Three cases of SARS-CoV-2 reinfection in The Netherlands: no clear evidence for increased pathogenicity

5.1.2e[1], 5.1.2e[2,3], 5.1.2e[4], 5.1.2e[1], 5.1.2e[3], 5.1.2e[3], 5.1.2e[3], 5.1.2e[3], 5.1.2e[5] and 5.1.2e[3]

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Summary

Patients with a cleared viral infection are generally assumed to have a period of relative protection for reinfection. Recently, the first reinfections with SARS-CoV-2 were reported, all with a disease free interval of several months. Here, we present three cases of SARS-CoV-2 reinfection in which we show, in contrast to previous reports, that reinfections can occur as early as several weeks after the initial infection. This is the shortest reported period to date. Negative RT-PCR in between the two episodes and sequencing analyses showing different virus strains in the first and second episode, confirm a reinfection. Serological analyses

were possible for two cases and both lacked an adequate antibody response to the initial infection. In conclusion, our observations suggest that reinfections might occur primarily in patients who fail to mount a proper antibody response to the initial infection. The clinical presentation of the reinfection may be markedly different from the primary infection.

Introduction

In January 2020 SARS-CoV-2 coronavirus was identified as the causative agent in a cluster of viral pneumonia cases in the region of Wuhan, China, later referred to as COVID-19 [1,2]. The first SARS-CoV-2 infected patient in the Netherlands was identified on February 27 2020 and as at October 26 2020 a total of 301.597 cases have been notified to the Dutch authorities [3,4]. A natural infection or vaccination for certain viruses is known to result in long-lived or lifelong protection to (re)infection with said viruses. However, recent work investigating seasonal coronaviruses showed that antibody protection for these viruses is relatively short-lived and reinfections can occur as early as 6 months after infection [5]. It is unclear if the same phenomenon and antibody dynamics apply to SARS-CoV-2 viruses, but reports on sequencing confirmed reinfections have been published from Hong Kong, Belgium, The Netherlands, South-America and the USA [6–9]. The time between reported clinical episodes in these patients was 142, 93, 59, 72 and 48 days respectively. Here, we present three cases of SARS-CoV-2 reinfections describing clinical symptoms and laboratory investigations.

Case descriptions

Case 1

On April 1st 2020, a 82 year-old man living in a long-term care facility presented to an in-house geriatrician with fever (38,7 °C) and upper respiratory complaints. His medical history included type 2 diabetes, hypertension, chronic heart disease and obesity. He was placed in isolation and a combined nose-throat swab taken on April 1st tested positive for SARS-CoV-2 RNA by RT-PCR (Cycle-threshold value (Ct) 23) [10]. He was treated with amoxicillin/clavulanic acid 500/125 mg 3 times a day for 7 days for a suspected respiratory tract infection.

On April 14th the pulmonary symptoms resolved and he was discharged from isolation on April 20th. On June 9th, the patient presented with complaints of diarrhoea. On examination, blood pressure was 148/67 mm Hg, a heart rate 56 beats per minute and an oxygen saturation of 92% while breathing ambient air. A combined nose-throat swab tested positive for SARS-CoV-2 RNA (Ct 30). During this episode the patient did not develop a fever and oxygen saturation remained stable at 92%. No additional treatment was required, but an isolation period of 2 weeks was maintained according to local infection prevention guidelines. The ccomplaints of diarrhoea resolved after 10 days.

Case 2

On April 12th, 2020, a 79-year-old man was admitted to the hospital, after a collapse at home. His medical history consisted of chronic heart failure, hypertension, diabetes and chronic obstructive pulmonary disease (COPD). At presentation, he was mildly dyspnoeic with a temperature of 37.9°C, blood pressure 107/72 mm Hg, heart rate 216 beats per minute. An electrocardiography showed atrial fibrillation. He had a respiratory rate of 22 breaths per minute and an oxygen saturation of 95%, while breathing ambient air. Auscultation of the lungs revealed rhonchi and crepitations on the right basal side. Chest radiography and remainder of the physical examination were without abnormalities. Laboratory testing showed a CRP of 104 mg/L with normal leukocyte count. A combined nose-throat swab tested positive for SARS-CoV-2 RNA by RT-PCR (Ct 15.9). The patient received supplemental oxygen at the general ward and was discharged from hospital on April 20. On May 4th, the patient was readmitted due to progressive drowsiness, dyspnoea and diarrhoea. Chest radiography showed an infiltrate of the right lung. Laboratory investigations showed CRP 312 mg/l and elevated leukocytes of 12.3x10^9/l.

