Management of COVID-19-related paediatric blood samples in a clinical haematology laboratory

There is currently limited knowledge about the transmission risks of the SARS-CoV-2 virus and its associated disease COVID-19 from routine clinical specimens. The first study to be published on the initial 41 cases of COVID-19 infections admitted in Wuhan detected SARS-CoV-2 RNA in the blood of 6/41 (15%) of patients. However, another study conducted on 1 070 clinical specimens collected from confirmed COVID-19 patients in China showed the highest positive rates of SARS-CoV-2 from real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) testing of respiratory specimens such as bronchoalveolar lavage, sputum and nasopharyngeal swabs (32%–93%). In contrast, only 1% of blood specimens and none of the urine specimens tested positive. Although the rates of viraemia appear to be low, it nonetheless poses a risk of potential respiratory transmission to laboratory staff via aerosolization of blood specimens during specimen processing steps such as centrifugation and vortexing. Paediatric specimens pose a particular challenge as automated analysers cannot handle small-volume samples from paediatric-sized tubes, necessitating manual handling of specimens. The Haematology Laboratory at the Department of Pathology and Laboratory Medicine, KK Women’s and Children’s Hospital, processes over 130 000 paediatric samples annually. Our laboratory received the first sample from a suspect COVID-19 patient on 22 January 2020 and processed samples from a confirmed COVID-19 patient on 5 February 2020. From January to March 2020, we processed approximately 2 070 samples from paediatric patients with suspected or confirmed COVID-19 infection. In this paper, we describe the specimen management policy for handling and processing COVID-19-related blood samples in our laboratory, and highlight the challenges of working with paediatric samples during this period.

Materials and methods

Risk assessment

Our laboratory had existing biosafety guidelines for specimen management from patients with emerging respiratory pathogens (SARS-CoV and MERS-CoV). In early January 2020, the Ministry of Health in Singapore alerted healthcare practitioners of the emergence of a novel respiratory infection in Wuhan. Following the announcement, we performed a series of risk assessments based on identification of potential hazards and available laboratory equipment and facilities. Our guidelines were regularly reviewed when documents from the Ministry of Health, Singapore Ministry of Health, Singapore, World Health Organization (WHO) and the Centres for Disease Control and Prevention (CDC) relating to laboratory biosafety when handling COVID-19 specimens became available. We reviewed each test offered in our laboratory and made the decision to either require consultations with the laboratory haematologists for tests with a higher risk profile or not to offer tests which could not be performed safely based on our risk assessments Table I. We redesigned the laboratory workspace such that dedicated analysers closest to the Class II biological safety cabinet (BSC) were used for COVID-related specimens in an area separated from the rest of the laboratory.

Use of appropriate paediatric tubes for specimen collection

Prior to the COVID-19 outbreak, paediatric blood specimens were collected in Becton Dickinson (BD) Microtainer EDTA microtubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). However, this required open-mode sampling on the Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan) analyser in use at our laboratory, and would have subjected staff to the risk of aerosol exposure. We had previously performed a validation study using the BD Microutainer MAP (Microtubule for Automated Process) Microtube (Becton, Dickinson and Company), which is an alternative collection tube that could allow automated sample piercing and analysis without cap removal. Early on in the outbreak, a decision was made to switch specimen collection tubes to the MAP Microtube, avoiding the need to manually handle specimens in open mode. Paediatric blood samples for coagulation assays are collected in 3·2% sodium citrate Greiner Bio-One MiniCollect® 9NC (Greiner Bio-One GmbH, Kremmen, Germany) tubes. Although the tube has a membrane cap allowing for automated closed-mode analysis, the coagulation analyser in use at our laboratory (STA Compact Max (Dinamica Stago, Asnières-sur-Seine, France)) is not equipped with the optional cap-piercing system. Specimens for blood gas analysis are collected in heparinized syringes (Becton Dickinson (BD) A-Line™ Blood Gas Analysis Syringe (Becton, Dickinson and Company)).

