

pulmonary, neurologic, and immunologic systems that may affect the course of the disease. Results from adults and even from pediatric studies should be extrapolated very carefully unless there is no neonatal data.¹⁵ For that purpose, we established a national neonatal database to follow all the neonates with confirmed COVID-19 in Turkey.

MATERIALS AND METHODS

We conducted a prospective multicentered cohort study among newborns with a reverse transcriptase–polymerase chain reaction (RT-PCR) proven SARS-CoV-2 infection in 24 neonatal intensive care units (NICU) in Turkey. The study was initiated upon approval by the Online Studies Scientific Steering Committee of the Turkish Neonatal Society, Institutional Ethical Review Board, and the Ministry of Health. We have accepted cases by using a secure online data registry system via electronic case report forms. The records between March 9th and June 15th were pooled together and analyzed.

Neonates who admitted to the hospital with symptoms of fever, cough, or tachypnea were tested by nasopharyngeal and oropharyngeal PCR swabs. The extraction of nucleic acids from the samples was performed by a rapid assay, namely Bio-Speedy SARS-CoV-2 qPCR Detection Kit (Bioeksan, Istanbul, Turkey) in the laboratories certified by the Ministry of Health. Neonates without SARS-CoV-2 via RT-PCR or whose mothers had been diagnosed with COVID-19 during pregnancy were excluded.

The management of neonates with the diagnosis of COVID-19 was in accordance with the proposals provided by the Turkish Neonatal Society and the Turkish Ministry of Health.^{16,17} We only hospitalized neonates with respiratory distress, feeding difficulty, or to rule out bacterial sepsis. Hospitalized neonates were isolated in separate NICU rooms, and all staff used disposable waterproof gowns, N95 masks, goggles/eye protection, and gloves before entering isolation rooms, abiding by hand hygiene precautions.

The decision about breast-feeding was made on a case-by-case basis after consulting with the parents. All neonates, whether admitted to NICU or not, were followed up, and broad contact tracing precautions were delivered by the Ministry of Health for at least 14 days.

The severity of COVID-19 was defined as asymptomatic, mild, moderate, severe, and critical.¹⁸ The diagnostic criteria were as follows:

Asymptomatic: without any clinical symptoms and signs.

Mild: symptoms of upper respiratory tract infection or fever, however, no feeding difficulty and no obvious hypoxemia, and no risk of late neonatal sepsis. These were the patients who followed up without hospitalization.

Moderate: any patient that needed to be hospitalized primarily due to feeding difficulty or risk of late neonatal sepsis, but no obvious hypoxemia or no need for nasal continuous positive airway pressure (nCPAP).

Severe: any patient with oxygen saturation <92% or need for nCPAP.

Critical: any patient that needed mechanical ventilation or disseminated intravascular coagulopathy or multiple organ dysfunction.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, IL). We presented continuous values as the mean \pm standard deviation (SD) or median (minimum–maximum) according to the homogeneity of the distribution, which was evaluated by the Kolmogorov–Smirnov test. We presented categorical values as the number and percentage, and analyzed them by the χ^2 test. The Student's *t* test was used for continuous variables with

normal distribution, and the Mann-Whitney *U* test and Kruskal–Wallis test were used for continuous variables which did not exhibit a normal distribution. Correlation between the independent parameters was investigated by bivariate (Pearson and Spearman) correlation analysis. A receiver operating characteristic (ROC) analysis was constructed to determine the best cutoff values to predict the outcomes. The estimated probabilities were used in the ROC analysis to calculate the area under the curve (AUC). A *p* value of < 0.05 was considered to indicate statistical significance.

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RESULTS

Thirty-seven neonates from 24 NICUs were admitted to the study. More than half of the cases were from Istanbul and Ankara; other cases were scattered throughout Turkey (Fig. 1). Demographic values and clinical characteristics at admission are summarized in Table 1. All of the cases were neonates admitted to the emergency department or neonatal/pediatric outpatient polyclinics. There was no asymptomatic case. The most frequent findings were fever, hypoxemia, and cough (49%, 41%, 27%, respectively). Two of the patients had congenital anomalies; 1 meningocele and another Down syndrome newborn with an atrioventricular septal defect. None of the other neonates had preexisting medical comorbidities. All of the neonates were born with APGAR scores 8 and more. Three (8%) patients were late preterms, and the remaining neonates (92%) were full term.

All parents were nonsmokers. Three of the neonates had parents working as a doctor or nurse (8%). Eighty-four percent of the neonates had at least 1 symptomatic contact, and 76% of contacts were proven COVID-19 cases. Laboratory findings of the patients are summarized in Table 2.

