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Clinical and laboratory analysis of two B.1.617.2 (Delta) SARS-CoV-2 variant outbreaks in the port of Antwerp

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Abstract

The B.1.617.2 SARS-CoV-2 (WHO Delta) variant, first detected in India, has shown a rapid global spread due to its high transmissibility. This lineage is currently the third most frequent variant in Belgium and has the potential to further increase in accordance with the replacement phenomenon observed in the UK. In May 2021 two ships that had recently arrived in the Port of Antwerp reported crew members with COVID-like symptoms. SARS-CoV-2 RNA was detected in nasopharyngeal swabs in 30 out of 45 skippers and the B.1.617.2 variant was identified via sequencing. Crew members were quarantined and repeatedly tested to assess the evolution of the cycle threshold (CT) values in the SARS-CoV-2 PCR. Their clinical condition was closely monitored using a digital monitoring tool. Despite being in good health and relatively young age, 11 crew members (36,6%) required hospitalization, with CT values of SARS-CoV-2 RNA being a good predictive factor for hospitalization. Furthermore, based on the CT values, our data indicate that patients infected with the Delta variant remain infectious for a longer period compared to the Alpha variant. This could mean that the currently recommended isolation and/or quarantine period might be too short for this particular variant and might need revision.

Introduction

Since the beginning of the SARS-CoV-2 pandemic in 2019 several variants have emerged worldwide of which 4 are currently classified as variants of concern (VOC) by the WHO. [1] Pango lineage B.1.617.2 was added to the VOC list most recently and was first detected in India in **December** 2020, differing itself from the other Indian variant or Kappa variant (Pango lineage B.1.617.1), by its T487K mutation and lack of E484Q mutation [2]. By late February 2021 the B.1.617.2 lineage had spread globally, being first detected in the UK on February 22nd, followed by the USA on February 23rd, and Singapore on February 26th. UK sequencing data indicate that B.1.617.2 is quickly outcompeting both its related subtypes and the previously dominant British variant, as preliminary data suggest that the B.1.617.2 lineage might be 50% more transmissible than B.1.1.7 [3-4]. **Latest data also suggests significantly increased risk of hospitalization within 14 days of specimen date for the B.1.617.2 lineage in comparison to the British variant (HR 2.61, 95% CI 1.56-4.36, p<0.001) [5].**

On April 30th an alarming message was send to the Antwerp health inspector from a chemical tanker in the Port of Antwerp, stating several crew members were ill and feverish. The vessel had left the United States mid-March, made one stop in France **mid**-April before arriving in Antwerp **one week later**. For 17 out of 23 tested crew members SARS-CoV-2 RNA could be detected in nasopharyngeal swabs **at day 1**, **and 3 more became positive during their quarantine**.

Source investigation revealed that 6 new crew members came aboard in France, 5 of which came directly from India by plane. These 5 sailors were tested negative by PCR in India prior to boarding. Upon arrival in France, no additional quarantine or PCR test was performed. Three crew members had already developed symptoms when boarding the chemical tanker.

On May 11th, a second vessel that had arrived in the Port of Antwerp reported that their cook was experiencing COVID-like symptoms. All 22 crew members were tested and in 8 nasopharyngeal swabs SARS-CoV-2 RNA could be detected **at day 1, 2 more became positive during their quarantine**. After further investigation, it appeared that on the 6th of May almost an entire new crew boarded the vessel in Bremerhaven. The majority of that crew, including the index patient, recently resided in India.

Further testing on the RNA of the samples from the crew members of both vessels revealed the presence of the L452R and P681R mutation (in the absence of delH69-V70, N501Y and E484K/Q) suggesting a B.1.617.2 lineage, making these the first cases of the B.1.617.2 (Delta) variant in Antwerp, which was confirmed by sequencing afterwards.

