Modeling lesion transition dynamics to clinically characterize mpox patients with clade I MPXV in the Democratic Republic of the Congo

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One-sentence summaries

The proposed approach classifies mpox patients into two groups by severity of lesions and predicts prognosis using individual viral load data.

Abstract

Coinciding with the global outbreak of clade IIb mpox virus (MPXV), the Democratic Republic of the Congo (DRC) recently experienced a rapid surge in mpox cases with clade I MPXV. On August 14, 2024, the WHO declared the continued cross-border spread of this clade in Africa a Public Health Emergency of International Concern (PHEIC). Clade I MPXV is known to be more fatal than clade IIb, but its clinical characteristics and prognosis differ between patients. Here, we used mathematical modelling to quantify temporal changes in total lesion counts during clade I MPXV infections, using data from a large cohort of patients with mpox in the DRC from 2007-2011. We further analyzed individuals' clinical data to explore predictive biomarkers of high lesion counts. Our analysis indicates that patients with mpox can be stratified into two groups according to lesion severity, and that viral load in peripheral blood at symptom onset may serve as a predictor for this classification (AUC=0.70). Our estimates also suggest substantial individual heterogeneity in the time period for which patients have lesions, ranging from 20 to 65 days. Understanding the severity and duration of lesions in different patients, as characterized by our approach, may contribute to more tailored treatment strategies and control measures in ongoing clade I mpox outbreaks.

Introduction

Mpox (formerly known as monkeypox) is a zoonotic disease caused by the monkeypox virus (MPXV) (*1, 2*). MPXV was first discovered in humans in the Democratic Republic of the Congo (DRC) in 1970, and is classified into two clades (clade I and clade II). Both clades are endemic in countries in Central and East Africa (clade I) and West Africa (clade II), and were historically mainly transmitted to humans from animals (*3-7*). A recent global mpox outbreak with clade IIb has spread via human-to-human transmission, emerging in Western countries in May 2022, and predominantly affecting men who have sex with men through sexually associated transmission (*8-10*). Initially rapid, the mpox outbreak was no longer considered a public health emergency of international concern (PHEIC) by May 2023. Nevertheless, following earlier concerns (*11, 12*), the number of mpox cases has substantially increased in the Western Pacific Region since April 2023, particularly in China. Moreover, the DRC has recently documented transmission via sexual contact of clade I MPXV (*13*), which has a crude case fatality ratio (CFR) of about 10% among historical cases infected with MPXV subclade Ia (*5, 6, 14, 15*), substantially higher than that of clade IIb during 2022 (<1%) (*16, 17*). On August 14, 2024, the WHO held an emergency committee meeting where they declared the rise and spread of the fatal mpox clade Ia a PHEIC for the second time (*18*). This declaration came in response to the increasing number of mpox cases and their ongoing cross-border spread in Africa, originating from the DRC and affecting at least 13 countries. As of August 2024, while 96% of the total cases have been reported in the DRC, several other African countries, including Burundi, Kenya, Rwanda, and Uganda, have reported their first-ever mpox outbreaks (*19, 20*).

Understanding disease progression is key for both treatment and outbreak control. Common symptoms of mpox include a skin rash or mucosal lesions accompanied by fever, headache, muscle aches, back pain, low energy, and swollen lymph nodes (*17, 21-24*). The skin lesions associated with mpox typically persist for 2 to 3 weeks and progress through seven stages: macules, papules, vesicles, and then pustules, followed by umbilication, scabbing, and

desquamation (*25, 26*). Current recommendations for patients are to refrain from direct skin contact from symptom onset until lesions have healed and scabs fall off (in other words, a symptom-based isolation rule (*27*)), given that person-to-person transmission of mpox can occur through direct contact with infectious skin or other lesions (*25, 26*). Notably, the viral load in skin lesion samples is markedly higher, by several orders of magnitude, and persists longer than the viral load in other body locations (*28, 29*). The number of skin lesions that a patient experiences during MPXV infection varies over time and between individuals, ranging from a few to over 1000 (*7, 22, 24*). Clinical features of the rash also differ depending on MPXV clade (*23, 28, 30, 31*). Thus, individual-level dynamics in the number of lesions provide evidence for determining tailored treatment and effective interventions. However, mpox lesion dynamics have not previously been characterized quantitatively, and in particular, individual heterogeneity is yet to be fully examined.

In this study, we quantify the lesion count progression (that is., the temporal change in total lesion counts at different stages) and find predictors of individual-level lesion severity, using a large longitudinal dataset that contains lesion counts, viral loads from various specimens, and other biomarkers from patients infected with clade I MPXV in the DRC during 2007–2011 (*24*). Lesion severity, in our study, is determined by lesion counts and the duration of the presence of lesions. Our analysis stratifies the study population into two groups by lesion severity (identifying severe progressors who experience high lesion counts and/or a prolonged duration of lesions), clinically characterizes them, and identifies clinical markers predicting the lesion severity group. We believe that our findings are relevant to the ongoing clade I mpox outbreaks, and may provide immediate implications for treatment decisions and control measures.

