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RESEARCH ARTICLE

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Effect of tecovirimat on healing time and viral clearance by emulation of a target trial in patients hospitalized for mpox

Valentina Mazzotta[1,2] | Alessandro Cozzi-Lepri[3] | Simone Lanini[1] | Annalisa Mondi[1] | Fabrizio Carletti[4] | Alessandro Tavelli[5] | Roberta Gagliardini[1] | Serena Vita[1] | Carmela Pinnetti[1] | Camilla Aguglia[1] | Francesca Colavita[2,4] | Paolo Faccendini[6] | Giulia Matusali[4] | Francesca Faraglia[1] | Alessia Beccacece[1] | Jessica Paulicelli[1] | Enrico Girardi[7] | Emanuele Nicastri[1] | Francesco Vaia[8] | Fabrizio Maggi[4] | Andrea Antinori[1]

[1]Department of Clinical and Research Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

[2]Doctoral School of Microbiology, Immunology, Infectious Diseases, and Transplants (MIMIT), University of Rome Tor Vergata, Rome, Italy

[3]Centre for Clinical Research, Epidemiology, Modelling, and Evaluation (CREME), Institute for Global Health, UCL, London, UK

[4]Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

[5]Department of Health Sciences, Clinic of Infectious Diseases, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy

[6]Pharmacy Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

[7]Scientific Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

[8]General Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

Correspondence
Valentina Mazzotta, Clinical and Research Infectious Diseases Department, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Via Portuense 292, 00149 Rome, Italy.
Email: valentina.mazzotta@inmi.it

Funding information
Ministero della Salute, Ricerca corrente Linea 1 e 2

Abstract

Tecovirimat is a treatment option for severe mpox, although randomized clinical trials are ongoing. The aim of the study is to assess the effect of tecovirimat on healing time and the extent of viral clearance by target trial emulation using observational data. Clinical and virological data of patients hospitalized for mpox were collected. Samples from the upper respiratory tract (URT) were grouped in two time points: T1 (median 6 days from symptoms onset) and T2 (median 5 days from T1). Patients were followed-up until recovery. Average treatment effect (ATE) in patients untreated versus treated with tecovirimat was estimated on time to healing and variation in viral load in URT, using a weighted and cloning analysis. Among the 41 patients included, 19 completed a course of tecovirimat. The median time from symptoms onset to hospitalization and to drug-starting was 4 days and 10 days, respectively. No improvement in healing time in treated versus untreated was observed. No difference by treatment group in time to viral clearance was detected by ATE fitted in a subset of 13 patients after controlling for confounders. We found

Valentina Mazzotta and Alessandro Cozzi-Lepri equally contributed.

no evidence for a large effect of tecovirimat in shortening healing time and viral clearance. While awaiting the results of randomized studies, the use of tecovirimat should be restricted to the clinical trial setting.

KEYWORDS
healing, mpox, tecovirimat, therapy, viral clearance

1 | INTRODUCTION

On July 23, 2022, the World Health Organization (WHO) declared mpox to be a Public Health Emergency of International Concern (PHEIC).[1] Among the 85 922 mpox confirmed cases registered worldwide as of February 15, 2023, only 96 deaths occurred,[2] and usually, the disease improved without any antiviral treatment. However, complications leading to hospitalization may occur, and the illness may last for several weeks, during which patients are forced into isolation.

The Centers for Disease Control and Prevention (CDC) suggested considering mpox treatment in people with severe disease or involvement of anatomic areas, which might result in serious sequelae, or in immunocompromised people or at high risk for severe disease.[3] Moreover, the recommended timing for the start of therapy is early after onset.

However, there are currently no proven therapeutics to shorten healing times in mpox. Tecovirimat (TPOXX) was authorized in the United States[4] and EU[5] for use against mpox based on promising results from initial studies in animals[6] and evidence of safety in healthy human volunteers[7]; tecovirimat is also the first choice treatment for mpox suggested by CDC.[3] In recent series, oral tecovirimat was reported as safe and well tolerated,[8–13] no worsening was observed in treated patients, and subjective improvement was reported after a median time of 3 days after treatment. However, the lack of a control group made it difficult to assess treatment effectiveness, and the limited supply did not allow the early use of tecovirimat. Furthermore, randomized controlled trials (RTCs) are still ongoing,[14–16] and there is an urgent need for robust evidence to guide clinical decision-making.[17] Nevertheless, the rapidly decreasing number of mpox cases worldwide will probably not allow RCTs to be concluded soon.

