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Impact analysis of SARS-CoV-2 vaccination in patients treated with monoclonal antibodies: A monocentric experience

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ABSTRACT

Background: Since the discovery of SARS-CoV-2, no treatment has been able to completely eradicate the virus. The study aimed to evaluate the virological and clinical impact of the vaccination in SARS-CoV-2 infected patients treated with monoclonal antibodies (mAbs).

Methods: This single-centre, observational, retrospective, real-life study was performed on SARS-CoV-2 symptomatic outpatients and inpatients treated with mAbs from March 2021 to November 2022 includes 726 patients. Each patient received available mAbs (bamlanivimab-etesevimab or casirivimab-indevimab or sotrovimab or tixagevimab-cilgavimab) according to the circulating virus strains. Age, comorbidities, vaccination status, death rates, duration of virological clearance, average length of stay, risk factors, and hospitalization or ICU admission were recorded.

Results: Of 726 patients with complete data analyzed (median age 64), 516 outpatients and 210 inpatients were included. Vaccination status was known for all participants: 74.4 % and 51.7 % were vaccinated against SARS-CoV-2 among inpatients and outpatients, respectively. A shorter duration of virological clearance was observed in the vaccinated group, with a median of 16 days (IQR 15–17), compared to 19 days (IQR 18–21) in the unvaccinated group [HR 1.21; $p < 0.032$]. Multivariate analysis of virological clearance also showed statistical significance with tixagevimab cilgavimab 300 mg/300 mg (HR 2.73, p value < 0.001). No significant difference was found in worsening [OR 1,29; $p = 0.57$] and mortality [OR 0.65; $p = 0.81$] rates between vaccinated and unvaccinated patients treated with mAbs.

Conclusions: Key findings include a shorter duration of virological clearance in vaccinated outpatients but no significant differences in worsening or mortality rates between vaccinated and unvaccinated patients treated with mAbs. The study suggests a potential synergistic role of mAbs in accelerating virological clearance in vaccinated patients with mild to moderate COVID-19, with differing effects in hospitalized patients. Therefore, it is essential to implement health surveillance in high-risk patients with comorbidities in order to identify early any variants that might otherwise escape neutralizing antibodies.

1. Introduction

The pandemic spread of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2), with its burden of morbidity and mortality due to Coronavirus Disease 2019 (COVID-19) [1], prompted regulatory agencies worldwide to grant transient and emergency authorization to

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various drugs for COVID-19 treatment in early 2020. Notably, SARS-CoV-2 monoclonal antibodies (mAbs) emerged as an innovative therapeutic against SARS-CoV-2 [2]. As vaccination campaigns reduced severe cases and hospitalizations [3], the Italian Pharmaceutical Agency (AIFA) authorized specific SARS-CoV-2 mAbs for mild-to-moderate COVID-19 cases: bamlanivimab (LY-CoV555; Eli Lilly) and etesevimab (Ly-CoV016; Eli Lilly), casirivimab/imdevimab (REGN-COV2; Regeneron Pharmaceuticals), sotrovimab (VIR-7831, GlaxoSmithKline and Vir Biotechnology, Inc.) and tixagevimab/cilgavimab (AZD7442, Astrazeneca) [4–6]. Firstly, tixagevimab/cilgavimab was approved for prophylaxis use (once every 6 months) in patients with an insufficient or absent serological response to SARS-CoV-2 vaccines. Subsequently, the Italian Medicines Agency (AIFA) approved the use of the drug for the treatment of COVID-19 in adults and adolescents who do not require supplemental oxygen and who are at an increased risk of the disease becoming severe, in accordance with the available indication [7]. All available SARS-CoV-2 monoclonal antibodies target different epitopes of the spike protein (receptor binding domain −RBD- or S2 subunit) blocking its interaction with the angiotensin converting enzyme 2 (ACE2) on human cells [8]. However, concerns arose with the emergence of new SARS-CoV-2 variants of concern (VOCs), particularly Omicron, impacting the efficacy of mAbs [9]. The first VOC of SARS-CoV-2 was Alpha (B.1.1.7) identified in late 2020, followed by Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), with the latter circulating since end of 2021: all VOCs, but in particular Omicron (B.1.1.529), showed a significative number of viral lineages designated BA.1, BA.2, BA.3, BA.4, and BA.5, which were further divided into severals sub-lineages [10,11]. The Omicron variant was demonstrated to exhibit greater transmissibility than other SARS-CoV-2 variants, including the Delta variant, which had previously been considered the most transmissible [12]. The main mutations showed by different VOCs are in the domains of Spike protein, leading to defective viral entry and a potential escape to neutralizing antibodies [13]. A comprehensive understanding of the function of viral and receptor genes is of paramount importance for the development of effective prevention strategies. Furthermore, the ongoing evolution of SARS-CoV-2 presents a challenge to the efficacy of available treatments [14]. Different studies showed that SARS-CoV-2 mAbs were effective in reducing the risk of disease progression [15–18], although more real-life data are needed to confirm their effectiveness, especially in the vaccinated population, also in the perspective to evaluate their possible future therapeutic role in a public health view. In light of the current epidemiological situation, further research is required to elucidate the impact of the various emerging sub-variants of the SARS-CoV-2 virus on disease progression. Consequently, we conclude that there is a clear need for enhanced health surveillance. In this retrospective, observational study (Mar 2021-Nov 2022) aims to evaluate the impact of SARS-CoV-2 vaccination on virological clearance, COVID-19 related hospitalization, ICU admission, and 30-days mortality in SARS-CoV-2 infected patients receiving monoclonal antibodies due to their risk for COVID-19 progression.