The patient had developed acute renal failure with a glomerular filtration rate (GFR) of 15 ml/min/1.73m2. He was admitted to the intensive care unit (ICU) on May 5th with a pneumonia and sepsis which required mechanical ventilation. Empiric antibiotic treatment was initiated with 1500 mg b.i.d. cefuroxime for five days and 200 mg b.i.d. ciprofloxacin for one day until exclusion of a *Legionella* infection. A combined nose-throat swab tested negative for SARS-CoV-2 RNA while a sputum sample taken on May 5 tested positive (Ct 27.2). A faecal sample tested positive for *C. difficile* toxin for which he received 1500mg metronidazole once a day for 10 days. Sputum, urine and blood cultures revealed no other pathogens. On May 7th the patient was transferred to the general ward. Sputum samples taken from May 9th onwards tested negative for SARS-CoV-2. The patient was discharged on May 15th, 2020.

Case 3

On April 14th, 2020 a 59-year-old man was admitted to the hospital with general complaints of fatigue, stomach ache, headache, muscle aches, chest pain and fever. He had no relevant medical history. In the days prior to admission he had become dyspnoeic. Laboratory investigations on admission were unremarkable. On examination, temperature was 39.2°C, blood pressure 139/100 mm Hg, heart rate 120 beats per minute and oxygen saturation 98% while breathing ambient air. Auscultation revealed rhonchi over both lungs and chest radiography revealed bilateral infiltrates. Laboratory investigations showed a CRP of 96 mg/L, thrombocytopenia (127 x10^6/ml) and lymphocytopenia (0.84 x10^6/ml). A combined nose-throat swab tested positive for SARS-CoV-2 RNA by RT-PCR (Ct 26.2). Empiric antibiotic treatment was initiated with intravenous ceftriaxone 2000mg per day. He was discharged from hospital on April 17th. On April 19 he was readmitted with progressive dyspnoea, nausea, vomiting and

fever. His temperature was 40.4°C, blood pressure 158/102 mm Hg, heart rate 106 beats per minute and oxygen saturation 87% breathing ambient air. Chest radiography revealed severe progression of infiltrates in both lungs. A combined nose-throat swab tested negative for SARS-CoV-2. Empiric antibiotic treatment consisted of ceftriaxone 2000mg q.d. and chloroquine (loading dose of 600 mg b.i.d. on the first day, followed by 300mg b.i.d for four days) as experimental treatment for COVID-19 pneumonia. Within 24 hours after admission the patient was transferred to the ICU for mechanical ventilation. A chest CT-scan revealed several pulmonary embolisms and patchy ground-glass opacities. A bronchoalveolar lavage taken on April 26th was positive for SARS-CoV-2 RNA (Ct 26.4). A follow-up sputum sample obtained on May 4 tested negative for SARS-CoV-2 RNA. Further ICU treatment was complicated by invasive pulmonary aspergillosis and the patient was discharged from the ICU after 50 days.

Sequencing

The full genomes of the SARS-CoV-2 strains present at the first (T1) and second (T2) episode for each of the three cases were determined by next generation sequencing (see supplemental materials for technical details). (Fig. 1, 2). The period between T1 and T2 sampling was 67, 22 and 12 days for case 1, 2 and 3 respectively. We observed 8, 7 and 5 nucleotide differences between strains for case 1, 2 and 3 respectively (supplemental data). Based on the commonly assumed mutation rate of 2 nucleotide substitutions per month, these differences exceed the number of expected differences assuming within-host evolution, suggestive for two independent infections [11]. Pangolin lineage determination [12] showed that in cases 2 and 3 reinfection occurred with a SARS-CoV-2 strain from a different genetic lineage further supporting a case of reinfection and makes prolonged shedding unlikely (Fig. 2). When compared to

all publicly available Dutch GISAID sequences, the T1 and T2 sequences did not cluster together for all three cases but were interspaced by multiple contemporary sequences, again supporting reinfection. (see supplemental figure 1).

Serology

Multiple sera of cases 2 and 3 (Fig. 1; Table 1) were analysed for the presence of a) total Ig directed at SARS-CoV-2 RBD (Wantai ELISA, Sanbio BV, Uden, The Netherlands); b) IgG directed at both the monomeric and trimeric SARS-CoV-2 S and N-proteins (in-house multiplex microarray [13]) and c) neutralizing antibodies directed to SARS-CoV-2 [14].

For case 2, sera were available from hospital admission of the first and second episode as well as a third serum taken 3 days after admission during the second episode. The T1 serum tested negative for total Ig SARS-CoV-2 as well as virus neutralization, while both T2 sera tested borderline positive at the cut-off with a ratio of 1,03 and 1,00 respectively. Seroconversion from T1 to T2 was observed for SARS-CoV-2 S-trimer IgG while no sero-responses were observed for monomeric S or the N-protein in both disease episodes. SARS-CoV-2 neutralizing antibodies were detected in both T2 sera (reciprocal titres of 1:15 and 1:30) but not the T1 serum.