Our study aimed to assess the difference in healing time and extent of viral clearance between patients treated and untreated with tecovirimat using observational data to emulate a hypothetical target trial.

2 | METHODS

2.1 | Study population

We included all adult patients with laboratory-confirmed mpox admitted at the Lazzaro Spallanzani National Institute for Infectious Diseases (INMI; Rome, Italy) from May 19 to September 29 and hospitalized for mpox.

Demographic, epidemiological, and clinical characteristics were collected at the time of diagnosis, and diagnostic testing for the mpox virus (MPXV) was performed.

The patients treated with tecovirimat received a course of 600 mg twice daily for 14 days: the decision regarding treatments was based on international medical consensus and the availability of drugs.

Participants were followed-up for at least 30 days from symptoms onset until complete clinical recovery or last clinical follow-up.

Data on MPXV viral load in samples collected during the hospitalization from the upper respiratory tract (URT), including oropharyngeal swabs and saliva, were recorded. Viral DNA was extracted by the automatic extractor QIAsymphony (Qiagen), and amplified using the real-time polymerase chain reaction (PCR) method targeting the tumor necrosis factor receptor gene, G2R. MPXV DNA concentration was measured using cycle threshold (Ct) values of the MPXV-specific PCR.

All patients provided written informed consent to participate in the study. The study was approved by the Ethical Committee of the Lazzaro Spallanzani Institute (MpoxCohort protocol: *"Studio di coorte osservazionale monocentrica su soggetti che afferiscono per sospetto clinico o epidemiologico di malattia del vaiolo delle scimmie (mpox)"*; approval number 40z, Register of Non-Covid Trials 2022).

2.2 | Statistical analysis

In the natural cohort, the characteristics of treated and untreated participants were compared using a chi-square test or Fisher exact test as appropriate for categorical variables and the Mann–Whitney test for continuous factors.

Because the time of symptom onset did not coincide with the date of treatment initiation, to assess the effectiveness of tecovirimat, in our target population, we used a counterfactual framework accounting for immortal time bias.

The primary endpoint for the comparison between treated and untreated patients was the time from the date of symptom onset to achieving complete clinical recovery by Day 21, defined as the healing of skin and mucosal lesions. Our secondary endpoint was the variation in Ct values in URT from a median of 6 days from the date of symptom onset (T1) to a median of 5 days after T1 (T2).

For the primary endpoint analysis, we aimed to emulate a parallel trial design. The treatment strategies were defined as to start or not

to start tecovirimat within 10 days from symptoms onset; this interval has been consequently chosen as the grace period in the analysis. The per-protocol effect of tecovirimat initiation within 10 days of clinical onset on the primary outcome was quantified by the differences between strategies in (i) not achieving clinical recovery by Day 21 (failure) and (ii) restricted mean survival times (survival time difference over a 21-days window).[18] We assumed that at hospital admission, all participants were equally likely to be offered treatment with tecovirimat. We created two clones of each participant, with one clone allocated to each strategy, hence doubling the size of our data set. The study arms in this newly created pseudo-population were, therefore, identical with respect to demographics and clinical characteristics at the time of entering the hospital, thus minimizing confounding bias at baseline. In each arm, participants' follow-up times have been censored when their treatment was no longer compatible with the treatment strategy for the arm (e.g., when there was a deviation from the planned protocol). In our analysis, this occurred for: (i) participants in the no tecovirimat arm (control) who received tecovirimat within 10 days from symptoms onset and whose follow-up was censored at their time of starting tecovirimat and (ii) participants in the treated arm who did not receive tecovirimat within 10 days from symptoms onset and whose follow-up was censored at 10 days (including participants who started tecovirimat after 10 days). For each participant, clinical recovery by Day 21 (if achieved at all) was attributed to the arm in which the participant was still uncensored at the time of the event (i.e., the arm the participant is compliant with). Because there are common causes of the probability of treatment initiation and that of clinical recovery, the artificial censoring introduced by cloning is usually informative. For the comparison of interest, we identified the following potential confounders: age, HIV status, and disease severity, defined as the presence of more than 20 skin lesions and/or involvement of anatomic areas which might result in serious sequelae (e.g., proctitis, pharyngotonsillitis, or ocular involvement). We use the inverse probability of censoring weights to control for the cloning-induced informative censoring bias. The 95% Cis was calculated using 100 bootstrap replicates.

