Received: 15 November 2022 | Accepted: 6 February 2023

DOI: 10.1002/jmv.28560

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Monkeypox outbreak in Genoa, Italy: Clinical, laboratory, histopathologic features, management, and outcome of the infected patients

Giulia Ciccarese[1] | Antonio Di Biagio[2,3] | Bianca Bruzzone[4] | Antonio Guadagno[5] | Lucia Taramasso[2] | Giorgio Oddenino[6] | Giorgia Brucci[2,3] | Laura Labate[2,3] | Vanessa De Pace[4] | Mario Mastrolonardo[1] | Francesco Broccolo[7] | Giacomo Robello[6] | Francesco Drago[6] | Matteo Bassetti[2,3] | Aurora Parodi[6]

[1]Department of Medical and Surgical Sciences, Unit of Dermatology, University of Foggia, Foggia, Italy

[2]Department of Specialist Medicine, Infectious Disease Clinic, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

[3]Department of Health Sciences, Division of Infectious Diseases, University of Genoa, Genoa, Italy

[4]Department of Health Sciences, Hygiene Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

[5]Pathology Unit, Ospedale Policlinico San Martino, University of Genoa, Genoa, Italy

[6]Department of Health Sciences, Section of Dermatology, DISSAL, University of Genoa, Ospedale-Policlinico San Martino, IRCCS, Genova, Italy

[7]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy

Correspondence
Giulia Ciccarese, Department of Medical and Surgical Sciences, Unit of Dermatology, University of Foggia, Viale Pinto 1, Foggia 71122, Italy.
Email: giulia.ciccarese@unifg.it

Abstract

Since May 2022, multiple human Monkeypox cases were identified in nonendemic countries, mainly among men who have sex with men. We aimed to report the features, clinical course, management, and outcome of the Monkeypox cases diagnosed in the Dermatology and Infectious Disease Units of the San Martino Hospital, Genoa, Italy. We performed an observational study of the Monkeypox cases diagnosed from July 1 until August 31, 2022, collecting clinical, laboratory, and histological data. We studied 16 Monkeypox-infected men (14 homosexual, 2 bisexual) with a median age of 37 years. Three were HIV-infected. All patients reported multiple sexual partners and/or unprotected sex in the 2 weeks before the diagnosis. Most patients had prodromal signs/symptoms before the appearance of the skin/mucosal eruption, consisting of erythematous papules/vesicles/pustules in the anogenital area, which tended to erode evolving into crusts and ulcers. Lesions were often associated with local and/or systemic symptoms. Histopathology showed overlapping features in all cases: epidermal ulceration and dermal inflammatory infiltrate consisting of lymphocytes and neutrophils with an interstitial and perivascular/peri-adnexal pattern and endothelial swelling. Concomitant sexually transmitted infections (STIs) (gonococcal/nongonococcal proctitis and anal high-risk human papillomavirus [HR-HPV] infection) were frequent. Four patients were hospitalized, and one received specific treatment. The overall outcome was good. At the follow-up visit, three patients presented skin scars. Our series confirms the features of the current Monkeypox outbreak; however, different from other studies, we found a considerable rate of concomitant STIs, such as anal HR-HPV

infection, that should be kept in mind because this persistent infection is the main cause of anal cancers.

KEYWORDS

erythematous papules, high-risk human papillomavirus infection, human Monkeypox infection, pustules, sexually transmitted infections, superficial ulcers of the anogenital site, vesicles

1 | INTRODUCTION

Monkeypox (MPX) is a DNA virus belonging to the *Poxviridae* family that was first identified in captive monkeys in 1958. A major concern for zoonotic pathogens is the evolution toward more transmissible or virulent forms in humans. Indeed, it is traditionally transmitted from animals (squirrels, rats, nonhuman primates, and other species) to humans, causing symptoms similar to those previously observed in smallpox patients, although less severe.[1] The first identification of MPX as a human pathogen occurred in 1970 in the Democratic Republic of the Congo. Human-to-human transmission can also occur through close contact with infectious material from skin lesions of an infected person, respiratory droplets in prolonged face-to-face contact, and fomites. Since the first human MPX case in 1970, many other human cases have been reported from rural, rainforest regions of central-west Africa. In 2003, the first MPX outbreak outside of Africa was reported in the United States and other sporadic cases have been registered in European and Extra-European countries in 2018, 2019, and 2021.[1,2]

Since early May 2022, multiple human MPX cases were identified in nonendemic countries. This new epidemic has surged mainly among men who have sex with men (MSM), rapidly reaching the proportions of a global health emergency during the summer 2022.[3,4] The European Centre for Disease Prevention and Control (ECDC) reported that the West African clade of MPX, which has been detected in Europe so far, has a mortality rate of 3.6% (estimated from studies performed in endemic African countries).[5]

New MPX cases are declining worldwide by the end of August 2022, a trend that can be traced back to the decrease in social activities after the summer holidays, a broader understanding of how MPX spreads, and the introduction of new MPX vaccination.[3] The World Health Organization (WHO) recommends MPX preventive vaccination for persons at high risk of exposure: MSM or persons who in the past 6 months have had more than one sex partner and/or a new diagnosis of one or more sexually transmitted infections (STIs), sex workers, health workers at risk (laboratory personnel working with Orthopoxviruses).[6]

While the clinical features of MPX infection have been largely described, the dermoscopic and histological features of MPX skin lesions have been poorly investigated, and data on the management of severe infections, the possibility of long-term sequelae, and cutaneous scarring over time are scant.[7–9]

The present study aimed to report the clinical, dermoscopic, and histopathological features of the MPX cases diagnosed from July to the end of August at the San Martino Hospital, Genoa, Italy, and to describe the clinical course, management, and outcome of the patients.

