Profiling of viral load, antibody and inflammatory response of people with monkeypox during hospitalization: a prospective longitudinal cohort study in China

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Summary

Background The dynamics of viral shedding and the specific humoral response against monkeypox virus (MPXV) have not been well characterized in patients across their disease course during hospitalisation. The aim of this study was to determine the viral load and the levels of antibodies against MPXV using longitudinal paired-collected samples from hospitalized patients.

Methods Patients who were hospitalised with mpox were recruited at Beijing Ditan Hospital Capital Medical University in China between June 2 and September 23, 2023. Paired samples, including samples from skin lesions, the oropharynx, saliva, faeces, urine, plasma, and serum, were serially collected at days 1, 3, 7, and 14 after admission until discharge. Not all of the patients had samples obtained at all of the timepoints. All the samples were analysed via quantitative PCR. Virus isolation was performed by using clinical samples and Vero cells. The presence of IgM, IgA, IgG, and neutralising antibodies (NAbs) against MPXV was evaluated. The first collected plasma sample was taken when the patient was hospitalised, and the levels of cytokines and chemokines were measured in the sample. The demographic data, smallpox vaccination status, history of known exposure to MPVX, HIV status and other clinical data were collected using a standard case report form.

Findings A total of 510 specimens were serially collected from 39 recruited people with mpox. Among all the samples, the skin lesions had the highest viral DNA detection rates and viral loads, and the saliva samples had the second highest rates and viral loads. One day before discharge, 85% of the dry scrabs (median Ct 28.2, range 19.0–38.3) and 70% of the saliva samples (median Ct 32.4, range 24.5–38.1) were positive for viral DNA, Of which, 23.1% of dry scrabs were positive in viral culture. The rate of viral DNA detection in the oropharyngeal, saliva, and faecal samples decreased with time, while the rates in the plasma, serum, and urine samples increased quickly before 10 days post symptom onset (PSO). The median days of appearance of MPXV-IgM, MPXV-IgA, MPXV-IgG, and NAb were at 8 (interquartile range [IQR] 7–9), 9 (7–10), 12 (9–15), and 12 (9–15) PSO, respectively. The IgM, IgA, IgG, and NAb titres increased with time. Between days 11 and 21 PSO, the NAb titres were lower in people living with HIV (PWH) than in people living without HIV (PWOH). Increased NAb titres were associated with decreased viral loads in the saliva ($r = 0.28$, $p = 0.025$), faeces ($r = 0.35$, $p = 0.021$), plasma ($r = 0.30$, $p = 0.0044$), and serum samples ($r = 0.37$, $p = 0.001$). Compared with PWOH, PWH had higher plasma levels of MIP-1α, MIP-1β, G-CSF, IL-4, and FGF-basic.

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Interpretation The high positive viral culture rate of clinical samples of patients when they are discharged from the hospital indicates that effective public health management strategies are needed for people with mpox. The low NAb titres and high levels of cytokines in PWH shows that earlier treatment is needed to control inflammation in high-risk populations.

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Keywords: Monkeypox virus; Viral load; Antibody; Temporal changes; Cytokines

Research in context

Evidence before this study

We searched PubMed and the medRxiv and bioRxiv preprints for articles published in English before November 30, 2023 with the terms "Monkeypox", "Orthopox virus" or "MPXV" and "viral dynamic" or "viral kinetics" and "antibody response" or "humoral immunity" or "antibody dynamics" or "antibody kinetics" and "cytokine and chemokine". Viral DNA has been detected in skin lesions, the oropharynx, the rectum, saliva, semen or the vagina, and the blood. Most of the reports described the presence of viral DNA and its levels in samples collected cross-sectionally or those collected at a few timepoints. A study from Spain described the viral shedding time by evaluating 77 patients who had self-collected samples taken from different body sites. However, all the patients were outpatients, and in that study, saliva was not tested. The kinetics of viral shedding throughout the disease course during hospitalisation have not been clarified. Previous studies have shown that the immune response during an acute monkeypox virus (MPXV) infection is mainly cross-reactive immunity induced by smallpox vaccination. A few studies have described the kinetics of antibodies against MPXV in patients; however, the relationship between the antibody responses and cytokine and chemokine responses and the human immunodeficiency virus (HIV) status have rarely been evaluated.

Added value of this study

In our study, the kinetics of the viral load, antibody response, and inflammatory cytokine production that develop during MPXV infection were elucidated. Our data provide current knowledge on viral shedding, humoral immune responses and host inflammatory responses to MPXV and the dynamics of these factors throughout the disease course during hospitalisation. The high viral load in saliva and positive in viral culture in dry scrabs before discharging emphasize the improvement of public health strategies for patients regardless HIV status.

Implications of all the available evidence

Our data have important implications, including implications related to the viral transmissibility, antiviral treatment, and vaccination strategies, for public health strategies in high-risk populations. These patients have a high viral load and positive rate in saliva across their disease course, which indicate noninvasive samples can be used to rapid and sensitive diagnostic tests for MPXV infection regardless of whether skin lesions are present. The presence of viral DNA in dry scrabs and saliva samples from convalescent patients before discharge emphasizes the need for improvements in public health strategies. The lower neutralizing antibody titers and higher cytokine levels in people living with HIV (PWH) compared to those in people living without HIV (PWOH) indicate the importance of early antiviral and supportive treatment, as well as preventive strategies, such as with vaccination.

Introduction

MPXV is a virus with zoonotic potential belonging to the Orthopoxvirus genus of the Poxviridae family.[1] In May 2022, human cases of MPXV infection were reported in European countries outside known endemic areas, and MPXV subsequently spread globally.[2,3] As of 23 February 2024, 93,921 laboratory-confirmed cases of mpox have been reported to the World Health Organization (WHO).[4] Approximately 92–98% of MPXV-infected patients were men who have sex with men,[5,6] and 52.7% were PWH.[4] The majority of severe patients had a history of HIV infection. A low CD4 cell count, especially when less than 200 cells/mm^3, was strongly associated with disease severity.[7,8] A report from a global case series suggested that immunity from either natural MPXV infection or vaccination is not completely protective against MPXV reinfection.[9] These data highlight the need for improved clinical management and disease control, as well as optimization of vaccination strategies for populations at high risk.