In the analysis of the secondary endpoint (Ct variation over time), to control for immortal time bias, we used a "matching on time" type of analysis.[19] In this approach, participants who initiated tecovirimat after hospitalization were matched to those who did not receive tecovirimat and were followed up for the same amount of time from the date of symptom onset. For example, if a participant started tecovirimat 5 days after the date of symptom onset (T1) and had a Ct value measured at T1, the patient was matched to another participant who had not received tecovirimat by 5 days who also had a Ct value measured at T1, and both were followed-up from T1 onwards until a second time point (T2) in which a second Ct value was available. We then compared the Ct variation over T1–T2 (in the log2 scale), again by emulating a parallel trial in which tecovirimat was the intervention of interest. The average causal effect of tecovirimat was estimated using marginal models in which, to control for the effects of age and disease severity, we modeled both the exposure (through inverse probability weighting) and the outcome (via regression) or both (doubly robust by means of augmented inverse probability weights). This secondary endpoint analysis was restricted to participants with a Ct value from samples collected from URT available at T1 and T2, and with a T1 value < 35. We also conducted a sensitivity analysis controlling for age and HIV status (numerical problems prevented the adjustment for all three identified confounders at the same time in this analysis).

3 | RESULTS

Forty-one hospitalized subjects with mpox were included as of September 29, 2022. Among the 41 patients enrolled, 19 completed a course of tecovirimat therapy. In Table 1 main characteristics of patients according to tecovirimat exposure were reported. All participants were male, and 95% were self-reported as men who had sex with men. The median age was 35 years (interquartile range [IQR] 32–39), and 78% were Caucasian. Only 3 (7.3%) received smallpox vaccine during childhood. Fifteen (36.6%) patients were living with HIV with a median CD4 cell count of 684 cells/mm^3 (IQR 471– 884), with no evidence for a difference between tecovirimat-treated and untreated individuals. Among the HIV-negative, 17% were on pre-exposure prophylaxis (PrEP) with antiretrovirals (Table 1). Overall, 95% of patients had systemic symptoms, and 25 (61%) were classified as having severe disease; 18 (43.9%) had more than 10 mpox cutaneous lesions, and main organ disease localizations were proctitis (26.8%), and pharyngotonsillitis (22%). In the original cohort, before cloning took place, the median time from symptoms onset to hospital admission was 4 days (IQR 2–6). The main reasons for hospitalization and treatment were mucosal inflammation and/or superinfection of the lesions and/or management of severe pain due to the lesions. The median time from symptoms onset to initiation of tecovirimat was 10 days (IQR 8–11). No deaths were observed, and the overall median time for clinical recovery was 21 days (IQR 17–26).

The emulation of a parallel trial design analysis in the pseudo-population showed that, although the risk of 21-day failure was 4.1% lower in participants who were treated with tecovirimat versus those who remained untreated, the 95% CI was large and did not exclude benefit or harm of treatment. Similarly, no evidence for a significant improvement in recovery time was observed in treated patients, with a mean of 14.7 days estimated for both the treated and untreated groups (Table 2).

A total of 122 URT samples were collected from 15 treated and 19 untreated patients. Among these, a subset of 13 patients (6 treated and 7 untreated) who had a T1 value < 35 and had a second sample at the following time T2, were included in the analysis. Main characteristics of these 13 patients are shown in Table S1 and were similar to those of the full cohort. T1 was a median (IQR) of 6 days (3–8) from the date of onset of symptoms, and T2 was 5 days (3–7) from T1. As a consequence of the matching, the timing of T1 and T2 was similar in treated and untreated. Overall, mean Ct values were

TABLE 1 Main characteristics of hospitalized patients with mpox according to the administration of tecovirimat.