2 | MATERIALS AND METHODS

We performed an observational study of the MPX cases diagnosed from July 1 until August 31, 2022, in the Dermatology Unit and Infectious Disease Unit of the San Martino Hospital, Genoa, Italy. The inclusion criteria were a laboratory-confirmed case of MPX infection, defined by a positive result on polymerase chain reaction (PCR) assay in a specimen from any anatomical site, and having performed a follow-up (dermatologic or infectious disease) visit at least 1 month after the diagnosis to assess the clinical outcome. MPX patients have been invited to contribute to the case series by their dermatologist/infectious disease specialist. Written informed consent was obtained for recording clinical, laboratory data, clinical/dermoscopic images, and to perform skin biopsies. All procedures were performed following the Declaration of Helsinki for research involving human subjects.

The swabs taken from skin/mucosal lesions, oropharynx, and/or rectum were analyzed at the Hygiene Unit (San Martino Hospital). The same analysis was performed on the blood of the consenting patients. MPX DNA was extracted from the clinical samples using the MagCore® Automated Nucleic Acid Extraction workstation (MagCore system) with the MagCore® Viral Nucleic Acid Extraction Kit (RBC Bioscience Corp.). A homemade real-time PCR using MPX-specific primers and probe was carried out for generic MPX virus DNA detection, as previously described.[10] Positive samples were confirmed using the Real-Star Orthopoxvirus PCR kit (Altona Diagnostics GmbH). The automated rapid molecular assay has also been performed to detect specifically MPX West African and MPX Congo Basin strains. STANDARD M10 MPX/OPX is a research use only multiplex real-time PCR test, which can be used both as first-level diagnostic screening and for the differential diagnosis of MPX virus strains from other Orthopoxvirus in positive specimens.

The following clinical data of the MPX patients were collected: sex, age, nationality, sexual orientation, unprotected sexual practices, travel history, comorbidities, HIV status, use of pre-exposure prophylaxis (PrEP) against HIV, vaccination against smallpox, symptoms onset date, signs and symptoms at the onset of the disease and at diagnosis; the number of the skin/mucosal lesions (less than 10; between 10 and 20; more than 20), presentation and site of the

lesions, skin/mucosal associated symptoms, enanthem, systemic symptoms, concomitant STIs, hospitalization, and clinical outcome. Clinical, dermoscopic images, and punch skin biopsy (4 mm) for histological examination were performed on consenting patients.

3 | RESULTS

We collected data from 16 patients. Demographic and clinical features are summarized in Table 1.

The MPX patients were all men with a median age of 37 years (interquartile range 25–39); 14 persons self-identified as MSM, and 2 as bisexual men. Three patients (19%) had a history of foreign travel in the month before the disease onset. Possible sexual exposure to MPX was referred by all patients: 4 patients had sexual exposure to an individual known to have MPX in the week before the diagnosis, and the other 12 had risk factors for STIs, such as multiple sexual partners and/or unprotected sex in the 2 weeks before the onset of symptoms. Three were HIV-infected patients (19%): two had high adherence to the antiretroviral therapies, with HIV-RNA < 50 copies/mL and CD4+ T cell count >500/mm^3 at the time of MPX diagnosis; one received the HIV diagnosis at the same time of the MPX diagnosis (RNA load of 2.6 × 105 copies/mL, CD4+ T-cells count of 414/mm^3, CD4+/CD8+ ratio 0.3). This latter patient presented us with several diffuse skin lesions (papules and vesicles) and a deep ulcer of the columella, probably deriving from the coalescence of multiple ulcerated vesicles of the nose, associated with a diffuse superficial lymph node enlargement. We treated this patient for MPX infection with intravenous cidofovir (250 mg in a single dose), without adverse effects.[11]

Four patients used PrEP (daily or on-demand). Only one patient had a history of smallpox vaccination in his childhood (just one dose): he developed a mild disease without prodromal/systemic symptoms and recovered without sequelae. All but three patients reported prodromal symptoms (most frequently fever, asthenia, and lymphadenopathy) before the appearance of skin/mucosal lesions. The mean time from onset of symptoms and sample collection for assessment of diagnosis was 7 days (range 2–20 days) (Table 2). Cutaneous/mucosal manifestations at diagnosis were observed in 14 patients with a wide spectrum of presentation and evolution stages. Two patients had no skin/mucosal manifestations at the time of diagnosis: the first presented for the PrEP follow-up appointment complaining of anal pain and admitting risky sexual behaviors in the last 2 weeks (unprotected sex with multiple casual partners in the previous 2 weeks); his stable partner complained of sore throat and reported the same risky behaviors. Their oropharyngeal and anal swabs for MPX DNA resulted in all positive.

In the 14 patients with cutaneous/mucosal manifestations, the most observed lesions were vesicles and/or pustules on an erythematous background (Figure 1A,B) and erythematous papules which tended to erode in the center, sometimes developing a crust, and evolving into superficial ulcers (Figure 1C,D). One patient had also a faint urticarial eruption on his trunk, legs, and extremities (Figure 1E) which had appeared for 5 days, simultaneously with the episodes of fever, with spontaneous resolution and recurrent episodes. Such urticarial eruption, from which the patient had never suffered before, spontaneously regressed during hospitalization.

The most involved site was the anogenital area (87%), where most swabs were collected (Table 2). MPX DNA was searched also in the serum of seven consenting patients during the acute illness, with five positive findings (Table 2).

Regarding the number of skin/mucosal lesions, most patients (62%) had less than 10 lesions, 25% had 10–20 lesions, and 3% had more than 20 lesions. One patient had a single genital/anal ulcer which could easily be misdiagnosed with other STIs. Enanthem consisting of erythematous, often confluent, macules of the hard/soft palate was observed in seven patients (44%) (Figure 1F). Cutaneous/mucosal symptoms were associated with symptoms in eight patients (50%). More specifically, involvement of the anorectal skin-mucosa was associated in six patients (60%) with itch or anorectal pain. Systemic signs/symptoms at MPX diagnosis were observed in six patients (37%), mainly consisting of fever and inguinal/cervical lymphadenopathy. Concomitant acute/chronic STIs were diagnosed in 8 of the 14 patients that were tested (57%): gonococcal and nongonococcal proctitis (five cases) and anal high-risk human papillomavirus (HR-HPV) infection (five cases) were the most frequent. Anal HR-HPV infection was associated with normal anal cytology in four cases and with low-grade anal intraepithelial neoplasia (LG-AIN) in one case.

