Hematological parameters and peripheral blood morphologic abnormalities in children with COVID-19

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Abstract

Objectives: The aim of this study is to evaluate the hematologic parameters and peripheral blood cell morphological changes in children with COVID-19 and compare them with those of children suspected but then confirmed to be negative for SARS-CoV-2. Methods: Thirty children were tested to be positive for SARS-CoV-2 and the remaining 40 were negative. Hemoglobin, leukocyte, neutrophil, lymphocyte, monocyte counts according to age-specific intervals, platelet, large unstained cell counts, and delta neutrophil index were recorded. Differential counts were formulated by manual counting and morphology of the blood cells were evaluated. Results: The mean leukocyte counts of the SARS-CoV-2 positive and negative groups were 7.0 ± 3.7x109/L and 10.4 ± 7.1x109/L, respectively ($p<0.05$). Nine (30%) children with COVID-19 had lymphopenia. Among children with COVID-19, absolute lymphocyte count was lower in those with pneumonia ($p<0.05$). Reactive lymphocytes were noted in 77.8% and 90% in the SARS-CoV-2 test positive and negative groups, respectively ($p>0.05$). Mean absolute neutrophil counts of the SARS-CoV-2 test positive and negative groups were 3.7±2.9 x109/L and 5.4±4.2 x109/L ($p<0.05$). Four patients (13.3%) with SARS-CoV-2 test positive had neutrophilia and seven (23.3%) had mild neutropenia. In the peripheral smear, vacuolated monocytes and dysplastic changes in neutrophils and platelets were noted in both groups. Conclusions: Leukocyte, neutrophil and monocyte counts were significantly lower in children with COVID-19 compared with symptomatic children without COVID-19. Lymphopenia, reactive lymphocytosis and dysplasia, could be noted in children with COVID-19. Further studies on hematological findings linked with the course of the disease in children are warranted.

Introduction

Coronavirus Disease 2019 (COVID-19) is an infectious disease with a rapid increase in cases and deaths since its first identification in Wuhan, China, in December 2019. The virus that causes COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Sore throat, high fever, shortness of breath, dry cough, headache, confusion, nausea, vomiting, diarrhea or loss of taste/smell are the most common manifestations of the disease (1, 2). COVID-19 testing includes either the detection of the virus itself by real-time reverse transcription polymerase chain reaction or those antibodies produced in response to infection (3). COVID-19 is predominantly prevalent among adults; patients under 18 years only account for 2% of the severely affected patients (4, 5).

Quantitative changes in blood cells, including leukocytes, lymphocytes, neutrophils, monocytes and platelets, have been reported in adults patients with COVID-19 (2, 6-8); moreover, the extent of such quantitative changes has been correlated with the severity of the disease in adults (6, 9). Normal (10), decreased (11) and increased (8) leukocyte counts have been reported. In general, decreased lymphocyte (11, 12) and increased neutrophil (2, 9, 13), counts have been noted in various studies. However, there is paucity of data on the hematologic parameters of children with COVID-19 (4). In a review article on COVID-19 in children, a Chinese article was quoted reporting that "the most routine blood examinations were normal" (4). In another study reporting 10 pediatric patients affected with the disease, "a few cases" were reported to have

leukopenia and lymphopenia (14). Moreover, although the morphological changes in peripheral blood cells have been reported in adults with COVID-19 (15), such changes, to our knowledge, have not been reported in children affected with the disease .

The aim of this retrospective study is to report the quantitative and qualitative changes of peripheral blood cells in children with COVID-19 and compare them with those of symptomatic children suspected but then confirmed to be negative for SARS-CoV-2.

Methods

Children admitted to the Emergency Department of Ministry of Health Ankara City Hospital Children's Hospital, which is a tertiary children hospital with 555 beds capacity, with symptoms including fever, sore throat, rhinorrhea, cough between 1-15 April 2020, were included in the study. All patients were tested for SARS-CoV-2 by quantitative real-time reverse transcription polymerase chain reaction from combined nasal and oropharyngeal swab samples. Complete blood count test and peripheral smear were done on admission of Emergency Department. Patients unable to obtain peripheral blood smear were excluded from the study. Thirty children were tested to be positive for SARS-CoV-2 and the remaining 40 symptomatic children were tested to be negative.

Complete blood count (CBC) was analyzed with Siemens ADVIA 2120i Hematology Analyzer with Auto slide (Siemens Healthcare Diagnostics, Erlangen, Germany); CBC results, including differential count results, were noted. Estimated absolute lymphocyte (ALC), neutrophil (ANC), monocyte (AMC) counts, were recorded from CBC results and were also calculated from peripheral smear specimens. Hemoglobin, leukocyte and neutrophil values were assessed according to age-specific intervals (16); ALC$<3x10^9$/L and ALC $<1.5x10^9$/L were accepted as lymphopenia in <12-month and >12-month old patients, respectively (16). Neutropenia was diagnosed with an absolute neutrophil count of $<1.5x10^9$/L (16). Neutrophil/lymphocyte ratio was calculated by the ADVIA 2120i analyzer. C-reactive protein values were also recorded.