Results

Description of cohort and study design

We used comprehensive clinical data collected from MPXV-infected patients at the remote L'Hopital General de Reference de Kole (Kole Hospital), in the rainforest of the Congo River basin of DRC, from March 2007 until August 2011. The data were collected as part of a study conducted jointly by the Institute National de Recherche Biomedical (INRB) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID) (*24*).

Of 244 patients diagnosed with MPXV infection, 228 tested positive by MPXV-specific PCR testing. Of the individuals who tested positive, 13 patients were excluded from our analysis due to uncertain timing of observational data collection. In our analysis, we studied lesion transition dynamics in 149 participants and virus dynamics in 151 participants, excluding 66 and 64 individuals, respectively, owing to incomplete longitudinal data on lesion counts and viral load (see **Fig S1**). The 228 patients who tested positive were predominantly male (63%), with age range 0–61 years (mean= 14.5, median= 13). 119 (52%) cases were of age ≥ 12, and 3 cases were fatal (CFR = 1.3%). The low CFR may be attributable to the low proportion of children under 5 or the rapid implementation of treatment guidelines during the survey period (*24*).

The dataset comprised lesion counts at different stages on various areas of the patients' bodies, as well as the MPXV genomic DNA load (i.e., viral load) in peripheral blood from the same individuals. Additionally, we integrated clinical laboratory data from hematology analysis, urinalysis, and clinical chemistry within the same patient cohort; **Table 1** shows summary statistics of these measured items for all individuals (total) and by the degree of disease severity (i.e. by stratified group G1 or G2, as characterized in the next section). Furthermore, the comprehensive clinical dataset for these patients included annotations for their sex, age, clinical symptoms, clinical signs, and exposure possibilities (outlined in (*24*)), as summarized in **Table S1** and **Supplementary Materials and Methods**.

Quantifying and stratifying lesion transition dynamics of MPXV-infected patients

Generally, mpox lesions can be classified into seven distinct stages: macules, papules, vesicles, pustules, umbilication, scabbing, and desquamation (*24, 25, 28*). As depicted in the distribution of lesion counts at various stages in **Fig S2**, the average lesion count at the macule, papule, vesicle, umbilicated, and pustule stages reached zero by approximately 20 days after onset of the first lesion, whereas for some individuals the count at the scab and desquamation stages persisted even beyond 40 days post-onset (**Fig 1A**). Nevertheless, there was substantial individual-level variation in the number of lesions and in the temporal dynamics of lesion transition (**Fig S2**).

To quantify the variation among MPXV-infected patients, we used a mathematical model that describes the dynamics of lesion transition. For model fitting, we merged the macule, papule, and vesicle stages into one stage, owing to the limited number of lesions observed at each stage and the lack of clinical relevance in distinguishing between these three stages (*32*). Additionally, considering that the infectious period persists until all scabs (or crusts) have fallen off (*25*), we excluded the desquamation stage from our analysis. Consequently, we modelled lesion counts using the compartments L_1, L_2, L_3, and L_4, in which L_1 represents lesions in the macule, papule, and vesicle stages, and L_2, L_3, and L_4 represent lesions in the pustule, umbilicated, and scab stages, respectively (**Fig S3**). We then characterized the transition dynamics among these stages at the individual level. We reconstructed the lesion transition dynamics for each participant in our cohort over a period of approximately 40 days after lesion onset. Individual-specific model fits are shown in **Fig S4** and estimated parameters are summarized in **Table S2**.

We performed an unsupervised clustering analysis using a dissimilarity-based random forest clustering approach (*33, 34*) to stratify the time-course patterns of lesion transition based on estimated individual-level parameters (see **Methods** in detail). The number of clusters optimized by the eigengap heuristic method described in **Fig S5** was two (that is, two groups of patients; G1 and G2). **Fig 1B** represents a two-dimensional Uniform Manifold Approximation and Projection (UMAP) embedding of these two groups. Using a different color for each group, we

also plotted the reconstructed individual transition dynamics (**Fig S6)**. The corresponding projected time course of each group in **Fig 1C** shows that the lesion transitions between stages progressed rapidly among individuals in G1 (n=42), whereas they were slower in the G2 participants (n=107). Our statistical tests showed that individuals in G1 had a significantly smaller area under the curve (AUC) of lesion counts for each stage compared with individuals in G2 ($P < 0.0001$; **Fig 1D**). Additionally, we observed statistically significant differences in the AUC of total lesion count and in the lesion duration (defined as time between lesion onset and disappearance) between patient groups ($P< 0.0001$; **Fig 1E**). Hereafter we classify G1 as “mild progressors” and G2 as “severe progressors” in our analysis.

As a sensitivity analysis, the equivalent analysis was performed with the number of clusters set at 3 (an alternative choice according to the eigengap heuristic method used in **Fig S5**). In this scenario, patients stratified into G2 could be further subdivided into two groups: G2-a (n=76) and G2-b (n=31) (**Fig S7A**). Although we observed differences in intermediate patterns within G2-a among the patients in G2, the dynamics of the scab disappearance stage (the cyan curve in **Fig S7B**) in G2-a and G2-b were comparable. More specifically, we found that individuals in G1 and G2-b had significantly smaller and larger AUCs of lesion counts in all stages, respectively, compared with individuals in the other groups ($P < 0.05$ or $P <0.0001$; **Fig S7C**). In contrast, there were no significant differences in lesion duration between patients in G2-a and G2-b patients (**Fig S7D**). These findings demonstrate that G2-a and G2-b are distinguished by the total number of lesions as well as by the lesion counts in each stage, rather than by lesion duration.

