Clinical and laboratory predictors of mpox severity and duration: an Italian multicentre cohort study (mpox-Icona)

Valentina Mazzotta,[a,p] Silvia Nozza,[b,c,p] Simone Lanini,[a,d] Davide Moschese,[e] Alessandro Tavelli,[f,*] Roberto Rossotti,[g] Francesco Maria Fusco,[h] Lorenzo Biasioli,[i] Giulia Matusali,[j] Angelo Roberto Raccagni,[c] Davide Mileto,[k] Chiara Maci,[c] Giuseppe Lapadula,[l] Antonio Di Biagio,[m] Luca Pipitò,[n] Enrica Tamburrini,[o] Antonella d'Arminio Monforte,[f] Antonella Castagna,[b,c,q] and Andrea Antinori,[a,q] for the mpox-Icona study group[r]

[a]Clinical Infectious Diseases Department, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy
[b]Infectious Diseases Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c]Vita-Salute San Raffaele University, Milan, Italy
[d]Infectious Diseases Clinic, Department of Medicine (DAME), University of Udine, Udine, Italy
[e]I Division of Infectious Diseases, ASST Fatebenefratelli Sacco, Luigi Sacco Hospital, Milan, Italy
[f]Icona Foundation, Milan, Italy
[g]Infectious Diseases Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy
[h]UOC Infezioni Sistemiche e dell'Immunodepresso, AORN Ospedali dei Colli, Naples, Italy
[i]Infectious Diseases Unit, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy
[j]Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy
[k]Laboratory of Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli Sacco, Luigi Sacco Hospital, Milan, Italy
[l]Fondazione IRCCS San Gerardo dei Tintori, University of Milano-Bicocca, Monza, Italy
[m]Infectious Diseases Unit, Clinic of Infectious Diseases, IRCCS Policlinico San Martino Hospital, University of Genoa, Genoa, Italy
[n]Infectious and Tropical Diseases Unit, Azienda Ospedaliera Universitaria Policlinico Paolo Giaccone, Palermo, Italy
[o]Infectious Diseases Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Catholic University of the Sacred Heart, Rome, Italy

Summary

Background Severe and prolonged mpox courses have been described during the 2022–2023 outbreak. Identifying predictors of severe evolution is crucial for improving management and therapeutic strategies. We explored the predictors of mpox severity and tested the association between mpox severity and viral load in biological fluids. We also analysed the predictors of disease duration and kinetics of inflammatory markers and described the viral presence and duration of shedding in biological fluids.

Methods This multicentre historical cohort study included adults diagnosed with laboratory-confirmed mpox diagnosis between May 2022 and September 2023 at 15 Italian centres. Patients were followed up from the day of diagnosis until clinical recovery. Biological fluids (blood, urine, saliva, and oropharyngeal and rectal swabs) were collected from each subgroup during the course of the disease and after healing. The primary outcomes were disease severity (presence of mucosal involvement, extended rash, or need for hospitalisation) and its association with the cycle threshold value (Ct-value, surrogate of viral load) in biological fluids, using standard linear and linear mixed-effect logistic regression models. Among the secondary outcomes, predictors of disease duration were assessed using a linear regression model.

Findings A total of 541 patients were enrolled, including four (0.74%) women, with a median age of 38 years (IQR 33–44). Among the 235 people living with HIV (PLWH) (43.44%), 22 (4.07%) had a CD4 count lower than 350 cells/μL. Severe mpox was reported in 215 patients (39.74%). No patient died. Multivariable analysis showed that, severe mpox was more likely among Caucasians (OR 1.82; 95% CI 1.14–2.90, p = 0.012) and patients who had an onset of fever (1.95; 1.27–2.99, p = 0.002), lymphadenopathy (2.30; 1.52–3.48, p < 0.001), sore throat (2.14; 1.27–3.59, p = 0.004), and peri-anal lesions (2.91; 1.93–4.37, p < 0.001). There was a significant difference (p = 0.003) between the median Ct-value in the upper respiratory tract for patients presenting with either mild (35.15; IQR 28.77–42.01) or severe infection (31.00; 25.00–42.01). The risk of developing severe disease decreased by approximately 5% per Ct increase (0.95; 0.91–0.98; p = 0.005). The disease lasted longer in the case of proctitis (+4.78 days; 1.95–7.61, p = 0.001), sore throat (+3.12; 0.05–6.20, p = 0.046), extended rash (+3.42; 0.55–6.28, p = 0.020), as well as in PLWH with a low CD4 count (+12.51; 6.79–18.22, p < 0.001).

*Corresponding author. Icona Foundation, Via Antonio di Rudinì, 8, Milan 20142, Italy.
E-mail address: alessandro.tavelli@icona.org (A. Tavelli).
[p]These authors equally contributed.
[q]These authors equally contributed.
[r]Full list in the Supplementary section.

Interpretation The identification of predictors of severe or prolonged disease and the direct association MPXV Ct-value in the upper respiratory tract and disease severity could be useful in establishing proper management and early treatment of new mpox cases.

Research in context

Evidence before this study

In 2022–2023, a global outbreak of mpox (formerly monkeypox) disproportionately affected men who have sex with men, and a substantial proportion lived with HIV. The clinical spectrum of mpox infections ranges from mild to severe, possibly leading to hospitalisation and, very rarely, death. We conducted a PubMed search for the terms "monkeypox, mpox AND severity, hospitalization" and "monkeypox, mpox AND kinetics, dynamics" to evaluate the existing data on possible predictors of mpox severity and virologic kinetics of infection. Studies identified various factors associated with clinical severity and outcomes, including viral clade, vaccination or infection history, baseline health status, the timing of medical care, concurrent diseases, and immunosuppression, particularly a low CD4 cell count or uncontrolled HIV viral load. Recently, the Mpox Severity Scoring System (MPOX-SSS) has been proposed as a clinical predictor of mpox severity. Moreover, mpox viral kinetics has been widely described, mostly focusing on transmission routes and viral infectivity. Most evidence indicates that higher viral loads occur during the early phases of mpox infection. However, limited data are available on viral shedding after clinical recovery, which may have implications for isolation recommendations.

Added value of this study

This study explored the factors associated with mpox infection severity and their association with the viral load (MPXV Ct-value) in body fluids. Moreover, this study assessed the predictors of mpox duration, analysed the kinetics of inflammatory markers, and described mpox virus (MPXV) DNA detection in body fluids following clinical recovery. The Caucasian race and presentation with fever, sore throat, lymphadenopathy, and peri-anal lesions could predict the severe evolution of the disease. Notably, high upper respiratory tract Ct-values in the first week of infection were a possible predictor of severe mpox infection. This might constitute a measurable laboratory predictor of unfavourable clinical evolution in the very early stages of infection, even among individuals not presenting with skin lesions. The predictors of prolonged mpox include mucosal involvement, diffuse skin rash and advanced HIV infection. These factors can help plan initial counselling and isolation strategies. Characterising the kinetics of C-reactive protein (CRP) and neutrophil-to-lymphocyte ratio (NLR) in severe cases, we observed that the mpox virus showed no tendency to cause specific organ damage. Furthermore, the study showed viral shedding in different anatomical sites after clinical recovery, although definitive interpretations of infectivity could not be drawn. From a clinical perspective, this study could guide risk stratification and therapeutic decisions, whereas, from a public health perspective, it could pave the way for possible containment strategies.