Hospitalization was required in four patients (25%) for the management of severe anorectal pain and treatment of soft tissue superinfection. Only one patient received MPX-specific treatment with cidofovir. The overall outcome was good in all patients; at the 1-month follow-up visit, three patients (19%) presented depressed pinkish/erythematous skin scars, two on the face (forehead and chin) and one on the penis (Figure 2A–C).

Depending on the lesion stage, dermoscopy of the skin lesions showed whitish structureless areas surrounded by a halo of perilesional erythema with a central yellowish surface (Figure 3A) or a reddish crust (Figure 3B).

Histopathology was available only for four patients who consented to undergo a skin biopsy on MPX skin lesions. Histology showed overlapping features in all cases: epidermal ulceration (Figure 4A) with necrosis of keratinocytes and neutrophils exocytosis (Figure 4B); dermal subacute inflammatory infiltrate consisting of lymphocytes and, mainly, neutrophils. The inflammatory infiltrate showed an interstitial and perivascular/peri-adnexal pattern with endothelial swelling (Figure 4C). Immunostaining demonstrated that the inflammatory infiltrate was predominantly composed of T CD3+ lymphocytes (Figure 5A) with an equal distribution of T CD4+ and CD8+ lymphocytes in the dermis (Figure 5B,C). Scattered B CD20+ lymphocytes were observed (Figure 5D).

TABLE 1 Demographic, clinical, and laboratory features of the studied patients.

Patient n°	Age	Nationality	Sexual orientation	foreign travel history	non infectious comorbidities	HIV status	Antiretroviral therapy	PrEP	Smallpox vaccine	Sexual contact with a confirmed MPX case	Prodromal signs/ symptoms	Skin/mucosal manifestation at diagnosis
1	27	Italian	homosexual	no	Crohn disease, atopic dermatitis	neg	no	yes	no	unknown	fever (38.5°C), myalgia, asthenia, headache, urticaria, mucorrhea, rectorrhagia, ano-rectal pain	erythematous vesicles on the hands, perianal superficial ulcers; urticarial lesions on the trunk and legs
2	37	Italian	homosexual	no	no	neg	no	no	no	unknown	asthenia, pharyngodynia, inguinal lymphade-nopathy	skin ulcer on the penis shaft, perianal superficial ulcers
3	33	Italian	homosexual	yes (Spain)	no	neg	no	yes	no	unknown	bilateral inguinal and cervical lymphade-nopathy	single ulcer on the shaft penis
4	39	Italian	homosexual	yes (United Kingdom)	no	neg	no	no	no	yes	asthenia, headache	perianal ulcerated papules, erythematous vesicles and papules on the trunk
5	29	Russian	homosexual	no	no	neg	no	no	no	yes	high fever (39°C), cervical lymphade-nopathy	ulcerated vesicles on the foreskin, confluent papules on the chin
6	25	Ecuadorian	homosexual	no	no	pos	bictegravir/ emtricita-bine/ tenofovir alafenamide	no	no	unknown	no	perianal erythematous vesicles; erythematous papules on the trunk

TABLE 1 (Continued)

Patient n°	Age	Nationality	Sexual orientation	foreign travel history	non infectious comorbidities	HIV status	Antiretroviral therapy	PrEP	Smallpox vaccine	Sexual contact with a confirmed MPX case	Prodromal signs/ symptoms	Skin/mucosal manifestation at diagnosis
7	33	Jamaican	homosexual	no	no	pos	dolutegravir/ lamivudine	no	no	unknown	fever	diffuse papulo-vesicles on the body surface
8	21	Ecuadorian	homosexual	no	no	neg	no	no	no	unknown	no	perianal superficial ulcers
9	67	Italian	bisexual	no	diverticulosis, hyper-cholesterolemia	neg	no	no	no	unknown	proctorrhagia	erythematous-crusted lesions on the arms, legs and perianal site
10	41	Ecuadorian	homosexual	no	epilepsy	neg	no	no	no	yes	anal pain	vesicles on the shaft and glans penis and on the perianal site
11	50	Italian	homosexual	no	no	neg	no	no	yes (1 dose)	unknown	no	vesicles on the shaft and glans penis
12	32	Italian	homosexual	no	no	neg	no	yes	no	unknown	anal pain	erythematous papule and vesicles on the entire body; perianal superficial ulcer
13	25	Brazilian	homosexual	no	no	pos	no	no	no	unknown	fever, diffuse lymphade-nopathy	vesicles diffuse on the entire body, nasal columella ulcer
14	38	Italian	bisexual	no	no	neg	no	no	no	unknown	fever, anal pain, tenesmus	vesico-pustules on the trunk
15	37	Italian	homosexual	yes (Indonesia, France)	Crohn disease	neg	no	yes	no	unknown	anal pain	no
16	24	Italian	homosexual	no	no	neg	no	no	no	yes	pharyngodynia	no

Patient n°	enanthem	n° of lesions	skin/mucosal symptoms	systemic signs/symptoms	concomitant STI	Hospital-ization	antiviral therapy for MPX	outcome
1	yes	10-20	itch, ano-rectal pain	low-grade fever (37.5°C), bilateral inguinal lymphadenopathy	gonococcal proctitis, anal HR-HPV (normal citology)	yes	no	resolution without scarring

(Continues)

TABLE 1 (Continued)