The dynamics of viral shedding in patients with mpox have been evaluated in several studies.[10,11] A study

in Italy showed that viral loads varied widely in different body fluids, and viral loads tended to be greater in oropharyngeal swabs, saliva, and stool.[10] In a study including 77 immunocompetent patients with mild disease, a contact isolation period of 3–6 weeks was suggested based on PCR data.[11]

Limited data are available on the human immune response after being infected with the MPXV. One study including three patients showed that immunoglobin (Ig) M, IgA, and IgG were detected within two weeks after symptom onset.[10] Then, the authors enlarged the cohort and assessed the dynamics of the antibody response in 18 patients. The results showed that NAbs against MPXV were detectable in the serum of these patients one week after symptom onset. However, the antibody responses of these patients were not significantly different regardless of their smallpox vaccination status, HIV status, or disease severity.[12] Moreover, data on cytokine levels in patients with mpox are limited. Overproduction of IL-2R, IL-10, and GM-CSF was observed in patients with severe disease.[13]

We aimed to clarify the dynamics of the viral load, antibody response, and plasma cytokine and chemokine response in patients with mpox, and we especially wanted to evaluate the impact of these in PWH. We evaluated viral DNA and specific IgM, IgA, IgG, and NAb responses using longitudinal samples collected from days 3 to 21 PSO and plasma cytokine and chemokine levels in 39 people with mpox.

Methods

Study design and participants

We performed a prospective cohort study to determine the viral and antibody dynamics in patients who suffered from acute MPXV infection and who were admitted to Beijing Ditan Hospital Capital Medical University in Beijing, China. Participants who were confirmed to be infected with MPXV were eligible if they were older than 18 years and had a PCR-confirmed MPXV infection. Patients were excluded if they had an alternative confirmed diagnosis that can fully explain the illness. Patients were enrolled and followed up until discharge between June 2 and September 23, 2023. Samples were collected from seven body sites, including skin lesions (vesicle fluid or dry scraping of scabs, some samples came from the same lesions, and others came from the different lesions), the oropharynx, saliva, faeces, urine, plasma, and serum by clinicians at days 1, 3, 7, and 14 after hospitalisation and until discharge. All patients were subjected to HIV-RNA and CD4 cell count testing when they were diagnosed with mpox. The participants provided written informed consent. The present study was approved by the institutional review boards of Beijing Ditan Hospital Capital Medical University (2023-025).

Case definition

Suspected cases and monkeypox virus nucleic acid test positive or culture isolated mpox virus using cell culture according to mpox Treatment Guide (2022 Edition, China) (http://www.nhc.gov.cn).

Data collection of mpox patients

Data on demographic and clinical characteristics were retrieved from electronic medical records. The clinical characteristics included disease severity, systemic symptoms, the number and location of the patient's lesions, and coexisting conditions.

Plasma isolation

Venous blood was collected from each patient at different time post symptoms onset during hospitalization and processed within 6 h to isolate plasma. Plasma was separated by centrifugation at 300×*g* for 10 min and stored at –80 °C until testing.

Nucleic acid extraction and viral DNA qualification using real-time PCR

Total nucleic acid extraction was performed from MPXV positive clinical samples, including skin lesions (vesicle fluid or dry scraping of scabs), oropharynx swabs, saliva, faeces, urine, plasma, and serum using the Gene Rotex96 automatic nucleic acid extraction apparatus (Tianlong, Xi'an, China). The elution volume of each sample was 80 μL. DNA concentration was determined using the Qubit dsDNA HS assay kit (Thermofisher, Cat#Q32854, Waltham, MA, USA).

Real-time PCR Detection Kit for Monkeypox Virus (Beijing Applied Biological Technologies Co., Ltd., Cat#D2361YH, Beijing, China) were used for rapid detection of Monkeypox Virus. The assay comprised of 5 μL nucleic acid, 18 μL nucleic acid amplification reaction solution Mix and 2 μL enzyme mixture. Monkeypox specific primers and probes target the MPXV F3L gene. The real-time RT-PCR assay was performed on a CFX96 Touch Real-Time PCR Detection System (BIO-RAD, Hercules, CA, USA) with cycling conditions of 95 °C for 30 sec, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s.

Enzyme-linked immunosorbent assay

MPXV-specific IgM, IgA, and IgG titres were evaluated using the enzyme-linked immunosorbent assay (ELISA). Briefly, MPXV infected Vero cells were lysed using RIPA buffer (Solarbio, Cat#R0010, Beijing, China) and were quantified using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Cat#23227, MA, USA). 0.5 μg lytic proteins per well in carbonate buffer (pH 9.6) were coated in high-binding 384-well plate (Corning, Cat#3700, NY, USA) overnight at 4 °C. Plates were washed with PBST (1 × PBS with 0.05% Tween-20) and incubated with 100 μL blocking buffer (1 × PBS with 2% bovine serum albumin [BSA, Sigma Aldrich,