Implications of all the available evidence

The identification of predictors of severe mpox disease could help guide management decisions, especially in the early phases of infection. The direct association between the upper respiratory tract Ct-values (surrogate of viral load) and mpox severity suggests its potential use as a laboratory tool, together with known predictive clinical factors, for early case management and to identify people at risk of severe disease. This may ease the timely beginning of antiviral treatment or indication for hospitalization, particularly among the most vulnerable people, such as those with advanced HIV infection. Finally, research efforts and continued international surveillance are paramount for improving current containment strategies and future responses to outbreaks among members of key populations.

Introduction

Since May 2022, more than 90,000 cases of mpox have been reported in 117 countries,[1] and, for the first time, cases and clusters have been described globally, both in non-endemic and endemic settings.

This unprecedented pattern of spread produced a global outbreak sustained by novel clinical presentations and new human-to-human transmission routes,[2,3] resulting in the novel classification of mpox as a sexually transmitted disease. Indeed, viable mpox virus (MPXV) DNA was detected in seminal fluid.[4,5]

This illness typically lasts for 2–4 weeks and usually heals spontaneously, although complications have been described.[6] According to the SHARE-net Clinical

Group,[3] 41% of infected individuals presented with mucosal involvement and the hospitalization rate was around 13%. The hospitalisation rate has been highly variable (ranging from 1% to 13%),[7] depending on the propensity and policies to use hospitalization as an isolation strategy, as in the early stages of 2022–2023 outbreak in the UK.[8] It is also influenced by clinical factors (such as HIV infection or other immunosuppressive conditions) or some demographic characteristics (ethnicities).[9,10] Factors previously described to be related to the clinical severity and outcome of human mpox include viral clade, prior vaccination or infection,[11] baseline health status, promptness of medical care received, concurrent diseases, and immunosuppression,[7] particularly for individuals living with HIV with CD4 count lower than 200 cells/μL or uncontrolled HIV viral load (VL).[12]

To quickly decide on the type of management (at home or in the hospital) and the early start of treatment, there is a need to explore factors associated with poor clinical outcomes, severe clinical evolution and longer disease duration. Moreover, the Centre for Disease Control and Prevention (CDC) recommended the end of isolation after lesions' healing,[13] however, some data on viral shedding suggest the presence of MPXV DNA in different body fluids after clinical recovery.[14,15]

This study aimed to explore the risk factors associated with the severity of the disease and its duration, including those associated with enhanced MPXV virulence, to evaluate virus detection in different body compartments after clinical resolution, and to describe the kinetics of inflammatory markers during disease.

Methods

Study design and setting

The Mpox-ICONA is a multicentre historical cohort study implemented within the Italian cohort naïve antiretrovirals (ICONA) clinical network. Since the beginning of the mpox outbreak, the ICONA Foundation has endorsed a study on mpox, exploiting its network of clinical centres, including people living with HIV (PLWH) and those without HIV infection (PLWoH) having a high risk of mpox infection.

ICONA is the largest clinical network for HIV in Italy. Over the last 27 years, it has conducted the largest Italian cohort of PLWH, which currently includes more than 21,000 patients enrolled in 64 clinical centers. ICONA networks retain considerable capabilities for biobanking and clinical data sharing. This represents an ideal study setting to assess diseases, such as mpox, that recognise an epidemiological network that overlaps with HIV.

Ethics

The master mpox-ICONA study protocol was centrally approved by the INMI Lazzaro Spallanzani Ethics Committee (approval number 42z, Register of Non-Covid Trials 2022) and notified to the Ethics Committee of each participating clinical centre.

Enrolment in the mpox-ICONA was voluntary, and all enrolled participants provided written informed consent.

Participating clinical centers provided a set of information gathered throughout the clinical management of patients with mpox infections and filled in an *ad-hoc* electronic database developed using RedCap.

Participants

All participants aged 18 years or more with a confirmed mpox diagnosis, by real-time polymerase chain reaction (RT-PCR) assay, between May 2022 and September 2023 were eligible for the analyses. All patients were followed up from the day of diagnosis until clinical resolution of the disease. A minority of clinical centres (n = 5) also shared information on biochemical markers of inflammation and organ damage collected during the course of the disease and collected body fluids after clinical resolution to assess the presence of MPXV DNA.

Variables and data source

Clinical recovery was defined according to Centre for Disease Control and Prevention guidelines as the fall of all the scabs and underneath re-epithelization for those with skin lesions and resolution of all symptoms.[16] Time to clinical resolution (disease duration) was calculated as the number of days from the symptom onset to clinical recovery. Patients who were completely asymptomatic were excluded from the disease duration analysis. We considered severe mpox as the presence, over the course of the disease, of either proctitis, pharyngitis, ocular involvement, extended rash (defined as having more than 20 skin lesions) or needing hospital admission.[17] The detection of MPXV DNA in biological fluids after clinical resolution was also evaluated. In this study, the term virulence was used to describe the direct association between MPXV Ct-value (proxy of viral load) and the severity of the disease.

All participants underwent a first visit at mpox diagnosis, and information was collected on epidemiological features, clinical presentation, and, for a subset of patients, repeated measures of MPXV DNA using CT-values in different anatomical sites, including samples from the upper respiratory tract (URT: oropharyngeal swab or saliva), from anorectal area (anal swab or stool), blood, semen, urine, and inflammation markers, including C-reactive protein (CRP) and neutrophil-to-lymphocyte ratio (NLR).

All information was collected by the medical doctor during the visit and recorded on the mpox-ICONA electronic case report form.

Laboratory method

Inflammation and organ damage markers were assessed using standard methodologies implemented in each clinical centre. MPXV DNA detection was conducted in Italian reference centres for mpox diagnosis using the available diagnostic procedures. As a surrogate for viral DNA levels in body fluids, we used the cycle threshold (Ct) values to study the extent of MPXV DNA shedding in different biological samples. Details of the methodologies used for the measurement of MPXV DNA using Ct values are reported in the Supplementary Materials. RT-PCR for mpox diagnosis has been performed in the following sites/specimens: skin lesions, oropharyngeal swabs, saliva, anal swabs, stool, blood, plasma, semen, and urine. Oropharyngeal, skin lesion and anal swabs were collected in viral transport medium; saliva samples were collected by passive drooling in a sterile container without transport medium; semen, stool, urine samples were collected using sterile containers while blood withdrawn were collected in EDTA tubes.

Objectives

The primary objective was to identify predictors of disease severity and their association with MPXV Ct-value in biological fluids.

