Diagnostic approach to monkeypox outbreak, a case-control study

Moretti, Marco; Heymans, Benjamin; Yin, Nicolas; Kaur, Simran; Libois, Agnes; Quoilin, Sophie; Martin, Charlotte

Published in:
International journal of STD & AIDS

DOI:
10.1177/09564624231152789

Publication date:
2023

Document Version:
Accepted author manuscript

TITLE OF THE STUDY

Diagnostic approach to monkeypox outbreak, a case-control study

Key Words

Monkeypox outbreak; Diagnostic; Real-Time PCR; HIV infection; Anogenital skin lesions.

Word count

Number of words (Abstract): 248

Number of words (Text): 3008

Number of figures: 2

Number of tables: 2

Abstract

Background: An international outbreak of the monkeypox (MPX) virus is ongoing with a different clinical presentation than previously reported.

Objective: A monocentric retrospective study was designed to investigate clinical predictors of confirmed MPX cases among a group of patients referred for MPX screening.

Furthermore, the additional value of performing a real-time polymerase chain reaction (RT-PCR) on multiple anatomical sites was analyzed.

Methods: Between 28/05/2022 and 22/07/2022, the medical records of patients referred for MPX screening were investigated. Patients with positive RT-PCR were defined as cases, while the ones with negative RT-PCR as controls. Multivariable regression analysis was performed to estimate predictors of MPX diagnosis.

Results: Among the 141 included patients, 85 (60%) had at least one positive RT-PCR for MPX. Carrying out RT-PCR only on the swab obtained by skin lesions sampling, 7 patients (7/85: 8%) would have been misdiagnosed. Multivariable regression analysis showed significant differences in the independent variables: “being men who have sex with men (MSM)”, “living with HIV”, “having multiple sexual partners in the last 3 weeks”, and “having skin lesions in the anogenital area” for prediction of MPX diagnosis. These four discriminants were used to create a score to improve diagnosis in patients screened for MPX.

Conclusion: MPX diagnosis was associated with being MSM, living with HIV, having multiple sexual partners, and presenting with anogenital skin lesions. In this study, the derived score had good sensitivity and specificity to predict MPX diagnosis. Finally, performing multi-site swabs for MPX RT-PCR might lower false negative rates.

TEXT

Introduction

Background

Monkeypox virus (MPXV) is responsible for a zoonotic disease called Monkeypox (MPX), which was first described in 1958 [1 - 3]. MPXV infects the human host through percutaneous, mucocutaneous inoculation, or respiratory droplets. After three to twenty-one days of incubation with a median of seven days, the disease classically begins with systemic symptoms followed by a maculopapular eruption [2 - 6]. Three different virus clades were identified: clade 1, formerly Congo-basin one, clade 2 and 3, which were previously defined as West-Africa clade [1, 2, 3, 7]. The clade 2 and 3 seem to give a lower case fatality rate. Limited outbreaks were reported also outside of the African continent [1, 8]. Nowadays, an international large outbreak is ongoing, particularly affecting Europe and the United States. The isolated responsible pathogen belongs to clade 3 and might be linked to previous clusters [5, 7, 9]. However, a recent phylogenomic study classified the MPXV, responsible for the present outbreak, in a divergent phylogenetic branch, suggesting an accelerated evolution [7]. Unusually, men who have sex with men (MSM) without any contact with African countries or infected animals seem to be the main disease target of the present outbreak. Transmission from person to person, mainly through sexual networks, might be the main spreading method [5, 6, 9]. Furthermore, most of the previous studies consider the swab of the skin lesions as the preferred sample to analyze [6, 10]. However, a throat swab may be performed in contacts of confirmed or highly probable MPX cases without any symptoms, and an anal swab might be considered for patients presenting with proctitis [6, 11]. Although Monkeypox seems to be frequent in people living with HIV and

might present in this population in atypical ways, no specific diagnostic methods are withheld in the guidelines [12]. Moreover, limited access to nucleic acid amplification tests is reported in middle and low incomes countries with 47% of the world's population lacking access to adequate diagnostic analyses [13]. Finally, supplemental investigations are required to better understand the diagnostic approach to MPX to promptly implement infection control policies.

Objectives

The current monocentric observational study has been designed to improve the diagnostic approach to MPX and develop a new diagnostic score for MPX diagnosis. Clinical predictors of MPX diagnosis were investigated in patients presenting with general symptoms and skin rash during the MPXV outbreak. Furthermore, diagnostic methods and their diagnostic accuracy for MPX diagnosis were analyzed in this study.