In this outbreak report we describe the trends in CT values over time by repeated nasopharyngeal swabbing combined with intensive daily clinical follow up of the sailors infected with the B.1.617.2 variant. Our data are of added value to the characterization of this emerging VOC as longer transmissibility is suspected and therefore might contribute to the discussion on current quarantine and/or isolation periods and testing strategy.

Methods:

Study design and population

This study is based on a cross-sectional study design, including **30** crew members from 2 different vessels, both arriving in the Port of Antwerp in May 2021. They were followed during their stay in Belgium over a time period of about 4 weeks. **Upon arrival in the Port of Antwerp, crew members were either put in isolation or quarantine depending on their PCR result. All were given a separate room, with daily follow-up.** Demographics of the study population are summarized in table 1.

Diagnostic techniques

Nasopharyngeal swabs were collected by the mobile testing team of the Antwerp University Hospital (coBUSters) in RNA/DNA shield solution (Zymo, Diagenode) and extracted using the Diagenode extraction kit on KingFisher (ThermoFisher). Amplification was performed with the Taqpath COVID-19 assay (ThermoFisher) on the QuantStudio real-time PCR system, detecting the ORF1ab, N and S gene. Based on the results of this assay, reflex PCRs were performed on the RNA extracts using VirSNip primer/probe reagents (TIB MOLBIOL) for detection of delH69/V70, N501Y, E484K, H655Y, L452R and P681R which are key deletions/mutations in the characterization of the current VOCS.

SARS-CoV-2 whole genome sequencing was performed on RNA extracts (Thermofisher extraction protocol on KingFisher) using the COVIDSeq test (Illumina) on NextSeq, according to the manufacturer's instructions. All diagnostic techniques were performed at the Antwerp COVID testing platform (real-time PCR) and at the clinical laboratory of Antwerp University Hospital (reflex PCR and sequencing).

Viral shedding

To evaluate the duration of viral RNA shedding, a nasopharyngeal swab was taken every few days from the crew members. The 23 crew members of the first vessel were tested 5 times: day 1, day 8, day 12, day 18 and day 24. For the second vessel 22 sailors were tested on 4 days: day 1, day 7, day 13 and 17. For the SARS-CoV-2 RNA positive samples, the CT values of the three different gene targets (ORF1ab, N1 and S) were documented. Low CT values or high viral loads in nasopharyngeal swabs are associated with a greater risk of transmission. [6] If a CT value increased above 27, **and no symptoms or elevated temperature were present** the patient was not tested further. In addition, **samples were taken at day 7 on vessel 2 for viral culture and** were send to the Belgian COVID national reference laboratory (UZ Leuven). For these specific samples, swabs were not collected in RNA/DNA shield solution but in Universal Transport Medium (UTM, Copan). To evaluate if the B1.617.2 variant might be more transmissible than the

B.1.1.7, the dataset **of the PCR-positive crewmembers (n=30)** was compared to a dataset from January 2021 containing PCR results of 22 patients infected with the B.1.1.7 variant of which a retest **at day 8** was performed. To analyze differences in delta CT values between the first test and retest for both variants a Mann Whitney test was performed using **MedCalc v17.5.5 software**.

Clinical symptoms and hospitalization risk

Besides regular testing, the health condition of all crew members was monitored using Telecovid. Telecovid is a digital tool, created by the Antwerp University Hospital (UZA) and partners, to closely observe non-hospitalized SARS-CoV-2 patients during their illness and recovery. Using this tool, our subjects were asked to complete a daily survey rating the presence and severity of symptoms like muscle pain, headache, coughing and other symptoms. In addition, their heart rate and oxygen saturation were measured **several times a day** using a medical grade oximeter which was connected to their cell phone (Bluetooth connection). Through Telecovid a large amount of information regarding the clinical evolution of all crew members in isolation could be collected, although the completion of the surveys was hampered by linguistic barrier. For those who needed hospitalization, data from the electronic patient files was collected.