The secondary objectives were to determine disease duration, describe the kinetics of inflammatory markers during the disease and detect MPXV in biological fluids after clinical recovery.

Statistics

This historical exploratory study analysed all eligible patients with confirmed MPXV infections. Formal sample size calculations were not performed.

Descriptive analysis was performed by calculating the distribution of patient characteristics in the whole sample. Endpoints for the primary objective were the diagnosis of severe mpox as previously defined, while for the secondary objectives, days between symptom onset and clinical recovery, CRP and NLR changes during the course of mpox infection in hospitalized subjects, and detection of positive MPXV DNA at different sites after clinical recovery.

Analysis for predictors associated with disease severity was carried out using the χ^2-test (univariable analysis) and logistic regression model (multivariable analysis). A backward stepwise approach was used to perform the final set of variables for multivariable analysis. HIV class as a 3-level categorical variable (i.e. PLWoH, PLWH with ≥350 CD4 cells/µL, and PLWH with <350 CD4 cells/µL) was included a priori in the multivariable model, and $p < 0.200$ was used as the cut off for variable selection in the multivariable model. A sensitivity analysis without patients with pharyngitis has also been performed. An analysis to assess the association between Ct-value and severe mpox was conducted on a subset of symptomatic patients with available MPXV Ct-value in the upper respiratory tract within seven days of symptom onset. Undetectable MPXV DNA in the upper respiratory tract was assigned to Ct values ≥ 42.01, representing the method's upper limit of the method. The Kruskal–Wallis test was used to assess the differences in the median Ct values between severe and mild infections. The association between severe disease (as a binary variable) and Ct value as a continuous variable was evaluated using a mixed-effect logistic regression model with a random intercept at the clinical center level. The same association was tested using samples from other body fluids collected during the first week after disease onset. The predictors associated with disease duration were analysed using the t-test (univariable analysis) and a linear regression
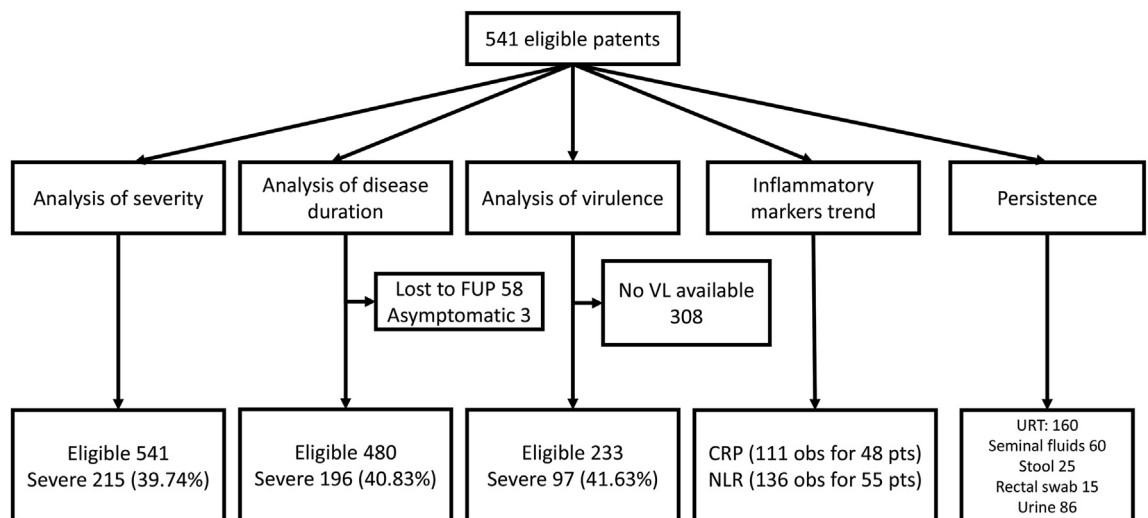

Fig. 1: Flowchart for patient enrolment and inclusion in the different analyses.

model (multivariable analysis) among symptomatic participants at presentation. For a subgroup of patients hospitalised with severe mpox, the temporal kinetics of inflammatory markers [including CRP and NLR] were assessed by linear mixed model for a small sample according to Kenward and Roger model.[18] Each model included the natural log of each marker as dependent variable and as covariates, the days since symptom onset (continuous) and disease severity (binary) and a random intercept at the patients and center level. The model parameters are presented both in natural log and exponential forms. The results were plotted in exponential form to facilitate interpretation. MPXV DNA detection and Ct-value in samples obtained from convalescent patients (from clinical recovery to 46 days after) are also described. Statistical analyses were performed using Stata software (v.18.0 MP). All p-values presented are two-sided, with $p < 0.05$ indicating statistical significance.

Funding

Results

Descriptive analysis and clinical outcomes

Patients' enrolment and inclusion in the different analyses are reported in Fig. 1.

Between May 2022 and April 2023, 541 patients were enrolled. Of these, only four were women. The median age was 38 years (IQR 33–44). There were 235 (43.44%) PLWH; of them, 22 (4.07%) presented with a CD4 count lower than 350 cells/μL, three participants were asymptomatic and identified through active surveillance of local clusters. Severe disease was reported in 215 individuals (39.74%); all the severe mpox patients had skin lesions. Hospitalisation was required in 81 (14.97%) patients: 27 (4.99%) presented with more than 20 skin lesions, 85 (15.71%) with pharyngitis, 107 (19.78%) with proctitis and seven (1.29%) with ocular lesion. No patient died. The main characteristics of the study population are summarised in Table 1.

Risk factors associated with severe disease

Analysis of the risk factors associated with severe mpox included the entire sample (n = 541). The results of the unadjusted and multivariable analyses are presented in Table 2. Multivariable analyses, showed that severe mpox is more likely among Caucasians (OR 1.82; 95% CI 1.14–2.90, p = 0.012) and among patients presenting

Patients features[a]	Num (N = 541)	%
Epidemiological features		
Age 46 years or more[b]	107	19.78%
Female	4	0.74%
Caucasian	404	74.68%
Previous smallpox vaccination	61	11.28%
Omo-bisexual	512	94.64%
Reported sexual transmission	502	92.79%
PLWoH	306	56.56%
PLWH with >350 CD4 cells/mmc	213	39.37%
PLWH with <350 CD4 cells/mmc	22	4.07%
Clinical picture		
Fever	350	64.70%
Lymphadenopathy	317	58.60%
Myalgia	60	11.09%
Sore throat	84	15.53%
Fatigue	73	13.49%
Headache	55	10.17%
Diarrhea	24	4.44%
Severe disease[c]	215	39.74%
Hospitalized[d]	81	14.97%
Pharyngitis	85	15.71%
Proctitis	107	19.78%
More than 20 lesions	27	4.99%
Ocular lesions	7	1.29%
Pattern of rash		
Peri-anal lesion	208	38.45%
Face lesion	174	32.16%
Palmar-plantar lesion	61	11.28%
Penile lesions	241	44.55%
Groin lesion	10	1.85%
Lower limbs lesion	86	15.90%
Upper limbs lesion	92	17.01%
No lesions	17	3.14%

Descriptive analysis was reported according to three groups of patients' characteristics, including epidemiological features, clinical presentation and pattern of rash. PLWH: people living with HIV; PLWoH: people living without HIV. [a]Three patients were asymptomatic and identified through active surveillance of local clusters. [b]Median age 38 years (IQR 33–44). [c]Presence of either proctitis, pharyngitis, ocular involvement, or extended rash (defined as having more than 20 skin lesions) or of needing hospital admission. [d]Eight patients were hospitalised for surveillance purposes and did not present with severe infection.