Seventy-three percent of the neonates were placed in an isolation room in the NICU; on the other hand, other patients were followed at their homes as 2 m away from their family members and abiding by personal protection precautions. The treatment modalities, disease severity, respiratory support need, hospitalization duration, complications, and imaging results of the patients are summarized in Table 3. Median hospitalization was around 11 days (minimum: 1, maximum: 35 days). A patient with Down syndrome and atrioventricular septal defect died due to neonatal acute respiratory distress syndrome (ARDS) and co-infection with MRSE (methicillin-resistant *Streptococcus epidermidis*) after 21 days of hospitalization in the NICU.

C-reactive protein (CRP) and prothrombin time (PT) levels were found to be higher in patients who needed supplemental oxygen or who were severe/critical (Table 4). A cutoff value of 5 mg/dL for CRP and 14 seconds for PT had a statistically significant sensitivity of 53% and 75%, and a specificity of 91% and 90%, to estimate oxygen requirement (*p* = 0.002 and *p* = 0.015, respectively). Additionally, the same cutoff values were statistically significant to estimate critical/severe cases with a sensitivity of 50% and 78%, and with a specificity of 91% and 100% (*p* = 0.01 and *p* = 0.002, respectively).

CRP and PT values were significantly correlated with oxygen supplementation (*r* values = 0.49, 0.65; *p* values = 0.002, 0.004 respectively) and with total nCPAP duration (*r* values = 0.45, 0.61; *p* values = 0.002, 0.008 respectively). Thirty-one neonates (84%)



FIGURE 1. Distribution of community-acquired neonatal coronavirus disease (COVID-19) cases in Turkey.

TABLE 1. Demographic and clinical characteristics of the neonates at admission

| Total Number of Patients: 37 | n (%) or Mean ± Standard Deviation |
|---|--|
| Sex (n) | Female: 18 (49); Male: 19 (51) |
| Place of living (n) | Rural: 1 (3) Urban: 36 (97) |
| Health worker parent (n) | Health worker: 3 (8) Others: 34 (92) |
| Age at admission (days) | 15.6 ± 7.7 |
| Birthweight | 3223 ± 553 g AGA: 32 (87) SGA: 2 (5) LGA: 3 (8) |
| Gestational age (weeks) | 38.3 ± 0.2 34 weeks 0 days–36 weeks 6 days: 3 (8) ≥37 weeks 0 days: 34 (92) |
| Delivery mode | Vaginal: 19 (51) Cesarean section: 18 (49) |
| Complaints and physical findings at admission | Fever 18/37 (49) Hypoxemia 15/37 (41) Cough 10/37 (27) Tachypnea 9/37 (24) Poor feeding 6/37 (16) Retractions 5/37 (14) Rale 5/37 (14) Diarrhea 2/37 (5) Nasal congestion, rhinorrhea 2/37 (5) Exanthema 1/37 (3) |
| Number of people in the house (including the infant) | 4.8 ± 1.9 |
| Contact history with a symptomatic person | 31 (84) |
| Contact history with a proven COVID-19 positive patient | 28 (76) |

n indicates number of the patients; SD, standard deviation; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age; COVID-19, coronavirus disease 2019.

had chest radiograph, whereas 5 neonates (14%) were evaluated by computed tomography (CT). Their findings are summarized in Table 3. Only 39% of the chest radiographs revealed a pathology, whereas 80% of the chest CTs were abnormal.

Targeted antiviral therapies were used in 12 (32%) of patients; however, a respiratory panel was obtained only from 5 of them (14%), and none of them revealed any accompanying virus. Two of the neonates also had fecal PCR tests, which were resulted in negative.

Medication rates for azithromycin, oseltamivir, and intravenous antibiotics were 38%, 32%, and 54%, respectively. Complications like myocarditis, disseminated intravascular coagulopathy, and multiple organ dysfunction were infrequent (8%, 5%, 3%, respectively). None of the patients had intracranial hemorrhage, QTc prolongation, or necrotizing enterocolitis. Azithromycin was used with a higher rate in patients who needed supplemental oxygen (64% vs. 26%, $p = 0.04$). There was no difference for hospitalization, oxygen requirement, disease severity, or myocarditis rates in accordance with sex, birth weight, gestational age, neutrophil or lymphocyte count, procalcitonin, IL-6 levels, oseltamivir usage, having neutropenia, or being premature ($p > 0.05$).

Nine mothers (22%) continued breast-feeding, whereas 22 neonates (52%) were fed by expressed milk. Only 11 neonates (26%) were fed by a formula.