This analysis suggests substantial individual variation in the infectious period of MPXV-infected patients, which is consistent with our recent findings indicating marked variations in the duration of viral shedding across different specimens for MPXV clade IIb B.1 (*35*). Hereafter, we considered two stratified groups (G1 and G2) as the default for our analysis and utilized the three groups (G1, G2-a and G2-b) as part of a sensitivity analysis.

To describe the difference in timing of lesion disappearance, we calculated the probability of detectable lesions over time by using the model with estimated parameters for each group (**Fig 1F**). We found that individuals in G1 had significantly shorter periods of lesion presence: there was a difference in the timing of lesion disappearance between G1 and G2 ($p<0.0001$ by log-rank test). In the total group (all analyzed cases), the probability of detectable lesions was 42% (95% CI: 35%-51%) at 31 days after lesion onset, consistent with a reported median duration of experiencing apparent lesions among mpox cases in the 2022 outbreak (*36*). All lesions in G1 patients had disappeared at 31 days after lesion onset, whereas the corresponding probability in G2 was 58% (95% CI: 49%-68%). In the total group, the probability of detectable lesions was less than 10% by 45 days after lesion onset. Of note, we found a significant difference ($p=0.04$) in the hospitalization period between groups G1 and G2 (**Fig 1G**). Patients in G1 required an average hospitalization of 19.2 days, compared to 23.7 days for those in G2.

Additionally, we compared our stratified groups with two major existing categorizations of MPXV-infected patients to assess reliability from a clinical standpoint: "Lesion severity categories" (*22*) based on the total number of lesions on admission day (individuals with <25, 25-99, 100-499, and >=500 lesions were categorized as 1, 2, 3, and 4, respectively) and "age categories" (*24*) (individuals <5, 5-11, and >=12 years old were categorized as 1, 2, and 3, respectively) (**Fig 1H**). Both categories were based on clinical investigations. We observed that the lesion severity categories generally aligned with our stratified groups, as patients in G1 typically had fewer lesions at admission, and those in G2 had more lesions. In our logistic regression analysis, using the number of lesions at hospital admission as the predictor and the stratified group as the outcome, we obtained a high Receiver Operating Characteristic-Area Under the Curve (ROC-AUC) value of 0.94. Maximizing the Youden Index from the ROC curves, we calculated a cutoff value of 107 lesions with 62% specificity and 87% sensitivity for predicting the stratified group that the patient belonged to. Notably, the cutoff value (107 lesion counts) closely matched the boundary between lesion severity categories 2 and 3 (100 lesion counts) (*25*). In terms of age categories, children under 5 years old generally exhibited a longer lesion duration and a larger

number of lesions, which aligns with previous reports indicating severe lesions in young children (*14, 24, 37*), but we did not find statistically significant differences between age groups.

Viral load at lesion onset may define lesion severity

Although the lesion severity categories are useful, it is practically (and ethically) challenging in clinical settings to always count all lesions appearing on the entire body of a patient (*38*). Recent studies have suggested that the diagnosis of small lesions is difficult even for clinicians and quite subjective (*39*). To provide more objective support for mpox diagnostics, we explored the relationship between lesion transition dynamics and individual-level viral load, as our recent study suggested that viral load in lesions is positively associated with the infectious period of a patient (that is, the duration of having culturable MPXV) for clade IIb MPXV (*29, 30, 40*).

In our cohort, individual viral load in peripheral blood over time was measured (*24*) (see **Methods**). All patients (n=151; **Fig S1**) exhibited a decaying viral load profile (**Fig 2A**), because the viral load is likely to have already peaked before the first measurement (that is, before patients present to the hospital) (*24*). Therefore, we used a decay model to reconstruct the best-fit viral load profile for each case, considering inter-patient heterogeneity. In **Fig 2B** and **Fig S8**, we depict the reconstructed individual dynamics aligned with the stratified groups G1 and G2. We compared four key features derived from the viral dynamics among the groups in **Fig 2C**: estimated viral load at lesion onset ($V(0)$, estimated by Eq. (5) using all measured viral load data) and disappearance (when scab count falls below 1), AUC of viral load above 10^{-2} genomes/mL, and the viral clearance rate (**Fig 2C**). Notably, we found that the onset viral loads and AUCs of viral load in G1 were significantly lower than those in G2 ($P \leq 0.01$), whereas the viral loads at lesion disappearance in G1 were significantly higher than those in G2 ($P \leq 0.0001$). Conversely, there was no apparent difference in the clearance rate among the groups. On an individual patient level, we observed positive correlations for the two main quantified lesion dynamics features (lesion duration and AUC of total lesion counts in **Fig 1E**) with the onset viral load in **Fig 2D** (0.39 and 0.50 Pearson's correlation coefficient, respectively, with a p-value of 0.001 in both cases). We also conducted separate correlation analyses for G1 and G2. We found statistically significant correlations in G2, whereas no significant correlations were observed in G1 (**Fig S9**). The low lesion counts in G1, likely below a "threshold," suggest that lesion counts above a certain

threshold may be associated with distinct viral load dynamics. Taken together, these findings suggest that the onset viral load may serve as a potential biomarker for predicting lesion transition patterns and lesion severity over the course of infection (discussed below). Additional correlation analyses between lesion dynamics and viral load dynamics are illustrated in **Fig S10**.