Table 1: Description of the 541 patients included in the analysis.

with fever (OR 1.95; 95% CI 1.27–2.99, p = 0.002), lymphadenopathy (OR 2.30; 95% CI 1.52–3.48, p < 0.001, sore throat (OR 2.14; 95% CI 1.27–3.59, p = 0.004), and peri-anal lesions (OR 2.91; 95% CI 1.93–4.37, p < 0.001). By contrast, mild presentation was more likely in patients with penile localisation (OR 0.52 95% CI 0.35–0.79; p = 0.002). We did not find any differences according to HIV status or CD4 count. To confirm that the association was driven mainly by the proportion of participants who presented with severe disease due to severe pharyngitis, we performed a sensitivity analysis by fitting the model without the

Patients feature	Severe		Unadjusted analysis				Multivariable analysis			
			Risk for severe disease			p-value	Risk for severe disease			p-value
	Yes	No	OR	95% CI			OR	95% CI		
Overall	215	326	–	–	–	–	–	–	–	–
Fever										
Yes	166	184	2.61	1.78	3.84	<0.001	**1.95**	**1.27**	**2.99**	**0.002**
No	49	142	base	–	–		base	-	-	
Lymphadenopathy										
Yes	150	167	2.2	1.53	3.16	<0.001	**2.3**	**1.52**	**3.48**	**<0.001**
No	65	159	base	–	–		base	-	-	
Myalgia										
Yes	27	33	1.28	0.75	2.18	0.377				
No	188	293	base	–	–					
Sore throat										
Yes	48	36	2.32	1.45	3.7	<0.001	**2.14**	**1.27**	**3.59**	**0.004**
No	167	290	base	–	–		base	-	-	
Fatigue										
Yes	25	48	1.2	0.68	2.09	0.302				
No	190	278	base	–	–					
Headache										
Yes	24	31	1.2	0.68	2.09	0.533				
No	191	295	base	–	–					
Diarrhea										
Yes	15	9	2.64	1.16	6.02	0.02				
No	200	317	base	–	–					
Peri-anal lesion										
Yes	116	92	2.98	2.08	4.27	<0.001	**2.91**	**1.93**	**4.37**	**<0.001**
No	99	234	base	–	–		base	-	-	
Face lesion										
Yes	75	99	1.23	0.85	1.77	0.271				
No	140	227	base	–	–					
Palmar-plantar lesion										
Yes	24	37	0.98	0.57	1.69	0.946				
No	191	289	base	–	–					
Penile lesions										
Yes	74	141	0.5	0.35	0.71	<0.001	**0.53**	**0.35**	**0.79**	**0.002**
No	167	159	base	–	–		base	-	-	
Groin lesion										
Yes	2	8	0.37	0	1.57	0.198	0.31	0.06	1.61	0.163
No	213	318	base	–	–		base	–	–	
Lower limbs lesion										
Yes	26	60	0.61	0.37	1	0.049	0.63	0.36	1.09	0.098
No	189	266	base	–	–		base	–	–	
Upper limbs lesion										
Yes	29	63	0.65	0.4	1.05	0.077				
No	186	263	base	–	–					
Age										
46++	30	77	0.52	0.33	0.83	0.006				
18–45	185	249	base	–	–					
Ethnicity										
Caucasian	172	232	1.62	1.08	2.44	0.021	**1.82**	**1.14**	**2.9**	**0.012**
Non-Caucasian	43	94	base	–	–		**base**	-	-	
Previous vaccination										
Yes	19	42	0.66	0.37	1.15	0.145				
No	196	284	base	–	–					

(Table 2 continues on next page)

Patients feature	Severe		Unadjusted analysis				Multivariable analysis			
			Risk for severe disease			p-value	Risk for severe disease			p-value
	Yes	No	OR	95% CI			OR	95% CI		
(Continued from previous page)										
Sexual transmission										
Yes	204	298	1.74	0.86	3.53	0.126				
No	11	28	base	–	–					
HIV class										
PLWoH	122	184	base	–	–		base	–	–	
PLWH >350 CD4	81	132	0.93	0.65	1.33	0.672	0.68	0.45	1.02	0.060
PLWH<350 CD4	12	10	1.81	0.76	4.32	0.181	1.49	0.57	3.91	0.431

In bold, predictors independently associated with severe disease. PLWH: people living with HIV; PLWoH: people living without HIV.

Table 2: Predictors of severe mpox disease by means of chi-square (unadjusted analysis) and multivariable logistic model.

37 patients with pharyngitis. This model demonstrates that the point estimates are nearly identical, with the p-value and standard deviation increasing due to the shrinking of the sample size (OR 0.95; 95% CI 0.91–0.99, p = 0.023).

Association between MXPV Ct-value in the upper respiratory tract and disease severity

The analysis was performed on a subset of 233 patients (97 with severe disease and 136 with mild disease) with the determination of MPXV DNA in the upper respiratory tract during the first week of the disease. The median Ct-value was 33.62 (IQR 27.35–42.01). Analysis with the Kruskal–Wallis test showed a significant difference (p = 0.003) between the median Ct-value for patients presenting with either mild (35.15; IQR 28.77–42.01) or severe infection (31.00; IQR 25.00–42.01) (Fig. 2A).

The mixed logistic regression model, adjusting for potential heterogeneity between clinical centres (Fig. 2B) provided evidence that the likelihood of severe disease was inversely proportional to the Ct-value and that the risk of developing severe disease decreased by about 5% per Ct-unit (OR 0.95; 95% CI 0.91–0.98, p = 0.005). We found no association between severity and MPXV Ct-values in other fluids (Supplemental Table S1).

Risk factors associated with disease duration

Time to disease resolution and analysis of risk factors associated with disease duration were carried out on 480 patients (88.7%) followed up since clinical recovery. Among these, the mean disease duration was 23.1 days (95% CI 21.9–24.3). Sixty-one patients were excluded from this analysis: three because they were asymptomatic at virological diagnosis and 58 because they did not return to clinical centres (lost to follow-up) (Fig. 1).

