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Original article

High prevalence of asymptomatic SARS-CoV-2 infection in a cohort of liver transplant recipients in central Italy

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ABSTRACT

Asymptomatic subjects account for 25 to 45% of SARS-CoV-2 infections, and in particular, subjects on mild immunosuppressive therapy may have symptoms masked and could spread virus for an extended period of time. To determine the cumulative incidence of symptomatic and asymptomatic SARS-CoV-2 infections and associated risk factors, we conducted a prospective clinical and serological survey in a cohort of 278 liver transplant recipients (LTRs) from Central Italy. Three different serology tests were performed every 4 months in 259 LTRs between April 2020 and April 2021: one based on raw extract of whole SARS-CoV-2 virus and two on specific viral antigens (nucleoprotein and receptor binding domain) to detect specific IgG, IgM and IgA. Hundred fifteen LTRs who reported symptoms or close contact with a SARS-CoV-2-positive subject, or had a positive serological result underwent molecular testing by standard screening procedures (RT-PCR on naso-pharyngeal swab).

Thirty-one past or active SARS-CoV-2 infections were identified: 14 had positive molecular test (64% symptomatic), and 17 had positive serology only (18% symptomatic). SARS-CoV-2 infection was not statistically related to gender, age, obesity, diabetes, renal impairment, type of anti-rejection therapy or time from transplant. Asymptomatic SARS-CoV-2 cases (61.3%) were more frequent in males and in those with glomerular filtrate rate >50 ml/min. Overall, the addition of repeated serology to standard diagnostic molecular protocols increased detection of SARS-CoV-2 infection from 5.1% to 10.9%. Anti-SARS-CoV-2 seroprevalence among our LTRs (11.2%) is comparable to the general population of Central Italy, considered a medium-impact area. Only one asymptomatic subject (6%) was found to carry SARS-CoV-2 in respiratory tract at the time of serological diagnosis.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly throughout the world since the first cases of coronavirus disease 2019 (COVID-19) were described in China. It has been hypothesized that infected persons who remain asymptomatic play a significant role in ongoing viral spread, but their actual number and effect remain uncertain. Asymptomatic persons seem to account for 25% to 45% of SARS-CoV-2 infections [1], and may transmit the virus for an extended period, perhaps more than 14 days [2]. Thus, the magnitude of asymptomatic SARS-CoV-2 carriers has become a global concern.

Solid organ transplant recipients (SOTRs) receive immunosuppressive treatment to avoid graft rejection, which could theoretically mask overt disease, and could therefore harbor the infection for prolonged periods. This hypothesis has not been yet clearly demonstrated.

Asymptomatic COVID-19 infections in liver transplant recipients (LTRs) are poorly studied, due to the difficulty to identify such cases.

Abbreviations: LTRs, liver transplant recipients; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; SOTRs, solid organ transplant recipients; MMF, mycophenolate mofetil; NPS, naso-pharyngeal swab

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The factors that are generally associated to mild COVID-19 in LTRs are a younger age and a low level of immunosuppression, in particular the absence of mycophenolate mofetil (MMF) [3,4]. On the contrary, LTRs on combined calineurine inhibitors/MMF treatment may have both antibody and cellular response to the virus blocked, and therfore prone to an increased SARS-CoV-2 spread, leading finally to more severe disease.

After an initial description of small cases series [5] obtained in select Transplant Centers, more robust data on frequency and clinical outcomes of COVID-19 in SOTRs emerged at the end of 2020 [6].

In particular, the Italian National Transplant Center published a nationwide study in which the data from the Italian surveillance system on SARS-CoV-2-positive cases were cross-referenced with those from the Italian Transplant Information System; the cumulative incidence (CI) of SARS-CoV-2 infection was calculated and the outcomes between SOTRs (kidney, liver, heart, pancreas and lung) and non-transplanted patients (non-SOTRs) were compared [7]. CI, also called incidence proportion, in epidemiology, estimates the risk that an individual will experience an event or develop a disease during a specified period of time. The authors reported a higher CI of SARS-CoV-2 infection in SOTRs than in non-SOTRs with a follow-up through September 30, 2020. This conclusion is in line with the common knowledge that subjects receiving chronic immunosuppressive therapy have a higher incidence of respiratory viral infections. Surprisingly, the same study found a lower CI of SARS-CoV-2 infection in LTRs compared to other organ transplant recipients, as well as compared to the general population. Furthermore, even COVID-19 mortality in LTRs was significantly lower compared to kidney transplant recipients [7]. The most plausible hypothesis to explain these results is the different immunosuppressive regimen in LTRs compared to other organ transplants; in fact, liver recipients are subject to milder immunosuppressive therapy and present greater immunological tolerance compared to other SOTRs [8]

The frequency of asymptomatic infections in LTRs is currently unknown. Much of the epidemiological data contained in the official registries is based on symptomatic cases, and they usually refer to patients with positive naso-pharyngeal swab (NPS) performed either due to respiratory symptoms or for close contact to a positive case [3].