2. Methods

2.1. Study design and objectives

A retrospective, observational, monocentric study was carried out at the Department of Infectious Diseases of Umberto I University Hospital in Rome, Italy, with the purpose to evaluate the virological (timing of clearance) and clinical (hospitalization, ICU admission, 30-days mortality) impact of vaccination in SARS-CoV-2 infected patients treated with mAbs due to high risk of disease progression. The study spanned a twenty-month period from March 2021 to November 2022, utilizing medical records. Monoclonal antibody against SARS-CoV-2 has been administered according to the predominant viral variants, based on available virological national surveillance data [19]. The choice of the monoclonal antibody type (intravenous or intramuscular single-dose) adhere to regulatory agency rules aligned with the circulating viral variants during the three epidemic waves in Italy: bamlanivimab-etesevimab (700 + 1,400 mg); casirivimab-imdevimab (600 + 600 mg; 1,200 + 1,200 mg; 4,000 + 4,000); sotrovimab (500 mg); tixagevimab-cilgavimab (300 + 300 mg). All type of mAbs were available for both SARS-CoV-2 infected in- and outpatients with the following exceptions: (1) tixagevimab-cilgavimab (300 + 300 mg) and casirivimab-imdevimab (600 + 600 mg) available only for outpatients; (2) casirivimab-imdevimab (4,000 + 4,000 mg) only for inpatients.

All patients with a SARS-CoV-2 RT-PCR positivity on nasopharyngeal swab (NPS) were evaluated by infectious diseases specialists at the Emergency Department of the Polyclinic Umberto I. Patients meeting specific criteria, including age (>18 years), body weight (>40 kg), (for females, pregnancy excluded) and having at least one risk factor for COVID-19 progression (Table 1) the administration of SARS-CoV-2 mAbs was proposed as inpatient or outpatient according to their ongoing clinical condition. Informed consent was obtained from all participants, and baseline and follow-up data were retrospectively collected from medical records. The study categorized participants based on COVID-19 clinical severity [20]. Severe cases were defined by criteria such as the presence at room-air of a SpO2 < 94 %, baseline PaO2/FiO2 ratio < 300 mmHg, respiratory rate > 30 breaths/min or radiology infiltrates affecting more than 50 % of lungs. The primary outcome was the impact of SARS-CoV-2 vaccination on the time to virological clearance (assessed trough PCR on NPS) in SARS-CoV-2 infected patients treated with mAbs, measured as days to clearance of SARS-CoV-2 starting from 5 days after the first molecular positivity. The secondary endpoints included COVID-19 progression and death from any cause within 30 days from SARS-CoV-2 mAbs administration. Disease progression was defined as worsening of respiratory symptoms requiring hospitalization for outpatients, and/or admission to intensive care units (ICUs) for inpatients. The study also explored the association of outcomes with various factors, including age, sex, comorbidities, types of mAbs used according to predominant viral variants, vaccination status, days between SARS-CoV-2 RT-PCR positivity on NPS and mAbs administration. The participant patients were considered vaccinated if they were receiving, within 120 days from mAbs administration, at least 2-doses of BNT162b2 or mRNA-1273 or ChAdOx1-S vaccines, or at least 1 dose of Ad26.COV2.S. Follow-up data were collected by phone calls up to 30 days after the SARS-CoV-2 mAbs administration. Regarding other COVID-19 medications, starting from February 2022 the prescription of oral antivirals (molnupinavir, ritonavir-nirmatrelvir) was allowed only to SARS-CoV-2 infected outpatients not receiving SARS-CoV-2 mAbs; thus, none of the participant patients have received oral antiviral drugs. Concerning COVID-19 inpatients, participants may have received a 3 to 5 days course of remdesivir, as well as dexamethasone if in need of oxygen, whereas anti-IL6 (Tocilizumab) or anti-JAK (Baricitinib) were prescribed only to severe/critical COVID-19 inpatients. No data on outpatients' home self-medication was available. For hospitalized patients, outcomes were categorized into the following groups: deaths attributable to COVID-19, deaths unrelated to COVID-19 and discharges. Data were managed anonymously using individual identification code. The study was carried out in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP), and received approval from the Ethics Committee of Sapienza, University of Rome (Protocol n. 6707).

2.2. Statistical analysis

Patients' characteristics for the whole population stratified by vaccination status and by inpatient/outpatient status were summarized by means of the frequencies and percentage for categorical variables or using quantiles for continuous variables. In univariate analysis, non-parametric tests were performed for comparisons between groups (Chi-Squared and Fisher Exact test in case of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test in case of

Table 1
Criteria for the prescription of SARS-CoV-2 monoclonal antibodies.

Exclusion criteria: −Unavailability of a nasopharyngeal swab for SARS-CoV-2 or negative result −Age < 18 years; −Weight < 40 kg; −Pregnancy or lactation status; −Attested allergy to monoclonal antibodies or their components
Inclusion criteria: −Nasopharyngeal swab (RT-PCR or third-generation antigenic test) positive for SARS-CoV-2 by < 10 days −Obesity (BMI>30) −Diabetes mellitus uncontrolled −Chronic renal failure (including hemodialysis or peritoneal dialysis). −Cardiovascular disease and/or vascular cerebropathy −Primary immunodeficiency −Secondary immunodeficiency (onco-hematologic patients, AIDS, etc.) −Neurodegenerative disease −Infusion of one of the anti-SARS-CoV-2 monoclonal antibodies (mAbs)

continuous variables). Regression models (Cox, linear and logistic) were used in univariate and multivariate analyses to assess whether the demographics and clinical parameters were associated with viral clearance, clinical worsening and/or death. Beta coefficient in case of linear model, Hazard ratio (HR) in case of Cox regression model and Odds Ratios (OR) in case of logistic model, were reported as parameter results of the regression models as well the 95 % Confidence Intervals. Subgroup analyses for vaccinated and unvaccinated patients as well as for in- and outpatients' status were performed to manage the interaction among variables. As only patients treated with monoclonal antibodies were included, no control group was available. For confronting the impact of mAbs administered on the study outcomes, we used Sotrovimab as reference for the comparison with other mAbs because of longer availability throughout the study period (Fig. 1). All tests were 2-sided, accepting $p < 0.05$ as statistically significant and confidence intervals were calculated at 95 % level. All analyses were performed using the R software (R Core Team 2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/).