Cat#9048-46-8, St Louis, MO, USA]) per well for 2 h at 37 °C. Plasma samples were diluted starting at 1/100 for 5 two-fold serial dilutions with 1 × PBS with 0.5% BSA (Sigma–Aldrich) and incubated for 1 h at 37 °C. After washing, peroxidase AffiniPure™ Goat Anti-Human IgM, Fc5μ fragment specific (Jackson Immuno Research, Cat#109-035-129, RRID#AB_2337588, West Grove, PA, USA), Peroxidase AffiniPure™ Rabbit Anti-Human Serum IgA (Jackson ImmunoResearch, Cat#309-035-011, RRID#AB_2339650), and [h]goat anti-human Fc specific polyclonal IgG (Sigma Aldrich, Cat#A1070, RRID#AB_257868, St Louis, MO, USA) antibodies were added to the plates at a dilution of 1/60,000 with 0.5% BSA. After 1 h of incubation at 37 °C, the plates were washed and developed with 50 μL TMB Two-Component Substrate solution in each well (Solarbio, Cat#PR1210). The reaction was stopped by adding 25 μL of stop buffer (Solarbio, Cat#C1058). Optical density at 450 nm (OD450) was determined with EnSight (Revvity, Waltham, MA). The area under the curve (AUC) was calculated using GraphPad Prism 10.1. The same amount of lytic Vero cell without MPXV infection was used as a negative control for each plasma sample. Titres of MPXV-specific IgM, IgA, and IgG were calculated by subtracting the background before further analysis. Cut-off values were determined by calculating the mean AUC of negative plasma plus 3-fold SD values, which were 116, 132, 126 for MPXV-IgM, MPXV-IgA, and MPXV-IgG, respectively.

Viral culture

MPXV DNA-positive samples were cultured for seven days in a 24-well cell culture plate using Vero cell (ATCC, CCL-81, RRID#CVCL_0059). About 100 μL of specimen fluid and 100 μL viral culture medium (Earle's minimum essential medium [EMEM] containing 2% heat inactivated fetal bovine serum [FBS]) were added to inoculate the monolayer cells in each well at 37 °C incubator for 1 h. The wells were monitored daily for the presence of cytopathic effect (CPE). Blind passage of CPE-negative cell was performed for two times and seven days for each passage. The negative CPE after three-time passages in general did not yield viable virus. All viral culture was performed in biosafety 3 laboratory. All the CPE-positive cell were lysed and performed nucleic acid extraction and viral DNA qualification using real-time PCR to confirmed the MPXV.

Plaque reduction neutralization test

A serial four-fold dilution of plasma samples (starting at 1:10) were pre-incubated with MPXV, which were isolated from skin lesion samples of Mpox patient in a biosafety level 3 laboratory, at 75 pfu determined by Vero cells (ATCC, CCL-81). After 1 h of incubation at 37 °C, the virus/plasma mixture was incubated with Vero cells in 12-well plates (Costar, Cat#3513, NY, USA). The virus/plasma mixtures were removed after 2 h and 1 mL fresh overlay-medium (DMEM containing 2% FBS, and 1% methyl cellulose) was added to each well. At three days post-infection, the plates were stained with crystal violet and the number of plaques were counted using Countstar Castor X1 (ALIT life science, Shanghai, China). For each plasma dilution, duplicate wells were used. Antibody titers were defined as the highest plasma dilution that resulted in >50% (PRNT50) in the number of virus plaques. The cut-off for a positive NAb titre was 1:10. When the neutralising antibody values were below the limit of detection, the neutralising antibody titer is defined as 1:5.

Cytokine and chemokine measurement

Plasma cytokines and chemokines were measured using Human Cytokine Standard 27-Plex Assays panel and the Bio-Plex 200 system (Bio-Rad, Cat#M500KCAF0Y, Hercules, CA, USA) in 39 patients when they were in hospital first day (PSO, median 8 [IQR 6–10]) according to the manufacturer's instructions. The plasma samples from 8 healthy adults were used as controls.

Outcomes

The primary outcomes were viral load; titres of IgM, IgA, IgG, and NAb at different timepoints PSO; and plasma cytokine and chemokine concentrations. The viral loads are expressed as the cycle threshold (Ct) values from real-time PCR. The titres of IgM, IgA, and IgG are expressed as the area under the curve (AUC). The cut-off value for the NAb titres against MPXV for the PRNT was 1:10. Secondary outcomes included viral load, antibody responses, and plasma cytokine and chemokine concentrations while accounting for HIV status. Other secondary outcomes included the demographic and clinical characteristics of the patients.

Statistical analysis

The demographic and clinical characteristics of the patients are presented as median and IQR for continuous variables and are expressed as absolute values along with percentages for categorical variables. A locally weighted scatterplot smoothing (LOWESS) method was used to portray the trends of viral load and antibody titres, viral detection rate, and cumulative seroconversion rate over time. The Mann–Whitney U test was used to compare the viral loads between skin lesions and the samples collected from other body sites, as well as to compare the patients' plasma cytokine concentrations with those of healthy donors. When the NAb titre measurements were below the limit of detection, the titres were set to 1:5. Spearman correlation analysis was performed for the correlation analyses between viral load (Ct value) and NAb titres and between

[h]Peroxidase conjugated.

the IgM, IgA, and IgG titres and the NAb titres involving all the samples collected at different time-points were utilized. A two-sided p value less than 0.05 was considered significant. All the statistical analyses were conducted using GraphPad Prism 10.1.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

Results

A total of 510 specimens, including 75 skin lesions, 79 oropharyngeal samples, 74 saliva samples, 47 faecal samples, 73 urine samples, 87 plasma samples, and 75 serum samples, were collected from 39 people with mpox who were recruited for this study. All the patients had clinically healed, and 37 (94.9%) patients were discharged on day 14 after hospitalisation. The demographic and clinical characteristics of the participants are listed in Table 1. The median age of the patients was 33 years (IQR 28–37), and all of the patients were male. The median interval between symptom onset and hospitalisation was seven days (IQR 5–9). A total of 20 (51.3%) patients were PWH, and all of them were compliant with regard to taking antiretroviral therapy (ART) after being diagnosed with HIV infection. One person with mpox was newly diagnosed with HIV infection when he was admitted. The CD4 cell count of the PWH ranged from 417 to 1369 cells/mm^3 (median 638, IQR 484–854). A total of 18/20 (90%) PWH had no detectable HIV RNA (Appendix p 3). The major clinical manifestations included skin rashes or mucosal lesions, fever, lymphadenopathy, and sore throat. Chronic hepatitis B was recorded in 2 (5.1%) patients based on their self-reports on admission and laboratory examinations after hospitalisation. Two (5.1%) of the 39 patients were born between 1972 and 1975, respectively, while the other 37 (94.9%) patients were born between 1981 and 2004.