Patient n°	enanthem	n° of lesions	skin/mucosal symptoms	systemic signs/symptoms	concomitant STI	Hospital-ization	antiviral therapy for MPX	outcome
2	yes	<10	anal itch	bilateral inguinal and cervical lymphadenopathy	N. gonorrhoeae and U.urealyticum proctitis, anal HR-HPV (normal citology)	no	no	resolution without scarring
3	no	<10	no	bilateral inguinal lymphadenopathy	anal HR-HPV (normal citology)	no	no	pink macule in the site of the previous ulcer
4	yes	<10	no	no	U.urealyticum proctitis	no	no	resolution without scarring
5	no	10-20	genital pain	high fever (39°C)	no	yes	no	depressed rose-colored scar on the chin
6	no	<10	no	no	urethritis from U. urealyticum, M. hominis, M. genitalium	no	no	resolution without scarring
7	yes	>20	no	no	proctitis from N.gonorrhoeae, M.hominis, U.urealyticum; urethritis from M.genitalium, anal HR-HPV (normal citology)	no	no	resolution without scarring
8	yes	<10	itch	no	proctitis from C.trachomatis and M.genitalium, HR-HPV (citology with LG-AIN)	no	no	resolution without scarring
9	no	10-20	no	no	no	yes	no	resolution without scarring
10	no	<10	no	no	not tested	no	no	resolution without scarring
11	yes	<10	no	no	not tested	no	no	resolution without scarring
12	no	>20	perianal pain	no	no	no	no	depressed erythematous scar on the forehead
13	no	10-20	anal and nasal pain	fever	HIV, primary syphilis	yes	intravenous cidofovir 250 mg	resolution without scarring
14	no	<10	no	fever	no	no	no	resolution without scarring
15	no	0	anal pain	no	no	no	no	resolution without scarring
16	yes	0	pharyngodynia	no	no	no	no	resolution without scarring

Abbreviations: HR-HPV, high-risk human papillomavirus; MPX, Monkeypox; STI, sexually transmitted infections.

TABLE 2 Features of the clinical samples collected from the studied patients.

Patient no.	Date of sample collection	Days from symptoms/signs onset	Samples	Results
1	July 8, 2022	5	Skin lesion swab	Pos
			Pharyngeal swab	Pos
			Anal/perianal swab	Pos
			Serum	Pos
2	July 11, 2022	7	Skin lesion swab	Pos
			Pharyngeal swab	Pos
			Anal/perianal swab	Pos
3	July 13, 2022	20	Skin lesion swab	Pos
			Pharyngeal swab	Neg
4	July 15, 2022	13	Skin lesion swab	Pos
			Anal/perianal swab	Pos
			Pharyngeal swab	Neg
5	July 12, 2022	3	Skin lesion swab	Pos
			Serum	Pos
6	July 15, 2022	7	Skin lesion swab	Pos
			Anal/perianal swab	Pos
			Serum	Pos
7	July 29, 2022	5	Skin lesion swab	Pos
			Pharyngeal swab	Pos
			Serum	Pos
8	August 6, 2022	7	Anal/perianal swab	Pos
			Pharyngeal swab	Neg
9	August 1, 2022	2	Skin lesion swab	Pos
			Anal/perianal swab	Pos
			Serum	Neg
10	August 5, 2022	4	Skin lesion swab	Pos
			Pharyngeal swab	Neg
11	August 3, 2022	17	Skin lesion swab	Pos
			Pharyngeal swab	Pos
12	August 5, 2022	8	Skin lesion swab	Pos
			Anal/perianal swab	Pos
			Pharyngeal swab	Pos
			Serum	Pos
13	August 12, 2022	2	Skin lesion swab	Pos
			Anal/perianal swab	Pos
			Pharyngeal swab	Pos
			Serum	Neg
14	August 17, 2022	6	Skin lesion swab	Pos
			Pharyngeal swab	Neg
15	August 31, 2022	7	Anal/perianal swab	Pos
			Pharyngeal swab	Pos
16	August 31, 2022	3	Pharyngeal swab	Pos

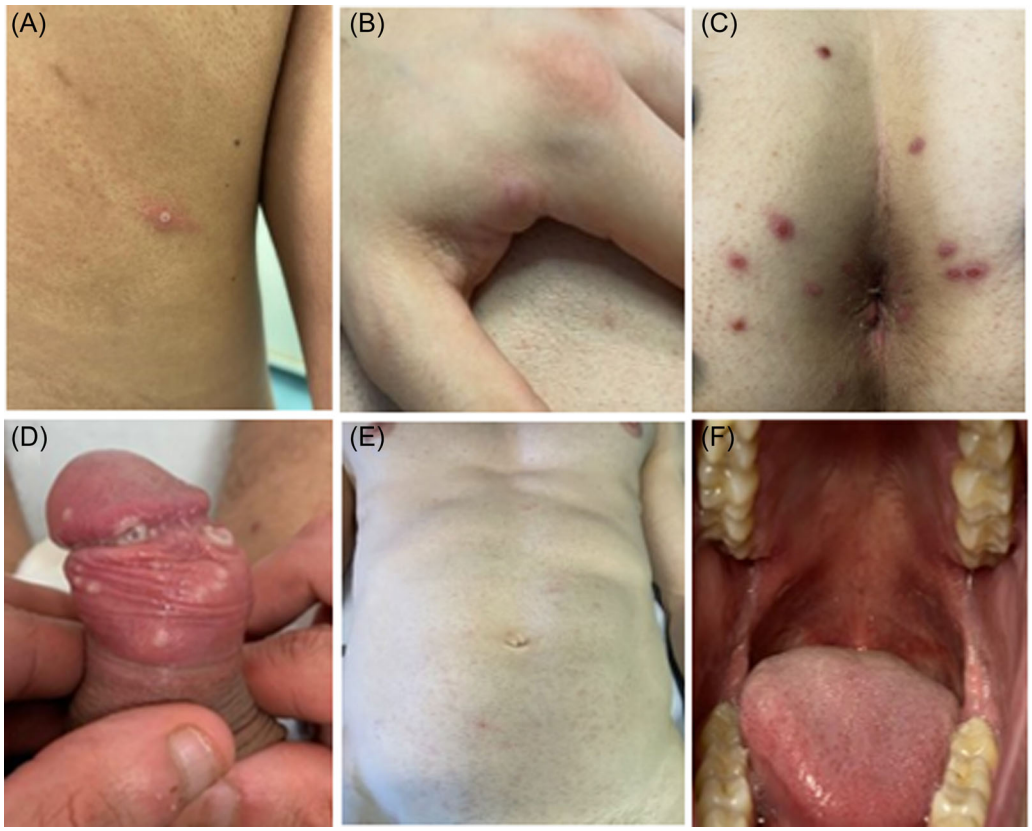

FIGURE 1 Vesicles and/or pustules on an erythematous background (A, B); erythematous papules eroding in the center (C), sometimes developing a crust, and evolving into superficial ulcers (D); faint urticarial eruption on trunk (E); erythema of the hard and soft palate (F).