3. Results

A total of 916 adult patients infected with SARS-CoV-2 were consecutively recruited for monoclonal antibody administration based on inclusion criteria; of these, 190 (20.7 %) were excluded due to lack of data. The study population comprised 726 adult patients (Fig. 2) with a median age was 64 (18–99) years and 47 % were females. Table 2 summarizes baseline characteristics of study participants according to the vaccination status. More than two-thirds (n = 516, 71 %) were outpatients; almost one-third (n = 233, 32.1 %) of the study population was unvaccinated. The majority of participants (n = 660, 91 %) reported at least one comorbidity recognized as a risk factor for severe COVID-19. Various mAbs therapies were prescribed, considering national surveillance data for viral variants [12]. The most frequently administered mAbs were Bamlanivimab/etesevimab (n = 212, 29 %) and Sotrovimab (n = 183, 25 %) (Table 2); the first was being prescribed early during the study period (latest administration in January 2022), while the second was introduced later (first administration in December 2021) and continuously administered during the study period. Regarding other COVID-19 treatments, among inpatients all received subcutaneous enoxaparin (prophylactic or therapeutic dose according to the clinical severity) and those needing oxygen have received dexamethasone, 51 %

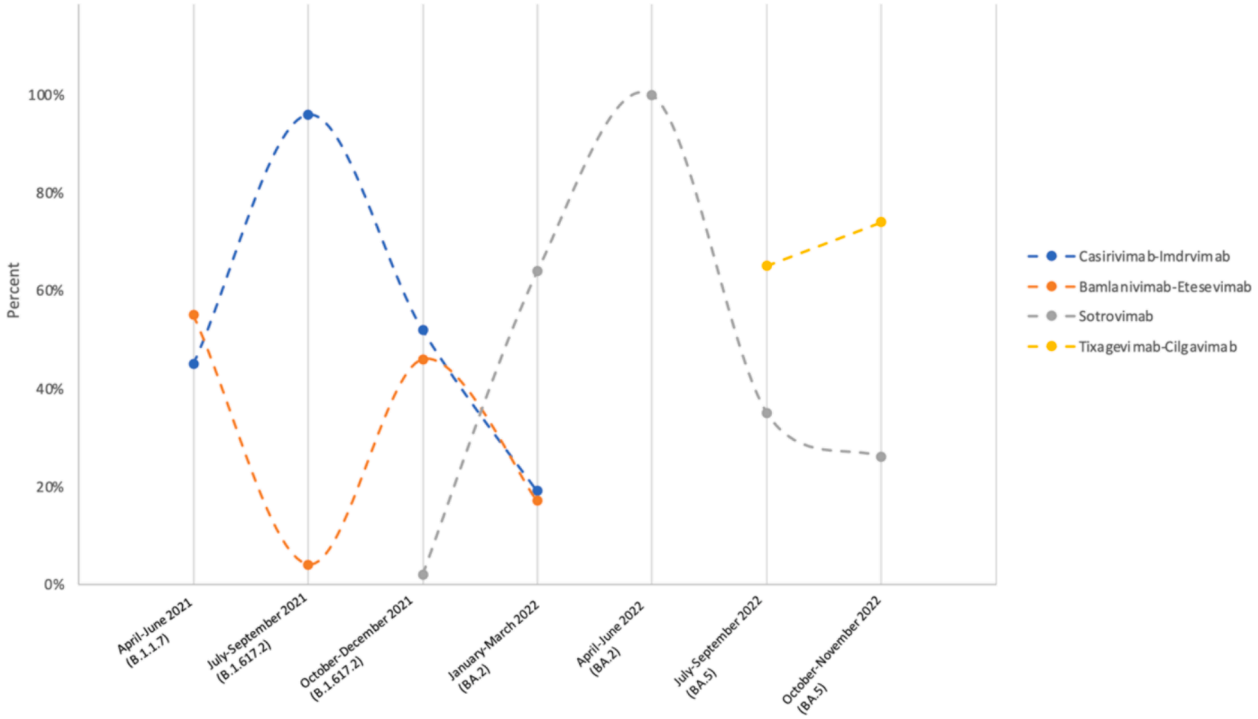

Fig. 1. Use of mAbs over time in accordance with the evolving SARS-CoV-2 circulating variants.

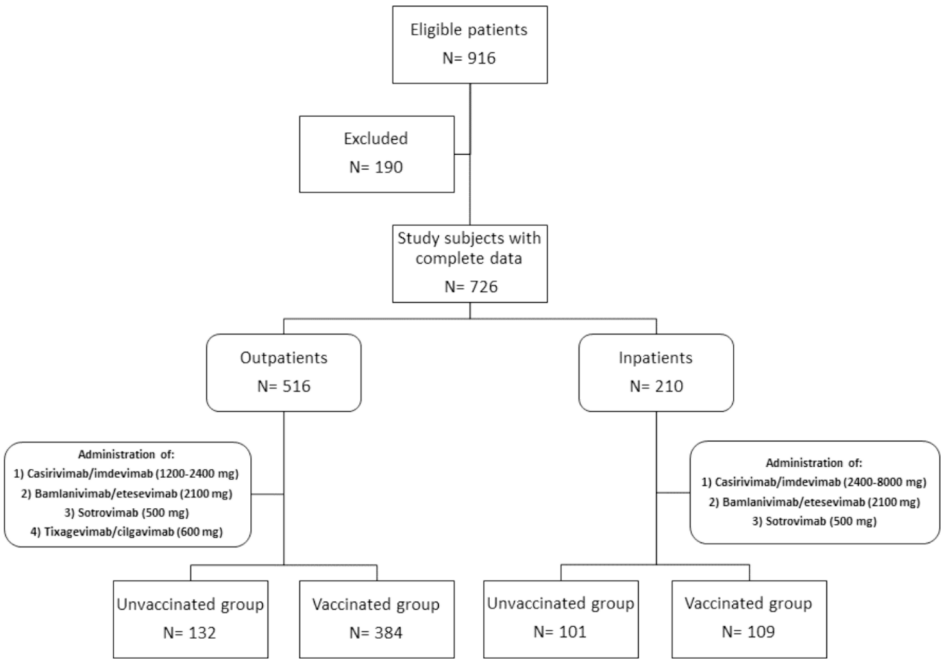

Fig. 2. Flow chart of the distribution of the study population.