The methods and sample sizes used for the detection of viral DNA, antibodies, and plasma cytokines and chemokines are described in the flow chart (Fig. 1). The sampling time differed among the patients due to differences in admission times; the earliest sample was taken on day 3 PSO, and the latest sample was taken on day 21 PSO.

The temporal changes in the rate of viral DNA detection in the different types of samples over time are shown in Fig. 2a. Within the first 7 days PSO, 13 (92.9%) of 14 skin lesion samples, 13 (92.9%) of 14 oropharyngeal samples, 12 (92.3%) of 13 saliva samples, 4 (80%) of 5 faecal samples, 6 (46.2%) of 13 urine samples, 7 (43.8%) of 16 plasma samples, and 5 (38.5%) of 13 serum samples tested positive on PCR. Among all the types of samples taken from days 8–14 to days 15–21 PSO, the highest rate of viral DNA was detected in the skin lesions (all $p < 0.05$), with 12 (85.7%) of 14 skin lesion samples testing positive on PCR on days 15–21 PSO. The saliva samples had the second highest positive rate and viral load among the samples, being only lower than those of skin lesions during the disease course. However, only nine patients had oral/pharyngeal lesions. On the discharge day and one day before discharge, 20 paired skin lesions (dry scrabs) and saliva were collected. Viral DNA was detected in 17 dry scrabs (median Ct 28.2, range 19.0–38.3) and 14 saliva samples (median Ct 32.4, range 24.5–38.1). These samples were further used for virus isolation. Three dry scabs with Ct values of 19, 25, and 24 had positive viral cultures, with Ct values of 15, 21, and 18 on MPXV PCR, respectively. The detection rate of viral DNA in the oropharynx and faeces decreased with time. However, the positive rate of viral DNA in the plasma, serum, and urine samples increased quickly before day 10 PSO and then decreased slightly (Fig. 2a). In this study, seven patients showed delayed positivization in their saliva, oropharyngeal, plasma, serum, faeces and urine, similar to the results reported in a previous study.[14]

The temporal changes in the viral load were similar to the temporal changes in the detection rate (Fig. 2b). The median Ct values of the skin lesions on days 1–7, 8–14, and 15–21 PSO were 27.4 (IQR 23.5–30.8), 26.4 (22.9–32.6), and 26.8 (23.7–34.5), respectively. The viral load in the skin lesions was greater than that in the other samples across the disease course, from admission to day 21 PSO (all $p < 0.05$) (Fig. 2b). Notably, the viral DNA detection rate and viral load in the saliva samples were second only to those in the skin lesions (Fig. 2a and b).

A total of 87 plasma samples were taken from 35 patients, four serial samples from three patients, three serial samples from 18 patients, two serial samples from seven patients, and one sample from the remaining seven patients. Based on the patients' self-reports, it was noted that two patients born in 1972 and 1975 had received smallpox vaccination (Vaccinia virus Tiantan strain, VTT) during childhood. Of the 87 plasma samples collected from 35 patients, 16 were collected on days 1–7 PSO, 51 on days 8–14 PSO, and 20 on days 15–21 PSO. We evaluated the seroconversion and titres of IgM, IgA, IgG and NAbs against MPXV. The median days of seroconversion of IgM, IgA, IgG, and NAb were 8 (IQR 7–9), 9 (7–10), 12 (9–15), and 12 (9–15) PSO, respectively (Fig. 3a). The cumulative seroconversion rate is shown in Fig. 3b. The seroconversion of IgM and IgA remained at high levels during our study period. The positive rates of IgG and NAbs increased quickly from days 3 to 10 and then increased slowly after day 11. IgM, IgA, IgG, and NAb against MPXV were detected in 94.3% (82/87), 88.5% (77/87), 69.0% (60/87), and 85.1% (74/87) of the plasma samples, respectively, as of day 21 (Fig. 3b). Of 35 patients with available plasma samples,