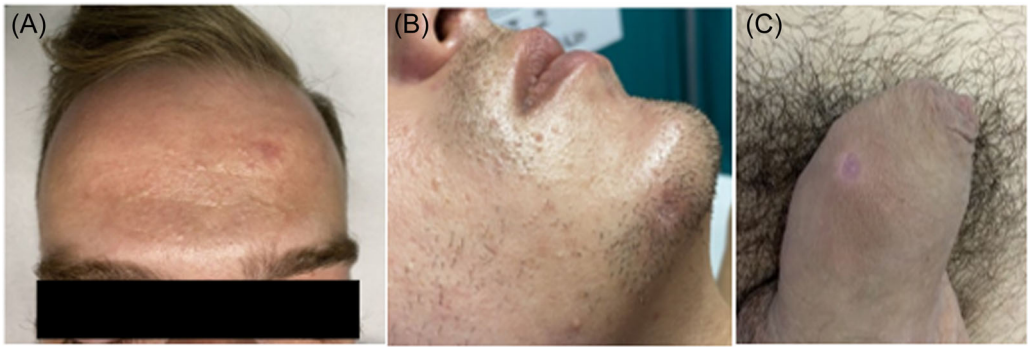

FIGURE 2 Depressed pinkish/erythematous skin scars, on the face (A, B) and one on the penis (C).

4 | DISCUSSION

Overall, the demographic and clinical features in our case series are in line with those observed in other studies on larger populations[7,12,13]: 100% of our patients were MSM or bisexual men, mainly Caucasians, and 19% were living with HIV. The spectrum of the skin/mucosal manifestation was wide: most patients had less than 10 lesions, especially in the anogenital area, in multiple stages of development and almost half of the patients had oropharyngeal involvement.[7,12,13] However, unlike other studies which often described cutaneous manifestations as the first and unique signs of MPX infection,[7,12] in our series, most of the patients (81%) reported prodromal signs/symptoms (fever, asthenia, and lymphadenopathies) before the skin eruption.

As previously reported,[7] the clinical presentation of MPX was similar between persons with HIV infection who were regularly taking antiretroviral therapy and those without HIV. Only one of the HIV-infected patients of our series was not taking antiretroviral therapy at MPX onset, because his HIV diagnosis was discovered simultaneously with MPX infection. Since people living with untreated HIV infection have been previously identified at higher risk of severe MPX,[14] this was the only patient that we treated for MPX infection.

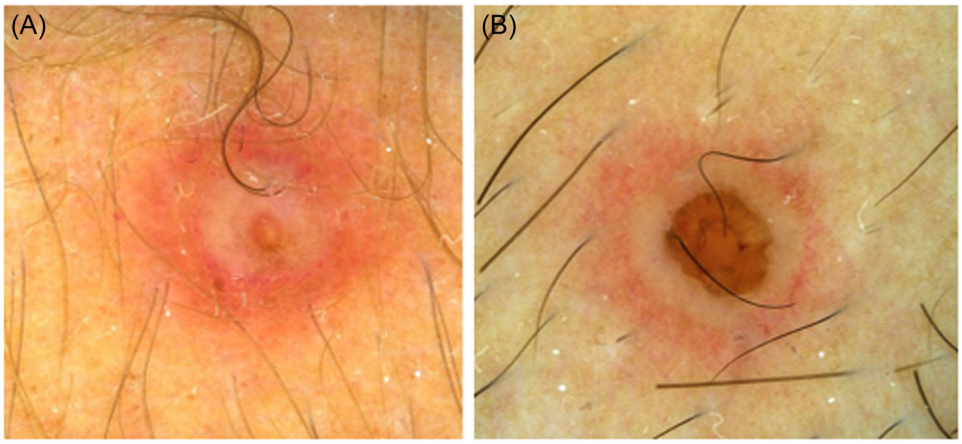

FIGURE 3 Dermoscopy showed whitish structureless areas surrounded by a halo of perilesional erythema with a central yellowish area (A) or reddish crust (B).

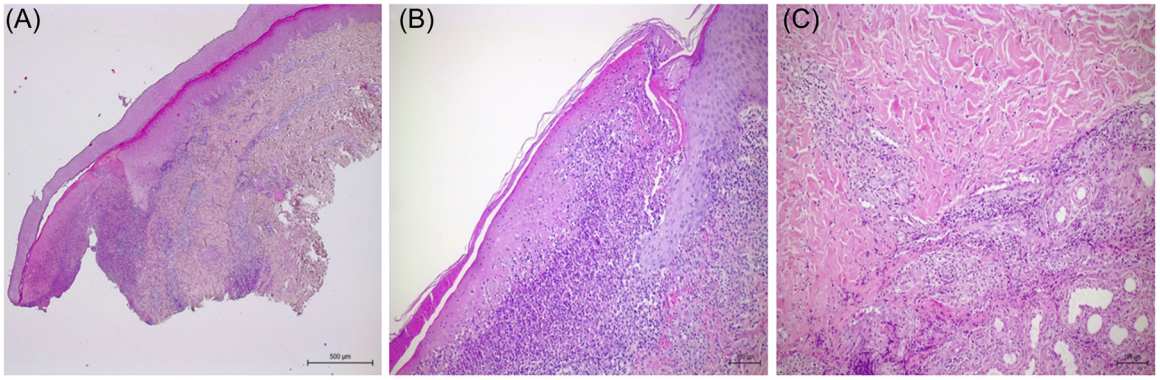

FIGURE 4 (A) Hematoxylin and eosin (H&E) stain, original magnification ×2.5: histopathology shows epidermal ulceration; (B) H&E stain, original magnification ×10: necrosis of keratinocytes and neutrophils exocytosis; (C) H&E stain, original magnification ×10: subacute dermal mixed inflammatory infiltrate consisting of lymphocytes and especially neutrophils with an interstitial and perivascular/periadnexal pattern and endothelial swelling.