The results of the unadjusted and multivariable analyses are presented in Table 3. Multivariable analyses found that more prolonged disease was experienced by patients presenting with lymphadenopathy (+2.47 days; 95% CI 0.11–4.83, p = 0.041), sore throat (+3.12 days; 95% CI 0.05–6.20, p = 0.046), proctitis (+4.78 days; 95% CI 1.95–7.61, p = 0.001) and extended rash (+3.42 days; 95% CI 0.55–6.28, p = 0.020). PLWH with a CD4 count lower than 350 cells/μL also experienced a more prolonged disease (vs. PLWoH: +12.51 days; 95% CI 6.79–18.22, p < 0.001).

Inflammatory markers kinetics among severe mpox infections

We analysed the temporal trend of variation in two routine markers of systemic inflammation, including 48 patients with 111 repeated measures of CRP (range 1–8, mean 2.3 observation per patient) and 53 patients with 136 repeated measures of NLR (range 1–8, mean 2.6 observation per patient). All participants included in the analysis were admitted to the hospital for severe mpox. The temporal kinetics of CRP and NLR are shown in Fig. 3A and B, respectively. Both markers showed a robust log–linear association with time, significantly above the upper standard limit during the first week after symptom onset and eventually normalised over the second and third weeks. Only one subject had a sudden increase in CRP level during the third week of mpox infection. This event was associated with a secondary bacterial infection of the skin. We also tried to explore the temporal kinetics of several other biochemical markers, including kidney function and transaminase, which could have been helpful for better understanding specific tissue damage during severe mpox. However, we found no evidence of clinically significant organ injury. In particular, only one subject had creatinine higher than 2 mg/dL which eventually normalized while other patients had a mild to moderate increase of ALT which was always below 200 UI/L (i.e. 5 times the upper normal limit). Results of liver and renal function markers are reported in Supplemental Figure S1.

Detection of MPXV-DNA in different anatomical sites post-clinical resolution

The detection of MPXV-DNA was assessed in a convenience sample of participants who returned to the
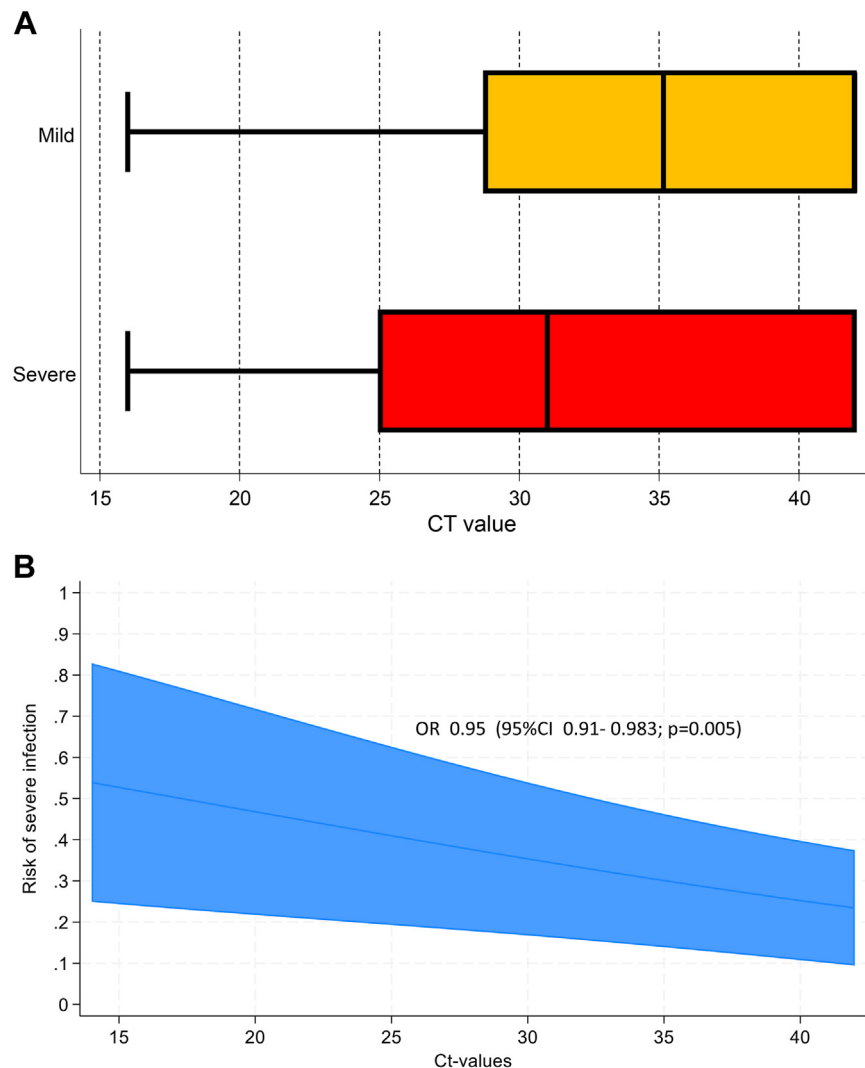
Fig. 2: (A) Kruskal–Wallis test for comparison of median cycle threshold (CT) values for MPXV in the upper respiratory tract during the first week of infection. The analysis includes a subset of 233 patients (97 with severe disease and 136 with mild disease). The analysis shows that patients with severe diseases have lower Ct values (i.e. higher viral load) than those with mild infection (p = 0.003); **(B)** Mixed logistic regression model to assess the association of disease severity with MPXV Ct-value in the upper respiratory tract. The model includes 233 patients enrolled in eight different centres and shows that the risk of severe infection decreases by 5% per unit-Ct increase (p = 0.005). This provides evidence that lower Ct-values (i.e. higher viral load) is strongly associated with the severity of diseases.

clinical centres after complete disease resolution. Upper respiratory tract specimens after disease resolution were available for 72 patients; 17 (23.6%) of them, collected in a time range from 0 to 46 days from resolution, were positive (Ct range 26.03–40.74). Seminal fluid specimens after disease resolution were available for 28 patients (38.9%); 12 (42.9%) were positive (Ct range 31.66–40.57; collection time range since resolution 0–46 days). Rectal swab specimens after disease resolution were available in 15 patients; of them, four (26.7%) were positive (Ct range 33.79–38.31; collection time range since resolution 0–32 days). Urine specimens after

disease resolution were available for 47 patients; three (6.0%) were positive, all collected on the same day of disease resolution (Ct range 33.75–36.13) (Supplemental Table S2).

Discussion

Our study identified predictors of mpox severity, such as Caucasian ethnicity, onset characterised by systemic symptoms (fever and lymphadenopathy), specific location of skin lesions (peri-anal), and sore throat.