Characteristic	Total (n = 39)	People living with HIV (n = 20)	People living without HIV (n = 19)
Age (year), median (IQR)	33 (28–37)	32 (28–37)	33 (26–37)
Sex, n (%)			
Male	39 (100%)	20 (100%)	19 (100%)
Female	0 (0%)	0 (0%)	0 (0%)
Coexisting conditions, n (%)			
Chronic hepatitis B	2 (5.1%)	1 (5.0%)	1 (5.3%)
Diabetes	0 (0%)	0 (0%)	0 (0%)
Cerebrovascular disease	0 (0%)	0 (0%)	0 (0%)
CD4vcell count (cells/mm^3), median (IQR)	/	638 (484–854)	/
Undetectable HIV RNA	/	18	/
Days from illness onset to hospitalization, median (IQR)	7 (5–9)	7.5 (5–10)	6 (4–9)
White-cell count (×1^{-9}/litre), median (IQR)	6.9 (5.8–8.8)	6.4 (5.5–9.9)	6.9 (5.6–7.9)
(4–10) × 10^9/litre, n (%)	32 (82.1%)	15 (75%)	17 (89.4%)
<4 × 10^9/litre, n (%)	0 (0%)	0 (0%)	0 (0%)
>10 × 10^9/litre, n (%)	7 (17.9%)	5 (25%)	2 (10.6%)
Lymphocyte count (×10^9/litre), median (IQR)	2.6 (2.1–3.2)	2.6 (2.0–3.1)	2.8 (2.2–3.3)
≥1.0 × 10^9/litre	38 (97.4%)	19 (95%)	19 (100%)
<1.0 × 10^9/litre	1 (2.6%)	1 (5%)	0 (0%)
Platelet count (×10^9/litre), median (IQR)	214 (160–259)	159 (139–239)	216 (157–282)
≥100 × 10^9/litre, n (%)	39 (97.5%)	19 (95%)	19 (100%)
<100 × 10^9/litre, n (%)	1 (2.5%)	1 (5%)	0 (0%)
Systemic symptoms (n, %)			
Fever (>38.3 °C)	29 (74.4%)	16 (80%)	13 (68.4%)
Lymphadenopathy	27 (69.2%)	15 (75%)	12 (63.2%)
Sore throat	8 (20.5%)	4 (20%)	4 (21.1%)
Headache	3 (7.7%)	2 (10%)	1 (5.3%)
Myalgia	2 (5.1%)	1 (5%)	1 (5.3%)
Diarrhoea	1 (2.6%)	1 (5%)	0 (0%)
Number of lesions (n, %)			
0	2 (5.1%)	2 (10%)	0 (0%)
1–4	14 (35.9%)	8 (40%)	6 (31.6%)
5–9	17 (43.6%)	6 (30%)	11 (57.9%)
10–19	5 (12.8%)	3 (15%)	2 (10.5%)
≥20	1 (2.6%)	1 (5%)	0 (0%)
Plasma Cytokine and chemokine concentration (pg/mL), median (IQR)			
MIP-1α	1.9 (1.5–2.4)	2.0 (1.8–2.4)	1.6 (1.3–2.2)
MIP-1β	138.8 (124.3–148.4)	145.9 (133.3–152.4)	135.5 (117.4–143.9)
G-CSF	97.4 (79.5–132.9)	124.1 (92.2–140.3)	85.4 (72.0–103.3)
IL-4	2.1 (1.7–2.6)	2.4 (1.9–3.0)	1.8 (1.7–2.3)
FGF-basic	6.4 (5.3–8.5)	6.9 (5.3–8.5)	5.3 (4.4–7.4)

IQR, interquartile range; MIP, macrophage inflammatory protein; G-CSF, granulocyte colony stimulating factor; IL, interleukin; FGF, fibroblast growth factor.

Table 1: **Patient demographic and clinical characteristics.**

34 had detectable NAbs. The kinetics of the IgM and IgA titres were similar, increasing from day 3 to day 14 PSO and then stabilizing between days 14 and 21 PSO. There were continuous increases in the IgG and NAb titres from 3 to 21 days PSO (Fig. 3c). The titres of IgM ($r = 0.40$, $p = 0.0001$), IgA ($r = 0.31$, $p = 0.0031$), and IgG ($r = 0.48$, $p < 0.0001$) were significantly correlated with the NAb titres (Appendix p 5).

When analysing factors potentially affecting the strength of the humoral response, we observed that the two patients with a history of smallpox vaccination had higher IgG and NAb titres than did those without prior smallpox vaccination (Fig. 3c). Notably, one of the two smallpox-vaccinated patients developed high IgA titres on days 6 and 8 (Fig. 3c). The titres of IgM, IgA, and IgG against MPXV were not significantly different between