Interestingly, one of our patients had an atypical cutaneous manifestation: in addition to erythematous vesicles and perianal superficial ulcers, he developed a faint urticarial eruption on the trunk and legs. To the best of our knowledge, such urticaria has never been described in association with MPX infection.

The rate of concomitant laboratory-confirmed STIs found in our series (57% of the tested patients) was higher than that described by other authors (29% in the work by Thornhill et al.[7] and 25% in the work by Orviz et al.[12]). The high number of simultaneous gonorrhea (especially gonococcal proctitis) confirmed the previous studies; however, we also found a not negligible fraction of patients (36%) with anal HR-HPV infection, associated with LG-AIN in one case. HPV as a concomitant STI with MPX was never described before but it should be kept in mind by healthcare providers because HPV is the most common STI and the prevalence of anal HPV infection is very high in MSM, especially in those living with HIV.[15–17] Indeed, anal HR-HPV testing, complementary to liquid-based anal cytology, can improve the diagnostic accuracy of screening for anal cancer.[18]

In our experience, also the number of hospitalized patients was higher compared to other case series[7,12]: the main reasons for hospitalization were the management of severe pain and the need for strict isolation. However, none of our patients were admitted to an intensive care unit nor developed serious complications, like myocarditis or encephalitis, that were rarely described in other recent studies on MPX outbreaks.[7,13] Even if the outcome was favorable for all patients, clinical resolution occurred with just one atrophic skin scarring in 19% of them, in line with another recent Italian study.[19] Dermoscopy, histopathology, and immunohistochemistry of the skin lesions in our series were comparable to the few data reported in other studies.[19,20] However, eosinophilic intracytoplasmic inclusion bodies referable to viral cytopathic changes of keratinocytes (spherical Guarnieri bodies), recently described[20] in agreement with data from the previous MPX outbreaks,[21,22] were not observed in our cases.

The clinical and social features observed in our study and similar reports on the 2022 MPX outbreak in non-African

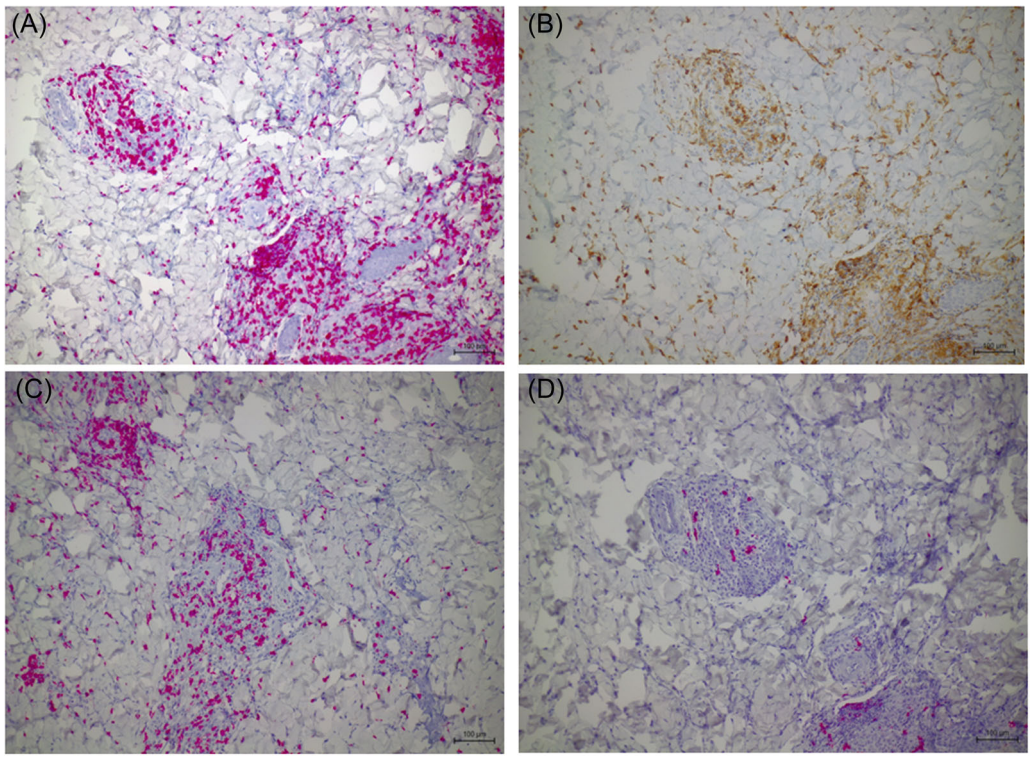

FIGURE 5 Immunostaining: (A) immunostaining for CD3 (clone LGV6); (B) immunostaining for CD4 (clone SP35); (C) immunostaining for CD8 (clone SP57); (D) immunostaining for CD20 (clone L26).

countries[7,12,13,19,20] have several differences from those of the "old" human MPX outbreaks that occurred in Africa in the last two to -three decades.[23–25]

During the 2017–2018 human MPX outbreak in Nigeria, most of the patients had genital rash and lymphadenopathies, more than half developed severe complications (sepsis, bronchopneumonia, encephalitis) and five patients died; moreover, the HIV-infected patients (most of which had failed or did not take antiretroviral therapy) had more prolonged illness and superinfections compared to HIV negative patients; sequelae included atrophic scars, hypertrophic scars, patchy alopecia, and deformity of facial muscles after healing of ulcerated facial lesions.[23]

Two older studies from Congo and Zaire reported that the MPX skin lesions (in number varying from a few to thousands) evolved all together through stages of macules, papules, vesicles, and pustules, before umbilication and desquamating,[24,25] unlike in the current MPX outbreak.