receiving Remdesivir, and only 2 patients have received baricitinib. Notably, for COVID-19 outpatients no concomitant oral antiviral and SARS-CoV-2 mAbs were allowed; thus, ensuring that all outpatients in this study did not receive oral antiviral drugs. Among inpatients, almost two-thirds (n = 144/210, 68.6 %) were initially hospitalized due to COVID-19, while the remaining (31.4 %) acquired SARS-CoV-2 during hospitalization for other medical reasons; additionally, 8 inpatients (n = 8/210, 3.8 %) were transferred to the ICU with only one surviving. Overall, we report no deaths among outpatients, and 28 deaths among inpatients, 57 % (n = 16/28) attribute to COVID-19, 53.6 % (n = 15/28) among unvaccinated; overall mortality in the study population was 3.8 % (n = 28/726), and 13.3 % (n = 28/210) for inpatients only. Among outpatients, only 18 (3.5 %) showed disease progression requiring hospitalization, concluding with full recovery. Patient's groups were non-homogeneous according to a descriptive analysis (data not shown). The univariate analysis assessing the impact of the vaccination status on the time to virological clearance and on death among SARS-CoV-2 mAbs-treated patients showed a significant difference for both outcomes ($p < 0.001$ and $p = 0.021$ respectively), whereas no difference was observed for disease progression (Table 3). Stratification according to the different monoclonal antibodies administered (Table 3A) showed that the mortality outcome lost statistical significance due to the limited number of events. The only outcome that remained statistically significant when stratifying by mAbs was virological clearance of sotrovimab. Vaccinated patients exhibited virological clearance at 15 days (interquartile range (IQR) 6–76), while unvaccinated patients demonstrated clearance at 20 days (IQR 9–58) (p-value = 0.018). Cox regression models were used to evaluate the effects of all baseline characteristics on the virological clearance, Hazard Ratio (HR) and confidence intervals at 95 % level were reported. Kaplan Meier plots were reported for the categorical variables deemed statistically significant. As shown in Fig. 3, the median time of virological clearance in the vaccinated group was 16 days (IQR 15–17), compared to 19 days (IQR 18–21) in the unvaccinated group (p-value = 0.00021). In patients with neurodegenerative diseases, the clearance time was 21 days (IQR 17–30), while the virological clearance time in patients without neurodegenerative diseases was 17 days (IQR 16–18) (p-value = 0.0017). Regarding the type of monoclonal antibody administered, sotrovimab 500 mg was found to be administered over a longer period, with a clearance time of 16 days (interquartile range (IQR) 14–18). Tixagevimab cilgavimab 300 mg 300 mg, had a shorter clearance time of 12 days (IQR 11–13) (p-value < 0.0001). The clearance times of the other monoclonal antibodies proved to be longer. Furthermore, these results were analyzed using the Cox model (see Table 4). The results showed that in patients treated with casirivimab imdevimab 4000 mg/4000 mg, the positivity rate at 28 days remained remarkably high at 37 %. This could happen due to the hospitalization of the patients, which led to a more complex clinical scenario. All patients treated with tixagevimab-cilgavimab showed a rapid virological clearance within 24 days. Variables identified as significant in the univariate Cox model included age (HR 1.0; 95 % IC 0.99, 1.00; p-value 0.035), patient status (HR 2.23; 95 % IC 1.85, 2.68; p-value < 0.001), vaccination status (HR 1.35; 95 % IC 1.15, 1.59; p-value < 0.001), neurodegenerative diseases (HR 0.61; 95 % IC 0.44, 0.83; p-value 0.002), interval between positive swab and mAbs (HR 0.99; 95 % IC 0.98, 0.99; p-value < 0.001) and the type of monoclonal antibody. Furthermore, among the monoclonal antibodies administered, only casirivimab imdevimab 4000 mg/4000 mg (HR 0.56; 95 % IC 0.41, 0.78; p-value < 0.001) and tixagevimab cilgavimab 300 mg/300 mg (HR 3.37; 95 % IC 2.48, 4.60; p-value < 0.001) retained statistical significance in the univariate analysis. The variables that remained statistically significant in the multivariate analysis were vaccination status (HR 1.21; 95 % IC 1.02, 1.43; p-value < 0.032), patient status (HR 2.05; 95 % IC 1.63, 2.58; p-value < 0.001) and the interval between positive swab and mAbs (HR 0.99, 95 % IC 0.98, 1.00; p-value < 0.001). Tixagevimab cilgavimab 300 mg/300 mg was the only mAb that confirmed statistical significance (HR 2.73, 95 % IC 1.99, 3.74; p-value < 0.001). Subsequently, univariate regression models for the time to virological clearance, disease progression, and deaths for any cause within 30 days. The time to virological clearance was the only study outcome with significant results at the univariate linear regression model (Table S1): independent variables with statistical significance at the univariate analysis were used for multivariate regression analyses. Overall, the multivariate linear regression model was significant only for the primary outcome, not for the secondary endpoints (Table S1). In the overall population, according to the multivariate analyses, the time to virological clearance correlated with being an outpatient (Beta = -7.7 days, 95 %CI −9.8,-5.6, p < 0.001), affected by chronic neurodegenerative diseases (Beta = 3.8 days, 95 %CI 0.74, 6.9, p < 0.015), and treated with Tixagevimab/Cilgavimab

Table 2
Baseline characteristics of overall population, unvaccinated and vaccinated subgroup.