Next, we explored whether an individual's risk group (G1 or G2) could be predicted by the onset viral load in peripheral blood. We used a logistic regression analysis with the onset viral load as the predictor variable and the stratified groups as the outcome variable, achieving an ROC-AUC of 0.70 (**Fig 2E**). Similarly, based on the Youden Index, we calculated cutoff values for the onset viral load of 4.61 ($\log_{10}$ genomes/mL) with 62% specificity and 87% sensitivity for predicting the stratified group that the patient belonged to. Notably, the threshold viral load significantly differentiated the duration of lesions ($p<0.0001$ by Mann-Whitney U-test): 28.0 and 35.2 days on average were required until lesion disappearance for patients with an onset viral load below and above 4.61 ($\log_{10}$ genomes /mL), respectively (**Fig 2F**). In our sensitivity analysis involving the three groups (G1, G2-a, G2-b), we were unable to achieve a high ROC-AUC for predicting G2-a (47%), which shows intermediate patterns, whereas we achieved a higher ROC-AUC of 68% for G2-b (**Fig S7E**).

We note that measuring viral load precisely at the onset of lesions presents substantial challenges in clinical settings. In this cohort, most patients came to the hospital up to 6 days after lesion onset (**Fig S11A**). However, sequential viral load measurements (at a minimum of two time points) enable the estimation of onset viral load. In our cohort, 80% of patients had blood samples collected on the first two consecutive days after admission (**Fig S11B**). To relax the requirement for individual viral load data, we therefore recalculated the initial viral load by applying an attenuation model to only the subset data of the first and second specimens (designated as $V^*(0)$) from each patient. Notably, even with $V^*(0)$ derived from this two-point estimation, the logistic regression analysis yielded a ROC-AUC of 0.66, compared to the original ROC-AUC of 0.70 (as shown in **Fig 2E**). Patients with $V^*(0)$ below 4.61 (log10 genomes/mL) showed lesion

resolution in a mean period of 32.9 days, while those above this threshold averaged 28.9 days (**Fig S11C**).

Last, additional laboratory data were incorporated to explore potential biomarkers for predicting mild and severe disease progression (see **Supplementary Materials and Methods**). However, the analysis did not identify any biomarkers that could replace viral load at onset as a predictor, and combining viral load data with other available clinical information did not enhance the predictability of lesion count progression.

Discussion

The declaration of a PHEIC is viewed as a crucial step in addressing the escalating mpox outbreaks in Africa and its potential as a global threat. The limited clinical insights into the disease progression of clade I MPXV may lead to delays in treatment and pose a challenge for allocating resources in hospital settings. We believe that our findings have immediate insights to support healthcare professionals preparing to treat mpox patients infected by clade I. In particular, the present study has investigated the dynamics of lesion severity at the individual patient level using a large cohort of mpox cases in the DRC from our previous study (*24*). We have identified groups of mild and severe progressors (G1 and G2); the G1 group was characterized by a lower lesion count and more rapid disappearance of lesions, whereas the G2 group exhibited a higher lesion count and slower disappearance of lesions. Our stratification also indicated that age may serve as a demographic characteristic affecting lesion dynamics, although the statistical power was not sufficient, in accordance with previous studies which suggested that younger age is associated with more likely severe outcomes, which determine the necessity and benefits of hospital-based care (*14, 24, 37*).

Limited research has been done on potential biomarkers for the severity of mpox, which could be used to monitor clinical progress and tailor treatment plans in individual patients (*41, 42*). In a recent study (*43*), some peptides were identified to be correlated with severity, although the plasma proteome analysis was confined to a small number of mpox patients. Another preliminary study showed that elevated viral loads in the upper respiratory tract during the first week following symptom onset were correlated with severe case (*44*). Although these studies provide insights into clade II MPXV, the present research focuses on clade I and our analysis with data from over 150 patients with mpox suggested that the viral load in peripheral blood at lesion onset may serve as a biomarker for predicting the progression of lesion severity over time. Specifically, it may be possible to differentiate between mild and severe progressors by comparing their viral load at lesion onset with a threshold value of 4.61 ($\log_{10}$ genomes/mL). Although our analysis showed moderate uncertainty in predicting an individual's lesion severity category (ROC-AUC=0.70 and

0.74 by the estimated onset viral load, $V(0)$, without and with clinical laboratory data), the onset viral load has practical advantages over purely symptom-based diagnostics, as it provides additional information to more objectively prioritize patients for either home treatment or hospitalization. Viral load data can be obtained during routine practice in clinical settings, such as when confirming a case with PCR testing. Our additional analysis with another estimated onset viral load, $V^*(0)$, derived from the same simple decay model but calibrated using only the first two consecutive samples after admission, showed the applicability of our approach under more data-limited conditions, despite a slight decrease in accuracy.