Regarding the previous smallpox vaccination, in our case series, only one 50-year-old patient had received a single dose of such vaccine in his childhood. This patient developed a mild disease without cutaneous/mucosal nor systemic symptoms and recovered without sequelae. Although some authors hypothesized that previous smallpox vaccination may confer a cross-immunity against MPX which may produce a milder disease compared to nonvaccinated persons,[26] the effectiveness of the smallpox vaccination in the current MPX outbreak remains to be assessed.[13] Indeed, most of our patients, as well as the others described in other studies, developed a mild disease, regardless of whether they have carried out smallpox vaccination in childhood or not.[7,13] In smallpox-vaccinated patients, especially in older subjects, immunity against smallpox (and, therefore, the cross-immunity against MPX) can undergo a progressive weakening with time.[7,26]

The current outbreak of human MPX infection suggests that some biological aspects of the virus may have changed. Even if Poxviruses had a lower mutation rate than RNA viruses, they have a large and flexible genome, allowing structural changes that result in gene loss or gene gain, modification of the viral phenotypes in addition to, we can suppose, the clinical features of the infection.[27] According to genome analysis, MPX is conventionally classified into two clades: the first was isolated from West Africa and has experienced a limited drift; the second is endemic to Central Africa and causes more severe and transmissible disease. A recent clade (MPX clade 3) has been described during the 2017–2019 outbreaks occurring outside Africa. Therefore, the West African strain category includes both clades 2 and 3. Phylogenomic analysis from the ongoing MPX outbreak confirms that the strain that is currently circulating descends from the clade 3 and thereby, the West African strain, as confirmed also by our molecular data of these clinical series. This viral phenotype shows a high number of mutations that raises concern about the increased capacity of adapting to humans and transmitting more easily.[28]

The reason why the current outbreak of MPX is mostly hitting MSM is not completely clear and communication about this aspect is arduous because of the risk of stigmatizing MSM. Among

US monkeypox cases with available data, 99% occurred in men, 94% of whom reported recent male-to-male sexual or close intimate contact.[29] The majority of cases continue to be detected in males between 18 and 50 years (87%), and primarily among MSM. Summer mass gatherings and specific sexual practices have facilitated the transmission of MPX among MSM groups until now.[30] Kupferschmidt reported that MSM have generally better relationships than heterosexual men with physicians, and are more inclined to get tested for STIs, allowing easier and faster diagnosis, therapies, and disease notifications.[31] Probably, not enough research on MPX infection has been performed in the heterosexual community. However, if many people outside the MSM community had MPX, more of them would have been highlighted in the statistics by now. MPX could have found a niche in the tightly connected sexual networks of the MSM community, spreading in ways that are more difficult to cross in the general population. Changing partners frequently and having several partners simultaneously can occur in all sexual networks, but if it happens within a *core* group of people, the virus transmission is facilitated.[31]

Sexual intercourse, even if not complete, clearly plays a role in virus transmission. MPX viral DNA has been found in the semen of some patients[8] but it is not sure that it is important for the contagion; skin-to-skin contact (not only in genito-anal sites), as happens for other STIs like herpes and scabies, could be enough. Skin-to-skin contact has also been considered responsible for MPX transmission from mother to child.[32]

In conclusion, our series confirms the main clinical and demographic features of the current MPX outbreak reported in the literature; however, differently from other studies, we described an atypical cutaneous manifestation consisting of recurrent episodes of urticarial skin eruption; moreover, among the concomitant STIs in MPX patients, we found a considerable rate of anal HR-HPV infection, that should be kept in mind because HR-HPV persistent infections are the main cause of anal cancers. HIV-positive patients have a significantly increased risk for anal cancer, even if the incidence of such cancer has recently increased also in the general population.

All healthcare providers, not only those serving STI patients, should receive specific training to recognize and manage MPX infection and to recommend vaccination for the prevention of MPX disease in vulnerable populations.

AUTHOR CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: *Study conception and design*: Giulia Ciccarese and Antonio Di Biagio. *Data collection*: Giulia Ciccarese, Antonio Di Biagio, Giorgia Brucci, Laura Labate, Lucia Taramasso, Matteo Bassetti, Giorgio Oddenino, and Giacomo Robello. *Analysis and interpretation of results*: Bianca Bruzzone, Vanessa De Pace, Antonio Guadagno, Giacomo Robello, Francesco Drago, Mario Mastrolonardo, Francesco Broccolo, and Aurora Parodi. *Draft manuscript preparation*: Giulia Ciccarese and Lucia Taramasso. All authors reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGEMENT

Open Access Funding provided by Universita degli Studi di Foggia within the CRUI-CARE Agreement.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on reasonable request.

ORCID

Antonio Di Biagio http://orcid.org/0000-0003-1436-5089
Lucia Taramasso http://orcid.org/0000-0002-6622-6358
Francesco Broccolo http://orcid.org/0000-0002-9737-0459

REFERENCES

1. World Health Organization. Monkeypox. Accessed October 23, 2022. https://www.who.int/news-room/fact-sheets/detail/monkeypox
2. Bunge EM, Hoet B, Chen L, et al. The changing epidemiology of human monkeypox—a potential threat? A systematic review. *PLoS Neglected Trop Dis*. 2022;16(2):e0010141. doi:10.1371/journal.pntd.0010141
3. European Centre for Disease Prevention and Control. Monkeypox situation update, as of 20 September 2022. Accessed October 23, 2022. https://www.ecdc.europa.eu/en/news-events/monkeypox-situation-update
4. Ministero della Salute. Malattie infettive. Vaiolo delle scimmie. Accessed October 23, 2022. https://www.salute.gov.it/portale/malattieInfettive/dettaglioSchedeMalattieInfettive.jsp?lingua=italiano%26id=254%26area=Malattie%20infettive%26menu=indiceAZ%26tab=1
5. Dhawan M, Priyanka, Choudhary OP. Emergence of monkeypox: risk assessment and containment measures. *Travel Med Infect Dis*. 2022;49:102392. doi:10.1016/j.tmaid.2022.102392
6. World Health Organization. Vaccines and immunization for monkeypox: interim guidance, 24 August 2022. Accessed October 23, 2022. https://www.who.int/publications/i/item/WHO-MPX-Immunization-2022.2-eng
7. Thornhill JP, Barkati S, Walmsley S, et al. Monkeypox virus infection in humans across 16 countries—April-June 2022. *N Engl J Med*. 2022;387(8):679-691. doi:10.1056/NEJMoa2207323
8. Raccagni AR, Candela C, Mileto D, et al. Monkeypox infection among men who have sex with men: PCR testing on seminal fluids. *J Infect*. 2022;85(5):573-607. doi:10.1016/j.jinf.2022.07.022.
9. Moschese D, Pozza G, Mileto D, et al. Isolation of viable monkeypox virus from anal and urethral swabs, Italy, May to July 2022. *Euro Surveill*. 2022;27(36):2200675. doi:10.2807/1560-7917.ES.2022.27.36.2200675
10. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods*. 2010;169(1):223-227. doi:10.1016/j.jviromet.2010.07.012
11. Labate L, Brucci G, Ciccarese G, et al. Nasal monkeypox virus infection successfully treated with cidofovir in a patient newly diagnosed with HIV. *Int J STD & AIDS*. 2022:9564624221141152. doi:10.1177/09564624221141152
12. Orviz E, Negredo A, Ayerdi O, et al. Monkeypox outbreak in Madrid (Spain): clinical and virological aspects. *J Infect*. 2022;85(4):412-417. doi:10.1016/j.jinf.2022.07.005
13. Vaughan AM, Cenciarelli O, Colombe S, et al. A large multi-country outbreak of monkeypox across 41 countries in the WHO European Region, 7 March to 23 August 2022. *Euro Surveill*.