Materials and methods

Study design

The design of the present study is retrospective, monocentric, and case-control. The case-control design was preferred to efficiently identify predictive factors for MPX diagnosis.

This study was elaborated following the STROBE checklist for reports of observational studies [14].

Setting

Patients presenting for suspicion of MPXV infection from 28th May 2022 to 22nd July 2022 at the University Hospital Saint Pierre (CHU Saint Pierre), an infectious diseases reference

center, were eligible for the present study. Patients were referred for MPX screening by the emergency department, the sexual health clinic of the CHU Saint Pierre, and general practitioners after a phone consultation with an infectious disease specialist.

Participants

Patients older than 16 years, fulfilling the criteria of MPX suspicion, and undergoing a specific Real-time Polymerase chain reaction (RT-PCR) for MPXV at CHU Saint Pierre within the chosen timeframe were eligible for this study. MPX suspicion was defined in the current study as a patient presenting with general symptoms (at least one of the following: fever, fatigue, headache, back pain, myalgia, and perspiration), and skin or mucosal eruption (vesicular-pustular eruption with at least the presence of a scab, or ulceration, or crusted lesion).

A confirmed MPX case was defined as a patient fulfilling the criteria of MPX suspicion and a positive RT-PCR for MPXV. The latter was carried out on a sample obtained by sampling the throat, skin lesions, and/or the anus of the affected subject. Nucleic acid extraction was performed on universal transport media-preserved swabs using either the Qiagen® DSP Virus/Pathogen Midi kit on QIAsymphony or the AltoStar® Purification Kit 1.5 on AM16. RT-PCR was then carried out following the MPXV generic assay described by Li *et al.* [15]. If multiple samples for one patient were sent for analysis (*eg.* skin lesions and pharyngeal swabs) and at least one of them turned out positive, then we considered the patient as a confirmed case of MPX. The study controls were defined as patients fulfilling the criteria of MPX suspicion, but without a positive RT-PCR for MPXV.

Variables definition

The main outcome was defined as being a confirmed MPX case (definition in the participants section of this article). This definition conforms with the Belgian national reference institute for epidemiology in infectious diseases, Sciensano [16]. The variable "being MSM" was defined as a male having sexual intercourse with another man, mentioned by the patient himself. The variable "multiple sexual partners" was defined as two or more different sexual partners in the last 3 weeks. The skin lesions were divided into the following anatomical zones: mouth, face and neck, chest and abdomen, back, inferior and superior limbs, and anogenital area. The variable "living with HIV" was defined as a medical history of HIV diagnosis. Patients with a new HIV infection were excluded from this variable.

Data sources

Epidemiological, clinical, biological, and microbiological data were independently collected from the medical records of eligible patients at CHU Saint Pierre by two study investigators (SQ and BH) to reduce the risk of sampling errors. In case of differences between the two authors, supplemental research of the patient's medical records was performed to verify the correctness of the data.

Potential study bias

Type II error could have affected study results as specific MPXV RT-PCR might have given false negative results. Sampling errors were prevented by the performance of swabs by trained medical staff (MM, BH, SK, and SQ). Furthermore, some of the samples analyzed in our center were also reviewed by a second university hospital (Laboratory of

Molecular Virology UZ Leuven, Belgium) to address potential analytic errors. Finally, most patients screened at CHU Saint Pierre underwent multiple site sampling (skin lesions, pharyngeal, and sometimes anal testing). These strategies were used to limit the risk of false negative results.

Study size

The study size for multiple regression analysis was calculated a priori. Accepting an alpha-error probability of 0.05, with the desired power of 80%, an effect size of 0.1, and 4 predictors in the model, the sufficient sample size was determined to be 125 patients.

Quantitative variables and statistical methods

Data are expressed as median and interquartile ranges for continuous variables, and as numbers and proportions for categorical variables. Initial analyses were performed to assess the sensitivity of skin lesions, pharyngeal, and anal swabs for the diagnosis of MPX. Thereafter, predictors for confirmed MPX cases were investigated. Independent predictors were all combined in a logistic regression to check which combinations, jointly, help predict the probability to belong to the confirmed MPX group. Based on significance and clinical interest some variables were retained and fitted in a multivariable logistic regression model. Finally, predictors found by the regression model were analyzed to estimate their diagnostic accuracy.