There are few data regarding SARS-CoV-2 seroprevalence among LTRs [9,10], and whether it is different from that of the general population or other immunosuppressed groups. Both these studies report data on a limited number of seropositive LTRs, since were censored in the beginning of the fall 2020, just before the second “wave” of the pandemic in Europe.

A repeated serology approach to a well-defined LTRs cohort may help discover asymptomatic subjects, and identify their clinical features.

In April 2020, shortly after the COVID-19 pandemic onset, we decided to perform a prospective study to determine the prevalence of SARS-CoV-2 infection and the CI in our cohort of LTRs. All patients were already followed at our Institute with regular screening for symptoms, SARS-CoV-2 antibody testing and NPS, as dictated by an internal protocol. In this study, we aimed to report the outcome of a serological one-year follow-up in 279 LTRs, before the start of anti-SARS-CoV2 vaccination program.

2. Material and methods

2.1. Study design

Monocenter, observational, prospective cohort study, to evaluate frequencies and features of serologic and molecular SARS-CoV-2 infections.

2.2. Patients and questionnaires

The Polo Ospedaliero Interaziendale Trapianti (POIT) cohort is composed of 278 LTRs from Central Italy, with regular follow-up visits during 2020 at the Hepatology Unit of Italian National Institute for Infectious Diseases L. Spallanzani in Rome. POIT is an Inter-Department Institution functionally linking the Infectious Diseases/Hepatology Division of the Italian National Institute for Infectious Diseases “L. Spallanzani” and the Transplant and Oncology Surgical Division of San Camillo General Hospital in Rome, Italy.

Between April 2020 and April 2021 all LTRs were regularly screened every three months through telephone-based questionnaire (Supplementary Table 1) or clinical visits to detect systemic, respiratory or gastrointestinal symptoms. Subjects reporting symptoms compatible with COVID-19 underwent NPS within 48hours to detect active infection.

If a patient reported a positive NPS (performed elsewhere) or a close contact with a SARS-CoV-2-infected subject, an additional screening questionnaire was administered.

2.3. SARS-CoV-2 antibody testing

All LTRs underwent anti-SARS-CoV-2 serology testing every four months. Whole-SARS-CoV-2 (crude extract virus) IgG, IgM and IgA levels were measured by ELISA according to manufacturer's instructions (DIESSE Diagnostica Senese S.p.a., Monteriggioni, Italy). The ratio between the optical density of the sample and the cut-off reagent (index) was calculated. Samples were scored positive (index>1.1), indeterminate (index between 1.1 and 0.9) and negative (index < 0.9).

Anti-nucleoprotein (anti-N) and anti-receptor binding domain (anti-RBD) IgG were also tested on samples collected between March 2021 and April 2021 (Architect® i2000sr Abbott Diagnostics, Chicago, IL, USA). Anti-N IgG were expressed as Arbitrary Units (AU)/mL and values ≥ 1.4 were considered positive. Anti-RBD IgG were expressed as Binding Arbitrary Units (BAU)/mL and values ≥ 7.1 were considered positive.

To increase specificity, SARS-CoV-2 serology was only considered significant when at least one of the following to conditions was met:

(1) The result obtained in two different classes of antibodies (IgG plus IgA, or IgG plus IgM or IgG plus anti-RBD or anti-N) was above the cut-off index of positivity.

(2) The result in a single antibody class was more than twice the cut-off index.

LTRs with positive or indeterminate anti-SARS-CoV-2 serology underwent NPS within 48 h.Based on serology and NPS results, LTRs were subdivided into 3 groups:

(a) “symptomatic SARS-CoV-2 infection” defined as positive NPS and/or significant serology result in the presence of typical symptoms.

(b) “asymptomatic SARS-CoV-2 infection” defined as positive NPS and/or significant serology result in the absence of typical symptoms.

(c) “uninfected” with no serological or molecular signs or of SARS-CoV-2 infection.