2022;27(36):2200620. doi:10.2807/1560-7917.ES.2022.27.36.2200620
14. Huhn GD, Bauer AM, Yorita K, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin Infect Dis*. 2005;41(12):1742-1751. doi:10.1086/498115
15. Rossotti R, Nava A, Baiguera C, et al. High prevalence of anal papillomavirus infection in men who have sex with men PrEP users. *Sex Transm Infect*. 2022:35882547. doi:10.1136/sextrans-2022-055447
16. Ciccarese G, Herzum A, Rebora A, Drago F. Prevalence of genital, oral, and anal HPV infection among STI patients in Italy. *J Med Virol*. 2017;89(6):1121-1124. doi:10.1002/jmv.24746
17. Drago F, Herzum A, Ciccarese G, Bandelloni R. Prevalence of oral human papillomavirus in men attending an Italian sexual health clinic. *Sex Health*. 2016;13(6):597-598. doi:10.1071/SH16155
18. Pisano L, Grandi V, Tiradritti L, et al. New screening strategy combining anal papanicolaou and human papillomavirus tests for human papillomavirus-related anal cancer: a prospective, single-center study. *Sex Transm Dis*. 2022;49(9):622-627. doi:10.1097/OLQ.0000000000001660.
19. Aromolo IF, Maronese CA, Avallone G, et al. Clinical spectrum of human monkeypox: an Italian single-centre case series. *J Eur Acad Dermatol Venereol*. 2022. doi:10.1111/jdv.18612
20. Maronese CA, Beretta A, Avallone G, et al. Clinical, dermoscopic and histopathological findings in localized human monkeypox: a case from northern Italy. *Br J Dermatol*. 2022;187(5):822-823. doi:10.1111/bjd.21773
21. Stagles MJ, Watson AA, Boyd JF, More IAR, McSeveney D. The histopathology and electron microscopy of a human monkeypox lesion. *Trans R Soc Trop Med Hyg*. 1985;79(2):192-202. doi:10.1016/0035-9203(85)90333-5
22. Bayer-Garner IB. Monkeypox virus: histologic, immunohistochemical and electron-microscopic findings. *J Cutan Pathol*. 2005;32(1):28-34. doi:10.1111/j.0303-6987.2005.00254.x
23. Ogoina D, Iroezindu M, James HI, et al. Clinical course and outcome of human monkeypox in Nigeria. *Clin Infect Dis*. 2020;71(8):e210-e214. doi:10.1093/cid/ciaa143
24. Centers for Disease Control and Prevention (CDC). Human monkeypox—Kasai Oriental, Democratic Republic of Congo, February 1996-October 1997. *MMWR Morb Mortal Wkly Rep*. 1997;46(49):1168-1171.
25. Jezek Z, Szczeniowski M, Paluku KM, Mutombo M. Human monkeypox: clinical features of 282 patients. *J Infect Dis*. 1987;156(2):293-298. doi:10.1093/infdis/156.2.293
26. Loconsole D, Sallustio A, Centrone F, et al. Monkeypox virus infections in Southern Italy: is there a risk for community spread. *Int J Environ Res Public Health*. 2022;19(18):11719. doi:10.3390/ijerph191811719
27. Xiang Y, White A. Monkeypox virus emerges from the shadow of its more infamous cousin: family biology matters. *Emerg Microbes Infect*. 2022;11(1):1768-1777. doi:10.1080/22221751.2022.2095309
28. Tiecco G, Degli Antoni M, Storti S, Tomasoni LR, Castelli F, Quiros-Roldan E. Monkeypox, a literature review: what is new and where does this concerning virus come from? *Viruses*. 2022;14(9):1894. doi:10.3390/v14091894
29. Centers for Disease Control and Prevention. Epidemiologic and clinical characteristics of Monkeypox cases—United States, May 17–July 22, 2022. Accessed November 15, 2022. https://www.cdc.gov/mmwr/volumes/71/wr/mm7132e3.htm
30. European Centre for Disease Prevention and Control. Monkeypox multi-country outbreak—second update. Accessed November 15, 2022. https://www.ecdc.europa.eu/en/publications-data/monkeypox-multi-country-outbreak-second-update
31. Kupferschmidt K. Why monkeypox is mostly hitting men who have sex with men. *Science*. 2022;376(6600):1364-1365. doi:10.1126/science.add5966
32. Alonso-Cadenas JA, Andina-Martínez D, García-García CJ, Gaitero-Tristán J, García-Ascaso MT, Torrelo A. Monkeypox disease in a breastfeeding infant. *Pediatr Dermatol*. 2022;40(1):214-215.doi:10.1111/pde.15145

How to cite this article: Ciccarese G, Di Biagio A, Bruzzone B, et al. Monkeypox outbreak in Genoa, Italy: clinical, laboratory, histopathologic features, management, and outcome of the infected patients *J Med Virol*. 2023;95:e28560. doi:10.1002/jmv.28560