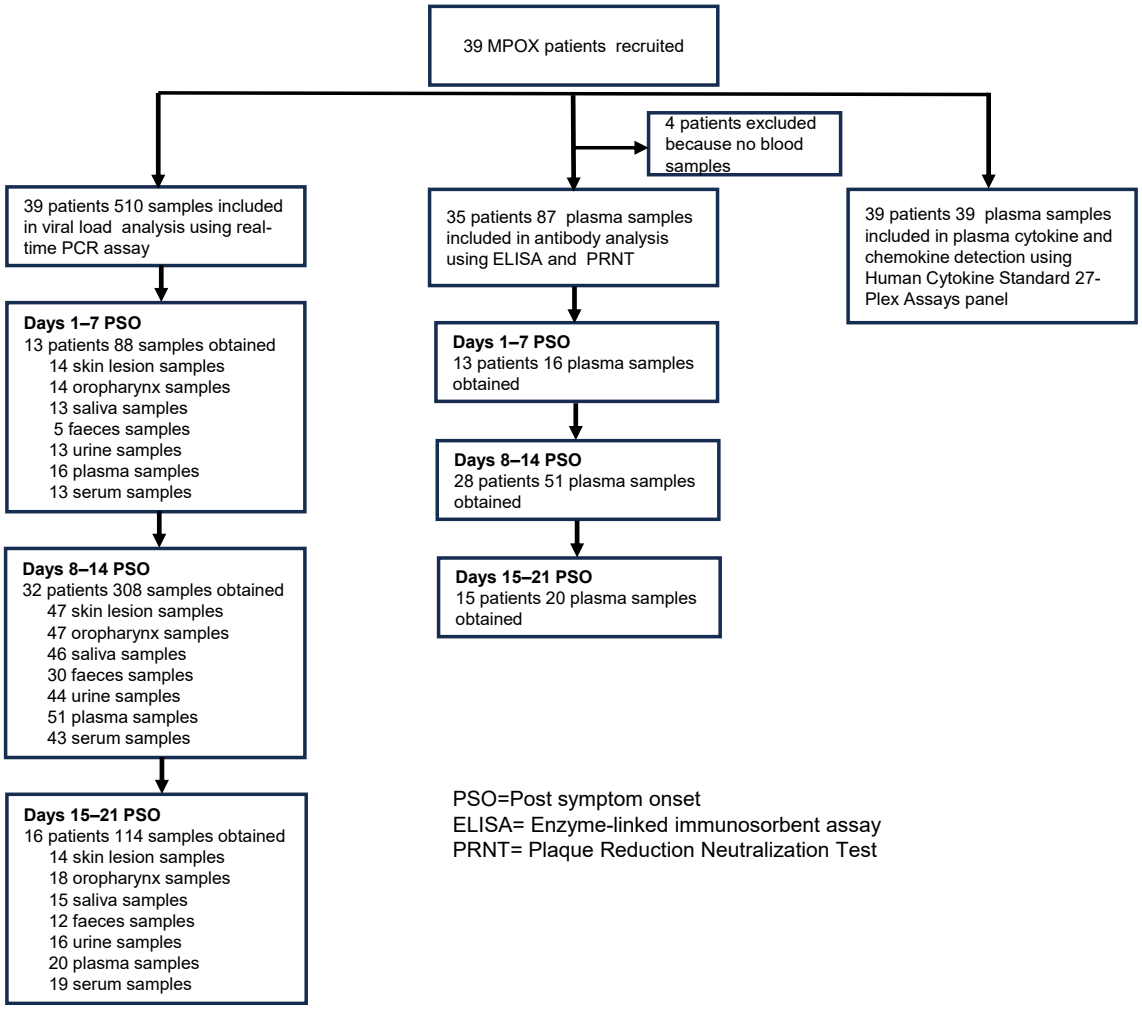

Fig. 1: **Flow chart of the study.**

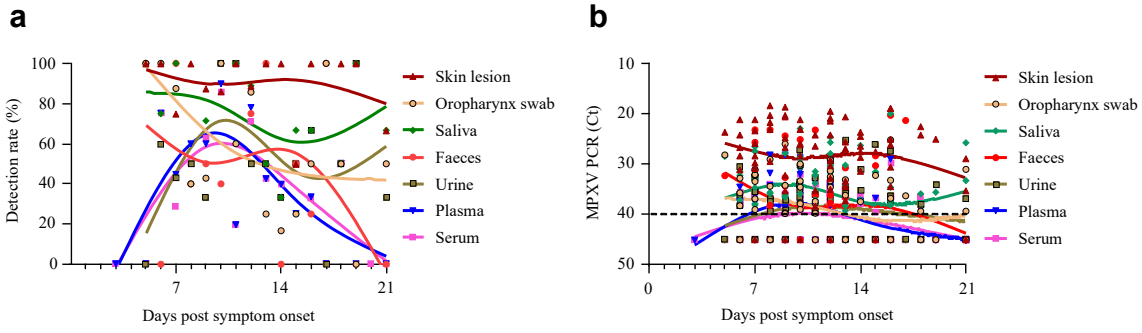

Fig. 2: **The kinetics of the load and detection rate for viral DNA and temporal trend curves for specimens collected from patients infected with monkeypox virus (MPXV).** (a) Fitted curve of the detection rate of viral DNA in skin lesion, oropharynx, saliva, faeces, urine, plasma, and serum samples by PCR on different days post symptom onset. The dots represent the PCR detection rates at each timepoint. (b) Dynamics of viral load in association with days post symptom onset in skin lesion, oropharynx, saliva, faeces, urine, plasma, and serum samples. The dots represent one sample per patient at each time point. The fitted curves were created by GraphPad Software.

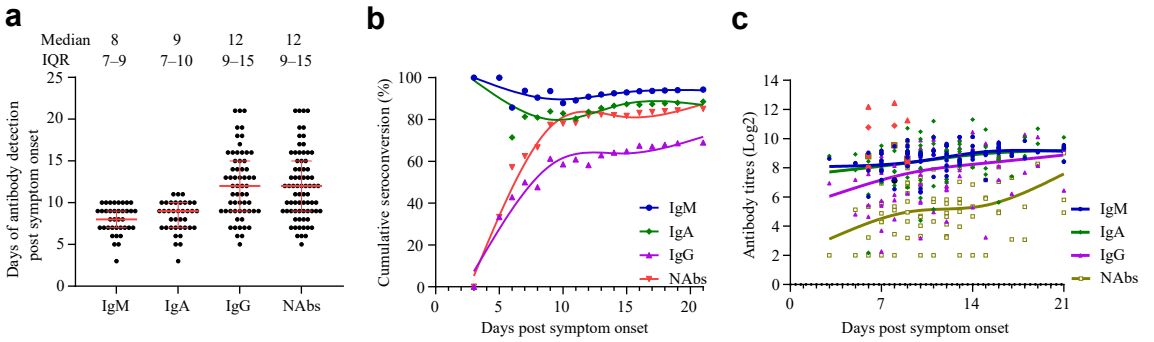

***Fig. 3:* Characteristics of plasma antibodies in patients infected with monkeypox virus (MPXV).** The detectable time (a) and cumulative seroconversion (b) of IgM, IgA, IgG, and neutralising antibodies against MPXV over time post symptom onset, as determined by ELISA (IgM, IgA, IgG) and PRNT (neutralising antibody) using plasma samples. (c) Temporal changes in IgM, IgA, IgG, and neutralising antibody titres against MPXV in plasma samples determined by the locally weighted scatterplot smoothing method. The antibody titres were log2 transformed. The dots in panels a and c represent one sample per patient at each time point. The dots in panel b represent the cumulative detection rates of antibodies in all the samples at each timepoint. The red dots shown in panel c represent the two patients, who were born in 1972 and 1975, respectively, who were vaccinated. Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test.

PWH and PWOH across the disease course during hospitalisation (Appendix p 6). The NAb titres did not significantly differ before day 10 PSO; however, the NAb titres were lower in PWH than in PWOH between days 11 and 21 PSO (Appendix p 6).

The association of the antibody levels with the viral load was evaluated. The NAb titre was not associated with the viral load in the skin lesions ($r = 0.14$, $p = 0.25$; Fig. 4a), oropharyngeal samples ($r = 0.22$, $p = 0.065$; Appendix p 7), or urine samples ($r = 0.035$, $p = 0.78$; Appendix p 7). However, increasing NAb titres were associated with lower viral loads in the saliva samples ($r = 0.28$, $p = 0.025$; Fig. 4b), faecal samples ($r = 0.35$, $p = 0.021$; Fig. 4c), plasma samples ($r = 0.30$, $p = 0.0044$; Fig. 4d), and serum samples ($r = 0.37$, $p = 0.001$; Appendix p 7).