All subjects provided written informed consent for repeated serology testing. The study protocol was approved by local Ethics

Table 1
Demographic and clinical features of the cohort composed by 274 LTRs: comparison between molecular- versus serology-confirmed SARS-CoV-2 infections, and total infected compared to uninfected LTRs. LT: liver transplant, BMI: body mass index, GFR: glomerular filtrate rate, HIV: human immunodeficiency virus, MMF: mycophenolate mofetil. Square brackets expess Inter-Quartile Range

Variable	Total (*N* = 274)	SARS-CoV2 NPS positive (*N* = 14)	SARS-CoV2 positive serology; NPS negative (*N* = 17)	*p*	SARS-CoV2 infected (*N* = 31)	SARS-CoV2 uninfected (*N* = 243)	*p*
Male Gender	218 (79.6%)	11 (78.6%)	14 (82.4%)	1	25 (80.6%)	193 (79.4%)	0.99
Age	62 [57–67]	60 [56–63]	61 [57–64]	0.99	61 [57–63]	62 [57–68]	0.97
Years from LT	8 [4–13]	5 [3–12]	7 [5–13]	0.55	6 [3–12]	7 [3–12]	0.66
Diabetes mellitus	88	7 (50%)	7 (41.2%)	0.72	14 (45.2%)	74 (30.5%)	0.11
Obesity (BMI≥30 Kg/m^2)	50	4 (28.6%)	3 (17.6%)	0.67	7 (22.6%)	43 (17.7%)	0.47
GFR <50 ml/min	58	4 (28.6%)	3 (17.6%)	0.67	7 (22.6%)	51 (21%)	0.30
HIV-infection	8	0 (0%)	1 (5.9%)	1	1 (3.2%)	7 (2.9%)	1
Curent Tacrolimus	256	14 (100%)	15 (88.2%)	0.49	29 (93.5%)	227 (93.4%)	1
Current Everolimus	31	2 (14.3%)	0 (0%)	0.20	2 (6.5%)	29 (11.9%)	0.55
Current MMF	142	7 (50%)	10 (58.8%)	0.51	17 (54.8%)	125 (51.4%)	0.85

Committee (approval n. 161bis/2020), and conformed to the Guidelines of the 1975 Declaration of Helsinki.

2.4. Statistical analysis

The cohort was considered "fixed", thus, the CI was calculated dividing the number of cases by the number of observed patients in follow-up.

Four patients in the cohort were lost to follow-up and excluded. Continuous variables were expressed as median and inter-quartile range (IQR), categorical variables as absolute numbers and percentage.

Differences in the 3 groups were analyzed in relation to selected demographical and clinical features.

Serologic follow-up was censored at the date of the last available serology, patient vaccination or death.

R statistical software was adopted for statistical analysis with Wilcoxon rank-sum, Chi-square or Fisher's exact tests. Statistical significance was defined as $p < 0.05$.

3. Results

Overall 274 LTRs were included (79.5% males, median age 62 years [IQR 57–67], range 22–80 years). Four patients were lost to follow-up and excluded from analysis. 47% of patients were transplanted for hepatocellular carcinoma resulting from hepatitis C- or hepatitis B-related cirrhosis. Median time from liver transplant was 8 years (IQR 413, range 1–35 years). Median serological follow-up was 12 months (IQR 12–12, range 4–13 years). Overall 31 LTRs had evidence of active or past SARS-CoV-2 infection (11.3%).

3.1. Molecular testing

During the study period, 115 patients (42%) underwent NPS at least once. Fourteen active SARS-CoV-2 infections (9 symptomatic and 5 asymptomatic) were diagnosed by NPS (5.1%).

3.2. Serology testing

SARS-CoV-2 serology was performed on 647 blood samples drawn from 259 LTRs; 158 (61%) were tested at least 3 times during the study period; 30 (11.6%) were tested only once at the end of follow-up (March 2021-April 2021). Among the 14 LTRs with a positive NPS, 12 also had a significant serology result (one missing, one negative).

Overall 29 LTRs (11.2%) had a significant serology result: 15 had IgG alone, 8 IgG+IgA, 5 IgG+IgM, and 1 IgA alone. Eighteen LTRs were re-tested within 3–6 months, and SARS-CoV-2 IgM and IgA were no longer detectable, while IgG persisted in 15 (83.3%).

SARS-CoV-2 serology screening allowed diagnosis of a single asymptomatic carrier plus 14 subjects with past infection (negative NPS).

Patients with serological diagnosis did not differ, regarding demographic and clinical characteristics, from NPS-confirmed cases, nor from the remaining "uninfected" LTRs (Table 1).