Prolonged cutaneous symptoms have been shown to be associated with clinically severe outcomes (*45*). This supports the development of adaptive treatments that account for the stratified time-course patterns of lesion transition, which may become feasible if patients can be classified into severity groups shortly after being diagnosed with MPXV infection. For patients classified in group G2 (severe progressors), combination treatment using tecovirimat with cidofovir, brincidofovir, or vaccinia immune globulin intravenous (VIGIV) may be considered [50, 51]. Establishing an early treatment plan is crucial for improved patient outcomes and efficient health care delivery.

Estimated durations of lesion presence by severity level can be used inform mpox outbreak control. The presence of lesions is thought to be a good proxy of infectiousness via direct skin-to-skin contact (*45*), as lesions can preserve higher numbers of infectious viral copies compared to other body fluids (*29, 40*). Our previous modelling study on viral load dynamics of clade IIb also suggested large individual variations in the duration of viral shedding in lesion samples (*46*). Taken together, the extent of individual heterogeneity in the duration of lesion presence, as well as in the viral load within lesions, highlights the importance of implementing individually tailored quarantine or self-isolation strategies.

Our study has several limitations. First, our analysis relied on patient data from a cohort of cases infected with clade I MPXV (precise genotype not measured) in the DRC, and thus caution should be taken when using our findings to inform treatment or interventions during

outbreaks of other (sub)clades or orthopoxviruses, such as the dominant MPXV clade in 2022 (clade IIb). Delays between infection and recognizable symptom onset, or between symptom onset and confirmation/hospitalization may differ by clade/virus (*47*) (and potentially by mode of transmission (*45, 48*)), and timely data collection may be crucial for effective implementation of adaptive treatment. Overall, our estimates of lesion count progression after onset were mostly consistent with epidemiological findings observed in 2022 (*23, 30*), and the added value of our analysis was to characterize the individual-level lesion severity – in particular, lesion counts and the duration of lesion presence. Our results may provide timely evidence for the control of the clade I mpox outbreaks currently surging in the DRC and surrounding countries, particularly for provinces affected by MPXV clade Ia (*49, 50*), as its higher CFR indicates the importance of swifter treatment compared to clade IIb (*5, 6, 14, 15*). Second, further investigation is needed on the association between lesion counts at each stage and the mpox viral load in peripheral blood, both in patients who have not been treated with antiviral drugs and in those receiving medications such as tecovirimat, cidofovir, and brincidofovir (*51-53*). This is particularly relevant in outbreak settings where these drugs may be administered to infected individuals and used prophylactically. For example, although tecovirimat is currently available only under emergency authorization, clinical trials assessing its efficacy in the treatment of mpox are underway (*54*). In fact, case studies of patients treated with tecovirimat show anecdotal improvement in symptoms and viral clearance (*55, 56*). Last, in our machine learning analysis, clinical laboratory data were available for only 120 patients because of missing measurements. The limited sample size may account for the lower-than-expected ROC-AUCs achieved using machine learning, despite the inclusion of the clinical data. These challenges highlight the need for larger datasets to be collected.

In conclusion, this study presents empirical evidence of heterogeneity in lesion transition dynamics and lesion severity among mpox cases. It also suggests that the viral load in peripheral blood at lesion onset may be linked to this heterogeneity. Given the absence of currently available sensitive, simple and rapid biomarkers, the onset viral load could potentially be used as a predictor of mpox skin lesion severity. Identifying risk factors for severe symptoms at the individual

level is crucial for improving treatment strategies and public health interventions against current and future outbreaks of mpox.

Materials and methods

Study design

This study analyzed a longitudinal dataset of patients infected with clade I MPXV in the DRC during 2007–2011 by developing a model to quantitatively explain the transition dynamics of lesions and identifying predictors of individual-level lesion severity. The prospective observational study of the clinical natural history of human MPXV infections at the remote Kole Hospital in the rainforest of the Congo River basin of the DRC, which was conducted from March 2007 until August 2011, was reviewed and approved by the Human Use Committee of the United States Army Medical Research Institute of Infectious Diseases (FY05-13) and the Headquarters, United States Army Medical Research and Development Command Institutional Review Board (IRB), Frederick, MD, USA, as well as the Ethics Committee at the University of Kinshasa School of Public Health (KSPH), Kinshasa, DRC (see (*24*) for details). The present study was approved by the ethics committee of Nagoya University (approval number: Hc 22-07).

Study data

Admission to the “Mpox isolation ward” was based on the following clinical diagnosis of human MPXV infection by hospital staff:

- Vesicular rash of various stages of development clustered on various parts of the body (regional monomorphism), typically first appearing on the face, hands, and feet with centrifugal distribution (pox-like rash)
- Fever of up to 38.5 to 40°C or a history of subjective fever, rash, lymphadenopathy, headache, malaise, or prostration in the past 2 weeks for which there is no attribution and have a history of one or more of the following exposures: handled or ate uncooked, freshly butchered meat, including meat of monkey, squirrel or other wild game during the 21 days prior to the onset of illness.

The study data were collected using physical case report forms. Data were then entered into a validated clinical data management system by data entry staff and reviewed for quality

control. Quality assurance was performed on 100% of patient records, further ensuring that hard copies and computer data matched. Data terminology synchronization was performed.