For case 3, sera were available over the course of the two episodes. The first sample taken at hospital admission during the first episode tested negative in the Wantai total Ig SARS-CoV-2 test as well as virus neutralization test. T2 serum taken after readmission to the hospital 5 days later, tested positive for total Ig SARS-CoV-2, with a ratio of 5,33 with still a negative virus neutralization test. Two additional sera taken 8 and 14 days after the initial hospital admission, during the second episode, showed strong positive results (ratio's 20,64 and

24,38) for total Ig SARS-CoV-2 with strong increases in virus neutralization titres (1:60 and 1:480 respectively). In addition, we observed a seroconversion to the SARS-CoV-2 S-trimer, S monomer as well as the N.

Discussion

There is broad interest in the phenomenon of reinfections as they can provide insight in the duration of protection after natural infection or vaccination. By definition, reinfection means a person was infected once, recovered, and then later became infected again. The period in between the two episodes is ideally characterised by a symptom-free period and/or a negative RT-PCR. Although the symptom-free period and negative RT-PCR indicate virus clearance, persistent shedding instead of a second infection cannot always be excluded due to detection limits. Therefore, sequencing of the virus genome of the first and second episode can provide valuable insights into the discrimination between persisting shedding of the initial infection versus an independent reinfection with an alternative virus strain.

Here, we described three confirmed reinfection cases, presenting with different clinical patterns. These case reports add to rapidly growing evidence of COVID-19 reinfection. Reinfection case 1 presented with a relatively long symptom free period, comparable to previous reports describing the first occurrences of SARS-CoV-2 reinfections [7–9]. However, only a short symptom-free period was reported for case 2 and 3, i.e. 22 and 12 days between detection of the two different virus strains respectively, with only 14 and 2 days between hospitalizations respectively. As both cases 2 and 3 had a negative PCR in the symptom-free episode, combined with the detection of a different SARS-CoV-2

lineage for T1 versus T2, it is likely that these cases indeed represent two independent infections acquired at different time points. Although it cannot be excluded that the initial infection occurred with two different virus strains (often referred to as co-infection), within our sequencing data we did not find any evidence of a mixed infection. This makes a dual infection highly unlikely in both cases, suggesting a second infection later in time, likely after the first infection was (mostly) cleared.

With regard to the clinical manifestation of the primary SARS-CoV-2 infections versus the secondary SARS-CoV-2 infections, we observed no consistent pattern. For case 1, the symptoms in both disease episodes were mild, with coughing in T1 and diarrhoea in T2 while no hospitalization was required. Cases 2 and 3 needed hospitalization in both disease episodes with an ICU admittance for mechanical ventilation in the second episode. Case 3, became RT-PCR negative in the second disease episode relatively quick compared to the length of hospital stay. Therefore, it remains unclear whether his extended hospitalization was due to the second SARS-CoV-2 infection or due to a disrupted immune response, illustrated by the occurrence of a pulmonary aspergillosis superinfection.

With the limited number of known reinfection cases, it is currently unclear at what time after the initial infection a reinfection could occur and what the role of protective antibodies prior to the second episode is or to what extent that depends on the individual patients underlying conditions. Current data suggest that antibodies to SARS-CoV-2 are induced to peak levels within weeks of infection, but there are variable data on how quickly they wane over time making individuals susceptible again. However, due to missing data it is not known whether reinfection cannot occur within first period post-illness due to

antibody protection in case of proper seroconversion and to what extent waning immunity increases the chance of reinfection. More detailed studies, following subjects over time, including serology are needed to address this question.

In addition to waning immunity, reinfections might occur because of an inadequate immune response after first infection. It has been suggested that patients with an asymptomatic or mild SARS-CoV-2 infection have a weaker immune response since their antibody titers are significantly lower than in patients with pneumonia [15]. An estimated 20% of infected individuals does not seroconvert to a detectable level after infection [16]. Immune compromising conditions could also diminish adaptive immune responses, possibly increasing the risk for reinfection. Most of the reinfection cases described to date, including the cases reported herein, had no known immune deficiencies [7–9]. However, in described reinfection cases there is often no serological data from between the first and second infection. Therefore, a correlation between presence of protective antibodies (or lack thereof) and reinfection is not possible. In the two cases described here for which serological analyses were possible, it seems likely that these patients did not mount an adequate antibody response (yet) to the initial infection as evidenced in the absent response in the serological assays. This could explain the susceptibility for a reinfection.

In general, reinfection cases are being picked up because of symptoms and are therefore biased towards detection of symptomatic cases. We are probably underestimating the number of mild or even asymptomatic reinfections in which immune responses against the first episode prevent from severe disease. The role of such mild cases in transmission needs to be explored, although a low viral

load combined with presence of antibodies during the second episode has been proposed to prevent transmission [17].

Prospective studies investigating the frequency of reinfections, the role of (waning) immunity and the clinical manifestations of the first and second episode should provide better insights into protection against reinfection and possible risk factors.

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