Results:

Study population

With the exception of one woman, all subjects of the B.1.617.2 groups were men with ages ranging between 19 and 59 years without any significant comorbidities.

The B.1.1.7 dataset was also gathered from clinical samples of the SARS-CoV-2 testing platform of Antwerp. These were the first B.1.1.7 (Alfa) variants detected in Antwerp in January 2021.

CT values at day 1

In the nasopharyngeal swabs of 17 of the 23 crew members of the first vessel SARS-CoV-2 RNA could be detected. For the second vessel 8 out of 22 crew members were tested positive for SARS-CoV-2. **An additional 5 crew member became positive during their quarantine period. The initial CT values of vessel 1, vessel 2 and the B.1.1.7-dataset can be found in table 2.** The average CT values (N-target) at day 1 were 15.6 [range 8-25] for vessel 1, 23.6 [range 19-34] for vessel 2 and 13.0 [range 8-21] for the British variant dataset. **Vessel 1 and the British variant dataset were both tested at the Antwerp COVID testing platform, vessel 2 was initially tested at the clinical lab of the University Hospital of Antwerp with a different PCR technique, which makes the evaluation in CT levels of vessel 2 not possible.**

All 3 targets (ORF1ab, N and S genes) could be detected in the samples from the positive tested crew members. In all samples the L452R and P681R mutation was detected. Pango lineage B.1.617.2 was confirmed by whole genome sequencing.

[Figure whole genome sequencing results?]

Viral shedding

On day 8 after testing positive, 13 out of the 17 positive crew members of vessel 1 had a second follow-up sample. On average the CT values (N-target) rose from 17.0 to 23.5. The CT values of each gene (ORF1, N, S) increased proportionally [figure 1]. Ten crew members of vessel 1 had an additional follow sample on day 12, and 7 had a follow-up sample on day 18 where a further increase in CT values was observed [figure 2] **One person who tested positive at day 1 had a sudden drop in CT-values at day 18 after an initial increase in CT-values (Day 1 CT 10.5, day 8 CT 17.5, day 12 CT 21.3, day 18 CT 10.9). This drop in CT-value did not coincide with a clinical deterioration.**

To compare the progress in CT values we used the dataset from January in which 22 patients infected with the B.1.1.7 variant were also retested on day 8. **The infected patients of vessel 1 showed an increase in CT value from 16.9 to 23.1.** According this dataset, B.1.1.7 variant infected patients showed an increase in CT value from 13.0 to 22.2 upon retesting at day 8. A rank Mann Whitney test revealed a **borderline** statistical significant difference between the 2 groups (P-value: 0.049) [Figure 3].

Unexpectedly, one of the sailors with a negative test at day 1 had a positive test result on day 13 while in quarantine.

At day 7, nasopharyngeal swabs were taken from **crew members of vessel 2** for viral culture to look for viable viruses. In **5 of the 6** crew member **who also tested positive at day 1**, viable SARS-CoV-2 viruses were found and grew positive on culture.

Symptoms and Hospitalization

At the first day of testing 17 out 30 crew members were feverish, 19 experienced coughing and 23 had tachycardia (>100 BPM), **clinical symptoms are summarized in table 3**. Hospitalization was indicated mostly because of fever **(>38.5°C),** desaturation **(SpO2 <95%),** or both. **Severe** respiratory problems seemed not to be a critical reason for hospitalization in this group of patients. The average duration of hospitalization was 5 days. All hospitalized sailors were relatively young with ages ranging between 19 and 41**(mean age = 31.3), which is in consonance with the age of the crew (vessel 1 mean age = 34.0, vessel 2 mean age = 39.4).** Out of 11 hospitalized sailors 6 needed oxygen with a maximum of 3 liters. Three hospitalized patients developed acute kidney failure which resolved after fluid administration.