Similarly, some authors recently developed the Mpox Severity Scoring System (MPOX-SSS)[19] based on the

Patients feature	Distribution		Unadjusted analysis				Multivariable analysis			
			Time to recovery (day)			p-value	Time to recovery difference (day)			p-value
	Num	%	Mean in days	95% CI			Mean variation in days	95% CI		
Overall	480	100.00%	23.10	21.93	24.27					
Fever										
No	170	35.42%	21.21	20.03	22.39	0.018	base	–	–	
Yes	310	64.58%	24.14	22.45	25.82		2.02	−0.48	4.51	0.113
Lymphadenopathy										
No	197	41.04%	21.29	20.00	22.58	0.011	base	–	–	
Yes	283	58.96%	24.36	22.60	26.12		**2.47**	**0.11**	**4.83**	**0.041**
Myalgia										
No	422	87.92%	23.21	21.92	24.51	0.607	base	–	–	
Yes	58	12.08%	22.28	20.12	24.43		−2.40	−5.92	1.11	0.180
Sore throat										
No	402	83.75%	23.03	21.69	24.38	0.804	base			
Yes	78	16.25%	23.44	21.44	25.44		**3.12**	**0.05**	**6.20**	**0.046**
Fatigue										
No	409	85.21%	23.05	21.72	24.38	0.837				
Yes	71	14.79%	23.39	21.40	25.39					
Headache										
No	429	89.38%	22.81	21.96	23.65	0.152				
Yes	51	10.63%	25.57	16.96	34.17					
Diarrhea										
No	461	96.04%	23.17	21.96	24.38	0.579				
Yes	19	3.96%	21.47	18.13	24.81					
Peri-anal lesion										
No	288	60.00%	22.50	21.44	23.57	0.219				
Yes	192	40.00%	23.99	21.55	26.44					
Face lesion										
No	314	65.42%	22.12	21.18	23.07	0.023	base	–	–	
Yes	166	34.58%	24.95	22.09	27.81		1.91	−0.59	4.40	0.134
Palmar-plantar lesion										
No	422	87.92%	22.82	21.55	24.08	0.192				
Yes	58	12.08%	25.17	22.16	28.18					
Penile lesions										
No	262	54.58%	22.69	20.77	24.62	0.455				
Yes	218	45.42%	23.59	22.44	24.73					
Groin lesion										
No	470	97.92%	23.01	21.83	24.20	0.314				
Yes	10	2.08%	27.2	20.11	34.29					
Lower limbs lesion										
No	401	83.54%	22.73	21.38	24.07	0.159	base	–	–	
Yes	79	16.46%	24.99	23.08	26.89		2.25	−0.84	5.33	0.153
Upper limbs lesion										
No	396	82.50%	22.77	21.42	24.11	0.221				
Yes	84	17.50%	24.68	22.57	26.79					
Ocular lesions										
No	474	98.75%	23.03	21.85	24.21	0.278				
Yes	6	1.25%	28.83	23.89	33.77					
Pharyngitis										
No	401	83.54%	22.43	21.58	23.27	0.011				
Yes	79	16.46%	26.52	20.83	32.21					

(Table 3 continues on next page)

Patients feature	Distribution		Unadjusted analysis				Multivariable analysis			
			Time to recovery (day)			p-value	Time to recovery difference (day)			p-value
	Num	%	Mean in days	95% CI			Mean variation in days	95% CI		
(Continued from previous page)										
Proctitis										
No	384	80.00%	22.14	21.30	22.98	0.001	base	–	–	
Yes	96	20.00%	26.94	22.18	31.70		**4.78**	**1.95**	**7.61**	**0.001**
More than 20 lesions										
No	456	95.00%	22.92	21.71	24.13	0.189	base	–	–	
Yes	24	5.00%	26.50	22.86	30.14		**3.42**	**0.55**	**6.28**	**0.020**
Age										
18–45	382	79.58%	22.65	21.74	23.55	0.134				
46 ++	98	20.42%	24.86	20.31	29.40					
Ethnicity										
Non-Caucasian	122	25.42%	22.78	21.08	24.48	0.753				
Caucasian	358	74.58%	23.21	21.75	24.67					
Previous vaccination										
No	424	88.33%	22.83	22.00	23.65	0.204				
Yes	56	11.67%	25.18	17.19	33.16					
Sexual transmission										
No	28	5.83%	22.29	18.69	25.88	0.733				
Yes	452	94.17%	23.15	21.93	24.37					
HIV class										
PLWoH	268	55.83%	22.29	20.77	23.82	0.000	base	–	–	
PLWH >350 CD4	192	40.00%	22.81	21.00	24.61		–0.52	–2.87	1.84	0.668
PLWH <350 CD4	20	4.17%	36.70	31.11	42.29		**12.51**	**6.79**	**18.22**	**<0.001**

In bold, predictors independently associated with longer duration of disease. PLWH: people living with HIV; PLWoH: people living without HIV.

Table 3: **Predictors of disease duration by means of t-test (unadjusted analysis) and multivariable linear regression model.**

number of active lesions, the anatomic extent of lesion involvement, the presence of confluent lesions, the presence of bacterial superinfection, the extent of mucosal areas affected, level of care, and analgesia requirement.

Knowledge of the features at clinical presentation possibly linked to the severe evolution of the disease could help clinicians manage patients. Distinctive presentations of severe mpox are represented by proctitis[20,21] and pharyngotonsillitis[22] that lead to hospitalisation and often require the intervention of other specialists because of possible fatal complications (such as tonsillar abscesses, airway obstruction and rectal bleeding), especially in PLWH.[23] Recognition of predictors of severe disease could also influence the decision to start treatment in the early stages of the disease. One reason for the failure to demonstrate the clinical and virological efficacy of tecovirimat is been speculated to be the delayed administration of the drug.[24] Early identification of individuals who may benefit from treatment and, consequently, starting treatment very early could increase the likelihood of an effect of currently available drugs.[25,26]

Importantly, our findings showed a direct association between the MPXV Ct-value measured in the first week after onset in the upper respiratory tract and disease severity. This result provides a possible measurable laboratory predictor of unfavourable clinical evolution in the early stages of the disease owing to the higher positivity rate of URT samples, even in patients without skin lesions.[27] Indeed, the association between MPXV Ct-value and disease severity was confirmed by sensitivity analyses after excluding patients with pharyngitis.

Viral kinetics have been widely described mainly to investigate the transmission route and infectivity of mpox, demonstrating the presence of MPXV in several biological fluids, with higher VL and positivity rates during the first two weeks of disease, and the duration of viable viral isolation from 14 days for saliva and urethra samples to 19 days for anorectal samples.[14,28,29]

Evidence that a higher VL in URT is associated with the most severe clinical presentation suggests that patients developing severe disease may be more efficient spreaders of infection.