Lesion counts were measured daily. An indelible marker was used by nurses to divide the patient's body into nine different skin regions and the oropharynx (when lesions involved the mouth or throat), for a total of 10 areas (head, left arm, right arm, hands, trunk front, trunk back, left leg, right leg, feet and oropharynx) when the latter was involved (see (*24*)). The daily lesion count was determined by summing lesions at all body locations on each study day. Results were audited by both an independent Clinical Monitor and the study team's quality control section.

In this study, we used the total number of lesions for each stage (macules, papules, vesicles, pustules, umbilication, scabbing, and desquamation) by aggregating the lesion count from all areas for each day of the study. Additionally, we incorporated viral load data, measuring the amount of MPXV genomic DNA in peripheral blood. Clinical laboratory data, obtained through hematology analysis, urinalysis, and clinical chemistry from the same group of patients, was also considered. Detailed information on data preparation can be found in (*24*).

Modeling lesion transition dynamics

In our recent report (*24*), we categorized lesions into seven different stages: macule, papule, vesicle, pustule, umbilicated, scab, and desquamation. Because of the limited number of macule, papule, and vesicle lesions for the purpose of parameter estimation and the lack of clinical relevance in distinguishing between these three stages, we merged these into one stage. The notation $L_1(t)$ therefore represents the total number of macule, papule, and vesicle lesions at time t since lesion onset ($t = 0$ represents the day at which a patient reported their first lesion appearance). Additionally, considering that the infectious period persists until all scabs or crusts have fallen off (*25*), we considered lesion counts in the umbilicated, pustule, and scab stages as $L_2(t)$, $L_3(t)$, and $L_4(t)$, respectively, while excluding lesion data from the desquamation stage. Subsequently, we developed the following mathematical model to describe the dynamics of lesion transitions in MPXV-infected patients:

$$\frac{dL_1(t)}{dt} = ge^{-ct}L_1(t) - a_1(1 - e^{-\tau_1 t})L_1(t), \qquad (1)$$

$$\frac{dL_2(t)}{dt} = a_1(1 - e^{-\tau_1 t})L_1(t) - a_2(1 - e^{-\tau_2 t})L_2(t), \qquad (2)$$

$$\frac{dL_3(t)}{dt} = a_2(1 - e^{-\tau_2 t})L_2(t) - a_3(1 - e^{-\tau_3 t})L_3(t), \qquad (3)$$

$$\frac{dL_4(t)}{dt} = a_3(1 - e^{-\tau_3 t})L_3(t) - dL_4(t). \qquad (4)$$

Here, we consider that the number of macule, papule, and vesicle lesions (L_1) increases at per lesion rate ge^{-ct} as the infection progresses, where g corresponds to the maximum growth rate of the number of lesions and c determines the rate at which this growth rate decreases over time due to viral clearance. It is assumed that the lesion counts in L_1 start to exponentially increase t_0 days from the self-reported time of first lesion onset and L_1 remains at 1 until that time ($L_1(t) = 1$ and $L_2(t) = L_3(t) = L_4(t) = 0$ for $t \leq t_0$). The differential equations above therefore apply when the time since lesion onset $t > t_0$. In those equations, the number of scab lesions (L_4) decreases at per lesion rate d, and the transition rates between the stages are denoted by $a_n(1 - e^{-\tau_n t})$ for $n = 1,2,3$ where a_n is the maximum transition rate and $1 - e^{-\tau_n t}$ sets the time dependence of delays between the stage transitions. The time-dependent transmission rates were assumed because we found that a model with constant transmission rates did not reproduce the lesion dynamics observed in some patients. We estimated the above parameters, g, c, a_n, d, t_0, τ_n for $n = 1,2,3$, by model fitting (see below).

Modeling decay dynamics of viral load and parameter estimation

To describe MPXV dynamics in peripheral blood, we used a simple decay model, which was previously used in a study of mpox (*35*):

$$\frac{dV(t)}{dt} = -\delta V(t), \qquad (5)$$

where the variable $V(t)$ is the viral DNA (genomes/mL) at time t and parameter δ represents the viral clearance rate. Note that the variable t again represents time after lesion onset; $t = 0$

is thus the date on which the first lesion was identified. We estimated the parameters of both the lesion transition and viral load models using a nonlinear mixed-effect modeling approach. The detailed inference procedures are provided in **Supplementary Materials and Methods**.

Unsupervised clustering and stratification of lesion transition dynamics

We extracted three key "features" of lesion transition dynamics for each individual: i) the average macule, papule, and vesicle lesion count increase rate ($\frac{1}{T}\int_{t_0}^{T+t_0} ge^{-ct}\,dt$) over 40 days ($T = 40$); ii) the average stage transition rates ($\frac{1}{T}\int_{t_0}^{T+t_0} a_n(1-e^{-\tau_n t})\,dt$ for $n = 1,2,3$), again with $T = 40$; and iii) the scab lesion decrease rate, d. For the purpose of stratification of the lesion transition dynamics, unsupervised random forest clustering based on each of five features was performed with number of clusters set to 4 (rfUtilities in R). Specifically, after a random forest dissimilarity (the distance matrix between all pairs of samples) was obtained, it was visualized by using UMAP in a two-dimensional plane and was stratified with spectral clustering (Python scikit-learn). The optimal number of clusters was determined using the eigengap heuristic method (*57*).

