Clinical Course of COVID-19 and Cycle Threshold in Patients with Haematological Neoplasms

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Abstract: The SARS-CoV-2 viral load in a respiratory sample can be inversely quantified using the cycle threshold (Ct), defined as the number of amplification cycles required to detect the viral genome in a quantitative PCR assay using reverse transcriptase (RT-qPCR). It may be classified as high (Ct < 25), intermediate (25–30) and low (Ct > 30). We describe the clinical course of 3 patients with haematological neoplasms who contracted COVID-19. None of them had been vaccinated. Firstly, a 22-year-old male with a refractory acute lymphoblastic leukaemia experienced an oligosymptomatic COVID-19 and had a Ct of 23 with an ascending curve. Another male, aged 23, had recently begun treatment for a promyelocytic leukaemia. He had a subacute course with high oxygen requirements. His Ct dropped from 28, when he only experienced fever, to 14.8, during the most critical period and on the edge of ventilatory support. Viral clearance was documented 126 days after the beginning of the symptoms. Finally, a 60-year-old male had received rituximab as maintenance

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therapy for a follicular lymphoma 3 months before contracting COVID-19. He had a fulminant course and required mechanical ventilation a few days later. We highlight the association between the course of CoViD-19 and the Ct. Viral shedding was longer than in immunocompetent hosts.

Introduction

SARS-COV-2 viral load in a respiratory sample can be inversely quantified using the cycle threshold (Ct). In a quantitative reverse-transcription polymerase chain reaction test (RT-qPCR), the Ct is understood as the number of amplification samples required to detect the viral genome above a colorimetric threshold (fluorescence). Therefore, the lowest number of cycles correspond to the highest viral load, and vice versa. A standard RT-qPCR executes a maximum of 40 cycles (Engelmann et al., 2021).

Even though the evidence gathered so far is non-conclusive, the Ct values from nasal swabs seem to correlate with the risk of death from the disease caused by the novel coronavirus SARS-COV-2, COVID-19 (Faíco-Filho et al., 2020), its clinical evolution (Rabaan et al., 2021), and the viral infectivity (La Scola et al., 2020).

We hereby describe the clinical evolution of 3 patients with previous haematological neoplasms, in different stages, who contracted, COVID-19 and correlated it with Ct values of their nasal swabs.

Case report

Case 1

A 22-year-old male, carrier of a refractory high-risk acute lymphoblastic leukaemia after two different treatments, had been discharged until bridge therapy for haematopoietic stem cell transplantation became available. In December 2020, at home, he experienced fever and odynophagia. He was diagnosed with mild COVID-19 with a PCR in nasal swab in a different medical centre, which is why we were not able to establish the initial Ct. He did not experience any other symptoms and there were no radiologic findings compatible with pneumonia. His symptoms lasted about 5 days.

20 days later, he was re-admitted to receive blinatumomab, a T-cell bispecific engager monoclonal antibody (BiTE) that acts binding T-cells and leukemic cells that express CD19, promoting the destruction of the latter (Einsele et al., 2020). He was asymptomatic and in excellent general conditions. The chest X-ray was normal. The absolute neutrophil and lymphocyte counts were 1,700 and 1,510/μl respectively. The remainder of the laboratory exam was unremarkable. He did not receive any specific treatment for COVID-19. In accordance with the sanitary protocols valid at that time, we obtained a follow-up RT-qPCR which was detectable with a Ct of 23. He received the drug with no complications.

A RT-qPCR performed a week later reported a Ct of 33. 7 days afterwards, 34 after symptom onset, viral clearance was documented with a negative test.

Case 2

A 23-year-old male with no past medical records was admitted for a new-onset, high-risk acute promyelocytic leukaemia in April 2021. This was his laboratory exam on admission: Haematocrit = 35%, haemoglobin = 12 g/dl, leukocytes = 43,870/µl (neutrophils = 21,935/µl, lymphocytes = 3,850/µl), platelets = 32,000/µl, prothrombin time = 56%, aPTT – activated partial thromboplastin time = 42 seconds, fibrinogen = 73 mg/dl. He started to receive the specific treatment with all-trans retinoic acid (ATRA), and, on the fifth day, the first infusion of idarubicin (AIDA protocol). 24 hours later he presented with fever. He had a positive RT-qPCR with an initial Ct of 28, and chest computed tomography (CCT) reported subtle sub-pleural ground-glass opacities compatible with viral infection (Figure 1). He had had no need for supplementary oxygen at first. Apart from transfusion support, he received convalescent plasma, in accordance with our institutional protocol at that time. He continued treatment for his leukaemia.