In our small sample group (n=30) of SARS-CoV-2 positive tested crew members there was a clear correlation between the initial CT value and the need for hospitalization: a CT-value below or equal to 15 (CT ≤ 15) was associated with 70% hospitalization (n=10), a CT-value higher than 15 and below or equal to 20 (15 < CT ≤ 20) was associated with 25% hospitalization (n=8), a CT-value higher than 20 and under or equal to 25 (20 < CT ≤25) was associated with 20% hospitalization (n=10) and with a CT below 25 (CT < 25) no hospitalizations were seen (n=2). In total 11 of the 30 patients (36.6%) needed hospitalization but none needed intensive care [figure 4].

Discussion

In May 2021 two ships in the Port of Antwerp experienced an outbreak with the B.1.617.2 (Delta) variant. This outbreak report investigates the 30 crew members infected with Sars-CoV-2 **delta variant** on both vessels.

In comparison to the B.1.1.7 dataset of January the CT values of the B.1.617.2 infected crew members increased less rapid over a 7 day period. This finding might implicate that the B.1.617.2 variant stays more transmissible for a longer time period. **Initial CT levels of the B.1.617.2 on vessel 1 were higher than those of the British dataset, this could bias our results in CT-progress. Other studies have confirmed that the B.1.617.2 lineage is more transmissible than B.1.1.7, but a longer period of viral shedding has not yet been identified as a possible mechanism** [3-4].

Another reason for increased awareness are the results of the viral culture results. **At day 7** after the initial testing, in **5 of the 6** tested crew member **who also tested positive at day one**, viable SARS-CoV-2 viruses could still be cultured. La Scola et al established the relationship between CT values and positive viral cultures [7]. CT values below 30 still held a 40% chance of positive growth on culture, CT values below 20 held a chance of 80% for positive growth on culture [8]. Viewing that 11 out of the 21 crew members tested at day 12-13 still had a CT below 25 after 12-13 days and 19 had CT-values below 30 raises concern that they are still infectious after 10 days.

Interestingly there was a correlation between the initial CT value and risk of hospitalization [figure y-z]. A CT value of less than 15 was associated with 70% risk of hospitalization and at a CT value above 25 no hospitalization was required [sample size 30]. **This correlation of CT-values and hospitalization risk is also described in other articles [8-9].**

The observed tachycardia, which was also observed in several crew members who did not need hospitalization, was possibly linked to an insufficient intake of fluids. Three of the hospitalized sailors developed an acute kidney failure that resolved after fluid intake.

Policy

Unexpectedly, one of the sailors with a negative test at day 1 had a positive test result on day 13. This is important, knowing that the current mandatory quarantine period for people with a high-risk contact in Belgium is only 10 days. Interestingly, in Melbourne, a similar problem with this variant occurred. An outbreak with 48 cases of the Indian variant caused the region to go in lockdown. The Index patient was a man that tested positive on the 12th day after a high-risk contact. [10] If an incubation period of more than 7 days is **not infrequent** for the Indian SARS-Cov-2 variant, a quarantine of 10 days and a retest on day 7 **would not be sufficient** to prevent the virus from spreading. A study

from Harvard Library mentioned a similar concern about the 10-day quarantine advice for the British variant. Their data offer evidence that SARS-CoV-2 variant B.1.1.7 may cause longer infections compared to non-B.1.1.7 SARS-CoV-2. However, the findings were only based on seven B.1.1.7 cases [11]. **Another study that compared 136 B.1.1.7 positive patients to 965 S-gen positive patients found a statistically significant (P = <0.0317) longer duration of PCR-positivity in the B.1.1.7 group (n = 136; median value = 16 days vs n =956; median value = 14 days)** [12]. **Our findings suggest that the currently advised quarantine period of 10 days may not be sufficient to completely prevent further transmission.**