Transport and labelling of COVID-related samples

Even before receiving specimens in the laboratory, close communication with clinical areas is essential to ensure safe
transport and appropriate labelling of specimens. At our institution, a disease outbreak task force (DOTF) was set up to co-ordinate management of suspect and confirmed COVID-19 patients, with representation from the laboratory to design protocols and communicate updates around laboratory-related issues. Specimens had to be double-bagged, labelled appropriately as COVID-19-related specimens and hand-delivered to the laboratory to avoid loss or misplacement of specimens. The pneumatic tube system was not used due to the risk of specimen loss and spillage.

Personal protective equipment

As there were limited supplies of face shields in our institution, a decision was made by hospital management to reserve the use of face shields for clinically high-risk situations, for example procedures involving suctioning of patients with confirmed COVID-19 infections. When handling and processing COVID-19-related blood samples in the laboratory, all staff must don personal protective equipment (PPE) including laboratory coats, disposable gloves, surgical masks and safety goggles which provide a good alternative for protection of the face and mucous membranes. All staff are reminded to practice good hand hygiene after processing samples and before leaving the laboratory. This policy applies round the clock.

Specimen handling and analysis

All COVID-related specimens are initially handled in the Class II BSC. Samples are carefully removed from specimen bags and manually disinfected with Mikrozid®AF (94% ethanol, Schülke & Mayr GmbH, Norderstedt, Germany) wipes with a minimum contact time of 2 min. Samples for full blood count are loaded directly into the dedicated Sysmex XN analyser located just beside the BSC. Blood films are manually prepared in the BSC and fixed in 100% methanol for 15 min before automated staining by a Hematek (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany) stainer. Samples sent for coagulation assays are decapped in the BSC and checked for clots with applicator sticks. The samples are centrifuged using a STI PlasmaPrep (Separation Technology Inc, Sanford, FL, USA) centrifuge located within the BSC. Plasma is then aliquoted

| Test                           | Specimen type                                      | COVID-19 status                           |
|--------------------------------|---------------------------------------------------|------------------------------------------|
| Full Blood Count               | Whole blood in EDTA                               | Test performed with use of enhanced biosafety practices |
| Peripheral Blood Film          | Whole blood in EDTA                               |                                          |
| Coagulation assays             | Whole blood in 3.2% sodium citrate                |                                          |
| Prothrombin time (PT)          |                                                   |                                          |
| Activated partial thromboplastin time (APTT) |                                                |                                          |
| International normalized ratio (INR) |                                               |                                          |
| Thrombin time (TT)             |                                                   |                                          |
| Fibrinogen                     |                                                   |                                          |
| Anti-Xa assay                  |                                                   |                                          |
| Blood Gas Analysis on I-Stat   | Whole blood in heparinized syringe                | Test performed with use of enhanced biosafety practices |
| Parameters assessed:           |                                                   |                                          |
| Sodium                         |                                                   |                                          |
| Potassium                      |                                                   |                                          |
| Ionized Calcium                |                                                   |                                          |
| Glucose                        |                                                   |                                          |
| Haematocrit                    |                                                   |                                          |
| pH                             |                                                   |                                          |
| pCO₂                           |                                                   |                                          |
| pO₂                             |                                                   |                                          |
| TCO₂                           |                                                   |                                          |
| HCO₃                            |                                                   |                                          |
| Erythrocyte sedimentation rate (ESR) |                                             | Discuss with Laboratory |
| Haemoglobin (Hb) electrophoresis | Whole blood in EDTA                              | Tests NOT performed* |

*Clinicians are to discuss the clinical urgency of these tests, and if possible to defer testing or to consider alternative tests until patients are tested negative for COVID-19.

†ESR and Hb electrophoresis tests are not performed for confirmed COVID-19 patients as testing involves open centrifugation and aspiration systems which are deemed to be high risk for aerosolization of samples.