DISCUSSION

We evaluated demographic characteristics, clinical course, prognostic factors, laboratory, and imaging results of 37 neonates with proven COVID-19. To the best of our knowledge, this is the most comprehensive study to present the clinical course of community-acquired neonatal COVID-19 in the literature.

The previous studies have shown that children present a milder clinical course with nonspecific viral symptoms, and children rarely need hospitalization and respiratory support, except

TABLE 2. Laboratory findings of the patients

| Parameter | Mean ± SD or Median (min-max) |
|--|----------------------------------|
| White blood cells (n = 37)/ μ L (N = 9100–34,000) | 10720 ± 4524 |
| Neutrophil count (n = 37)/ μ L (N = 2500–5800) | 3975 ± 2949 |
| | Neutropenia*: 22% |
| Lymphocyte count (n = 37)/ μ L (N = 1500–3000) | 4935 ± 2298 |
| Thrombocyte count (n = 37) × 10 ³ / μ L (N = 150–400) | 322 ± 133 |
| Hematocrit (Htc) (n = 37) % (N = 35–65) | 40.2 ± 8.1 |
| Hemoglobin (Hb) (n = 37) gr/dL (N = 11.1–17.4) | 13.9 ± 2.6 |
| C-reactive protein (CRP) (n = 37) mg/L (N = 0–5) | 2.1 (0.1–69.2) |
| | High CRP: 27% |
| Interleukin-6 (IL-6) (n = 7) pg/mL (N = 0.1–44.4) | 48.6 (1.5–796) |
| | High IL-6: 57% |
| Procalcitonin (n = 20) ng/mL (N = 0–0.15) | 0.14 (0.05–70.0) |
| | High procalcitonin: 50% |
| Alanine aminotransferase (ALT) (n = 37) U/L (N = 10–40) | 27.0 ± 19.6 |
| Aspartate aminotransferase (AST) (n = 37) U/L (N = 22–71) | 44.3 ± 23.3 |
| γ -Glutamyl transferase (GGT) (n = 16) U/L (N = 13–147) | 79.0 ± 54.5 |
| Lactate dehydrogenase (LDH) (n = 29) U/L (N = 170–580) | 404.6 ± 139.2 |
| Blood Urea Nitrogen (BUN) (n = 35) mg/dL (N = 3–12) | 9.7 ± 7.0 |
| Creatinine (n = 37) mg/dL (N = 0.03–0.50) | 0.39 ± 0.19 |
| Albumin (n = 30) g/dL (N = 1.9–4.9) | 3.4 ± 0.4 |
| Creatinine kinase (CK) (n = 25) U/L (N = 5–130) | 113 (6–427) |
| Troponin (n = 16) ng/mL (N = 0–126) | 40.2 (0.01–304) |
| Prothrombin time (PT) (n = 20) sec (N = 11–14) | 13.5 ± 2.5 |
| Partial thromboplastin time (PTT) (n = 20) sec (N = 33.0–47.8) | 35.2 ± 9.7 |
| International normalized ratio (INR) (n = 20) (N = 0.86–1.22) | 1.2 ± 0.4 |
| Fibrinogen (n = 20) mg/dL (N = 82–383) | 243 ± 95 |
| D-Dimer (n = 20) mg/L FEU (N = 0.11–0.42) | 1.2 (0.06–8.76) |

SD indicates standard deviation; N, normal values.

*Neutropenia: < 1,500/ μ L.

young children, particularly infants, who were vulnerable to COVID-19 infection.^{18,19} However, all neonates presented in our cohort were symptomatic, and fever and cough were the most common presenting symptom comparable to children series.^{18,19} Diarrhea, nasal congestion, and rhinorrhea were rare manifestations similar to adult and children studies, and generally were together with respiratory distress symptoms.^{5,6,20,21} Cutaneous manifestations may accompany and remain after other symptoms have disappeared; however, we only observed 1 neonate with exanthema, which was not chronic.^{22,23}

Contrary to the limited published reports, 35% of the cases were severe, and among them, 3% was critical, whereas more than 70% of them were admitted to the NICU. Criteria for hospitalization for neonates are usually more flexible than older children, especially to rule out neonatal sepsis. On the other hand, nearly 40% needed supplemental oxygen, 15% needed noninvasive ventilation modalities, and 1 died after 21 days of mechanical ventilation. Therefore, the high hospitalization rate was not conclusive, but the course of the disease in our series was more severe than previously reported children series.^{18,19} Dong et al reported that severe cases in children are usually among infants, although it is not clear how many of them were neonates.¹⁸ Five case reports, 2 case series with 3 community-acquired neonatal patients in each have been reported, and the prognosis of all these neonates was favorable.^{8–14} Götzinger et al reported 582 children in a multicenter study around Europe, and found that being younger than one month had a 5.06 odds ratio for requiring NICU admission.²⁴