In this outbreak report it was revealed that the 5 crew members originating from India who came aboard in France were insufficiently tested. These 5 sailors had only taken one negative PCR test in India prior to boarding the plane. Upon arrival in France, no additional quarantine or PCR test was carried out, which means no measures were taken to account for a longer incubation time. Of note is that 3 sailors had even already developed symptoms when boarding the chemical tanker, these index patients also tested positive at the first testing in Antwerp. **By law sailors do not have to undergo quarantine upon entering a country, regardless of their previous whereabouts in the last 14 days, even when returning from VOC-risk areas.** This case specifically clarifies the need for strict quarantine and testing policies on vessels, considering rapid spread that occurs in these environments due to multiple facilitating factors, **such as small non-ideally ventilated confined spaces and living close to each other[13]**. C**onditions on vessels mimic a household situation**, where it is known that there is a higher risk of transmission compared with that in other indoor situations [3]. Crew members often reside in smaller spaces that are not ideally ventilated, close to each other and not wearing masks. **An outbreak report from early 2020 investigating 712 Sars-CoV-2 infections on a cruise ship indicated a link between the food service crew and the number of infections, as they have frequent contact with passengers and fellow crew members** [14]. **In our case it was also the cook on vessel 2 that was the first crew member to experience symptoms. Another article describes the effect of small enclosed spaces, dining areas and shared lavatory facilities on vessels** [15]. **It also found a limited effectiveness of quarantine of the index patients on the ship and the wearing of surgical masks. Increased ventilation, the use of HEPA filters and ultraviolet germicidal irradiation in the ventilation system could theoretically reduce air contamination** [15]. This case clarifies the **pivotal** need for strict quarantine and testing policies on vessels, considering rapid spread that occurs in these environments. **Possible measures for this specific profession could be a 14 day quarantine and another PCR test in the land of arrival after 14 days**.

A recent study by Public Health England (PHE) investigated the effectiveness of COVID-19 vaccines against symptomatic disease with the Indian variant. [16] They concluded that 2 doses of the COVID-19 vaccines are highly effective against the B.1.617.2 variant. On the other hand, effectiveness after one dose of vaccine was notably lower with B.1.617.2 cases 33.5% compared to B.1.1.7 cases 51.1%. **None of the crew members of this report were vaccinated at the time of infection.** At this moment only **31%** of the Belgian population is vaccinated with both doses[17]. Therefore, it is important to vaccinate as much as possible and as fast as possible to prevent people from getting sick because of the **Delta** variant.

Limitations

This study has several limitations. **Sample size of this study only comprised of 30 patients.** Crew members of both vessels were tested at slightly different intervals which made it not possible to join the laboratory data of the 2 **vessels** together. **In addition the initial samples of vessel 2 at day 1 were performed with a different PCR test and could thus not be used for comparison of CT-progress.** An additional limitation is the loss in follow-up of several crew members, 6 out of the 25 positive crew members at day 1 did not have a follow up sample at day 7-8. **These limitations** reduced the amount of subjects which we could compare to the B.1.1.7 (Alfa) dataset. A limitation of the B.1.1.7 (Alfa) dataset is the lack of clinical data. **There were also no viral cultures performed 10 days after initial positivity, positive viral cultures after 10 days could have supported our advice of a longer quarantine period.**

Fundings-conflict of interest

None to declare.

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Figures & tables

Table 1: demographics of study population.

	Vessel 1	Vessel 2	Dataset B.1.1.7
sample size	20	10	22
male	20	9	13
female	0	1	9
Age			
mean	34.0	39.4	33.3
range	[19-59]	[30-48]	[4-59]

Table 2: Initial CT-values of vessel 1, vessel 2 and the B.1.1.7-dataset. Vessel 1 and the British variant dataset were both tested at the Antwerp COVID testing platform, vessel 2 was tested at the clinical lab of the University Hospital of Antwerp.