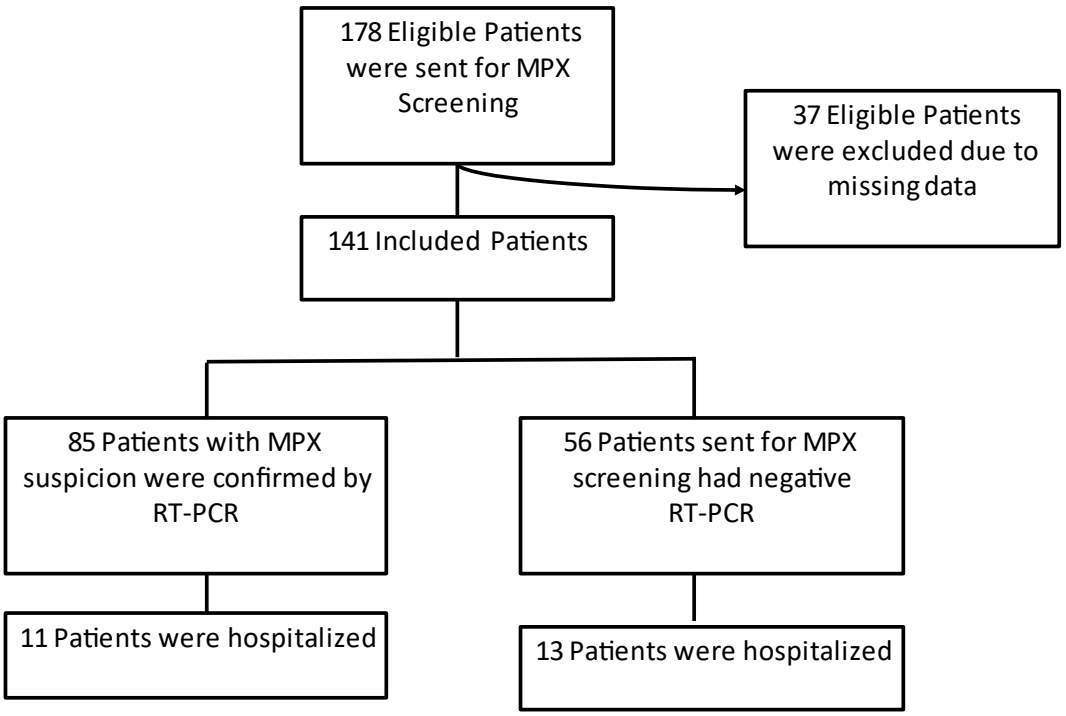
Power calculation was carried out with G*Power, Version 3.1.9.4 [17]. Further analyses were performed with IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp, released in 2011.

Results

Participants

Initially, 178 patients were eligible for this study. Due to incomplete data, 37 patients were excluded. Finally, 141 patients were enrolled in the current study. The study flowchart is presented in Figure 1.

Figure 1



Descriptive Data

Among the 141 included patients, 85 (60%) had at least one positive RT-PCR for MPXV and were defined as confirmed cases. The remaining 56 patients (40%) had no positive RT-PCR and were considered the control group. The vast majority of the included patients were male (95%) with a median age of 34 (IQR: 28-40) years old. The main characteristics among the confirmed MPX group were being MSM (n:73 - 89%), having more than one sexual partner in the last 3 weeks (n:56 - 73%), presenting with anogenital skin lesions (n:64 - 80%), fever, and adenopathy. Among the control group, the most prominent features were skin lesions on the chest/abdomen (n:25 - 49%), and inferior and superior limbs (n:30 - 59%). The baseline characteristics of the patients included in this study are illustrated in Table 1. The hospitalization rate among the MPX group was 13% (n:11), with the main reason for admission being pain management (n:5). Other admission reasons in the MPX group were a diagnostic work-up necessitating hospitalization (n:3), the impossibility of home isolation (n:2), and the treatment of a bacterial superinfection (n:1). The diagnosis of MPX lead to a de novo HIV diagnosis in one of the hospitalized MPX confirmed cases. Among the control group, 13 patients (23%) were hospitalized, most of them being admitted because of the impossibility of home isolation until the result of the RT-PCR for MPXV (n:9). Less frequent reasons for admission in the control group were a diagnostic process requiring hospitalization (n:3), and the concomitant respiratory insufficiency due to a pulmonary embolism (n:1). The reported diagnoses in the control group were illustrated in supplementary materials Table 1. The most common differential diagnosis was bacterial skin infection (n:12 - 21%) with three patients diagnosed with ecthyma and two with folliculitis.