The cumulative SARS-CoV2 seroprevalence in LTRs was 11.2% at the end of observation period.

3.3. Symptomatic and asymptomatic SARS-CoV-2 infections in LTRs

Twelve symptomatic COVID-19 cases were identified during follow-up (9 by positive NPS, 3 by significant serology). Six patients (50%) required hospitalization and 3 died (25%) from respiratory complications. Nine patients (75%) were receiving combined calcineurine inhibitor + MMF immunosuppressive treatment. Ten symptomatic patients underwent serology testing and 100% showed a significant result.

Nineteen LTRs had evidence of active or past SARS-CoV-2 infection in the absence of current or significant respiratory, gastrointestinal or systemic symptoms (Table 2). Eight patients (42%) were receiving combined calcineurine inhibitor + mycophenolate mofetil MMF immunosuppressive treatment.

Female gender and GFR< 50 ml/min were significantly associated to symptomatic versus asymptomatic infections (Table 3).

Regarding gender, 18/25 males with active or past SARS-CoV-2 infection were asymptomatic (72%) versus 1/6 females (16.7%, $p = 0.022$).

Among 6 subjects with baseline GFR< 50 ml/min, 5 (83.3%) reported current or previous COVID-19-related symptoms at the moment of diagnosis, and only one was asymptomatic (16.7%, $p = 0.007$).

3.4. Cumulative incidence and monthly distribution of SARS-CoV-2 infections

Overall 24/31 cases (77.4%) were diagnosed during the so called "second wave" of the epidemic (October 2020-April 2021). The monthly distribution of cases in the cohort reflected the epidemic curve in Lazio Region (Fig. 1).

The CI of NPS-confirmed cases was 5.1% over 13 months. Adding symptomatic and asymptomatic cases diagnosed by serology increased the CI to 6.2% and 11.2%, respectively.

The ratio of serologically detected infections to confirmed cases was 1.6.

Table 2
Liver transplant recipients with asymptomatic SARS-CoV-2 infection LT: liver transplant, FK: tacrolimus, MMF: mycophenolate mofetil, Anti-RBD: anti-SARS-CoV2 receptor binding domain antibodies, Anti-N: anti SARS-CoV2 nucleoprotein antibodies, DM: diabetes mellitus, Obesity: body mass index >30 Kg/m^2, HIV: human immunodeficiency virus, COPD: chronic obstructive pulmonary disease, AH: autoimmune hepatitis, CKD: chronic kidney disease.

Pt ID	Age	Years from LT	Month of diagnosis	Anti-rejection therapy	Molecular t esting	First positive serology	3–6 Months serology follow-up	Co-morbidity
ID 21	62	18	04/2021	FK	pos	IgG, Anti-RBD	n.a.	DM, obesity, cardiac
ID 24	64	11	01/2021	FK	pos	Negative	negative	DM, COPD, obesity
ID 25	68	3	03/2021	FK+MMF	neg	IgG, anti-N, anti-RBD	n.a.	DM
ID 38	62	6	01/2021	FK	neg	IgG+IgA	n.a.	DM, HIV
ID 49	64	7	02/2021	FK	neg	IgG+IgA	n.a	None
ID 71	61	12	06/2020	FK	neg	IgG	IgG	None
ID 80	60	1	11/2020	FK+MMF	pos	IgG+IgA	IgG	None
ID 87	60	5	01/2021	FK+MMF	neg	IgG+IgA	Anti-RBD, Anti-N	DM
ID 96	55	3	04/2021	FK+MMF	neg	IgG+Anti-RBD	n.a.	DM
ID 104	43	12	05/2020	FK	Neg	IgG+IgM	IgG	None
ID 131	64	4	03/2021	FK	Neg	IgG+Anti-RBD	n.a	COPD
ID 138	70	16	05/2020	FK+MMF	neg	IgG	IgG	None
ID 141	58	11	11/2020	EVER+MMF	pos	IgG	Anti-RBD, Anti-N	None
ID 176	58	8	11/2020	MMF	Neg	IgG	negative	Obesity
ID 180	61	6	11/2020	FK	pos	Negative	Anti-RBD	DM
ID 181	61	15	10/2020	FK	Neg	IgA	negative	DM
ID 198	62	6	01/2021	FK	Neg	IgG	IgG+anti-RBD	DM
ID 208	64	8	04/2021	FK+MMF	Neg	Anti-RBD	n.a.	AH, obesity
ID 224	58	7	12/2020	FK	neg	IgG	Anti-RBD	AH

Table 3
Demographic and clinical features of LTRs with SARS-CoV2 infection: comparison between symptomatic and asymptomatic. LT: liver transplant, HCC: hepatocellular carcinoma, BMI: body mass index, GFR: glomerular filtrate rate, MMF: mycophenolate mofetil. Evaluated comorbidities*: diabetes mellitus, obesity, GFR <50 ml/min, cardiopathy, active smoking, chronic obstructive pulmonary disease.