The plasma levels of several cytokines, including FGF basic ($p = 0.023$), G-CSF ($p = 0.0010$), IFNγ ($p = 0.0012$), IL-1β ($p = 0.0004$), IL-1ra ($p < 0.0001$), IL-2 ($p = 0.0001$), IL-6 ($p = 0.037$), IL-8 ($p = 0.0031$), IL-10 ($p = 0.038$), IL-15 ($p = 0.018$), IP-10 ($p < 0.0001$), MIP-1α ($p < 0.0001$), RANTES ($p = 0.0004$), and TNFα ($p = 0.014$) (Appendix p 8), were greater in patients than in healthy donors (HD). The levels of Eotaxin, GM-CSF, IL-4, IL-5, IL-9, IL12p70, IL-13, IL-17, MCP-1, MIP-1β, PDGF-BB, and VEGF were similar between patients infected with MPXV and HDs. The concentrations of IL-4, MIP-1α, MIP-1β, FGF-basic, and G-CSF were greater in PWH than in PWOH (Table 1) and the HD (data not shown).

Discussion

In this study, we characterized the temporal changes in viral load and antibody titres in people with mpox across the disease course while they were hospitalised. The detection rate of viral DNA and viral load were highest in skin lesions across the disease course during hospitalisation. The skin lesions contained the highest viral load and positive rate than the other types of samples, and the saliva samples had the second highest viral load and positive rate. More than 60% of the saliva samples were still positive for viral DNA even in the third week PSO. The percentage of samples positive for viral DNA among the oropharyngeal and faeces samples was high at the very early PSO and then decreased rapidly after the second week PSO. The dynamics of the viral load in the plasma, serum, and urine samples were similar, with positive results detected mainly within the second week PSO. The humoral responses occurred in the first week. The titres of IgG and NAbs increased continuously over time, while the titres of IgM and IgA remained high after the third week PSO. An increase in NAb titre was related to a decrease in the viral load in saliva, faeces, plasma, and serum. The NAb titres of PWH patients increased slowly and had lower NAbs titres that were found at the convalescence stage compared with PWOH. PWH had higher cytokine concentrations than PWOH. MPXV infections in two patients with a history of VTT vaccination in childhood boosted higher IgG and NAbs titers compared to other patients.

During hospitalisation, the patients received clinical care in the isolation unit where measures were taken to administer antibiotics for skin lesions and to perform symptomatic and supportive treatment. Clarifying the amount of viral DNA and its shedding time at different body sites during hospitalisation is critical for clinical management. Taken together, our data and those of other reports indicate that the DNA of MPXV is more

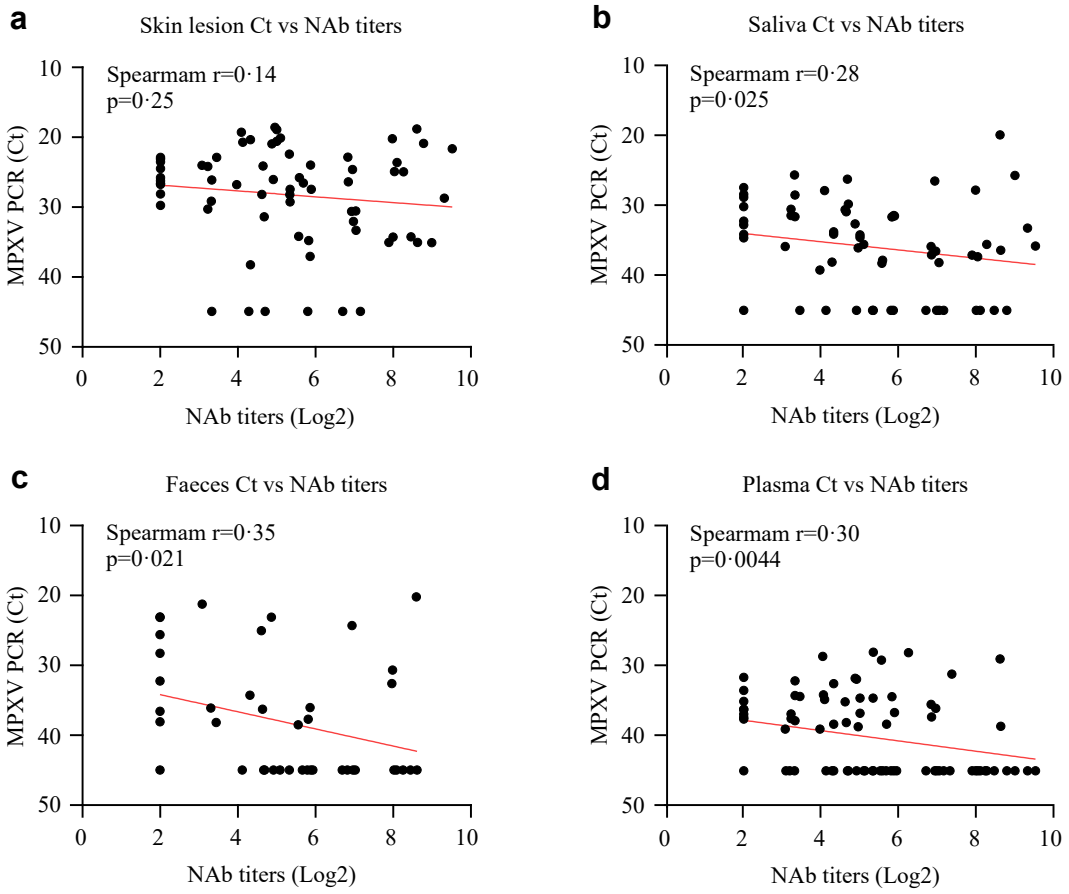

Fig. 4: **The correlation between neutralising antibody titres and viral load**. The correlation between neutralising antibody titres and viral load in skin lesions (a), saliva (b), faeces (c), and plasma (d). The dots in the panels represent one sample per patient at each time point. The neutralizing antibody titres were log2 transformed.