Predictors of longer disease duration include mucosal involvement, extended skin rash, and advanced
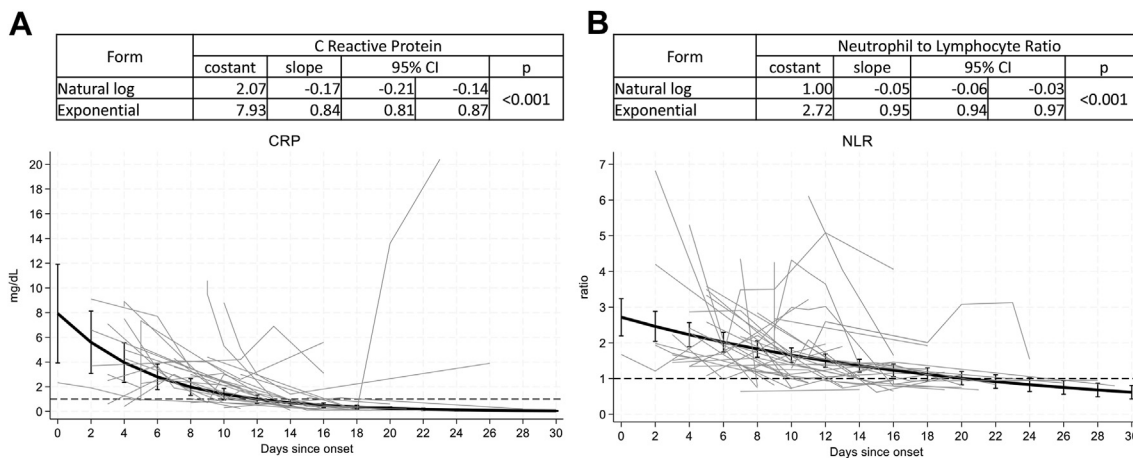
A

Form	C Reactive Protein				
	costant	slope	95% CI		p
Natural log	2.07	-0.17	-0.21	-0.14	<0.001
Exponential	7.93	0.84	0.81	0.87	

B

Form	Neutrophil to Lymphocyte Ratio				
	costant	slope	95% CI		p
Natural log	1.00	-0.05	-0.06	-0.03	<0.001
Exponential	2.72	0.95	0.94	0.97	

Fig. 3: Decay model according to Kenward and Roger mixed effect model. Black solid lines represent estimates and relative 95% CI. Black dashed lines represent normal upper values. Model parameters are reported both as natural log (additive association) and exponential (multiplicative association). The constant value represents the estimate at the time of symptom onset, and the slope represents an estimate of the decay coefficient over time. A) Exponential decay model for C reactive protein (CRP) between day 0 and 30 since symptoms onset. The model shows that the CRP average is 7.93 mg/dL at the onset of symptoms, eventually dropping by a multiplicative coefficient of 0.84 per day (p < 0.001). B) Exponential decay model for neutrophil-to-lymphocyte ratio (NLR) between day 0 and 30 since symptoms onset. The model shows that the NLR average is 2.72 at symptom onset and eventually drops by a multiplicative coefficient of 0.95 per day since symptom onset (p < 0.001).

HIV infection. A severe and prolonged course of mpox in PLWH with low CD4 counts has been described.[12,30–32] Mucosal immunity is altered in PWH, leading to an impaired mucosal barrier that predisposes PWH to systemic inflammation and opportunistic infections.[33] Mpox, in particular, given its frequent mucosal route of infection, behaves as an opportunistic infection in severely immune-depressed patients with a prolonged course and severe mucosal involvement due to a lack of a host immune response.[34] Our results confirm published literature, including the identification of other features at the onset that predict long-lasting disease. This could have implications for counselling during the first evaluation and managing isolation.

Moreover, we found that viral shedding may occur at several anatomical sites even after clinical recovery, as reported in previous case series.[35] Some studies have highlighted that viral clearance from biological fluids mainly occurred within four weeks of the disease onset.[14,27,28,36,37] Although, in very few individuals, long-term shedding was observed in the semen and URT.[38] Notably, we found a limited number of positive samples with Ct value < 35. Although this finding may suggest the unlikelihood of contagiousness[39,40] after recovery, as previously reported,[14] in the absence of viable virus analysis, we cannot establish the possible infectivity of these samples.

Finally, our analysis of inflammatory and biochemical parameters in this setting suggests that even severe mpox presenting with sudden onset, is a self-limiting systemic infection with no tendency to produce specific organ damage. Notably, only 4% of the cohort comprised immunocompromised individuals. Therefore, the generalisability of the results should be confirmed for this category. However, secondary bacterial infections or sepsis can occur during mpox, leading to organ damage and necessitating specific antibiotic therapies.[41–43]

These results should be interpreted with caution in light of the nature of the study design. Indeed, we report the results of explorative analyses on a historical data set lacking a homogeneous follow-up. This may have introduced a potential selection bias that reduced the generalisability of our results. We also used the Ct-values as a surrogate of viral load in this study, while it is worth noting that real-time PCR does not provide quantitative results as it does not use normalized controls. Moreover, our results pertain to Clade IIb MPXV infection that caused the global outbreak in 2022–2023; therefore, they are unlikely to be generalisable to MPXV Clade I cases, such as those of the 2023–2024 outbreak in the Democratic Republic of Congo.[44] In addition, we note that we did classify people as having severe mpox infection based on a definition which is not validated, given the lack of universal consensus on the definition of disease severity. Finally, the explorative nature of the study might have neglected several exposures associated with the clinical evolution of the disease.

Conclusions

The present study identified predictors of severe or prolonged disease using an observational analysis of mpox cases. In cases of proctitis, sore throat, lymphadenopathy, and disseminated skin lesions, mpox may

last longer, as observed also in PLWH with a low CD4 count. Conversely, the Caucasian race and presentation with fever, sore throat, lymphadenopathy, and peri-anal lesions could predict a severe evolution of the disease. Although the MPXV shows no tendency to cause specific organ damage, it behaves like a virulent pathogen with a direct association between VL (evaluated with MPXV Ct-value as surrogate markers) in the upper respiratory tract, disease severity and with viral shedding that may last after clinical recovery at several anatomical sites, albeit with uncertain persistent infectivity. In the current epidemiologic scenario, characterised by sporadic cases or limited clusters in unvaccinated or vaccinated people, this information may be useful for the proper management of new mpox cases, as well as for identifying people at risk of progression to severe or prolonged disease who are, potentially eligible for antiviral treatment or hospitalisation.