CRP and PT are ubiquitous tests, and we found that they may be applicable to differentiate disease severity. Patients who had CRP values below 5 mg/L and PT values lower than 14 seconds were less likely to be severe/critical or need oxygen. Although the sensitivity of CRP and PT was not good enough to predict the progression, they were helpful to rule out severe/critical disease due to

their high specificity. As far as we know, the prognostic importance of these biomarkers has not been reported in neonates before. Whitaker et al found that children with shock had higher CRP levels compared with those without shock during COVID-19.²⁵

The death of our one case might be due to underlying chronic cardiovascular conditions or immunologic dysregulation of Down syndrome. Espinosa hypothesized that patients with Down syndrome should be considered as high-risk patients due to immune dysregulation and possible exacerbated cytokine release syndrome.²⁶ To the best of our knowledge, only 1 case series had been reported with 3 Down Syndrome patients in it, but none of them were neonates.²⁷

Most of the components of the immune system are immature and developmentally regulated in neonates, which results in transient immunodeficiency during early infancy.²⁸ Host immune response to viral infections mainly relies on the responses of respiratory tract cells, innate immune cells, and acquired immune mechanisms.²⁹ Neonates are not mature enough for most of these factors. These may put newborns susceptible to infection; on the other hand, maybe make them less vulnerable to severe lung injury caused by immune responses. The immature immune system of premature neonates may prevent them from a cytokine storm, which is an essential factor for disease severity and mortality in COVID-19 patients.³⁰

SARS-CoV-2 enters the human cells via angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in several organs such as lungs, intestine, heart, and kidney.^{31,32} Higher expression of ACE2 in children may have a protective role in the COVID-19 pathogenesis.³³ Further studies are needed in neonates related to ACE2 receptors and immune responses to COVID-19.

The household contact rate in neonates (76%) was lower than previous findings in children (98.69%).³⁴ Moreover, some neonates were the only COVID-19 positive members in their families, similar to some reports.¹¹ Neonates may get infected even in controlled NICU

TABLE 3. NICU follow up of neonates with community-acquired COVID-19

| | Yes | No | Details, Median (min–max) |
|--|--|----------|---|
| NICU admission | 27 (73%) | 10 (23%) | Total NICU stay: 11 (1–35) days Male: 13/19 Female: 14/18 No asymptomatic cases |
| Disease severity | Mild 10 (27%) Moderate 11 (30%) Severe 13 (35%) Critical 3 (8%) | | |
| IV antibiotic treatment | 20 (54%) | 17 (46%) | Total: 8.2 (2–15) days |
| Azithromycin treatment | 14 (38%) | 23 (62%) | Total: 5.6 (3–10) days QTc prolongation: none |
| Oseltamivir treatment | 12 (32%) | 25 (68%) | Total: 5.2 (5–7) days |
| Corticosteroid treatment | 4 (11%) | 33 (89%) | |
| Hydroxychloroquine treatment | 2 (5%) | 35 (95%) | Total: Both 5 days QTc prolongation: none |
| IVIG treatment | 1 (3%) | 36 (97%) | Gestational Age: 37 weeks |
| Surfactant treatment | 1 (3%) | 36 (97%) | AVSD, Down syndrome, MRSE |
| Myocarditis | 3 (8%) | 34 (92%) | |
| DIC | 2 (5%) | 35 (95%) | |
| Multiple organ dysfunction | 1 (3%) | 36 (97%) | AVSD, Down syndrome, MRSE infection all in one patient |
| Apnea | 1 (3%) | 36 (97%) | Gestational age: 39 weeks |
| Oxygen requirement | 15 (41%) | 22 (59%) | Total: 72 (1–504) h |
| nCPAP requirement | 6 (16%) | 31 (84%) | Total: 24 (1–144) h |
| Mechanical ventilation | 1 (3%) | 36 (97%) | Total: 494 hours (only 1 patient) |
| Positive chest radiograph finding (n = 30) | 11 (36%) | 19 (64%) | Consolidation/infiltration: 11/31(36%) Atelectasis: 0 Pleural effusion: 0 |
| Positive chest CT finding (n = 5) | 4 (80%) | 1 (20%) | Ground glass opacities: 2/5 (40%) Consolidation with surrounding halo sign: 1/5 (20%) Bilateral pulmonary lesion: 3/5 (60%) Fine mash shadow: 0 Tiny nodules: 0 Unilateral pulmonary lesion: 0 |
| Mortality | 1 (3%) | 36 (97%) | AVSD, Down syndrome, MRSE infection all in one |