177 The missing data for the following: “having sexual intercourse with a positive case in the
178 last 3 weeks”, “frequentation of lesbian, gay, bisexual, transgender, and questioning
179 (LGBTQ) clubs in the last 3 weeks”, and “lesions in the mouth” amounted to 15 - 20%.
180 Within other parameters, missing data were less than 10%.

181 ***Table 1***

Parameters	**Overall study population (n=141)**	**Population features considering confirmed MPX as outcomes**	
		MPX patients (n=85)	***Non-MPX affected patients (n=56)***
Age, year	34 (28 - 40)	33 (28 - 38)	34 (27 - 45)
Sex, male (%)	134 (95%)	85 (100%)	49 (87%)
MSM, yes (%)	91 (71%)	73 (89%)	18 (39%)
Vaccination for Smallpox, yes (%)	11 (9%)	5 (7%)	6 (11%)
Travel history in the last 3 weeks, yes (%)	51 (40%)	37 (47%)	14 (30%)
***Sexual intercourse with a positive case in the last 3 weeks, yes (%)**	29 (26%)	20 (30%)	9 (20%)
***Frequentation of LGBTQ clubs in the last 3 weeks, yes (%)**	41 (37%)	36 (56%)	5 (11%)
Multiple sexual partners in the last 3 weeks, yes (%)	65 (52%)	56 (73%)	9 (19%)

Fever, yes (%)	62 (49%)	50 (64%)	12 (25%)
Myalgia, yes (%)	53 (43%)	40 (53%)	13 (28%)
Headache, yes (%)	59 (44%)	39 (51%)	20 (40%)
Sore throat, yes (%)	39 (33%)	25 (34%)	14 (30%)
Back pain, yes (%)	7 (6%)	7 (9%)	0 (0%)
Asthenia, yes (%)	45 (36%)	30 (39%)	15 (32%)
Proctitis, yes (%)	35 (25%)	29 (34%)	6 (11%)
Adenopathy, yes (%)	51 (40%)	41 (55%)	10 (20%)
***Skin lesions in the mouth, yes (%)**	19 (18%)	16 (25%)	3 (7%)
Skin lesions on the face/neck, yes (%)	51 (40%)	30 (37%)	21 (41%)
Skin lesions on the chest/abdomen, yes (%)	52 (39%)	27 (33%)	25 (49%)
Skin lesions on the back, yes (%)	48 (37%)	25 (31%)	23 (46%)
Skin lesions on the superior limbs, yes (%)	64 (48%)	34 (42%)	30 (59%)
Skin lesions on the inferior limbs, yes (%)	49 (37%)	23 (27%)	26 (51%)
Skin lesions in the anogenital area, yes (%)	83 (64%)	64 (80%)	19 (38%)
Living with HIV, yes (%)	36 (26%)	29 (34%)	7 (13%)
Patient on PREP, yes (%)	30 (22%)	23 (27%)	7 (13%)

Concomitant *Syphilis* infection, yes (%)	10 (8%)	8 (10%)	2 (5%)
Concomitant gonococcal infection, yes (%)	6 (5%)	3 (4%)	3 (6%)
Concomitant *Chlamydia* infection, yes (%)	7 (6%)	5 (7%)	2 (5%)

RT-PCRs sensitivity data

Considering a confirmed MPX case as a patient with MPX suspicion and at least one positive RT-PCR for MPXV, we could estimate the sensitivity of the molecular test performed on the skin lesions, pharyngeal, and anal swabs. A RT-PCR for MPXV carried out on skin lesion swabs was obtained for 80/85 confirmed MPX cases with a calculated sensitivity of 91%. A pharyngeal swab for molecular testing was asked for 69/85 positive MPX patients, and its estimated sensitivity was 74%. 54% (n:20) of patients presenting with a sore throat had positive RT-PCR for MPXV on throat swabs. Furthermore, 42/85 confirmed MPX patients were tested with RT-PCR performed on an anal swab, and its estimated sensitivity was 95%. 91% (n:20) of the patients presenting with proctitis had a positive RT-PCR for MPXV on anal swabs. Supplementary materials Table 2 pictures the comparison between the results of RT-PCR for MPXV carried out on skin lesions and the confirmed MPX cases. Seven MPX patients had negative RT-PCR performed on skin lesions swabs and positive molecular tests for MPXV which were performed on throat or/and anal swabs.