Characteristics	Treated with Tecovirimat	Untreated	*p*-Value*	Total
	N = 19	*N* = 22		*N* = 41
Age, years, median (IQR)	38 (34–46)	33 (29–39)	0.017	35 (31–39)
Smallpox vaccination, *n* (%)	1 (5.3%)	2 (9.1%)	0.643	3 (7.3%)
Caucasian, *n* (%)	15 (78.9%)	17 (77.3%)	0.898	32 (78.0%)
MSM[a], *n* (%)	18 (94.7%)	21 (95.5%)	0.916	39 (95.1%)
Use of Chemsex, *n* (%)	0 (0.0%)	2 (16.7%)	0.166	2 (8.7%)
HIV+, *n* (%)	7 (36.8%)	8 (36.4%)	0.975	15 (36.6%)
CD4 count[b], days, median (IQR)	714 (471–1323)	661 (390–823)	0.487	684 (471–884)
Use of PrEP[c], *n* (%)	3 (15.8%)	4 (18.2%)	0.841	7 (17.1%)
Route of transmission, *n* (%)			0.373	
Recent reported sexual intercourses	18 (94.7%)	21 (95.5%)		39 (95.1%)
Household	0 (0.0%)	1 (4.5%)		1 (2.4%)
Systemic symptoms, *n* (%)	19 (100.0%)	20 (90.9%)	0.183	39 (95.1%)
Number of skin lesions, *n* (%)			0.740	
0–4	6 (31.6%)	9 (40.9%)		15 (36.6%)
5–10	4 (21.1%)	4 (18.2%)		8 (19.5%)
11–20	6 (31.6%)	4 (18.2%)		10 (24.4%)
21+	3 (15.8%)	5 (22.7%)		8 (19.5%)
Pharyngotonsillitis, *n* (%)	6 (31.6%)	3 (13.6%)	0.172	9 (22.0%)
Proctitis, *n* (%)	6 (31.6%)	5 (22.7%)	0.529	11 (26.8%)
Ocular lesions, *n* (%)	2 (10.5%)	1 (4.5%)	0.469	3 (7.3%)
Severity of disease[d], *n* (%)	12 (63.2%)	13 (59.1%)	0.793	25 (61.0%)
Concurrent STIs, *n* (%)	11 (73.3%)	9 (69.2%)	0.814	20 (71.4%)
Gonorrhea	1 (5.3%)	0 (0.0%)		1 (2.4%)
Syphilis	4 (21.1%)	4 (18.2%)		8 (19.5%)
Other	6 (31.6%)	5 (22.7%)		11 (26.8%)
Time from onset to hospital admission, days, median (IQR)	5 (2–6)	4 (2–6)	0.462	4 (2–6)
Time from onset to treatment start, days, median (IQR)	10 (8–11)			10 (8–11)
Clinical recovery, days, median (IQR)	23 (18–29)	20 (16–23)	0.053	21 (17–26)

Abbreviations: IQR, interquartile range; STIs, sexually transmitted infections.

[a]Men who have sex with men.

[b]Available only in HIV+ participants.

[c]Pre-exposure prophylaxis for HIV.

[d]More than 20 skin lesions and/or presence of ocular lesions and/or pharyngotonsillitis and/or proctitis.

*Chi-square or Mann–Whitney *U* test as appropriate.

25.6 (SD 5.05) and 30.7 (SD 6.6) in the raw scale and 4.65 (0.30) versus 4.91 (0.35) in the log2 scale at T1 and T2, respectively. The variation over T1–T2 is expressed as the value at T2 minus the value at T1 so positive changes indicate that the participants had a decrease in viral load at T2 (increase in Ct value). In the unadjusted analysis, such increase was lower in the treated 0.13 log2 (0.53) versus untreated 0.37 (0.50) suggesting poor virological potency of treatment, although not statistically significant (unpaired *t*-test $p = 0.41$). Results were confirmed by the trial emulation analysis which, after controlling for confounding, showed no evidence for a

TABLE 2 Results of emulation of a parallel trial design with the target population of participants admitted to the hospital for mpox.

Original cohort	21-day failure (%)	95% CI[a]	Recovery days		95% CI[a]	
Tecovirimat arm[b]	8.3	0.0	33.3	8.4	1.9	9.2
No Tecovirimat arm	22.2	0.0	55.9	11.7	7.6	12.7
Differences[c]	*−13.9*	*−5.2*	*+21.0*	*−3.3*	*−8.8*	*+0.4*
Emulated cohort	**21-day failure (%)**	**95% CI***		**Recovery days**	**95% CI***	
Kaplan–Meier						
Tecovirimat arm	12.5	0.0	50.5	14.7	12.5	14.9
No Tecovirimat arm	15.4	0.0	31.4	14.7	12.4	14.9
Differences[d]	*−2.9*	*−29.2*	*+33.5*	*−0.01*	*−0.11*	*+0.13*
Weighted Kaplan–Meier						
Tecovirimat arm	9.8	0.0	45.4	14.7	12.5	14.9
No Tecovirimat arm	13.9	0.0	29.3	14.7	12.4	14.9
Differences[e]	*−4.1*	*−29.2*	*+30.8*	*−0.02*	*−0.11*	*+0.12*

Note: It was assumed that treatment initiation was based on the following confounders: age, disease severity, and HIV status. Inverse probability of censoring weights was used to control for the cloning-induced informative censoring bias.