IV indicates intravenous; CT, computed tomography; nCPAP, nasal continuous positive airway pressure; DIC, disseminated intravascular coagulation; MRSE, methicillin-resistant streptococcus epidermidis; IVIG, intravenous immunoglobulin; AVSD, atrioventricular septal defect.

environments.⁷ On the other hand, community screening and contact tracing studies have revealed a low number of infections in children compared with adults, suggesting that they are less likely to acquire SARS-CoV-2 than adults.^{35–37} However, it is not clear if these studies include neonates in the screening group. The transmission of the SARS-CoV-2 to neonates might be easier than previously extrapolated.

Only one-third of the cases had positive chest radiograph findings, which were consolidation or infiltration. Nearly 60% of the neonates had a typical appearance on CT with ground-glass opacities or consolidation with surrounding halo sign. Ground-glass opacities were the most common finding as previously reviewed and found as high as 62.4% in children by Shelmerdine et al³⁸ A total of 20% of patients had no abnormalities on chest CT which was similar to results by Xia et al³⁹ We suggest that CT should be preserved for selected cases, because of the possible long-term harmful effects of the radiation.

TABLE 4. CRP, PT, and PTT levels of patients according to their supplemented oxygen, nCPAP/MV requirements, and disease severity

| | CRP Level | P | PT | P |
|-------------------|---------------------------|--------|------------------|--------|
| Oxygen | (–) n = 22 0.9 (0.1–8.6) | 0.002* | 11.9 (10.1–17.2) | 0.012* |
| | (+) n = 15 5.8 (0.3–69.2) | | 15.2 (11.7–18.0) | |
| nCPAP or MV | (–) n = 31 1.4 (0.1–18) | 0.005* | 12.0 (10.1–17.2) | 0.008* |
| | (+) n = 6 34.5 (0.3–69.2) | | 16.9 (14.9–18.0) | |
| Mild + moderate | n = 21 1.0 (0.01–8.6) | 0.01* | 11.7 (10.1–13.9) | 0.001* |
| Severe + critical | n = 16 4.5 (0.1–69.2) | | 15.0 (11.7–18.0) | |

Values are given as median (minimum–maximum).
CRP indicates C-reactive protein; PT, prothrombin time; PTT, partial thromboplastin time; nCPAP, nasal continuous positive airway pressure, MV: mechanical ventilation.
*Values are significant.

Feeding neonates with breastmilk in NICU during COVID-19 is a great challenge. NICU visits, especially if the mother is also infected and transferring expressed milk to the NICU, are all delicate issues that should be carefully planned without putting NICU staff and other neonates in the NICU under risk. Many proposals recommend breast-feeding after taking all possible precautions.^{17,40} We think it was through the august efforts of the NICU staff that 74% of the neonates in this study continued to be fed by breastmilk.

Several limitations need to be highlighted. This study does not cover asymptomatic neonates or possible atypical cases with symptoms other than the respiratory system and fever. All prognostic factors and clinical data presented here belong to the patients seeking medical attention. The cases may be biased toward more severe illness and not likely representative of the distribution of severity of SARS-CoV-2 infection in neonates generally. Unless a comprehensive screening is made in neonates, there is no foolproof way of understanding the whole picture.

Although all of the NICUs were following the proposal provided by the Turkish Neonatal Society and the Turkish Ministry of Health, treatment and follow-up suggestions are not strict. The knowledge and experience are doubling each day about Covid-19, and designing a stringent protocol would be unethical.

Most of the patients do not have a respiratory PCR panel to assess coinfection. It is mostly due to the unavailability of the test at all centers. We could not exclude the possibility of coinfection and use targeted antiviral therapies as requested in all cases.

CONCLUSION

To the best of our knowledge, all neonatal series published up to now was on the risk of vertical transmission, and no outpatient series was reported specifically for neonates. Clinical

characteristics of community-acquired neonatal COVID-19 differ from other age groups. Oxygen and noninvasive ventilation requirement are frequent, although mechanical ventilation is rarely needed in symptomatic patients. Neutropenia is common; however, it is not associated with prognosis. Neonates who were severe/critical and needed respiratory support had higher CRP and PT levels. Underlying cardiovascular disorders or Down syndrome may be a risk factor for mortality.

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