198 ***Predictors for confirmed MPX cases (main results)***

199 By performing initial logistic regression, the independent variables: "living with HIV"
200 (OR: 3.48, 95% CI: 1.40 - 8.65; p-value: 0.007), "being MSM" (OR: 12.62, 95% CI: 5.07 -
201 31.38; p-value < 0.001), "having multiple sexual partners in the last 3 weeks" (OR: 11.56,
202 95% CI: 4.79 - 27.90; p-value < 0.001), "fever" (OR: 5.36, 95% CI: 2.41 - 11.93; p-value <
203 0.001), "adenopathy" (OR: 4.46, 95% CI: 1.94 - 10.27; p-value < 0.001), and "anogenital
204 skin lesions" (OR: 6.53, 95% CI: 2.96 – 14.40; p-value < 0.001) were retained for statistical
205 and clinical significance. Thereafter, the variable "fever" and "adenopathy" were left out as
206 they lost statistical significance in multivariable logistic regression. The final multivariable
207 model was fitted with the other retained independent variables. Table 2 illustrates the
208 multivariable regression model of the present study, adjusted odds ratio are presented in the
209 table. The probability to be in the group of confirmed MPX cases increased in MSM,
210 patients living with HIV, the one with multiple sexual partners in the last 3 weeks, and
211 patients presenting with anogenital skin lesions.

212 ***Table 2***

Term	Odds ratio	95% confidence interval	p-value
(Intercept)	0.31	-	< 0.001
MSM	5.28	1.37 - 20.36	0.016
Living with HIV	7.13	1.51 - 33.59	0.013
Multiple sexual partners	9.27	2.39 - 35.98	0.001

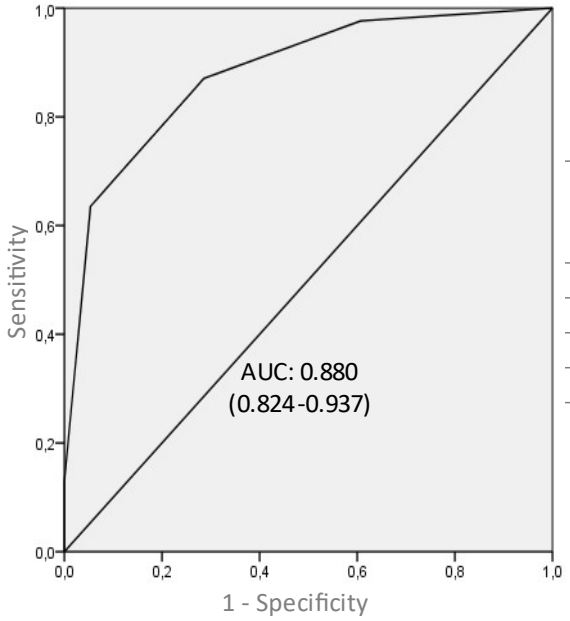
in the last 3 weeks			
Anogenital skin eruption	10.67	3.02 - 37.76	< 0.001

213

214 ***Diagnostic accuracy of the regression analysis discriminants to predict MPX diagnosis***
215 ***(other analysis)***

216 The four predictors identified by the multivariable regression analysis were used to build a
217 score to improve MPX diagnosis in patients screened for this condition. A ROC curve was
218 computed with the sum of the four different predictors, as the test variable, and "being a
219 confirmed MPX case", as the state variable (yes or no), to analyze the most interesting cut-
220 off for diagnostic accuracy. Whenever a cut-off of 2 or more discriminants was chosen, the
221 sensitivity and specificity to predict MPX diagnosis were respectively 87% and 71%
222 (Figure 2). If the cut-off was lowered to 1 or more predictors, the sensitivity increased to
223 98% with a negative predictive value of 92%.