Highlighted lesion transition dynamics of stratified groups by PLS-DA

Our analysis employed Partial Least Squares Discriminant Analysis (PLS-DA) utilizing a PLS1 algorithm. This method generates PLS components that maximize the covariance between lesion counts and the two stratified groups, effectively reducing dimensionality. For a comprehensive understanding of the PLS-DA algorithm, readers may refer to (*58*). In our implementation, data were systematically reorganized at each time point to emphasize the differences in lesion counts between the two stratified groups.

Classifying the stratified groups with a logistic regression

We used logistic regression to construct a predictive model for stratified groups, aiming to ascertain the relationship between these groups and viral load at lesion onset. In this model,

we used the viral load at lesion onset as the predictor variable, with the classification of the stratified group as the outcome variable. We assessed the model performance using AUC scores. Youden index was employed to determine the cutoff value, and the cutoff value was set to the point that maximizes the distance to the diagonal.

Statistical analysis

When necessary, variables were compared among different groups using analysis of variance (the Mann-Whitney U test and Fisher's exact test, for more than two numerical or categorical variables, respectively) with false discovery rate correction, or the Mann-Whitney U test (for two numerical variables) with Bonferroni correction. A significance level of $\alpha = 0.05$ was used for all tests, and two-sided testing was applied. All statistical analyses were performed using R (version 4.2.0).

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AUTHOR CONTRIBUTIONS

SI and FM designed the research. TN carried out the computational analysis. SI, FM, JWH, and PRP supervised the project. All authors discussed the research and contributed to writing and editing the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no conflicts of interest associated with this manuscript.

Code availability

The codes to reproduce the main results in this study have been deposited in Zenodo at 10.5281/zenodo.15567886

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was approved by the ethics committees of Nagoya University (hc22-05).

Supplementary Materials

The PDF file includes:

Materials and Methods

Figures S1 to S12

Tables S1 to S4

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Figure 1 | Stratification and characterization of lesion transition dynamics: (A) Time course plot of the average lesion count for each stage (macule, papule, vesicle, pustule, umbilicated, scab, and desquamation) across all patients (n=215). **(B)** UMAP of stratified lesion transition dynamics based on the estimated parameters. Data points represent individual participants and are colored according to group (i.e., G1: blue, n=42; G2: red, n=107). **(C)** Time-course patterns highlighted by Partial Least-Squares Discriminant Analysis (PLS-DA), which discriminates each group from the others. All lesion count values for each time were reconstructed to highlight the difference between lesion stages. The mean (solid line) and mean ± 1 standard deviation (shaded area) calculated based on these reconstructed values are shown, colored according to stage (L_1, L_2, L_3, and L_4: orange, magenta, light purple, and cyan). **(D)** AUC of lesion counts at each stage for each stratified group, calculated based on the reconstructed lesion transition dynamics. **(E)** AUC of total number of lesions (left) and lesion duration from lesion onset to disappearance (right), calculated based on reconstructed lesion dynamics. For **(D)** and **(E)**, statistical significance was calculated using the pairwise Mann-Whitney U test and p-values were corrected by Bonferroni's method (P values; NS, > 0.05, $*$, $\leq$ 0.05, $**$, $\leq$ 0.01, $***$, $\leq$ 0.001, and $****$, $\leq$ 0.0001). **(F)** Kaplan-Meier survival curves of lesion disappearance for each group based on reconstructed lesion dynamics. Solid lines and shaded areas indicate the survival probabilities and 95% confidence intervals, respectively. The blue, red, and black colors correspond to G1, G2, and the total group, respectively. The table below the figure shows the number and percentage of survivors in each group at each time point. **(G)** The distribution of the hospitalization period is shown for stratified groups G1 and G2. **(H)** Confusion matrices on the relationship between the stratified group and lesion severity score (top panel, individuals with <25, 25-99, 100-499, and >=500 lesions) as well as between stratified group and age category (bottom panel, individuals <5, 5-11, and >=12 years old).

Figure 2 | Relationship between lesion transition dynamics, peripheral blood viral load and clinical laboratory data: (A) The measured individual decay dynamics of mpox viral load (based on genomic DNA) in peripheral blood samples obtained from the DRC cohort (n=151). **(B)** The individual-level mpox decay viral load, reconstructed using the mathematical model, plotted aligned with the stratified groups (G1 and G2: blue and red). **(C)** Comparison of predicted viral load at onset, viral clearance rate, AUC of viral load in peripheral blood, and viral load at lesion disappearance among the stratified groups. **(D)** Correlations between the features of individual-level lesion transition dynamics (lesion duration and AUC of total lesion counts) and those of individual-level decay viral load dynamics (viral load at lesion onset and disappearance). **(E)** The ROC curves for a generalized linear model (GLM) logistic regression with the viral load at lesion onset for predicting stratified group G1 as positive or G2 as negative. The corresponding ROC-AUCs are displayed in the bottom right of each panel. FPR and TPR mean false positive rate and true positive rate, respectively. **(F)** Comparison of lesion duration between two groups classified based on the threshold value (Low: below 4.61 $\log_{10}$ genomes/mL, High: above 4.61 $\log_{10}$ genomes/mL).