Demographic characteristics	Sample size (N=726)	SARS-CoV-2 Unvaccinated (N=233)	SARS-CoV-2 Vaccinated (N=493)	p-value
Age, years – median (range)	64 (18–99)	63 (25–97)	64 (18–99)	0.50
Sex, n (%)				0.23
Male sex	374 (51.5 %)	112 (48.1 %)	262 (53.1 %)	
Female sex	352 (48.5 %)	121 (51.9 %)	231 (46.9 %)	
Patient status, n (%)				**<0.001**
Outpatient	516 (71.1 %)	132 (56.6 %)	384 (77.9 %)	
Inpatient	210 (28.9 %)	101 (43.4 %)	109 (22.1 %)	
Comorbidity*, n (%)				
None	66 (9.1 %)	28 (12 %)	38 (7.7 %)	0.081
Obesity (BMI>30)	76 (10.5 %)	31 (13.3 %)	45 (9.1 %)	0.11
Cardiovascular and/or cerebrovascular diseases	354 (48.8 %)	116 (49.8 %)	238 (48.3 %)	>0.99
Chronic respiratory diseases	105 (14.5 %)	32 (13.7 %)	73 (14.8 %)	0.79
Chronic renal failure	66 (9.1 %)	9 (3.9 %)	57 (11.5 %)	**0.001**
Neurodegenerative diseases	49 (6.7 %)	23 (9.9 %)	26 (5.3 %)	**0.032**
Immunodeficiency (primary or secondary) §	127 (17.5 %)	36 (15.4 %)	91 (18.5 %)	>0.99
Chronic hepatopathy	12 (1.7 %)	4 (1.7 %)	8 (1.6 %)	>0.99
Hemoglobinopathy	0 (0 %)	0 (0 %)	0 (0 %)	>0.99
Diabetes	108 (15 %)	35 (15 %)	73(15 %)	
Interval between positive swab and mAbs administration [Days] (IQR)	3 (0–128)	4 (0–121)	3(0–128)	0.085
Monoclonal antibody administered, n (%)				**<0.001**
Sotrovimab (500 mg)	183 (25.2 %)	38 (16.3 %)	145 (29.4 %)	
Casirivimab-Imdevimab (600 mg 600 mg)	25 (3.4 %)	5 (2.1 %)	20 (4.1 %)	
Casirivimab-Imdevimab (1200 mg 1200 mg)	187 (26 %)	92 (39 %)	95 (19 %)	
Casirivimab-Imdevimab (4000 mg 4000 mg)	52 (7.2 %)	26 (11.2 %)	26 (5.3 %)	
Bamlanivimab-Etesevimab (700 mg 1400 mg)	212 (29.2 %)	70 (30 %)	142 (28.8 %)	
Tixagevimab-Cilgavimab (300 mg 300 mg)	67 (9.2 %)	2 (0.9 %)	65 (13.2 %)	
Concomitant administration of iv antiviral therapy (only inpatients)				
Remdesivir	107 (14.7 %)	55 (23.6 %)	52 (10.5 %)	0.46

*Only comorbidities considered as risk factor for COVID-19 clinical progression have been included.
§Solid Organ Transplant patients (all but one vaccinated).

(Beta = -4.7 days, 95 %CI −7.6,-1.8, p = 0.001). Moreover, considering the non-homogeneity of study groups, a linear regression multivariate sub-analysis for outpatients and for inpatients showed significant results only for the primary outcome (Table S2). The analysis on inpatients did not show significant results, while among outpatients the time to virological clearance was associated with being vaccinated (Beta = -2.5 days; 95 %CI −3.9,-1.0, p < 0.001) and having received Tixagevimab/Cilgavimab (Beta = -4.0 days, 95 %CI −6.1,-1,9, p < 0.001) (Table S2).

Table 3
Outcomes by vaccination status.

Characteristics	Sample size, N= 726	Unvaccinated, N= 233	Vaccinated, N = 493	p-value
Time to virological clearance, median (range)	17 (2,128)	19 (2,128)	16 (2, 76)	**<0.001**
Disease progression, n (%)	26 (3.6%)	7 (3.0%)	19 (3.9%)	0.67
Death, n (%)	28 (3.9%)	15 (6.4%)	13 (2.6%)	**0.021**

4. Discussion

Since the beginning of the pandemic, many drugs have been evaluated for their potential treatment and prevention of COVID-19. Nowadays, no treatment capable of eradicating SARS-CoV2 infection has been found. The available treatment options for anti-SARS-CoV-2 infection are limited to three main categories: intravenous drugs (remdesivir), oral antiviral combinations (nirmatrelvir + ritonavir) and mAbs.

Monoclonal antibodies are widely used due to their favorable benefit/risk ratio, high safety profile, and tolerability. Nevertheless, all clinical efficacy data for mAbs have been derived from randomized controlled trials (RCTs) in cohorts that were unvaccinated, minimally immunocompromised, and evaluated when there was circulating virus susceptible to neutralization by both mAbs [21]. Moreover, mAb-based therapies have demonstrated diverse responses between inpatients and outpatients, due to the absence of clarity regarding the distinction between the severity of SARS-CoV-2 infection and the exacerbation of a pre-existing inflammatory condition. As observed by Focosi et al. (2024) [22], it is therefore imperative to monitor the effects of mAbs in these patient subgroups, which currently represent the majority of mAb prescriptions, through real-world studies.

Our data suggest reduced virological clearance in vaccinated outpatients with mild to moderate COVID-19 at risk of developing severe disease. No statistically significant variations were observed among inpatients with a complex medical history. Therefore, our study suggests a potential synergistic role of mAbs in vaccinated patients with mild to moderate COVID-19. The hospitalization and ICU admission rates were similar between the two groups (outpatients and inpatients) at 3.5 % and 3.8 %, respectively. However, there was a significant difference in mortality rates, with 13 % of inpatients experiencing mortality (only 57 % due to COVID-19), compared to none among outpatients. Therefore, our data showed that 43 % of patients died due to comorbidities and factors unrelated to COVID-19. This finding may have an impact on the mortality rate and could explain why it is slightly higher. Additionally, a few patients required a short-term increase in oxygen supply, but there were no complications requiring tracheostomy interventions. The study showed that several factors are associated with an increase in virological clearance, including the time between a positive NPS and mAbs infusion. Inpatients spent approximately 8 days longer than outpatients, while patients with neurodegenerative disorders took about 4 days longer. On the other hand, vaccinated outpatients achieved NPS negativization 2.5 days earlier than inpatients. Overall, in a multivariate analysis with a Cox regression model, vaccinated patients exhibited a more rapid virological clearance compared to unvaccinated patients (p-value: 0.032).

The principal limitations of the study include its single-centre design, which may introduce bias into patient selection, retrospective nature, the limited number of hospitalized patients, and the absence of stratification according to the SARS-CoV-2 variant. Consequently, the variant was indirectly defined based on the dominant one in Italy at the same time. The absence of resistance sub-lineage monitoring represents a limitation of numerous clinical studies, including our investigation, as

Table 3A
Outcomes by mAbs and vaccination status (N=726).