224 ***Figure 2***



Diagnostic accuracy of ≥ 2 regression analysis discriminants for prediction of MPX diagnosis	
Sensitivity	87%
Specificity	71%
Positive predictive value	82%
Negative predictive value	78%

Discussion

Key results and interpretation

Within the first two months of the recent MPXV outbreak, the data of 141 patients with suspicion of MPX were investigated to improve the diagnostic approach to this disease. These patients underwent a complete anamnesis and the specific RT-PCR, with 85 patients being confirmed MPX cases. Within this study, confirmed MPX patients were significantly more likely to be MSM, report multiple sexual partners in the 3 weeks before the screening, have a medical history of HIV, and present with anogenital skin lesions than controls. In line with the study results, more than three-quarters of the MPX patients, within an international and a Spanish large MPX series, were MSM and reported previous multiple sexual partners [6, 9, 18]. Furthermore, the percentage of patients living with HIV, among the MPX cases in the present study and previous case series, was similar [6, 9]. Patients

238 living with HIV seem to have no more severe MPX disease course in this and other series
239 [19]. A possible explanation is the complete viral suppression observed in most of the
240 patients followed at our institution. Nonetheless, data on clinical stage, CD 4 count, and
241 viral load of included patients living with HIV were not collected in the present study.
242 More data is needed in immunodeficient patients with HIV affected by MPX, and a
243 cautious personalized patient approach should be considered [19, 20]. Finally, the two
244 largest published series of MPX patients found that anogenital skin lesions were the most
245 common skin manifestation [6, 9, 21]. In the present study, skin eruptions on the superior
246 and inferior limbs were the most common features within the control group.

247 Through the four predictors derived by regression analysis, a score was developed to
248 predict MPX diagnosis. In the present study, whenever no diagnostic discriminants were
249 present, MPX could be almost excluded with high sensitivity and 92% negative predictive
250 value. Furthermore, two or more predictors had good sensitivity and specificity for MPX
251 diagnosis. The World Health Organization recommends only nucleic amplification tests for
252 the diagnosis of MPX, as the other diagnostic tools, like serology, lacks specificity [22].
253 Even though efforts worldwide are made to improve the availability of diagnostic tests,
254 there is still a substantial unmatched need [13, 23]. In places with molecular testing
255 restrictions and/or ongoing development programs of laboratory capacity expansion, the
256 application of this simple score might improve diagnostic and guide infection control
257 policies.

258 The present study also analyzed the interest of multiple site testing through MPXV RT-
259 PCR performed on skin lesions, pharyngeal and anal swabs. A MPXV RT-PCR performed

on skin lesions is universally regarded as the main screening modality for MPX, and whether skin eruption is absent, a throat swab should be considered [9, 10, 11, 22]. However, in the current study, 8% of the confirmed MPX cases would have been missed by the performance of only skin lesions RT-PCR. Furthermore, RT-PCR performed on anal swabs had the best sensitivity in this study. Finally, a recent study found three asymptomatic MPXV carriers through the examination of anal and oropharyngeal samples of MSM and patients living with HIV followed up at a Belgian sexual health clinic [24]. Altogether, routinely molecular tests for MPXV carried out on multiple sites swabs may increase diagnostic accuracy.

Strengths and study limitations

One of the strengths of this study is the rigorous data collection of the examined patients, and also the case-control design, allowing a comparison of the two groups. To the best of our knowledge, this is the first case series investigating discriminants for confirmed MPX cases among patients screened for this condition and proposing a diagnostic score. Finally, the results of the present study enforce the evidence-based diagnostic approach to MPX.

This study has some limitations. First, due to the retrospective design of the current study, some confounding factors may have influenced our results. Sampling bias might have affected the study results as only patients referred to our center for screening were included. Patients with atypical presentation of MPX might be underrepresented as they might be infrequently sent for testing. Furthermore, type II error might have altered the results of this study, even though efforts were made to limit false negative MPXV RT-PCR. Among

patients in the control group with negative MPXV RT-PCR, no alternative diagnosis was found in 25% of the patients. While the missing data within the patient's characteristics: "having sexual intercourse with a positive case in the last 3 weeks", "frequentation of LGBTQ clubs in the last 3 weeks", and "lesions in the mouth" were between 15 and 20%. The missing data within the other parameters were limited. They were not computer generated, as no further analyses were performed with the three variables mentioned above and their interpretation was cautious.

Generalisability

The study setting is the current outbreak of MPXV in Europe. The clinical application of the suggested diagnostic score should be cautiously interpreted in a setting with different epidemiology. Prospective large studies are required to validate this study's results before clinical application.

Conclusion

In the present study, MPX confirmed diagnosis was associated with being MSM, living with HIV, having multiple sexual partners, and anogenital skin eruption at presentation. A simple score comprising these four predictors could improve MPX case detection among screened patients. The clinical application of the study results might strengthen the diagnostic approach to MPX, especially in setting with limited or no access to molecular testing, and guide infection control policies. Nonetheless, future trials should confirm study results before their implementation.