Table I. List of haematology and coagulation tests offered in the Haematology Laboratory at KK Women’s and Children’s Hospital.
into Eppendorf tubes which are packed in a clean biohazard bag and hand-carried to the STA Compact Max coagulation analyser for analysis. A splash guard was installed beside the analyser for safe opening of the Eppendorf tubes which are then loaded into the sample drawer of the analyser. Point-of-care testing for blood gases in paediatric patients at our institution is handled mostly at clinical areas by staff in appropriate PPEs using I-Stat devices (Abbott Point of Care Inc, Chicago, IL, USA). However, specimens for blood gas analysis which includes measurement of the haematocrit (Table I) can also be analysed in the laboratory using the CG8 + cartridge on the I-Stat device, with all analysis performed within the BSC.

Specimen disposal and waste management

All consumables used during processing of COVID-related samples are immediately discarded into the original specimen bags, double-bagged and disposed into a biohazard waste bin. When testing is completed, all specimens are double-bagged with new biohazard bags and stored in a locked container for three days before disposal in the biohazard waste bin. When a suspect patient tests positive for COVID-19 from rRT-PCR of respiratory samples, the Haematology Laboratory is informed by the Molecular Microbiology Laboratory within the same laboratory service. Samples from the patient are retrieved and autoclaved the next working day before disposal. Work surfaces inside the BSC are disinfected with Mikrozid® AF wipes after each use. The Sysmex XN analyser is decontaminated daily with a proprietary 5% sodium hypochlorite solution (CellClean™) when performing daily maintenance and shutdown procedures. The Stago Compact Max analyser is decontaminated weekly with a higher strength of sodium hypochlorite (0.5%) using the routine maintenance protocol. Biological liquid waste from both analysers is decontaminated with 5% sodium hypochlorite for at least 30 min before disposal.

Discussion

The management of COVID-related specimens from paediatric patients poses unique challenges. Samples from children need to be collected in small-volume tubes, which may not always be suitable for automated analysis by analysers. Repeated blood sampling is also a challenge in young children, necessitating upfront discussion between clinicians and the laboratory haematologist as to the timing and availability of tests, so that repeated blood-taking procedures are minimized. It is now recognized that children with COVID-19 infections present with less severe symptoms compared to adults, with a case series of more than 2 000 children reporting that 13% of confirmed cases were asymptomatic.7 A six-month-old infant with confirmed COVID-19 infection identified through family screening treated at our institution remained asymptomatic despite detectable viraemia.8 Compared to adults, clinical identification of potential COVID-19-infected children may not be as obvious, adding to the risk of laboratory staff unknowingly handling a blood sample with SARS-CoV-2. This is why we have reiterated to staff in our laboratory on the need to adhere to standard precautions for all samples, including the use of PPE such as laboratory coats, surgical masks, gloves and fastidious attention to hand hygiene. In this letter, we have shared our experience and challenges with management of COVID-19-related blood specimens from paediatric patients, and hope that this can serve as a guide for other laboratories who need to handle similar specimens.

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References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395:497–506.
2. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. J Am Med Assoc. 2020. https://doi.org/10.1001/jama.2020.5766
3. Wong JEL, Leo YS, Tan CC. COVID-19 in Singapore – Current experience. Critical global issues that require attention and action. J Am Med Assoc. 2020;323:1243–4.
4. Ministry of Health, Singapore. Interim biosafety guidelines for laboratories and personnel handling samples or materials associated with the 2019 novel Coronavirus (2019-nCoV). 2020. https://www.moh.gov.sg/docs/librariesprovider7/biosafety-faq/moh-cir-no-17_2020_24jan20_interimbiosafetyguidelines2019_ncoov.pdf Accessed 21 March 2020.
5. World Health Organization (WHO) (12 February 2020) Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV) https://www.who.int/docs/defaultsource/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2 [Accessed 21 March 2020].
6. Centres for Disease Control and Prevention (CDC) Interim Laboratory Biosafety Guidelines for Handling and Preparing Specimens Associated with Coronavirus Disease 2019 (COVID-19); 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/biosafety-guidelines.html Accessed: 13 April 2020.
COVID-19 infection in patients with sickle cell disease

Severe acute respiratory syndrome coronavirus 2, also known as COVID-19, has spread to 184 countries, with almost 1.5 million cases (as of mid-April 2020) since first reported.1 The clinical features of this disease are not completely understood; however, severe illness is thought to occur predominantly in adults with advanced age and those with underlying comorbidities.2 Sickle cell disease (SCD), an immunocompromised condition, puts patients at higher risk for respiratory infections and subsequent pulmonary complications such as acute chest syndrome (ACS).3 Here, we present a case series of four SCD patients who were found to be positive for COVID-19 and describe our approach to management.