Abbreviation: CI, confidence interval.

[a]The 95% CIs were calculated using 100 bootstrap replicates.

[b]Tecovirimat was administered at the dosage of 600 mg twice daily for 14 days.

[c]These differences are prone to both confounding and immortal-time biases.

[d]These differences are prone to informative censoring.

[e]These differences account for all types of biases under the assumptions detailed in the methods.

TABLE 3 Potential cycle threshold (Ct) changes (log2 scale) over T1–T2 and average treatment effect (ATE) from fitting a linear regression model.

Potential Ct changes (log2 scale) over T1–T2[a] and ATE from fitting a linear regression model				
	Mean in treated with tecovirimat (95% CI)	Mean in untreated (95% CI)	ATE[b] (95% CI)	*p*-Value
Treated versus Untreated				
IPWs	0.25 (−0.02, 0.53)	0.41 (0.08–0.73)	−0.16 (−0.64, 0.33)	0.529
Double Robust	0.38 (0.13–0.64)	0.41 (−0.01, 0.84)	−0.03 (−0.58, 0.53)	0.920
Regression adjustment	0.38 (0.00–0.76)	0.37 (0.01–0.74)	0.01 (−0.56, 0.57)	0.979

Abbreviations: ATE, average treatment effect; CI, confidence interval.

[a]T1 was a median of 6 days after symptoms onset; T2 a median of 5 days after T1.

[b]Weighted for age and HIV status.

difference in the potential changes over T1–T2 by treatment arm and our estimate of the average treatment effect (ATE) was consistent with no difference by treatment group, again with large 95% CI around these estimates (Table 3). Specifically, using the double robust approach, our point estimate for the ATE was −0.03, with a 95% CI ranging from −0.58 to 0.53. Thus, our data are compatible with an equal change in CT values over T1–T2 for treated and untreated as well as with tecovirimat decreasing the CT value (ATE < 0) and tecovirimat increasing the risk (ATE > 0). The width of the CI shows that we had insufficient data for a precise estimation of the ATE but differences in CT of 0.58 or larger in favor of tecovirimat are unlikely.

4 | DISCUSSION

In our trial emulation analysis, we found no evidence for a large effect of tecovirimat on recovery time or viral replication after 5 days in participants who were hospitalized with mpox. The emulation analysis was based on a couple of firm points regarding the choice of the primary outcome and target population used. At this point in time, given the low case fatality rate of mpox and the required isolation during the presence of active skin lesions, time to recovery could be considered the primary clinical outcome to be also used in randomized trials.[16] Our target trial population was restricted to hospitalized patients because the use of

tecovirimat is currently only suggested for persons with severe mpox.[3] In the absence of the results from randomized studies, our results represent a valuable source of evidence for the effectiveness of tecovirimat in this setting.

Similarly to other disease models,[20–22] characterizing the early stage of infection as the viral response phase, also for mpox, a timely initiation of tecovirimat is believed to be critical for the effectiveness of the treatment. This is because tecovirimat is an antiviral, and it is reasonable to assume that a prompt reduction in viral replication would lead to quicker clinical recovery.

Our previous, uncontrolled, descriptive data on treated mpox patients showed a progressive decline in MPXV viral load in the course of antiviral treatment[10]; nevertheless, in other cohorts[23,24] of untreated patients, viral shedding also occurred mainly during the first two weeks of the disease after which it naturally declined. In our present analysis, there was no evidence for a difference in viral load reduction after an average of 12 days from the date of symptoms onset when comparing treated versus untreated mpox patients after controlling for potential immortal and confounding bias.