Declarations

Ethical approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was conducted in accordance with the study protocol, the Declaration of Helsinki and applicable regulatory requirements. The local Institutional Review Board and Ethics Committee of the CHU Saint Pierre approved the protocol (Ethics Committee aggregation number: O.M.007). In view of the retrospective nature of the study, which did not demand a deviation from standard clinical care, and the fact that all data was anonymized, informed consent from the patient or the next of kin was not essential.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All other authors declare that they have no competing interests in relation to the contents published in this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

MM: concept, study design, data analysis and interpretation, writing and revision, BH: study design, data collection, writing and revision, NY: data collection, writing and revision, SK: data collection, writing and revision, AL: writing and revision, SQ: data collection, writing and revision, CM: writing and revision. All authors have given final approval of the version to be submitted

Acknowledgements

We would like to express gratitude for the logistic support offered by the infectious diseases coordinating study team of CHU Saint Pierre and the study nurses.

References

[1] E. M. Bunge, B. Hoet, L. Chen, F. Lienert, H. Weidenthaler, L. R. Baer *et al.* The changing epidemiology of human monkeypox-A potential threat? A systematic review. *PLoS Negl Trop Dis.* 2022 Feb 11;16(2):e0010141. doi: 10.1371/journal.pntd.0010141

[2] N. Sklenovská, M. Van Ranst. Emergence of Monkeypox as the Most Important Orthopoxvirus Infection in Humans City. *Front Public Health*. 2018 Sep 4;6:241. doi: 10.3389/fpubh.2018.00241

[3] E. M. Beer, V. B. Rao. A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. *PLoS Negl Trop Dis*. 2019 Oct 16;13(10):e0007791. doi: 10.1371/journal.pntd.0007791

[4] P.-Y. Nguyen, W. S. Ajisegiri, V. Costantino, A. A. Chughtai, C. R. MacIntyre. Reemergence of Human Monkeypox and Declining Population Immunity in the Context of Urbanization, Nigeria, 2017-2020 *Emerg Infect Dis*. 2021 Apr;27(4):1007-1014. doi: 10.3201/eid2704.203569

[5] A. Adalja, T. Inglesby. A Novel International Monkeypox Outbreak. *Ann Intern Med*. 2022 May 24. doi: 10.7326/M22-1581

[6] J. P. Thornhill, S. Barkati, S. Walmsley, J. Rockstroh, A. Antinori, L. B. Harrison *et al*. Monkeypox Virus Infection in Humans across 16 Countries - April-June 2022. *N Engl J Med*. 2022 Jul 21. doi: 10.1056/NEJMoa2207323.

[7] J. Isidro, V. Borges, M. Pinto, D. Sobral, J. D. Santos, A. Nunes *et al*. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nat Med*. 2022 Aug;28(8):1569-1572. doi: 10.1038/s41591-022-01907-y.

[8] S. E. F. Yong, O. T. Ng, Z. J. M. Ho, T. M. Mak, K. Marimuthu, S. Vasoo *et al*. Imported Monkeypox, Singapore. *Emerg Infect Dis*. 2020 Aug;26(8):1826-1830. doi: 10.3201/eid2608.191387

[9] J. I. Martínez, E. G. Montalbán, S. J. Bueno, F. M. Martínez, A. N. Juliá, J. S. Díaz *et al*. Monkeypox outbreak predominantly affecting men who have sex with men, Madrid, Spain, 26 April to 16 June 2022. *Euro Surveill*. 2022 Jul;27(27):2200471. doi: 10.2807/1560-7917.ES.2022.27.27.2200471

[10] A. Peiró-Mestres, I. Fuertes, D, Camprubí-Ferrer, M. Á. Marcos, A. Vilella, M. Navarro *et al*. Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022. *Euro Surveill*. 2022 Jul;27(28):2200503. doi: 10.2807/1560-7917.ES.2022.27.28.2200503.

[11] UK Health Security Agency Guidance (Last updated 5 December 2022). Accessed 6st December 2022. https://www.gov.uk/guidance/monkeypox-diagnostic-testing

[12] J. O'Shea, T. D. Filardo, S. B. Morris, J. Weiser, B. Petersen, J. T. Brooks. Interim Guidance for Prevention and Treatment of Monkeypox in Persons with HIV Infection - United States, August 2022. *Centers for Disease Control and Prevention - Morbidity and Mortality Weekly Report*. 2022 Aug; 71(32);1023-1028.