Sotrovimab 500 mg, N = 183				**Tixagevimab - Cilgavimab 300 mg 300 mg, N = 67**			**Casirivimab - Imdevimab 600 mg 600 mg, N = 25**			**Casirivimab - Imdevimab 1200 mg 1200 mg, N = 187**			**Casirivimab - Imdevimab 4000 mg 4000 mg, N = 52**			**Bamlanivimab - Etesevimab 700 mg 1400 mg, N = 212**		
Characteristic	**Unvaccinated, N=38**	**Vaccinated, N=145**	**p-value**	**Unvaccinated, N=2**	**Vaccinated, N=65**	**p-value**	**Unvaccinated, N=5**	**Vaccinated, N=20**	**p-value**	**Unvaccinated, N=92**	**Vaccinated, N=95**	**p-value**	**Unvaccinated, N=26**	**Vaccinated, N=26**	**p-value**	**Unvaccinated, N=70**	**Vaccinated, N=142**	**p-value**
Time to virological clearance, median (range)	20 (9,58)	15 (6,76)	**0.018**	12.5 (10,15)	12 (4,24)	0.79	18 (11,37)	15 (7,28)	0.21	20 (5,128)	18 (7,57)	0.41	25 (8,42)	22 (2,60)	0.44	17 (2,59)	17 (3,47)	0.45
Disease progression, n (%)	0 (0 %)	8 (5.5 %)	0.30	0 (0 %)	6 (9.2 %)	>0.99	0 (0 %)	0 (0 %)		3 (3.3 %)	1 (1.1 %)	0.59	2 (7.7 %)	2 (7.7 %)	>0.99	2 (2.9 %)	2 (1.4 %)	0.85
Death, n (%)	0 (0 %)	4 (2.8 %)	0.68	0 (0 %)	0 (0 %)		0 (0 %)	0 (0 %)		8 (8.7 %)	3 (3.2 %)	0.19	3 (12 %)	5 (19 %)	0.70	4 (5.7 %)	1 (0.7 %)	0.075

this laboratory method is not typically integrated into routine clinical practice. It would have been more appropriate if, during the design and development phase of mAbs, the sub-lineages and patient subgroups, particularly the immunocompromised, could have been given greater attention, in accordance with the review by Focosi D. et al. (2023) [23]. In accordance with the authors' observations, the emergence of immune escape from treatment with anti-Spike mAbs, defined as the emergence of a mutation that drives resistance in at least 20 % of the sequences in a given host at a given time point has been identified as a prevalent and concerning phenomenon. This observation suggests that this phenomenon may be associated with the use of mAbs in immunocompromised hosts. The authors identified 32 publications detailing 216 cases that included various variants of concern (VOC). The incidence of emerging resistance to treatment was found to range from 10 % to 50 %. The majority of resistance events observed in the treatment cohort were present in immunocompromised patients. However, our data did not demonstrate a notable increase in viral clearance in this patient population.

Furthermore, in order to compensate for the lack of a control group for mAbs, a multivariate analysis was conducted using sotrovimab 500 mg as the reference drug for inpatients and tixagevimab/cilgavimab for outpatients. No mAbs significantly reduced NPS negativization time when compared to Sotrovimab among inpatients. However, in the multivariate analysis conducted on outpatients, comparing each monoclonal antibody to the reference drug tixagevimab/cilgavimab, it was demonstrated that tixagevimab/cilgavimab reduced the negativization time by 5 days compared to sotrovimab, and 5.6 days compared to bamlanivimab/etesevimab. The remaining mAbs showed a statistically significant increase in days to clearance of SARS-CoV-2 starting 5 days after the first positive NPS. The *meta*-analysis by Alhumaid et al. [24] confirms that tixagevimab/cilgavimab maintains most of its activity against sublineages BA.4 and BA.5, suggesting lower susceptibility to Omicron variants and potential efficacy during the current epidemiological wave (BA.5). However, its activity may be reduced for BA.1, BA.1.1, and BA.2. Furthermore, Tao K. et al. [25] conducted a systematic review and *meta*-analysis of more than 50 studies on commercially authorized monoclonal antibodies. According to the authors, the first two authorized mAbs combinations, bamlanivimab/etesevimab and casirivimab/imdevimab, were largely inactive against the Omicron BA.1 and BA.2 variants. Sotrovimab showed a median reduction in activity against Omicron BA.1 of 4.0-fold (IQR: 2.6 to 6.9) and a median reduction in activity against Omicron BA.2 of 17-fold (IQR: 13 to 30). The combination tixagevimab/cilgavimab showed a median reduction in activity against Omicron BA.1 of 86-fold (IQR: 27 to 151) and a median reduction in activity against Omicron BA.2 of 5.4-fold (IQR: 3.7 to 6.9). In a recent study by Planas D. et al. [26], the activity of mAbs against the new variants was analyzed. Casirivimab/imdevimab and tixagevimab/cilgavimab lost any antiviral efficacy against BA.2.75.2 and BQ.1.1, whereas sotrovimab remained weakly active against the variants. Therefore, regarding the primary constraint to the application of mAbs is associated with the diminished susceptibility to emerging viral strains, it would be prudent to assess the inclusion of an antiviral agent. Overall, although vaccination has reduced the healing time frame in outpatients, older inpatients and people with pre-existing medical conditions remain at greater risk. This is likely due to the fact that vaccine effectiveness decreases more rapidly in these groups, as confirmed by Tang F. et al. [27]. Additionally, the prolonged interval between vaccine and mAbs administration could have led to a lower vaccine response. Our study data indicates that administering monoclonal antibodies within the first five days of illness can provide the greatest therapeutic benefit, confirming their effectiveness ($p < 0.0001$). A recent observational study by Nevola R. et al. [28] examined almost one thousand patients treated with mAbs. The study found that vaccinated patients had a significantly shorter clearance time of SARS-CoV-2 from the nasopharyngeal swab compared to unvaccinated patients (median: 13 vs 17 days; $p < 0.0001$). However, the authors observed no