Variable	Symptomatic SARS-CoV2 infections (N = 12)	Asymptomatic SARS-CoV2 infections (N = 19)	p
Male Gender	7 (58.3%)	18 (94.7%)	0.022
Age >60 years	5 (41.7%)	14 (73.7%)	0.13
Years from LT ≥5	6 (50%)	15 (78.9%)	0.13
Diabetes mellitus	4 (33.3%)	10 (52.6%)	0.46
Obesity (BMI>30 Kg/m^2)	2 (16.7%)	5 (26.3%)	0.68
GFR <50 ml/min	6 (50%)	1 (5.2%)	0.007
Comorbidities* (≥3)	4 (33.3%)	4 (21.1%)	0.66
Current MMF	9 (75%)	8 (42.1%)	0.14

4. Discussion

Initial cases of COVID-19 in LTRs in Italy were identified in March 2020 [5]. Four months later, the CI of COVID-19 in this population was 0.63%, slightly higher than in the Italian adult population (0.4%) [7]. Thirteen months later, at the end of the second epidemic wave, CI in Italy reached 7.05% with more than 4 million confirmed SARS-CoV2 infections [2]. In April 2021, a Spanish nationwide study reported CI 8.37% in LTRs, higher than the general Spanish population (3.12%) [3].

In our Italian LTR cohort, SARS-CoV-2 infection CI was 5.03% in NPS-confirmed cases on April 15th 2021, compared to 8.78% found in the adult population of Lazio Region (3.488.589 inhabitants over 40 years of age) measured on the same day [11]. Therefore, our results confirm lower SARS-CoV-2 infection CI in LTRs than in the non-transplant population, as described in the Italian nation-wide study [7].

By adding the cases diagnosed by serology, without NPS confirmation, the CI increased to 11.2%. Similarly, in a prospective serosurvey of German LTRs censored in August 2020, CI of NPS-confirmed cases was 1%, increasing to 3.7% when positive serology cases were added [9]. A Recent meta-analysis and systematic review calculated a 4.7% seroprevalence in the European general population by the end of 2020, with an 8.3 ratio of serologically detected infections to NPS-confirmed cases [12]. Data on seroprevalence in the general population of Lazio Region are lacking; however, published studies on medium-impact areas similar to Lazio reported a 5% seroprevalence in August 2020 among healthy adult workers living in Northern Italy, with a 3:1 ratio to NPS-confirmed cases [13]. Among Swiss students a 7.8% cumulative seroprevalence was found at the end of 2020, with an 8:1 ratio to NPS-confirmed cases [14]. Thus, as expected, SARS-CoV-2 infections calculated only by positive NPS are under-estimated both in the general population and in LTRs.

Our study included the peak of the second wave of the epidemic, just before the start of the vaccination campaign thus explaining the higher calculated seroprevalence compared to other studies in LTRs at earlier time points of the pandemic [9,10].

Data on antibody response in LTRs after SARS-CoV-2 infections are conflicting. In a Spanish study, a significantly lower incidence and reduced levels of specific antibodies with a more pronounced antibody loss were observed in LTRs at 3 and 6 months after COVID-19, in comparison to immunocompetent subjects [15]. This may help to explain the underestimated seroprevalence observed in previous studies [9,10]. On the contrary, in our study, all but one of the NPS-confirmed cases produced a detectable SARS-CoV-2 antibody response, that was maintained in the following 3–6 months, similarly to what was recently reported in other small LTRs series [4,16]. These differences might be mainly due to the different sensitivity of single or repeated testing, as well as to the type of antibody assays used. Thus, the impact of patient-associated differences (i.e. the type of immunosuppressant or the comorbidities) on contracting and clinical presentation of SARS-CoV-2 infection, can better be evaluated in settings, like monocentric studies, where identical methods are adopted.

