



Detection of the genetic material of SARS-CoV-2 coronavirus and recurrence of infection symptoms in patients after a previous double negative result by RT-qPCR

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Abstract

Introduction. For over a year, the entire world has been struggling with the COVID-19 pandemic caused by the SARS-CoV-2 coronavirus. The gold diagnostic standard is the detection of specific fragments of the virus genome using molecular biology techniques (RT-qPCR) performed during the most intensive replication, i.e. within 10 days of the onset of infection symptoms. The time needed to eliminate the virus from the system has not been precisely defined. Obtaining reactive results of RT-qPCR tests in people recognized as convalescent after the previous negative result of the molecular test is becoming an increasingly common problem.

Case report. We analyzed the case of a patient who had been discharged home without clinical symptoms, after obtaining two negative RT-qPCR results, which took place 23 days after confirming his infection. After 6 days from the date of discharge, the patient returned with symptoms typical of COVID-19 and obtained a positive RT-qPCR test result.

Conclusions. There may be many reasons for obtaining a reactive test result for the presence of coronavirus in the material from the body of a person considered to be convalescent. Clinical data is still being collected and research is ongoing. In addition to false-positive or false-negative results of the tests performed, it is assumed that the possible cause of this phenomenon may be the excretion of dead cells from the body that contain inactive virus particles, which may be a natural part of the healing process and is not infectious.

Key words

SARS-CoV-2, re-detectable positive, RT-qPCR, detection, recurrence, COVID-19

INTRODUCTION

The new SARS-CoV-2 coronavirus, belonging to the beta-coronavirus group, causes a set of clinical symptoms known as COVID-19. It has been almost a year since the first case of the infection was recorded in the city of Wuhan, located in Hubei Province, China. Subsequently, the disease spread around the world, prompting the World Health Organization to declare a pandemic state on 11 March 2020, which is still ongoing. In Poland, the first confirmed case of infection with the virus that causes COVID-19 appeared on 4 March 2020.

Common symptoms of infection are fever, cough and shortness of breath. The clinical spectrum of the disease caused by the new coronavirus is quite wide, ranging from asymptomatic cases, through cases with mild symptoms, to cases of severe pneumonia, often leading to the development of acute respiratory failure and ending in the patient's death.

In virological diagnostics of SARS-CoV-2 coronavirus infections, methods of molecular biology, antigen tests and serological tests are used. Molecular tests reveal the infection at the earliest, while tests detecting antigens and antibodies give reliable results later, which is associated with the occurrence

of the so-called serological window (10–14 days). According to the recommendations of the World Health Organization, the gold standard here is identification of the genetic material of the virus using the RT-qPCR method (polymerase chain reaction with real-time reverse transcription), based on the detection of specific fragments of coronavirus genes in various samples from the body. The preferred material for testing is currently a nasopharyngeal swab. The type of clinical material and the technique of its collection significantly affect the reliability of the obtained result [1,2]. The optimal time to collect the material and conduct a molecular test is the period of the highest replication of the virus in the respiratory epithel, which usually occurs on the 4th-7th day after the onset of clinical symptoms. Therefore, RT-qPCR is used especially in the early stages of diagnostics of cases suspected of infection [1]. When interpreting the result of the molecular examination, one should take into account the overall clinical picture and the results of additional tests of the patient, especially computed tomography of the chest, in which changes typical of COVID 19 can be visualized and often allows confirmation of the diagnosis, or suggests the suspicion of obtaining a false negative result of the RT-qPCR test performed [3]. Sometimes, the presence of virus particles can also be detected 1–3 days before the onset of clinical signs of infection. The time when the virus should be eliminated from the system has not been precisely defined [4].

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CASE REPORT

A 60-year-old patient was admitted to the Observation and Infection Department due to infection with the SARS-CoV-2 coronavirus confirmed by a molecular test. In the diagnosis, the Cobas test was used. Unfortunately we do not have information about which variant of SARS-CoV-2 was detected in the patient. So far, he has been treated for aplastic anaemia and schizofrenia. He received pharmacotherapy with prednisone, methylprednisolone and quetiapine.

On admission, the patient reported tiring non-productive cough and episodes of exercise dyspnea with a severity of grade 2 on the mMRC scale. Physical examination revealed a slight weakening of the alveolar murmur in the lower fields of both lungs, but without any significant deviations from the norm. The saturation level was 97%. Laboratory tests showed increased inflammatory marker – CRP 44,3 mg/l [0.00–5.00 mg/l] and features of pancytopenia – WBC 1.9 K/uL [4.00–10.00], RBC 2.61 M/uL [4.50–5.90], PLT 32 K/uL [140–440]. Based on the available medical data, the patient's condition at admission can be described as medium, with the probability of a severe course of the disease as high. Due to the overall clinical picture and the results of additional tests, as well as the lack of treatment options, the patient was transferred to a homonymous hospital. After improvement and stabilization of the patient's general condition, he was transferred back to the initial department. On the 13th day after confirmation of the infection in the patient, a swab from the nasopharynx was taken in order to perform a control test for the presence of the SARS-CoV-2 coronavirus genetic material, which was dictated by the Regulation of the Minister of Health, valid until 1 September 2020, according to which the condition for the termination of isolation of the patient was, among others, obtaining a non-reactive result of the molecular test twice. The result of the first examination was negative, while the presence of coronavirus RNA was detected in the sample of material collected after 24 hours, which obligated the extension of the hospitalization period despite the lack of clinical symptoms. Earlier, an immunochromatographic test was performed to detect antibodies against the SARS-CoV-2 coronavirus, confirming the presence of IgG immunoglobulins. Molecular examination of the swab from the nasopharynx was repeated. Negative results were obtained twice with an interval of 24 hours. The patient was discharged home in good general condition without clinical symptoms on day 23 from the diagnosis of coronavirus infection.