All patients' peripheral blood smear specimens (May-Grünwald-Giemsa stain x100) were evaluated for neutrophil, thrombocyte and erythrocyte morphology and differential counts were formulated by manual counting including reactive lymphocyte and immature myeloid cells (band, metamyelocyte and myelocyte). the peripheral smears was blindly evaluated to the SARS-CoV-2 test result. The correlation between the reactive lymphocyte count in manual peripheral smear and large unstained cells (LUC), which reflects the activated lymphocytes and peroxidase-negative cells as measured by ADVIA 2120i, was analyzed. Moreover, the correlation between the percentage of immature myeloid cells in peripheral smear and delta neutrophil index (DNI) was assessed; DNI reflects immature myeloid cells and is calculated by subtracting polymorphonuclear leukocyte count in nuclear lobularity channel from neutrophil and eosinophil count in myeloperoxidase channel, both measured by the ADVIA 2120i analyzer.

The data were expressed as mean ±SD. Student's t-test was used to compare the means. Pearson correlation was used to assess the relationship between two quantitative, continuous variables. A value of $p<0.05$ was considered significant.

Results

The mean age of the whole cohort was 8.11±5.71years (4 months-17 years). Thirty-five patients (50%) were male; of the 30 SARS-CoV-2 test positive patients, 15 (50%) were male. In the whole cohort (n=70), the most common presenting symptoms were fever (65.7%) and cough (60%). In the SARS-CoV-2 test positive and negative groups, 17 (56.7%) and 19 negative patients (47.5%) had pneumonia, respectively. None of the patients required treatment in the intensive care unit.

Complete blood count parameters of the SARS-CoV-2 test positive and negative groups were given in Table 1. Six patients (20%) with SARS-CoV-2 test positive and 11 patients (27.5%) with test negative were anemic according to age-specific intervals. Of note, a SARS-CoV-2 test positive 14-year-old Afghan patient with a diagnosis of aplastic anemia had pancytopenia before COVID-19.

The mean leukocyte counts of the SARS-CoV-2 test positive and negative groups were $7.0\pm3.7x10^9$/L and $10.4\pm7.1x10^9$/L, respectively ($p<0.05$). Within the SARS-CoV-2 test positive group, two patients (6.7%) had leukocytosis and four patients (13.3%) had leukopenia, according to age-specific intervals. The mean ALC of COVID-19 patients were $2.7\pm2.3x10^9$/L; nine (30%) of them had lymphopenia, according to age-specific intervals. Twelve patients (30%) in the SARS-CoV-2 test negative group had also lymphopenia. The ALCs of COVID-19 patients with and without pneumonia were $2.1\pm0.9x10^9$/L and $3.4\pm2.9x10^9$/L, respectively ($p<0.05$). Reactive lymphocytes (Fig 1) were noted in 85.1% of the peripheral smears of the whole cohort; these figures were 77.8% and 90% in the SARS-CoV-2 test positive and negative groups. The mean absolute reactive lymphocyte (ARL) counts of SARS-CoV-2 test positive and negative groups were, $0.7\pm0.7x10^9$/L, $1.2\pm1.3x10^9$/L, respectively ($p<0.05$). The mean LUC counts of SARS-CoV-2 test positive and negative groups were $0.3\pm0.2x10^9$/L and $0.2\pm0.23x10^9$/L, respectively ($p>0.05$). No correlation between LUC count and ARL count was noted. The respective mean absolute neutrophil counts were 3.7 ± 2.9 $x10^9$/L and 5.4 ± 4.2 $x10^9$/L ($p<0.05$). Four patients (13.3%) with SARS-CoV-2 test positive and 10 patients (25%) with negative result groups had neutrophilia. Seven (23.3%) COVID-19 patients had mild neutropenia, whereas 3 (7.5%) patients with test negative had neutropenia. The mean neutrophil/lymphocyte ratios of SARS-CoV-2 positive and negative groups were 2.7 ± 2.9 and 2.9 ± 4.2, respectively ($p>0.05$). Immature myeloid cells (>2% in blood smear) were noted in 13 children (23%) in the COVID-19 group. All COVID-19 patients' DNI were <0.4. The mean AMC was $0.4\pm0.3x10^9$/L in the SARS-CoV-2 positive and $0.6\pm0.5x10^9$/L in the SARS-CoV-2 negative patients ($p<0.05$). Leukoerythroblastic reaction was not noted in any children in the whole cohort.