[13] C. Boodman, D. L. Heymann, R. W. Peeling. Inadequate diagnostic capacity for monkeypox-sleeping through the alarm again. *Lancet Infect Dis*. 2022 Nov 16;S1473-3099(22)00744-7. doi: 10.1016/S1473-3099(22)00744-7.

[14] E. von Elm, D. G. Altman, M. Egger, S. J. Pocock, P. C. Gøtzsche, J. P. Vandenbroucke. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008 Apr;61(4):344-9. doi: 10.1016/j.jclinepi.2007.11.008.

[15] Y. Li, V. A. Olson, T. Laue, M. T. Laker, I. K. Damon. Detection of monkeypox virus with real-time PCR assays. *J Clin Virol*. 2006 Jul;36(3):194-203. doi: 10.1016/j.jcv.2006.03.012.

[16] Belgian national epidemiological reference database "Sciensano". Accessed 1st August 2022. https://www.sciensano.be/en/health-topics/monkeypox.

[17] F. Faul, E. Erdfelder, A. G. Lang, A. Buchner. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007 May;39(2):175-91. doi: 10.3758/bf03193146.

[18] K. Kupferschmidt. Why monkeypox is mostly hitting men who have sex with men. *Science*. 2022 Jun 24;376(6600):1364-1365. doi: 10.1126/science.add5966.

[19] Y. Ortiz-Martínez, G. Zambrano-Sanchez, A. J. Rodríguez-Morales. Monkeypox and HIV/AIDS: When the outbreak faces the epidemic. *Int J STD AIDS*. 2022 Jul 13;9564624221114191. doi: 10.1177/09564624221114191.

[20] D. de Sousa, J. Patrocínio, J. Frade, C. Correia, J. Borges-Costa, P. Filipe. Human monkeypox coinfection with acute HIV: an exuberant presentation. *Int J STD AIDS*. 2022 Jul 13;9564624221114998. doi: 10.1177/09564624221114998.

[21] N. L. Bragazzi, J. D. Kong, N. Mahroum, C. Tsigalou, R. Khamisy-Farah, M. Converti *et al*. Epidemiological trends and clinical features of the ongoing monkeypox epidemic: A preliminary pooled data analysis and literature review. *J Med Virol*. 2022 Jun 12. doi: 10.1002/jmv.27931.

[22] World Health Organisation, Monkeypox (Last updated 19 May 2022). Accessed 6st December 2022. https://www.who.int/news-room/fact-sheets/detail/monkeypox.

[23] C. C. Tao, X-J. Lim, A. A. Nordin, C. C. Thum, S. Sararaks, K. Periasamy *et al*. Health system preparedness in infectious diseases: perspective of Malaysia, a middle-income country, in the face of monkeypox outbreaks. *Trop Med Health*. 2022 Nov 21;50(1):87. doi: 10.1186/s41182-022-00479-4.

[24] I. De Baetselier, C. Van Dijck, C. Kenyon, J. Coppens, J. Michiels, T. de Block *et al*. Retrospective detection of asymptomatic monkeypox virus infections among male

sexual health clinic attendees in Belgium. *Nat Med*. 2022 Aug 12. doi: 10.1038/s41591-022-02004-w.

Figure

Figure legend

Figure 1: Study flowchart; MPX: monkeypox; RT-PCR: real-time polymerase chain reaction

Figure 2: Diagnostic accuracy of the four regression analysis discriminants for prediction of MPX diagnosis; on the left, ROC curve of the four discriminants found by multiple regression analysis (being MSM, HIV infected, having multiple sexual partners in the last 3 weeks, and having anogenital lesions) for prediction of MPX diagnosis; on the right, diagnostic accuracy measures of them, the cut-off of ≥2 predictors was found by ROC curve; AUC: area under the curve; MPX: monkeypox; MSM: men who have sex with men.

Table

Table legend

Table 1 - Baseline characteristics of the study population; data are expressed as median and interquartile range for continuous variable and numbers and proportions for categorical variables; * : missing values are between 15 and 20%; LGBTQ: lesbian, gay, bisexual,

452 transgender, and questioning; MPX: monkeypox; MSM: men who have sex with men;
453 PREP: pre-exposure prophylaxis.
454
455 Table 2 - Multivariable logistic regression analysis for the prediction of confirmed MPX
456 cases; Adjusted oddsratio are presented in the table; MPX: monkeypox; MSM: who have
457 sex with men; '-' is used for 'not applicable'.