No risk factors were found to be directly associated to active or past SARS-CoV-2 infection in our cohort (Table 1). In particular, time from transplant and ongoing intensive immunosuppression, were not found to be associated with infection. MMF therapy was previously found to be associated with impaired antibody response after SARS-CoV-2 infection [4]. We failed to confirm this hypothesis, since all but one of the NPS-confirmed cases in the cohort, showed a significant antibody response. MMF treatment and time from liver transplant <5 years were relatively more frequent in LTRs with symptomatic infection, but these data failed to reach statistical significance, probably due to insufficient sample size. This may represent the major weakness of our study.

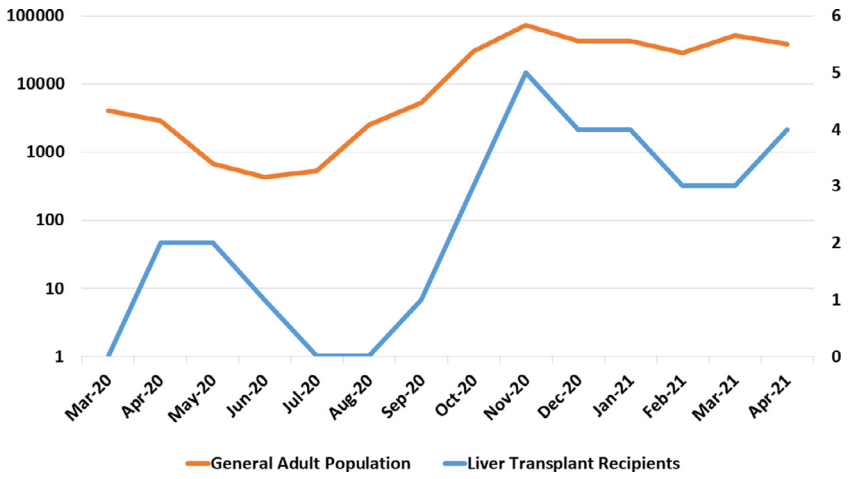

Fig. 1. SARS-CoV-2 infections in a cohort of liver transplant recipients and in the general adult population of Lazio Region.

Most of the SARS-CoV-2 infections identified in our LTRs cohort (61.3%) were asymptomatic. This finding confirms that, during a much longer observational period and with a higher number of cases, a substantial percentage of infections are unrecognized as preliminarily reported in the German serosurvey [9]. This is in contrast with the observation in a Spanish LTRs cohort, censored in October 2020 before the onset of the third COVID-19 epidemic wave, of a single asymptomatic case out of 9 serological or molecular confirmed infections [10].

SARS-CoV-2 asymptomatic infections induce global concern since they can possibly transmit the virus for an extended period of time [2]. In our study, with repeated serologic screening in a potential high-risk population, it was only identified a single asymptomatic subject with positive molecular NPS. This result is in line with the observation that asymptomatic infections harbor transient and low viral amount in the respiratory tract [17]. In fact, respiratory, gastrointestinal or systemic symptoms are also often reported to be mild and transient in LTRs [9,18].

An association of chronic kidney disease and comorbidities to severe manifestations of COVID-19 in immunocompetent subjects [19] and in LTRs [20] was recently shown. We confirmed in our LTRs cohort, considering 6 major comorbidities, a role of impaired renal function in the development of COVID-19 symptoms during of SARS-CoV-2 infection.

Regarding demographic factors, we observed that male gender was associated to asymptomatic infections compared to symptomatic COVID-19 cases. However, we are aware that this result may be biased by the under-representation of female gender in our cohort.

One of the strengths of our study is that data were prospectively collected, based on repeated NPS and regular blood sampling, on different antibodies assays and including all the LTRs in the cohort. Therefore, the loss of information and selection bias could be very limited. We believe the 11.2% prevalence of NPS- or serology-confirmed cases found in our study reflects the natural history and cumulative incidence of SARS-Cov2 in LTRs during the first year of epidemic. The study was terminated just prior to anti-SARS-CoV-2 vaccination program in LTRs, which will hopefully minimize infections in this population. However, vaccination will also introduce a further confounding factor in the complex field of interplay between immune response and SARS-CoV-2 pathogicity.

5. Conclusion

In conclusion, our study demonstrated a higher number of asymptomatic than symptomatic SARS-CoV-2 cases in LTRs during the first year of the pandemic, and hypothesized features associated to asymptomatic infections. Only one asymptomatic subject (6%) was found to be a viral carrier at the time of repeated serological screening, suggesting a low prevalence among immunosuppressed LTRs.

Adding serologic testing to regional or nationwide studies may help identify predictors and therapeutic strategies in LTRs, and substantially change the incidence and lethality calculations in this population.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.liver.2021.100064.

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