Case 1
A 32-year-old male, haemoglobin (Hb) SS, history of recurrent vaso-occlusive crises (VOC), ACS and chronic lower extremity ulcers presented to the emergency department (ED) with a typical VOC. He was afebrile and his pulse oximetry was 97% in room air. After admission, he was treated with intravenous (IV) morphine and fluids. He had a fever of 38.5°C on the second day of hospitalisation. A nasopharyngeal swab ordered because of a dry cough and a sore throat was COVID-19 positive, and a chest x-ray (CXR) that day showed plate-like atelectasis above the left lower lobe, suggesting developing pneumonia. He was treated with ceftriaxone (later changed to piperacillin-tazobactam) and azithromycin for seven days, along with hydroxychloroquine (200 mg twice daily) throughout the hospital stay. Starting the second day of this hospitalisation, supplementary oxygen via nasal cannula was initiated at two litres per minute after the oxygen saturation declined to 88% in room air, increased to four litres per minute later on and subsequently was intubated due to increasing oxygen requirements in the intensive care unit (ICU). After receiving a simple transfusion (1 unit) followed by an exchange blood transfusion in the ICU, his condition started to improve. He was extubated after 4 days and discharged home after 13 days of hospitalisation (Table 1).

Case 2
A 37-year-old female, HbSβ++, with a history of ACS, frequent VOC and venous thromboembolism presented to the ED with typical VOC pain. She was afebrile in the ED with a negative CXR after complaining of subjective fevers at home. After admission, she was treated with IV morphine and fluids, but a nasopharyngeal swab ordered due to upper respiratory symptoms (nasal congestion) was positive for COVID-19. During the hospitalisation, her pain gradually improved. She remained afebrile and did not require any supplementary oxygen. Her laboratory values were stable, with the exception of her white blood cell (WBC) count declining to a nadir of 2.3 × 10⁹/µl. She has discharged home after 8 days.

Case 3
A 22-year-old female, HbSS, with a history of ACS, frequent VOC and asthma presented to the ED with severe pain and nausea, vomiting and diarrhoea for one day. She was afebrile in ED, but had one episode of fever to 39.25°C after admission. The COVID-19 test, ordered due to the gastrointestinal symptoms, was positive. She was treated with IV morphine and started on ceftriaxone for suspected ACS due to the fever episode, although she had stable oxygen saturations (>95%). She defervesced on the second day of hospitalisation, her pain gradually improved and she was discharged after 2 days of stay.

Case 4
A 41-year-old male, HbSC, with history of bilateral hip avascular necrosis (AVN) and a pulmonary embolism (PE) presented to the ED with worsening hip pain. He developed a cough and dyspnea one week earlier and was diagnosed with COVID-19 infection at another institution, but left against medical advice. After admission he was treated with IV morphine and fluids for a VOC. Throughout this hospitalisation, he was afebrile and did not develop any respiratory symptoms. His pain continued to improve and he was discharged after 4 days.

It is reported that the COVID-19 infection disproportionately affects more African Americans than other ancestries,4 which may impose a higher risk in patients with SCD, especially considering the compromised immune system of this patient population. We tested 14 SCD patients for COVID-19 and three tested positive at our institution. Despite different SCD genotypes, the four patients all have history of

7. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. Pediatrics. 2020. https://doi.org/10.1542/peds.2020-0702
8. Kam KQ, Yung CF, Lin RTP, Mak TM, Maiwald M, Li J, et al. A well infant with Coronavirus disease 2019 (COVID-19) with high viral load. Clin Infect Dis. 2020. https://doi.org/10.1093/cid/ciaa201