On day 19 from symptom onset, he began to require supplementary oxygen, first with a low-flow nasal cannula. He began to receive a 10-day course of dexamethasone 6 mg qd at this point. Ct on a follow-up nasal swab had dropped to 14.8. The most critical moment, from the respiratory point of view, was observed on the 30th day since symptom onset. He needed oxygen delivered through a high-flow non-rebreather mask. His hemogram reported a total leukocyte count of 1,080/µl, 820/µl neutrophils, and 100 lymphocytes/µl. The CCT demonstrated progression of the lung infiltrates (Figure 2), which coincided with the Ct nadir (13.8).

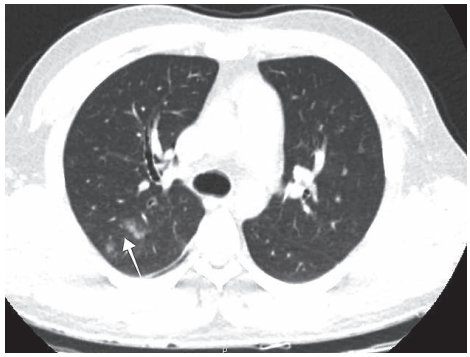

Figure 1 – Patient 2: Chest computed tomography on admission. Mild ground-glass opacities are observed (white arrow). The patient had no supplementary oxygen requirement. Ct (cycle threshold) was 28.

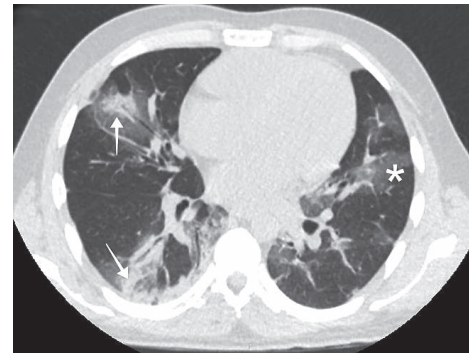

Figure 2 – Patient 2: Chest computed tomography on Ct (cycle threshold) nadir (13.8), on 30th day since symptom onset. Remarkable progression of lung ground-glass opacities (asterisk), with areas of parenchymatous consolidation (white arrows). The patient required oxygen through a non-rebreather mask.

He continued to need oxygen supplementation, regressively after 3 days with the maximum supply, for a total of 17 days. 71 days after symptom onset, RT-qPCR was still positive, but Ct was Ct. Viral clearance was confirmed on day 126 after symptom onset (approximately 4 months). He had no respiratory sequelae.

Case 3

A 60-year-old male had been successfully treated for a grade-IV follicular lymphoma with 6 cycles of R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), and, after 6 months, he had a negative PET (positron emission tomography)/computed tomography. He had received the most recent maintenance treatment with rituximab 3 months before the hospital admission presented.

He consulted for a 7-day history of cough and myalgia, and fever on the day he attended to the hospital. He was febrile, hemodynamically stable and required oxygen administered by a low-flow nasal cannula. The most remarkable laboratory results were leukopenia (3,020/μl, with 2,310 neutrophils/μl and 870 lymphocytes/μl) and renal failure (creatinine clearance = 31 ml/min/1.72 m^2). The CCT showed peripheral ground-glass opacities with predominance of both lung bases and superior lobes (Figure 3). The RT-qPCR for SARS-COV-2 genome was positive with an initial Ct of 10. Dexamethasone 6 mg qd was initiated. The patient progressively required greater oxygen supplementation. A follow-up CCT obtained a week later demonstrated radiologic progression of the bilateral ground-glass opacities with sub-pleural predominance. 20 days after symptom onset he required

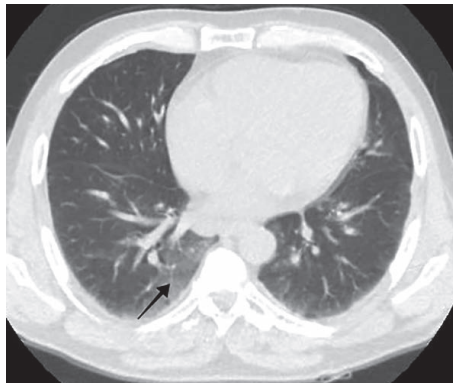

Figure 3 – Patient 3: Chest computed tomography on admission (day 7 since symptom onset), when he needed oxygen through low-flow nasal cannula. Ct (cycle threshold) was 10. Subpleural ground-glass opacities with basal predominance (black arrow).