Then, on day 6 after the end of hospitalization, the patient returned to the Emergency Room due to a fever of up to 39°C and severe dyspnea that persisted for 4 days. Physical examination showed no abnormalities. There was also no decrease in saturation (which remained above 97%). On the other hand, laboratory tests revealed increased inflammatory marker – CRP 55.8 mg/l [0.00–5.00], deterioration of the parameters of the red blood cell system – RBC 3.48 K/uL [4.50–5.90], HGB 8.2g/dl [14.00–18.00], thrombocytopenia – PLT 35 K/uL [140–440] and features of liver damage – AST 46 IU/l [0–40], bilirubin 3.42 mg/dl [0.00–1,20], and a high level of D-Dimers – 8129.53 ng/mL [normal range <500]. Chest X-ray showed inflammatory and congestive changes in both lungs. Empirical antibiotic therapy was implemented (ceftriaxone).

During hospitalization, the patient's general condition deteriorated. A follow-up chest X-ray showed signs of

progression of inflammatory changes and the presence of fluid in the right pleural cavity. The diagnostics was extended to include chest tomography, which did not show changes in the lung tissue typical for COVID-19, and additionally the fluid in the peritoneal cavity within the scope of the examination was visualized. Due to the observed high values of D-Dimers – 7291 ng/mL (normal range <500) and dyspnea at rest, an angio-CT examination of the chest was performed which did not show thrombus in the pulmonary arteries. The patient underwent drainage of the pleural cavity in the Department of General Surgery and, due to increasing ascites, also drainage of the peritoneal cavity in order to evacuate the fluid, which improved the patient's general condition. The course of further hospitalization was uneventful.

During the hospitalizations, the patient did not receive antiviral drugs or convalescent plasma.

DISCUSSION

Redetection of the genetic material of the SARS-CoV-2 coronavirus in patients after the clinical symptoms of the infection have resolved, and the results of the RT-qPCR test have proved negative twice for samples collected at 24-hour intervals, are becoming an increasingly common clinical problem [5]. This is important as the number of people considered cured of COVID-19 is also constantly increasing. It is worth noting that it is not fully known whether in such cases there is a recurrence of the infection, or whether other causes of this condition should be sought for. This is also important from epidemiological point of view because the infectivity of these patients for the environment is not known [6].

Based on an analysis of the general scientific database conducted mainly in China in Ccoronavirus may affect between 2.4% – 69.2% of people after obtaining a previously negative result twice [2]. The mean time from discharge to re-determination of the presence of SARS-CoV-2 in upper respiratory secretions was 1–38 days [7].

In the patient described above, infectious symptoms in the form of fever up to 39°C and dyspnea appeared about 3 days after discharge from the Observation and Infectious Ward. The performed RT-qPCR test turned out to be reactive. At this point, it was necessary to consider possible causes. One of the initial hypotheses was the possibility of obtaining a false-positive result, which could be related to the patient's exposure to virus particles persistent on surfaces in his environment, or contamination of the material sample after its collection from the patient. In addition, a positive RT-qPCR test result may also be the result of cross-reactions with other human coronaviruses [7].

After discharge from hospital the patient remained at home and his sanitary and epidemiological situation is not known. It is also not known whether there were any infected people in his vicinity who could have spread the virus. In the case of this patient, however, the theory of a false-positive result is unlikely due to his clinical condition indicating the features of an active infection.

On the other hand, we can consider a situation in which the double negation of the results of molecular tests performed 24 hours apart before the end of hospitalization was false negative. The false-negative rate can range from 3–41% [2]. The risk of their occurrence may be related, *inter alia*,

to improper collection of material for testing, improper storage and further transport. In addition, the risk factors for obtaining false-negative results may also occur at the stage of the RT-qPCR test itself, which may include the presence of reaction inhibitors in the collected sample, inadequate process temperature or the use of contaminated laboratory reagents, also after their expiry date [1]. In the described patient, pre-laboratory errors resulting from the need to transport diagnostic material to a laboratory more than 80 km away can be considered. Such risk should be considered despite ensuring and following appropriate sample protection procedures.