easily detected in skin lesions than in other samples due to the high viral loads found in skin lesions.[15,16] In our study, viral DNA was detectable by qPCR even on day 21 PSO, the longest observational period in our study. We found that the overall percentage of viral DNA-positive saliva samples was 73%, with an average Ct value of 32.2. Only eight patients with oral/pharyngeal lesions had been sampled and tested positive on MPXV- DNA, the other 26 cases with viral DNA in saliva had no detected oral/pharyngeal lesions/across the disease course during hospitalisation. In a study from Spain, all 12 patients were positive for viral DNA in their saliva samples.[17] In another study from Spain, MPXV DNA was detected in 85% (35 of 41) of the saliva samples collected in the first week post viral infection, and infectious MPXV could be detected in 67% (22 of 33) of the saliva samples.[18] Such findings furtherly indicate that MPXV would shed in saliva samples. Although not all patients with detectable MPXV-DNA in saliva had oral/pharyngeal lesions, however, the oral/pharyngeal lesions might not to be excluded as the cause for saliva positive PCR detection. In these published reports, saliva samples were collected only during a limited period during the disease course. In our study, saliva samples were serially collected, and we found that the saliva samples were still positive for viral DNA, with a median Ct of 32.4 (range of 24.5–38.1) one day before patient discharge. It has been confirmed that viable virus can be cultured from saliva with a Ct below 27.7,[19] while 50% of the clinical samples were positive on viral culture at a Ct of 34.1 (95% confidence intervals 32.1–37.4).[20] These findings further emphasized the importance of the shedding time of infectious viruses. Currently, the criteria of discharging patients with mpox were mainly according to the alleviation of clinical symptoms. The presence of infectious virus in samples from patients who were being discharged indicates the necessary for intensive improvement of the management strategies of patients with mpox after discharge.

The antibody response to MPXV was elicited after 14 days of PSO in most patients regardless of their HIV status, indicating that antibody responses were induced in the acute stage after MPXV infection. The seroconversion of IgM and IgA antibodies against

MPXV was induced at the early stage after infection, while the levels of IgG and NAbs significantly increased over time. All these data indicate that the antibody response can develop regardless of the patient's HIV status and vaccination status, as previously reported.[21,22] However, we observed that the NAb titres were lower in PWH than in PWOH, which indicates that HIV-driven immune depression might impair antibody responses.

The correlation between NAb titres and viral loads is not fully understood in people with mpox. In our study, NAb titres were detectable in the first week and then continuously increased as of day 21 PSO within the study period. There were no significant differences in the NAb levels between PWH and PWOH before day 10 PSO. However, the NAb titres were lower in PWH than in PWOH between days 11 and 21. When PWH were coinfected with other viruses, such as HCV and measles virus, their antibody titres against viruses causing coinfections slowly increased.[23,24] Plasma cell and memory B-cell disorders in PWH may impact the production of antibodies.[24,25] The titre of NAbs was negatively related to the viral load in saliva, faeces, plasma, and serum samples but was not related to the viral load in skin lesions, the oropharynx, or urine. These findings suggest that NAbs against MPXV might not be sufficient for viral clearance during disease progression, which has been confirmed in SARS-CoV-2-infected patients.[26] Innate immunity and cellular immunity must contribute to recovery.[27–29]

Johnston and his colleagues reported a prominent T helper 2 (Th2) response and a dampened Th1 response after MPXV infection.[13] We observed that people with mpox had high plasma levels of IL-1β, IL-6, IL-2, IL-8, IL-15, IP10, IFNγ, and TNFα, which could cause the activation of T-helper-1 (Th1) cells and result in an inflammatory response. Moreover, PWH had higher concentrations of MIP-1α, MIP-1β, G-CSF, and FGF basic than PWOH, suggesting the association of cytokine storms with the patient's HIV status. MPXV infection caused an increase in the level of IL-10, a T-helper-2 (Th2) cytokine, which suppresses inflammation. However, further studies are needed to explore the detailed pathophysiologies of cytokines with respect to disease severity.

There are several limitations in our study. First, the subjects in this study were mild mpox inpatients, and the characteristics of our study patients might not represent the characteristics of patients with severe mpox. Second, only 39 patients were recruited from unique hospital in our study. Our findings should be further validated by involving more hospitals and patients, which could improve the generalizability of our results. Third, not all of the patients in our study had samples collected at different timepoints for PCR and antibody analyses. Fourth, the median duration from symptom onset to hospitalization was 7 days. Further studies are needed to clarify the viral load and antibody dynamics in patients with severe infections, as well as in the short term from illness onset to hospitalization.

In summary, we evaluated the dynamics of the viral load, humoral response and host inflammation in mild mpox inpatients during hospitalization. The shedding of MPXV-DNA in skin lesions and saliva samples before discharge emphasizes the need for improvements in the public health strategies for people with mpox. The lower NAb titres and higher cytokine concentrations in PWH provided insights into the MPXV transmissibility, antiviral treatment, and vaccination strategies in high-risk populations.

Contributors

JW, LR, and RJ conceived and designed the study and take responsibility for data integrity and accuracy of the data analysis. YL and JZ did the literature review. LG, LC, QZ, DL, MF, XC, YX, XW, RG, ZL, and GW performed experiments. LG, QZ, and LR did the analysis. LG and LR drafted the manuscript. RS, XC, ZG, HZ, YZ, and NH collected the data of demographic and characteristics, and clinical samples. LG, QZ, and XW verified the underlying data in the study. All authors read and edited the manuscript, approved the final version, had full access to all the data, and had final responsibility for the decision to submit for publication.

Data sharing statement

Data sharing restrictions apply to the availability of the data; therefore, they are not publicly available. However, data are available from the authors on reasonable request and with permission from the authors' institutions.

Declaration of interests

We declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105254.

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