The mean platelet count was $268\pm89x10^9$/L in the SARS-CoV-2 positive and $339\pm178x10^9$/L in the SARS-CoV-2 negative group ($p<0.05$) (Table 1). In the whole cohort, thrombocytopenia was detected in four patients; one of them was the patient with aplastic anemia and COVID-19; the remaining three were in the SARS-CoV-2 negative group. Thrombocytosis was noted in one patient only. The mean platelet volume (MPV) was comparable between the two groups; with no child noted to have increased MPV. The mean C-reactive protein level of the SARS-CoV-2 positive group was significantly lower when compared with that of SARS-CoV-2 negative group ($p=0.006$).

In the peripheral smears of a few children affected by COVID-19, vacuolated monocytes (n=3; 13.3%) and dysplastic changes such as hypergranulation/lobulation abnormalities in neutrophils (n=11; 36.7%) were noted (Figs. 2, and 3). The respective figures within the non-COVID-19 group were 4 (10.0%) and 9 (22.5%). Although the MPV values were within the normal range, 20% of the COVID-19 patients had giant platelets (Figure 4); however, giant platelets were also noted in 27.5% of the negative patients.

Discussion

In adults, the most common hematological findings of COVID-19 include lymphocytopenia (11, 12), neutrophilia (2, 13), mild thrombocytopenia (7) and, less frequently, thrombocytosis (9, 17). However, there is paucity of data on hematological findings in affected children (4, 13). In our study, the majority of the SARS-CoV-2 infected children had normal leukocyte count, but 13.3% had leukopenia and 6.7% had leukocytosis, according to age-specific intervals. Despite normal leukocyte count, lymphopenia and neutrophilia were noted in 30.0% and 13.3% of children affected with the disease, respectively. Seven patients with SARS-CoV-2 test positive (23.3%) had neutropenia; this figure was 7.5% in the test negative group. Neutropenia has not been previously reported in adults affected with the disease.

The presence of reactive lymphocytes has been occasionally reported in adults with COVID-19. Fan et al. reported a few lymphoplasmacytoid reactive lymphocytes in peripheral blood of lymphopenic patients with COVID-19 (7). It is well documented in the literature that in response to stress, atypical reactive lymphocytes that are characterized by nuclear and cytoplasmic distortion appear in the blood (18). Reactive lymphocytes were detected in 85.1% of our patients in the whole cohort, 77.8% in the test-positive and 90% in the test-negative groups. The mean ARL count was lower in the COVID-19 positive group, which was statistically different from the COVID-19 negative group. Leukoerythroblastic reaction that reflects the immature erythroid and immature myeloid cells circulating in the peripheral blood has also been reported in adults with COVID-19 (19). However, we did not observe leukoerythroblastosis in the whole cohort, but

we noted bands and metamyelocytes in 23% of the COVID-19 patients' peripheral smear. We detected the lack of concordance between LUC/DNI as measured by the counter and manual counting. This addresses the importance to assess peripheral blood smears.

Thrombocytopenia was detected in only one patient with aplastic anemia and COVID-19; thrombocytosis was not noted in any patient. In a meta-analysis, low platelet count has been associated with the increased severity of the disease and increased mortality in adults with COVID-19, thus serving as an indicator of worsening illness during hospitalization (9). The absence of thrombocytopenia in our series may be related to better clinical prognosis of the disease in children.

Zini et al. from Italy reported marked morphological abnormalities in neutrophil lineage and platelet morphology in adults with COVID-19, mainly very large, usually hyperchromatic platelets, both in patients with thrombocytosis and thrombocytopenia (15). We noted some nonspecific dysplastic changes in peripheral smear of affected children. Giant platelets were noted in 20% of children with COVID-19. Vacuolated monocytes, hypergranulated neutrophils and pseudo Pelger-Huet abnormality were also seen in 13%, 7% and 30% of COVID-19 infected children, respectively. In patients with COVID-19, upregulation of pro-inflammatory cytokines in the blood, including interleukin (IL)-1, IL-6, TNF, and interferon γ has been reported (5). Dysregulation of immunological environment may have an important role in the pathogenesis of myelodysplastic syndromes (20). We do not have the data for pro-inflammatory cytokines in the blood and we speculate that the dysplastic changes of blood cells in our series might be related to those altered cytokines.

In conclusion, leukocyte and neutrophil counts were lower in children with COVID-19 compared with children with similar symptoms. Lymphopenia and reactive lymphocytosis, dysplastic changes of granulocytic lineage and giant platelets on peripheral smear, although not specific, could be noted in children with COVID-19. Further studies on hematological findings linked with the course of the disease in children are warranted.

Conflict of Interest: The authors have indicated they have no potential conflicts of interest to disclose.

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Figure Legends:

1: Reactive lymphocytes (x100) 2: Lobulation abnormalities (x100) 3: Hypergranulation and hypolobulation in a neutrophil (x100) 4: Giant platelet and platelet anisocytosis (x100)

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`Table 1 covid -19.docx` available at https://authorea.com/users/320139/articles/454868-hematological-parameters-and-peripheral-blood-morphologic-abnormalities-in-children-with-covid-19