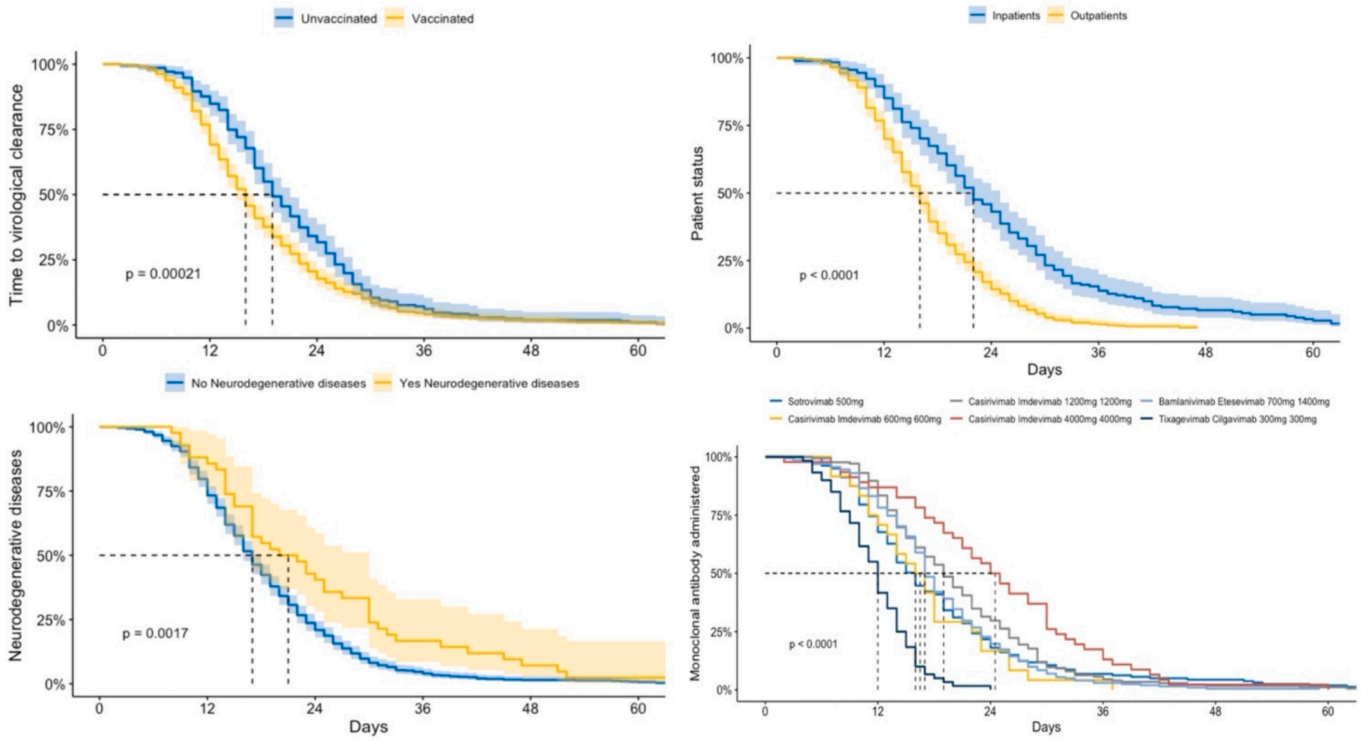

Fig. 3. Kaplan-Meier curves for vaccination status, patient status, neurodegenerative disorders and monoclonal antibodies.

significant difference between the monoclonal antibodies used in terms of hospitalization (p = 0.345). Furthermore, the study found no significant difference in mortality rates between vaccinated and unvaccinated patients (2.1 % vs 2.2 %, respectively; p = 0.925). These results suggest that vaccination does not offer a protective effect on mortality in patients receiving mAb treatment. However, mAbs themselves have been shown to reduce mortality rates. Therefore, the use of mAbs may partially compensate for the vaccine's lack of effect on mortality in these patients. A hypothesis of variability in response to mAbs and reduced vaccine protection can be attributed to the presence of certain mutations in new subvariants (with very high transmission rates). Such mutations have shown to be associated with reduced neutralization of the action of mAbs and vaccine-induced antibodies even in fully vaccinated individuals. Furthermore, patients with comorbidities are more susceptible to SARS-CoV-2 infection, as indicated by the review by Zhang et al. (2022) [29]. Among comorbidities, cardiovascular disease (CVD) has been observed to be the most prevalent in patients at increased risk of COVID-19 infection [30]. A recent analysis of the LEOSS registry [31]

Table 4
Univariate and multivariate regression Cox models for time to virological clearance (N=726).

		Univariate model				Multivariate model
Characteristics	HR	95 % CI	P-value	HR	95 % CI	P-value
Age	1.0	0.99, 1.0	**0.035**			
Male (reference)	–	–	0.85			
Female	1.01	0.87, 1.18				
Inpatients (reference)	–	–		–	–	
Outpatients	2.23	1.85, 2.68	**<0.001**	2.05	1.63, 2.58	**<0.001**
Vaccine	1.35	1.15, 1.59	**<0.001**	1.21	1.02, 1.43	**0.032**
Comorbidity	0.83	0.64, 1.08	0.17			
Obesity (BMI>30)	1.12	0.88, 1.43	0.37			
Cardiovascular and/or cerebrovascular diseases	0.92	0.74, 1.15	0.46			
Chronic respiratory diseases	1.06	0.85, 1.32	0.60			
Chronic renal failure	1.05	0.81, 1.38	0.70			
Neurodegenerative diseases	0.61	0.44, 0.83	**0.002**			
Solid organ transplant patients	1.24	0.79, 1.97	0.35			
Immunodeficiency (primary and secondary)	0.92	0.74, 1.15	0.46			
Chronic hepatopathy	1.08	0.60, 1.96	0.80			
Hemoglobinopathy	0.84	0.35, 2.01	0.69			
Diabetes	0.92	0.74, 1.15	0.46			
Interval between positive swab and mAbs administration	0.99	0.98, 0.99	**<0.001**	0.99	0.98, 1.0	**< 0.001**
Sotrovimab 500 mg (reference)	–	–		–	–	
Casirivimab-Imdevimab 600 mg 600 mg	1.22	0.79, 1.88	0.37	1.01	0.65, 1.56	0.97
Casiriviamb-Imdevimab 1200mg 1200 mg	0.82	0.66, 1.02	0.077	1.04	0.83, 1.31	0.73
Casirivimab-Imdevimab 4000 mg 4000 mg	0.56	0.41, 0.78	**<0.001**	0.97	0.67, 1,41	0.87
Bamlanivimab-Etesevimab 700 mg 1400 mg	1.01	0.82, 1.25	0.89	0.90	0.73, 1.12	0.36
Tixagevimab-Cilgavimab 300 mg 300 mg	3.37	2.48, 4.60	**<0.001**	2.73	1.99, 3.74	**< 0.001**
Remdesivir	1.00	0.74, 1.34	>0.99			