The negative result was obtained on the 23rd day after confirming the infection using the RT-qPCR method which, with the additional complete resolution of the infection symptoms and the regression of changes in imaging tests and improvement of laboratory parameters, would also allow rejection of the hypothesis that the obtained negative result was false. In the available literature, there are reports that the genetic material of SARS-CoV-2 coronavirus remains in the body for a period much longer than the duration of clinical symptoms and abnormalities in additional tests. The fact is that the exact time when the virus should be completely eliminated, which is related to the lack of its detection with available tests, has not been clearly defined [4]. At this point, it should also be noted that an important aspect related to the diagnostic process of SARS-CoV-2 infection and important in the interpretation of the obtained result is also the type of test used during the examination. The accuracy of commercially available tests is assessed using the concept of sensitivity (positive percent agreement, PPA) and limit of detection (LOD)[1]. It is known that none of the tests available on the market reaches the level of 100%, neither in terms of sensitivity nor specificity [3]. A negative result of RT-qPCR in the case of an epidemiological history indicating contact with an infected person and the occurrence of symptoms typical of COVID-19, is not a diagnostically binding result, but requires verification by re-taking a swab and subjecting it to another molecular examination [8].

The clinical picture and the results of additional tests obtained during the end of the presented patient's first stay in hospital allowed consideration of the result of the molecular examination as reliable, and to give him the status of a recovered patient and discharge him home.

A group of scientists from the University of Coimbra, Portugal, conducted a retrospective study on a cohort of 210 patients, which revealed that the time from confirming the presence of viral genetic material to the first negative RT-qPCR test result was 7–46 days, on average, and was similar in both genders. At the same time, it was estimated that approximately 70% of patients waited more than 20 days to obtain the first negative molecular test result indicating the elimination of the virus from the body. In 30% of people, this period was over 40 days [9]. The reasons for the prolonged elimination of the virus from the infected person's body are unknown; it is assumed that the patient's disease is

The patient in the presented Case Report, due to an underlying disease – aplastic anaemia – for many years had been administered oral systemic steroid therapy using prednisone and methylprednisolone. It can therefore be suspected that this was the cause of the impaired elimination of the pathogen from the patient's body. Additionally, the patient's age played an important role because, according

to the observations so far, people over the age of 50 have a tendency to a prolonged presence of virus particles [11].

The features of an active infection with a rather severe course raise the question of the possibility of re-infection. It seems that this hypothesis requires the rejection of the time criterion, as the symptoms appeared within a fairly short period of time. In addition, it is worth noting that on the 13th day after confirmation of infection with the coronavirus causing COVID-19, an immunochromatographic test for antibodies against SARS-CoV-2 was performed, which confirmed the presence of IgG immunoglobulins, confirming that the infection has passed and suggesting that the patient should acquire immunity to infection with this pathogen. As the test used was a qualitative test, the level of antibodies produced is not known; therefore, it cannot be assessed whether it was sufficient to protect against another infection. Studies show that the peak production of immunoglobulins responsible for resistance to re-infection occurs 4 weeks after the onset of symptoms, while their level in the initial stage of the disease is rather low [11].

From the epidemiological point of view, it is important to determine the infectivity status of patients with a long period of virus elimination from the body and their detectability in molecular tests [9]. RT-qPCR testing of material collected from the nasopharyngeal cavity of patients, which is most commonly used in virological diagnostics, does not allow for differentiation between the detection of live viruses or only 'dead' fragments [12]. Cell cultures from samples taken from the body of such patients were negative, which proves that the positive molecular test result is due to the presence of an inactive form of the virus in their secretions, which reduces the likelihood of contagiousness in these people. In a study carried out in Korea involving 285 patients, no cases of infection were found after contact with people with positive RT-qPCR results [7].

There is also an assumption that prolonged elimination of the virus from the system as well as a positive result of the RT-qPCR test after negating the material sample may be carried out by the therapeutic process itself, which may cause a temporary inhibition of viral replication, which is manifested by the lack of detectability of its genome in molecular tests.

However, there is no complete elimination of pathogen particles that circulate in the body, and under favourable conditions, i.e. after the end of the treatment and the cessation of the effects of the pharmacological agents used, the virus replication reactivates and increases again [13]; hence, the re-test result becomes reactive. Although the presented patient did not receive antiviral treatment during hospitalization, he underwent chronic steroid therapy for underlying diseases, which was intensified during his first stay in hospital, which could have affected the course of infection and the diagnostic process.

Summing up, it is worth noting that the observation of patients considered convalescents, who again obtained a positive result of the molecular test for SARS-CoV-2 coronavirus infection, is very important. Clinical data and multicentre retrospective studies which will allow to accurately explain the pathophysiology of this phenomenon are still being collected. In the light of the collected data, excluding false-positive or false-negative results of the tests performed, the most probable cause is the presence of fragments of the virus particle in the body's cells, which are excreted, as shown by molecular tests giving a positive result.

Most likely, however, the virus is no longer able to infect other people from the environment, which is very important from the epidemiological point of view.

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