CT-Vessel	Values 1	B.1.617	
subject	ORF1AB	N	S
1	14,9	12,4	15,9
2	18,4	18,4	18,2
3	18,1	18,1	18,4
4	22,8	22,8	23,4
5	13,9	13,9	13,7
6	11,3	8,2	11,5
7	29,6	23,6	30,4
8	12,7	9,8	12,6
9	20,3	18,3	21,5
10	10,5	9,0	10,5
11	13,4	11,5	13,3
12	14,8	13,1	14,6
13	22,6	22,9	23,3
14	14,1	12,7	14,3
15	13,5	10,5	13,2
16	24,4	22,5	27,4
17	20,8	19,1	20,2

CT-Vessel	Values 2	B.1.617.2	
subject	ORF1AB	N	S
1	23,2	24,2	Nt
2	18,6	19,7	Nt
3	24,2	25,8	Nt
4	22,3	23,6	Nt
5	19,5	20,5	Nt
6	20,6	21,8	Nt
7	17,9	19,1	Nt
8	33,9	34,3	Nt

CT-Dataset	Values	B.1.1.7	
subject	ORF1AB	N	S
1	11,9	10,1	0,0
2	14,9	10,8	0,0
3	15,6	13,0	0,0
4	14,7	13,8	0,0
5	13,6	12,0	0,0
6	15,2	12,1	0,0
7	12,1	9,6	0,0
8	13,0	10,8	0,0
9	19,8	18,4	0,0
10	13,7	11,0	0,0
11	17,2	14,1	0,0
12	24,2	21,6	0,0
13	16,9	14,2	0,0
14	21,0	21,4	0,0
15	17,0	14,6	0,0
16	13,9	11,1	0,0
17	14,5	10,4	0,0
18	12,8	10,0	0,0
19	9,9	8,0	0,0
20	13,9	12,1	0,0
21	15,2	13,6	0,0
22	16,5	14,3	0,0

Figure 1: CT-values at day 1 and 8, displaying the progress for the orf1-, N- and S-genes.

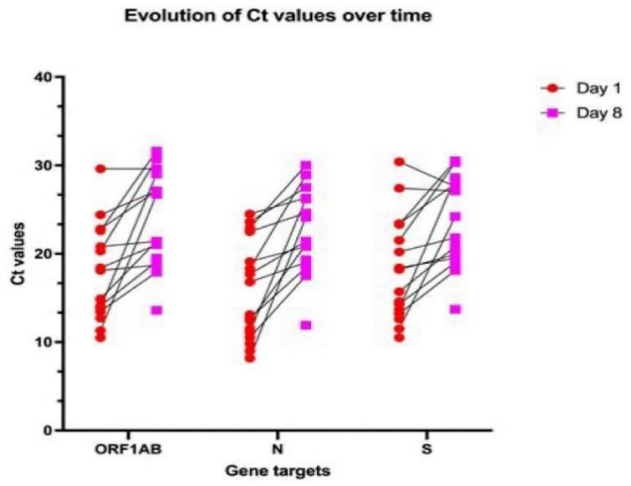

Figure 2: CT-values of vessel 1 tested repeatedly at day 8, 12, 18 and 24.