revealed that in all classes of disease severity, more than 50 % of patients with COVID-19 suffered from cardiovascular comorbidities, which were the most prevalent comorbidities associated with the disease. Our data (48.8 %) are consistent with the findings of previous studies. Nevertheless, despite cardiovascular disease being the most prevalent in patients at increased risk of SARS-CoV-2 infection, a univariate analysis using a Cox regression model demonstrated that CVD had no impact on disease progression and time to virological clearance [HR: 0.92, (0.74, 1.15) 95 % CI, p = 0.46].

The only statistically significant underlying comorbidity was neurodegenerative disease. We have demonstrated that patients with neurodegenerative disorders who were treated with mAbs had longer virological clearance [HR: 0.61 (95 % CI: 0.44–0.83; p = 0.002)], in accordance with the study by Montalvan V. et al. [32]. However, a complete understanding of the correlation between pre-existing neurodegenerative disorders and time to achieve virological clearance has not yet been achieved, and further research is warranted to elucidate the underlying mechanisms of this association. Additionally, the authors have discovered that additional factors, such as advanced age and disease severity, may contribute to this occurrence. The observed increase in virological clearance may be associated with the existence of multiple pathways by which the virus penetrates the nervous system. The review by Tyagi et al. [33] highlighted the virus's capacity to traverse the blood–brain barrier and the existence of ACE2 receptors on glial cells in cortical neurons, which could facilitate the neurological manifestations of SARS-CoV-2. Consequently, the olfactory lobes may also serve as the primary gateway for the virus to enter the brain, leading to the intensification of the inflammatory process.

In any case, the relationship between pre-existing neurodegenerative disorders and time to virological clearance is still under investigation, and further research is needed to fully understand this event. It would be of interest to reproduce our study by stratifying patients according to comorbidities. This approach could yield new insights, as evidenced by the findings in the neurodegenerative patient group. It is reasonable to hypothesize that similar observations may emerge in other high-risk patient populations, including transplant patients and those with onco-haematological conditions. Consequently, further investigation is required to assess the impact of these findings on specific patient subgroups. The study analyzed a heterogenous cohort of patients infected with SARS-CoV-2 who were treated with mAbs, which included both inpatients and outpatients, as previously vaccinated and unvaccinated individuals. The combination of all these characteristics makes the study rich in variables, which are rarely found in the plethora of previously published articles on the same topic. As a result, the study contributes to the expansion of knowledge acquired so far on a little-known viral agent. Therefore, our results support the existing literature on the efficacy of mAbs in patients with mild to moderate Covid-19. In patients with complex clinical conditions, however, the efficacy of mAbs is limited. In consideration of these results, it would be prudent to pursue further investigation through pharmacoeconomic studies to determine whether the observed synergy between mAbs administration and vaccination, predominantly in this subgroup of patients, can justify the impact on healthcare spending in order to ensure the sustainability of the system.

5. Conclusions

Our study showed reduced virological clearance in vaccinated outpatients with mild to moderate COVID-19 at risk of developing severe disease. No statistically significant variations were observed among inpatients, likely due to the complexity of their clinical conditions and frequent comorbidities. Indeed, there is currently a paucity of evidence regarding the efficacy of vaccination in high-risk groups, including cancer patients, diabetics, and individuals with cardiovascular, neurodegenerative, renal, or hepatic diseases. Therefore, further specific studies on these populations are recommended. The low rates of hospitalization, ICU admission, and mortality, regardless of vaccination status, suggest a potential key role for mAbs in reducing disease progression. Furthermore, mAbs are most effective when administered within five days of disease onset. Unlike its impact on mild cases, the combination of vaccination and mAbs may not have a synergistic effect in reducing the severity of COVID-19. Furthermore, any variants that might escape neutralizing antibodies need to be identified and characterized at an early stage, so that strategies can be developed and implemented in the future to produce highly effective vaccines targeting specific variants. Consequently, in order to prevent the spread of SARS-CoV-2, we propose the implementation of an intensified, global programme of health surveillance, with particular emphasis on the monitoring of frail patients. The latter represent a high-risk cohort and, if not adequately managed, may significantly increase hospitalization and mortality rates, with consequent implications for health and economic outcomes.

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Ethical aspects

This study was reviewed and approved by Ethics Committee of Sapienza, University of Rome, with the approval number: 6707, dated 13th March 2022. All patients provided written informed consent to participate in the study and for their data to be published. Data were collected anonymously, each patient was assigned an identification code. All data collected was treated in accordance with current privacy regulations and Good Clinical Practice (GCP).

CRediT authorship contribution statement

Nicola Perrotta: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Luigi Angelo Fiorito:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Cristiana Leanza:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Silvia Di Bari:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Gianfranco Casini:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Rossella Gentile:** Software, Methodology, Formal analysis, Data curation, Conceptualization. **Roberta Vescovo:** Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Alfonso Piciocchi:** Software, Methodology, Formal analysis, Data curation. **Camilla Ajassa:** Supervision. **Giancarlo Iaiani:** Validation, Supervision, Methodology, Conceptualization. **Enrica Maria Proli:** Visualization, Validation, Supervision. **Gianluca Russo:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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