Our analysis has a number of limitations. First of all, treatment was not randomly allocated and was initiated a number of days after the date of clinical onset/hospitalization. The delay in treatment initiation was due, in the first instance, to the fact that hospitalization typically occurred several days after clinical onset. In addition, once the patient was in the hospital, treatment initiation could have been further delayed by limited drug availability for two main reasons: (i) the drug is typically available only for large stocks, and our single-center study has a limited sample size; (ii) the high cost of the drug. Indeed, in our cohort, the average delay in starting treatment was 10 days after the date of clinical onset. This is consistent with the data of other similar reports showing an average delay of 7–21 days for treatment initiation after hospitalization.[8–10] This delay in treatment initiation appeared to have led to an artificial beneficial effect of tecovirimat in the original cohort analysis, which indeed showed a larger difference between arms for the main outcomes of risk of failure by Day 21 and length of clinical recovery. However, immortal time and confounding bias were minimized by our cloning and weighting approach to analysis, which showed a largely attenuated difference by study arm. In addition, by using a fixed grace period of 10 days for treatment initiation, our analysis cannot address the question of whether greater effectiveness of tecovirimat might be achieved by, for example, initiating therapy earlier. The approach to analysis represents a valid and well-established approach to minimize this source of bias within the framework of trial emulations.

Second, our study, especially for the analysis of the virological endpoint, has a very limited sample size and, consequently, needed more statistical power to detect a difference between arms. Although there seems to be no evidence for a difference by arm from looking at our point estimates, there was a large uncertainty around the estimates, and both benefit and harm could not be excluded with 95% confidence.

Third, URT sample collection and storage varied by participants and over time, and therefore the analysis of the virological endpoint could be conducted only on a small subset of the study population. However, the selection appeared to be fairly random, and the characteristics of the included population were similar to those of the whole cohort. In addition, there were only 5 days on average between the two samples, and it is unknown if it might be a sufficient time to detect a difference. A more classic length of stay outcome could not be evaluated because the time of discharge was influenced by other factors besides clinical recovery (e.g., sample collection, extended isolation, and patients' difficulty in keeping it at home). Similarly, we did not consider endpoints as the resolution of pain because there was no standardized collection of pain grades or types of analgesic treatment. Safety endpoints were not evaluated in this analysis; however, no interruptions were observed and the drug was well tolerated. Last but not least, as usual when using observational data, no matter how sophisticated the analysis might be, we cannot rule out unmeasured confounding bias.

In conclusion, our analysis carries no evidence for a large effect of tecovirimat in reducing viral load and time to clinical recovery in mpox patients compared to no treatment. Despite all the mentioned limitations, our careful analysis of observational data represents one of the valuable current sources of evidence to guide clinical decisions. While awaiting more solid data coming from randomized comparisons, we believe that the use of tecovirimat should be restricted to patients enrolled in clinical trials.

AUTHOR CONTRIBUTIONS

Valentina Mazzotta, Alessandro Cozzi-Lepri, and Andrea Antinori contributed to the study concept and design, analysis, and interpretation of data. Simone Lanini contributed to the critical revision of the manuscript for important intellectual content. Alessandro Tavelli, Camilla Aguglia, Jessica Paulicelli, and Alessia Beccacece acquired the data. Valentina Mazzotta and Alessandro Cozzi-Lepri drafted the manuscript. Alessandro Cozzi-Lepri did the statistical analysis. Annalisa Mondi, Carmela Pinnetti, Roberta Gagliardini, Serena Vita, and Francesca Faraglia reviewed the manuscript. Fabrizio Carletti, Francesca Colavita, Giulia Matusali, and Fabrizio Maggi were responsible for the virological data. Valentina Mazzotta and AA are guarantors. All authors reviewed the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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CONFLICTS OF INTEREST STATEMENT

All authors declare no conflict of interest for the present study. The corresponding author (CC) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported, no important aspects of the study have been omitted, and any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Data were recorded as part of routine activities and from database of the observational study approved by the Ethical Committee of the Lazzaro Spallanzani Institute (MpoxCohort protocol: *"Studio di coorte osservazionale monocentrica su soggetti che afferiscono per sospetto clinico o epidemiologico di malattia del vaiolo delle scimmie (Monkeypox)"*; approval number 40z, Register of Non-Covid Trials 2022). Data have been analyzed anonymously.

ORCID

Valentina Mazzotta http://orcid.org/0000-0002-0240-7504
Annalisa Mondi http://orcid.org/0000-0003-4096-2610

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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