References
1. *2022 mpox outbreak global map | mpox | poxvirus.* CDC; 2024. published online April 23 https://www.cdc.gov/poxvirus/mpox/response/2022/world-map.html. Accessed May 24, 2024.
2. Antinori A, Mazzotta V, Vita S, et al. Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. *Euro Surveill.* 2022;27:2200421.
3. Thornhill JP, Barkati S, Walmsley S, et al. Monkeypox virus infection in humans across 16 countries - April-June 2022. *N Engl J Med.* 2022;387:679–691.
4. Raccagni AR, Candela C, Mileto D, et al. Monkeypox infection among men who have sex with men: PCR testing on seminal fluids. *J Infect.* 2022;85:573–607.
5. Lapa D, Carletti F, Mazzotta V, et al. Monkeypox virus isolation from a semen sample collected in the early phase of infection in a patient with prolonged seminal viral shedding. *Lancet Infect Dis.* 2022;22:1267–1269.
6. Petersen E, Kantele A, Koopmans M, et al. Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. *Infect Dis Clin North Am.* 2019;33:1027–1043.
7. Mitjà O, Ogoina D, Titanji BK, et al. Monkeypox. *Lancet Lond Engl.* 2023;401:60–74.
8. Ward T, Overton CE, Paton RS, Christie R, Cumming F, Fyles M. Understanding the infection severity and epidemiological characteristics of mpox in the UK. *Nat Commun.* 2024;15:2199.
9. Henao-Martínez AF, Orkin CM, Titanji BK, et al. Hospitalization risk among patients with Mpox infection-a propensity score matched analysis. *Ther Adv Infect Dis.* 2023;10:20499361231196683.
10. Garneau WM, Jones JL, Dashler GM, et al. Risk factors for hospitalization and effect of immunosuppression on clinical outcomes among an Urban cohort of patients with mpox. *Open Forum Infect Dis.* 2023;10:ofad533.
11. Hazra A, Zucker J, Bell E, et al. Mpox in people with past infection or a complete vaccination course: a global case series. *Lancet Infect Dis.* 2024;24:57–64.
12. Mitjà O, Alemany A, Marks M, et al. Mpox in people with advanced HIV infection: a global case series. *Lancet Lond Engl.* 2023;401:939–949.
13. Isolation and Infection Control At Home. *Mpox | poxvirus.* CDC; 2023. published online Sept 27 https://www.cdc.gov/poxvirus/mpox/clinicians/infection-control-home.html. Accessed May 24, 2024.
14. Suñer C, Ubals M, Tarín-Vicente EJ, et al. Viral dynamics in patients with monkeypox infection: a prospective cohort study in Spain. *Lancet Infect Dis.* 2023;23:445–453.
15. Towns JM, Lim CK, Chow EPF, et al. Persistence of monkeypox virus at oral and rectal sites. *Lancet Microbe.* 2023;4:e210.
16. *Clinical recognition | mpox | poxvirus.* CDC; 2023. published online Sept 21 https://www.cdc.gov/poxvirus/mpox/clinicians/clinical-recognition.html. Accessed May 24, 2024.
17. Mazzotta V, Cozzi-Lepri A, Lanini S, et al. Effect of tecovirimat on healing time and viral clearance by emulation of a target trial in patients hospitalized for mpox. *J Med Virol.* 2023;95:e28868.
18. Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics.* 1997;53:983–997.
19. Zucker J, McLean J, Huang S, et al. Development and Pilot of an mpox severity Scoring System. *J Infect Dis.* 2024;229:S229–S233.
20. Bruzzesi E, Raccagni AR, Mileto D, et al. Isolated monkeypox proctitis among men who have sex with men. *Infection.* 2023;51:1597–1598.
21. Mazzotta V, Scorzolini L, Falasca L, et al. Lymphofollicular lesions associated with monkeypox (Mpox) virus proctitis. *Int J Infect Dis.* 2023;130:48–51.
22. Pinnetti C, Mondi A, Mazzotta V, et al. Pharyngo-tonsillar involvement of Mpox in a cohort of men who have sex with men (MSM): a serious risk of missing diagnosis. *J Infect Public Health.* 2024;17:130–136.
23. Silva MST, Coutinho C, Torres TS, et al. Mpox severity and associated hospitalizations among people with HIV and related immunosuppression in Brazil. *AIDS Lond Engl.* 2024;38:105–113.
24. *Treatment information for Healthcare Professionals | mpox | Poxvirus.* CDC; 2023. published online April 4 https://www.cdc.gov/poxvirus/mpox/clinicians/treatment.html. Accessed May 24, 2024.
25. Aldred B, Lyles RH, Scott JY, et al. Early tecovirimat treatment for mpox disease among people with HIV. *JAMA Intern Med.* 2024;184:275–279.
26. Nguyen BT, Marc A, Suñer C, et al. Early administration of tecovirimat shortens the time to mpox clearance in a model of human infection. *PLoS Biol.* 2023;21:e3002249.
27. Meschi S, Colavita F, Carletti F, et al. MPXV DNA kinetics in bloodstream and other body fluids samples. *Sci Rep.* 2024;14:13487.
28. Colavita F, Antinori A, Nicastri E, et al. Monkeypox virus in human body sites and fluids: evidence for transmission. *Lancet Infect Dis.* 2023;23:6.
29. Kim H, Kwon R, Lee H, et al. Viral load dynamics and shedding kinetics of mpox infection: a systematic review and meta-analysis. *J Travel Med.* 2023;30:taad111.
30. Pinnetti C, Cimini E, Mazzotta V, et al. Mpox as AIDS-defining event with a severe and protracted course: clinical, immunological, and virological implications. *Lancet Infect Dis.* 2024;24:e127–e135.

31 Govind A, Lazarte SM, Kitchell E, et al. Severe mpox infections in people with uncontrolled human Immunodeficiency virus. *Clin Infect Dis*. 2023;76:1843–1846.

32 Ogoina D, Dalhat MM, Denue BA, et al. Clinical characteristics and predictors of human mpox outcome during the 2022 outbreak in Nigeria: a cohort study. *Lancet Infect Dis*. 2023;23:1418–1428.

33 Heron SE, Elahi S. HIV infection and Compromised mucosal immunity: oral manifestations and systemic inflammation. *Front Immunol*. 2017;8:241.

34 Shin H, Shin H, Rahmati M, et al. Comparison of clinical manifestations in mpox patients living with HIV versus without HIV: a systematic review and meta-analysis. *J Med Virol*. 2023;95:e28713.

35 Adler H, Gould S, Hine P, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect Dis*. 2022;22:1153–1162.

36 Candela C, Galli L, Rizzo A, et al. Mpox DNA clearance in semen over 6-month follow-up. *J Med Virol*. 2023;95:e29259.

37 Palich R, Burrel S, Monsel G, et al. Viral loads in clinical samples of men with monkeypox virus infection: a French case series. *Lancet Infect Dis*. 2023;23:74–80.

38 Pettke A, Filén F, Widgren K, et al. Ten-week follow-up of monkeypox case-patient, Sweden, 2022. *Emerg Infect Dis*. 2022;28:2074–2077.

39 Lim CK, McKenzie C, Deerain J, et al. Correlation between monkeypox viral load and infectious virus in clinical specimens. *J Clin Virol*. 2023;161:105421.

40 Paran N, Yahalom-Ronen Y, Shifman O, et al. Monkeypox DNA levels correlate with virus infectivity in clinical samples, Israel, 2022. *Euro Surveill*. 2022;27:2200636.

41 Di Bari S, Mondi A, Pinnetti C, et al. A case of severe mpox complicated with Streptococcus pyogenes sepsis in a patient with HIV infection. *Pathogens*. 2023;12:1073.

42 Studemeister L, Pai S, Walsh K, Cooper J. Acute tonsillitis due to monkeypox. *J Emerg Med*. 2023;64:211–213.

43 Brown L, Delaney C, Hainsworth A. Monkeypox-associated proctitis and rectal wall perforation. *BMJ Case Rep*. 2023;16:e252168.

44 Mpox (monkeypox) - Democratic Republic of the Congo. https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON493. Accessed May 24, 2024.