin time (APTT) ([37.5+-4.6] vs [34.2+-5.1];P=.057),fibrinogen (FIB) ([3.1+-0.7] vs [2.9+-0.6]), antithrombin III (ATIII) ([105.2+-9.7] vs [106.7+-11.1]) and D-dimer (0.32[0.22-0.42] vs 0.32[0.26-0.42]) showed no statistical difference (Table 2). In addition, there were no statistically significant differences in indicators related to liver and kidney function, troponin I (Supplemental Table 1).

12 of 24 (50%) in control group and 10 of 14 (71.4%) in RP group tested humoral immune function.There was no statistical difference in IgG, IgA, IgM, C3c and C4 between the two groups (Table 3).10 of 24 (41.7%) in control group and 14 of 14 (100%) in RP group tested T lymphocyte subclassification by flow cytometry. Percentage and count of $CD3^+$,$CD4^+$,$CD8^+$ and $CD4^+/CD8^+$ ratio were not statistically different (Table 4).

Discussion

According to data reported by our hospital,[5] RP patients have clinical characteristics of younger age and milder symptoms, and according to the 39 pediatric patients we observed in previous follow-up, most of them are mild symptoms, and there is no severe patient. Our study found that children have a higher incidence of RP compared with adults. However, there was no statistically significant difference between the RP group and the control group in terms of age, gender distribution, clinical symptoms, CT positive findings, and incidence of co-infection. Children with RP have a higher proportion of family cluster infections.[9-10] According to our observation, other patients in the family are generally cured first and close contacts were also without RP during followed-up, which can basically rule out the possibility of infecting children from other family members. However, it is still unknown whether children with RP are still infectious, because the children have to be isolated at home for 2 weeks after discharge and almost have no contact with the outside world.This also indicates us that SARS-CoV-2 is extremely resilient.[11]It seems to coexist peacefully with humans. In any case, disinfection of household environment and hand hygiene must be the top priority for disease prevention and control.[12]

The underlying risk factors of high incidence of RP and the viral clearance mechanism in children are still not completely understood.[13] T lymphocyte and phagocyte function may play an important role.T lymphocytes help clear viruses and regulate innate immune system responses.Chu et al demonstrated that MERS-CoV infection can induce T cells apoptosis through the activation of external and intrinsic apoptotic pathways.[14]Coleman et al found that the depletion of $CD4^+$ T cells, $CD8^+$T cells or macrophages had no effect on the replication of MERS-CoV in infected lungs of mice.[15] Earlier studies have revealed that SARS-CoV,which share the same cell entry receptors with SARS-CoV-2,could infect immune cells,including T lymphocytes, monocytes,and macrophages.The $CD4^+$ and $CD8^+$ T cells counts decreased at the onset of illness.[16-17]These studies indicated that the reduction of T cells is likely to contribute to the continuous replication and RP of SARS-CoV-2. However, our investigation did not observe this similar change and no significant difference between the two groups. After analysis,there may be the following reasons. First, the sampling time point may be in the early stage of the disease, the children's immune system is imperfect, and

the response is relatively slow and lagging. This may also explain why children are asymptomatic or mild, but the incidence of RP is high.[3,5] Second, the sample size is small, and there are many missing values in the control group, which may be different from the true level.These also suggest that $CD4^+$ and $CD8^+$ T cells count may not be suitable to be predictors of RP.

The average WBC level of pediatric patients in our investigation is totally within the normal reference range, which is consistent with related reports,[18] but WBC in the RP group is significantly higher than the control group.As far as we know, there is still no accurate answer to why the WBC in the RP group is higher than the control group. However, PCT, hs-CRP, IL-6 are within the normal range and there is no difference between the two groups.Obviously,the impact of co-infection is negligible.We speculate that this may be related to the weak response of the innate immune system and the reduction of WBC depletion of the RP group.

The coagulation cascade is activated during viral infections. This response may be part of the host defense system to limit spread of the pathogen.[19] A good balance between host coagulation and viral infection can improve pathological disease outcomes.[20] However, excessive activation of the coagulation cascade can be deleterious. Tissue factor (TF) appears to be the major activator of the coagulation cascade during viral infection.[19] Tang et al found that non-survivors of COVID-19 showed significantly higher levels of D-dimer, longer PT and APTT.We also observed a similar change. Compared with the control group, the PT and APTT of the RP group were prolonged, but there was no significant difference in FIB, ATIII, and D-dimer. This suggests that the activation of the exogenous coagulation pathway with TF as the starting point may be involved in the process of immune clearance of SARS-CoV-2,and it is milder in children. At present, there is still too much unknown, and the specific role and mechanism of the activation of coagulation cascade in SARS-CoV-2 clearance need to be further explored.

There are several limitations in our retrospective cohort study. First, due to the small sample size of the single-center research hospital, logistic regression analysis cannot be used to control confounding factors. Second, the children may be in different stages of COVID-19 when they are admitted to the hospital. Third,some children's humoral immune function and lymphocyte subclassification data by flow cytometry are missing, which may not reflect the true difference between the two groups.Therefore,these results should be carefully interpreted owing to potential selection bias and residual confounding. Larger cohort studies from other cities in China and other countries may also be needed to provide further data support.

Conclusions:

Family cluster infection,higher WBC and longer PT are the main risk factors for RP in the recovered COVID-19 children.Early activation of coagulation and WBC may be involved in SARS-CoV-2 clearance.,but the specific role and mechanism need to be further explored.

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