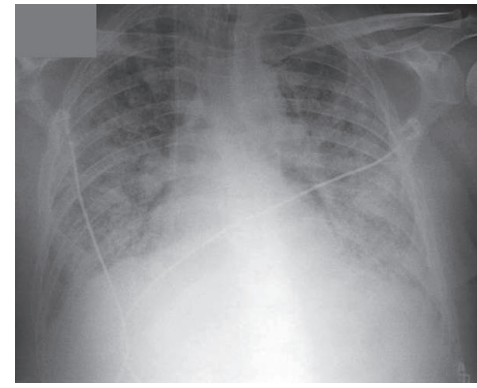

Figure 4 – Patient 3: Chest X-ray which shows near complete involvement of lung parenchyma by patchy infiltrates. This film was obtained the day before he died.

mechanical ventilation and died on the 40th day. Figure 4 shows multiple bilateral opacities on X-ray obtained the day before death.

It is important to highlight that none of these patients had been vaccinated for COVID-19.

Discussion

Patients with leukaemias, lymphomas and myelomas are the most susceptible to severe forms of COVID-19, and have a higher mortality rate than the general population and even higher than other oncologic patients with solid tumours (Lee et al., 2020). The American Society of Hematology reported a 28% mortality rate in a registry of 250 patients with haematological neoplasms (Wood et al., 2020). Local data gathered in Argentina concluded a 20.8% mortality rate (Basquiera et al., 2021).

One multicentric and retrospective study developed in New York (Westblade et al., 2020) included patients with and without cancer and classified the SARS-COV-2 viral load, according to the Ct value, in high (Ct < 25), intermediate (25–30) and low (Ct < 30). Moreover, *in vitro*, it has not been possible to achieve viral transfection using respiratory samples with Ct > 34 inoculated to Vero E6 cells, suggesting that patients with this Ct value or higher are no longer contagious (La Scola et al., 2020). Viral shedding in immunocompromised patients tends to be longer than in the general population. It has been reported as long as 151 days after symptom onset (Choi et al., 2020).

In case 3, with a catastrophic end, it is of supreme importance to remember that rituximab, an anti-CD20 monoclonal antibody, causes a prolonged B-cell depletion, secondary hypogammaglobulinemia, and the consequent infectious complications. There has been a reasonable concern for patients who require this treatment during the COVID-19 pandemic. A small, retrospective study of 49 patients who had received this drug, for any medical reason, and contracted COVID-19, reported that 63.2% had to be admitted, 24.5% required intensive care, and 32.7% died (Levavi et al., 2021).

The interpretation of Ct has its limitations since several factors may influence the result (Rabaan et al., 2021). They have been classified as pre-analytic (collection technique, type of specimen, sampling time and viral kinetics), analytic (internal control, type of RT-qPCR, purity of reagents, pipetting defects) and post-analytical (interpretation of results).

Even though Ct has not been formally and universally validated for routinary use (IDSA, 2021), it could be useful in individual cases when certain clinical decisions must be made. In the city of Buenos Aires, Argentina, the local Ministry of Health stated that, for immunocompromised patients with COVID-19, isolation may be safely ended when, after 21 days from symptom onset, Ct value is greater than 35, provided the test is positive (Buenos Aires Ciudad – Gobierno de la Ciudad Autónoma de Buenos Aires, 2021).

Last but not least, it is noteworthy that Ct and viral load are not the only laboratory determinations that can be used as clinical prediction tools in COVID-19. Two SARS-CoV-2 structural proteins elicit the generation of antibodies: nucleocapsid protein (N) and spike glycoprotein (S). The latter also functions as a viral attachment and fusion protein, enabling virus binding and entry via de angiotensin-converting enzyme 2 (ACE-2) (Murrell et al., 2021). Both of them can be quantitatively measured in serum and have shown to correlate with disease severity and intensive care unit admission, although correlation was higher for S (Ogata et al., 2020). On the other hand, sensitivity of antigen tests varies depending on the viral load, as was reported by Ford et al. (2021). A sensitivity greater than 90% was achieved only with samples with Ct values lower than 29 cycles (Ford et al., 2021). This singularity place quantitative antigen tests at a disadvantage for a comprehensive course predictive tool.

Conclusion

We have presented 3 patients with haematologic neoplasms who contracted COVID-19 and had different clinical courses, ranging from a practically eventless disease to a fulminating pneumonia, whose Ct values accompanied the disease development.

We could not avoid mentioning that our study is a limited case series of the most representative patients we have encountered since the beginning of the COVID-19 pandemic, rather than original research from which definitive conclusions could be derived.

In accordance with other authors' experience, we believe that Ct is a potentially useful tool which could guide therapeutic decisions and monitor the course of COVID-19, especially in patients with haematologic neoplasms.

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