Table 1. Summary of clinical laboratory data on the admission day of 149 patients with mpox for lesion transition analysis

	Unit	Total (n=149)	G1 (n=42)	G2 (n=107)	Adjusted P value †
Chemistry					
Glucose	MG/DL	93.3 [20.9]*	87.3 [16.6]	95.5 [22.0]	0.218
Blood urea nitrogen	MG/DL	8.23 [5.22]	6.18 [2.78]	9.08 [5.75]	0.013
Creatinine	MG/DL	0.72 [0.50]	0.84 [0.80]	0.67 [0.30]	1.000
Uric acid	MG/DL	4.60 [1.80]	4.44 [1.40]	4.67 [1.94]	1.000
Calcium	MG/DL	8.82 [0.80]	8.60 [1.29]	8.91 [0.49]	1.000
Albumin	G/DL	2.79 [0.55]	2.89 [0.88]	2.76 [0.37]	1.000
Total protein	G/DL	7.72 [0.98]	7.67 [1.33]	7.74 [0.81]	1.000
Alanine aminotransferase	U/L	23.6 [10.7]	21.4 [9.52]	24.5 [11.0]	1.000
Aspartate aminotransferase	U/L	44.5 [22.4]	39.5 [24.8]	46.4 [21.2]	0.020
Alkaline phosphatase	U/L	118 [45.6]	116 [52.1]	119 [43.0]	1.000
Total bilirubin	MG/DL	0.62 [0.19]	0.61 [0.20]	0.62 [0.19]	1.000
Gamma glutamyl transferase	U/L	40.6 [36.9]	32.4 [19.3]	43.7 [41.3]	1.000
Amylase	U/L	79.3 [102]	75.1 [40.1]	81.0 [117]	1.000
Complete blood count					
Neutrophils	%	38.8 [13.1]	33.5 [12.8]	40.8 [12.8]	0.004
BANDS	%	1.17 [2.98]	0.65 [1.90]	1.37 [3.28]	0.646
Lymphocytes	%	41.2 [15.7]	46.7 [13.3]	39.1 [16.0]	0.058
Atypical lymphocytes	%	0.78 [1.33]	0.63 [1.25]	0.84 [1.36]	1.000
Metamyelocytes	%	0.01 [0.08]	0 [0]	0.01 [0.10]	1.000
Myelocytes	%	0.27 [0.73]	0.10 [0.38]	0.34 [0.82]	0.929
Monocytes	%	5.55 [6.61]	5.30 [6.84]	5.64 [6.56]	1.000
Eosinophils	%	11.5 [6.48]	12.8 [6.93]	11.0 [6.27]	1.000
Basophils	%	0.29 [0.59]	0.30 [0.46]	0.28 [0.63]	1.000
White blood cell	10^3/UL	10.7 [5.15]	9.29 [4.26]	11.3 [5.36]	0.090
Red blood cell	10^6/UL	4.50 [0.71]	4.45 [0.78]	4.52 [0.69]	1.000
Hemoglobin	G/DL	11.6 [2.02]	11.4 [2.22]	11.7 [1.95]	1.000
Hematocrit	%	34.7 [5.84]	34.5 [6.33]	34.8 [5.66]	1.000
Mean corpuscular volume	FL	77.9 [7.72]	79.1 [8.99]	77.5 [7.19]	1.000
Mean corpuscular hemoglobin	PG	25.9 [2.85]	25.8 [3.24]	25.9 [2.72]	1.000
Mean corpuscular hemoglobin concentration	D/DL	33.2 [1.89]	32.6 [1.64]	33.4 [1.94]	0.269
Platelet	10^3/UL	275 [154]	244 [118]	286 [165]	1.000
Malaria smear					
Peripheral blood parasite detection	-	1 (1)**	1 (0.7)	0 (0)	1.000
Urinalysis					
Urine glucose	-	17 (11)	5 (3.4)	12 (8.1)	1.000
Bilirubin	-	112 (75)	29 (20)	83 (56)	0.112

Ketones	-	75 (50)	20 (13)	55 (37)	1.000
Specific gravity***	-	110 (74)	34 (23)	76 (51)	0.358
		26 (17)	5 (3.4)	21 (14)	
Occult hematuria	-	31 (21)	8 (5.4)	23 (15)	1.000
Urine pH	-	6.41 [1.05]	6.59 [1.10]	6.34 [1.02]	1.000
Proteinuria	-	114 (77)	31 (21)	83 (56)	1.000
Urobilinogen	-	56 (38)	12 (8.1)	44 (30)	1.000
Nitrites	-	30 (20)	6 (4.0)	24 (16)	0.627
Leukocytes esterase	-	37 (25)	11 (7.4)	26 (17)	1.000

† Statistics for G1 (mild progressors) and G2 (severe progressors) performed by Mann-Whitney U test and Fisher's exact test with FDR.
* mean [S.D.].
** No. of positive (%).
*** The top row shows the number of people in specific gravity level 2 and the bottom row shows the number of people in specific gravity level 3.