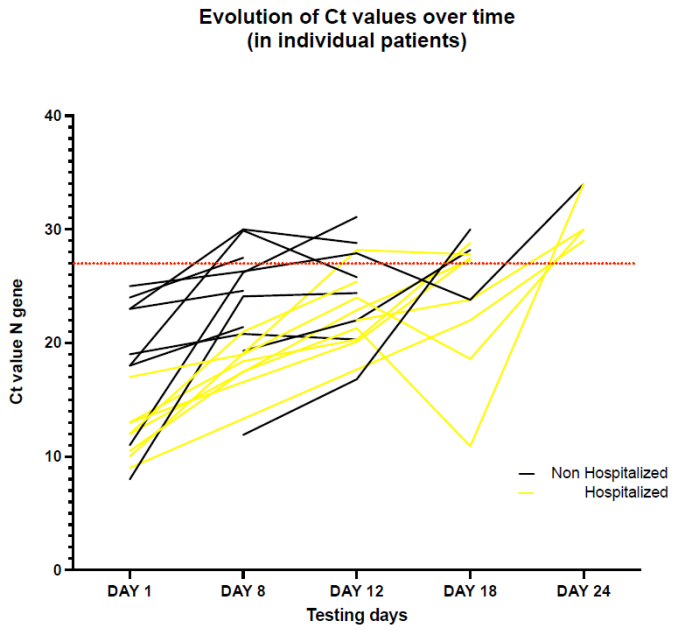

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Figure 3: A rank Mann Whitney comparing the difference in CT-increase (delta-CT) after 7 days between vessel 1 (B.1.617.2) and the British dataset (B.1.1.7 dataset) [P-value: 0.049].

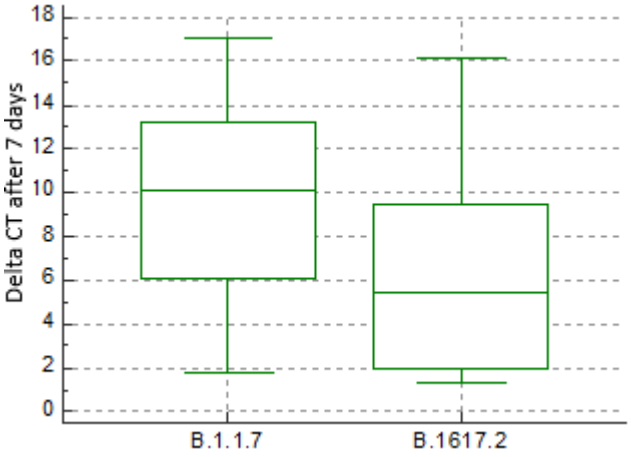

Table 3: clinical symptoms of crew members from vessel 1 and 2.

	vessel 1	vessel 2
sample size	20	10
Clinical signs at inclusion		
fever	15	2?
cough	16	3?
fatigue	17	2
headache	17	2
other	5	3
hospitalized	9	2
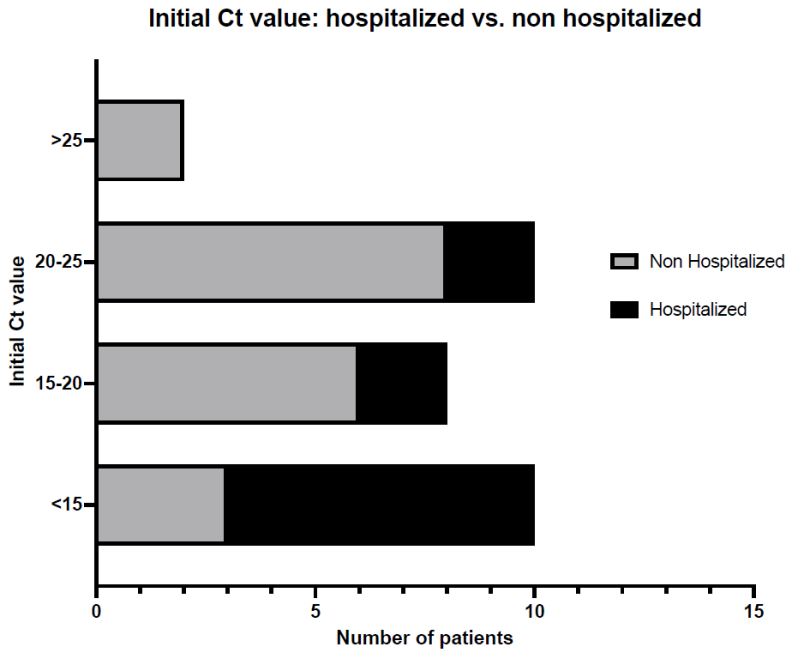
Figure 4: correlations of initial CT-value and hospitalization.