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Case Report

Lymphofollicular lesions associated with monkeypox (Mpox) virus proctitis

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ABSTRACT

In the recent 2022 monkeypox (Mpox) global outbreak, cases have been mostly documented among men who have sex with men. Proctitis was reported in almost 14% of cases. In this study, four Mpox-confirmed cases requiring hospitalizations for severe proctitis were characterized by clinical, virological, microbiological, endoscopic, and histological aspects. The study showed the presence of lymphofollicular lesions associated with Mpox virus rectal infection for the first time.

Introduction

Since May 2022, almost 74,000 human cases of monkeypox (Mpox) virus infection have been reported in non-endemic countries, involving predominantly men who have sex with men (MSM) [1,2]. The current Mpox outbreak presents a distinctive pattern from the cases previously described [3–5]. Proctitis, characterized by anorectal pain, anal discharge, perianal lesions, and occasional rectal bleeding [6,7], was reported in between 14% and 25% of cases [1,8]. Nevertheless, pathological features involving the colonic tissues were poorly investigated. Here we describe four hospitalized patients for severe Mpox proctitis, focusing on the endoscopic and histopathological patterns.

Case presentations

Four patients with a confirmed diagnosis of Mpox [9] admitted from May 16 to September 15, 2022, for severe proctitis or proctocolitis, to the Lazzaro Spallanzani National Institute of Infectious Diseases, in Rome, Italy, were included. All patients were MSM with a median age of 31.5 years (interquartile range [IQR] 26.75-35.75). Demographic, clinical, and laboratory characteristics are summarized in Supplementary Table 1. Two patients (Pt 3 and Pt 4) were HIV positive, both on antiretroviral therapy (ART) with good viroimmunological status (clusters of differentiation [CD4] >500/μl; HIV-RNA undetectable). Three patients had a history of previous STIs and all reported unprotected anal intercourse within ten days (median incubation period 7 days (IQR 7-7.75) before the disease onset. The clinical picture was characterized in all patients by systemic symptoms and skin lesions first localized in the anogenital area and later in the rest of the body. Real-time polymerase chain reaction (PCR) revealed Mpox DNA in all oropha-

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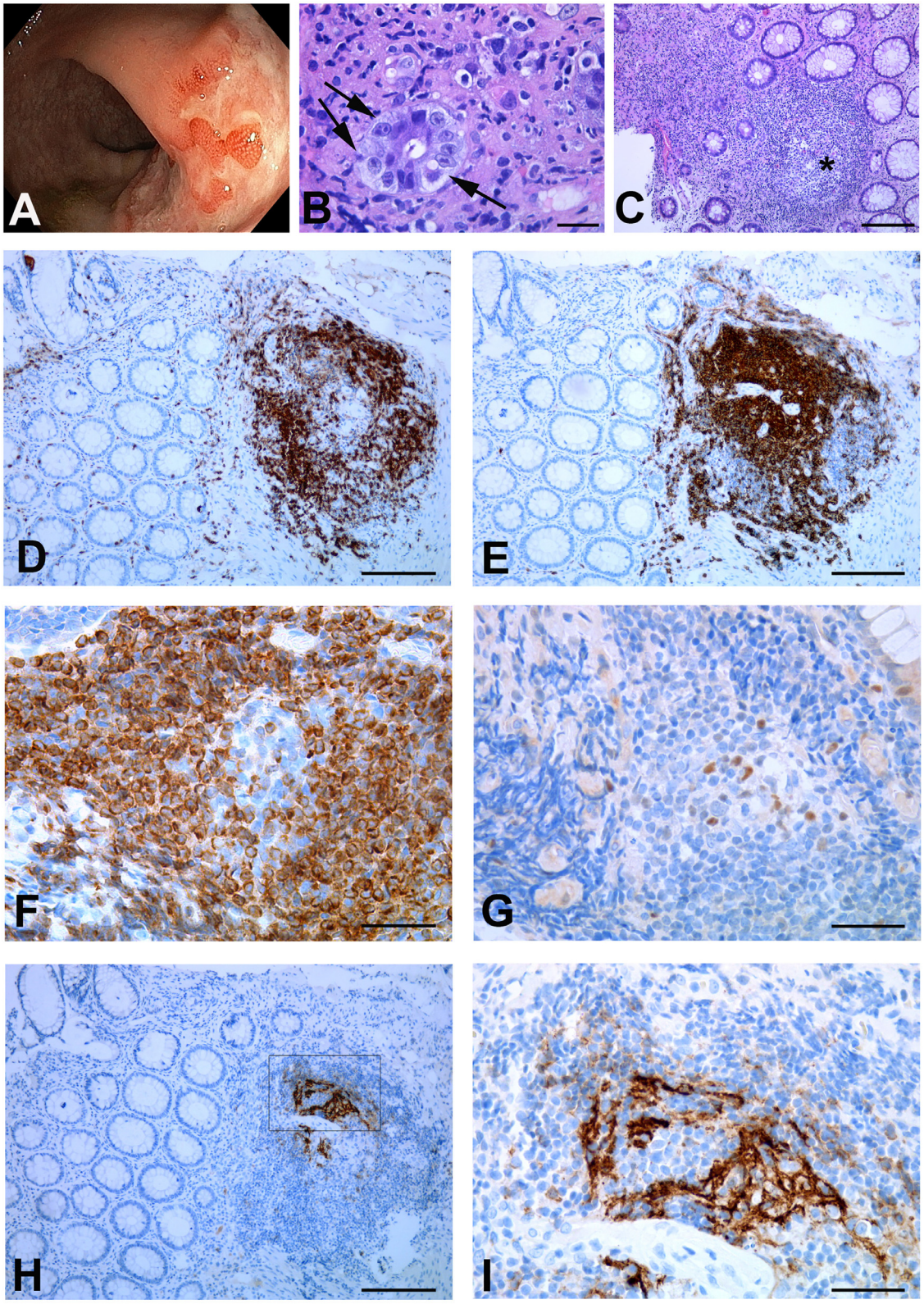

Figure 1. Endoscopic and histopathological findings. (a) Representative endoscopic image shows edematous rectal mucosa sprinkled with small surface erosions. (b,c) Representative micrographs of hematoxylin and eosin stained sections: (b) Crypt epithelial cells displaying signs of viral cytopatic changes: loss of cell polarity, nuclei dislocation with irregular contours and prominent nucleoli, vacuolization of cytoplasm (arrows); (c) Intramucosal isolated hyperplastic follicle showing PTGC, with irregular demarcation of germinal center and mantle zone (light zone and dark zone) (asterisk). (d-i) Immunohistochemical characterization of PTGC: (d,e) prominent and irregular lymphoid follicle display dispersion of CD3 positive T cells (d), and B cells CD20 positive extending into the germinal center (e); (f,g) germinal center appear predominantly composed of mantle zone B lymphocytes that are BCL2 positive cells (f), with few residual germinal center cells, highlighted by BCL6 positivity (g); (h,i) CD23 immunolabeling shows disruption of follicular dendritic cells network into clusters (i, higher magnification of squared area in h). Bars: b = 50 µm; c = 100 µm; d, e, h = 200 µm; f, g. i = 50 µm. CD, clusters of differentiation; PTGC, progressive transformation of the germinal centers.

ryngeal swabs and fecal samples. Three patients showed concurrent infections: two patients tested by real-time PCR on rectal biopsy resulted positive for *Chlamydia trachomatis* and Human Papillomavirus respectively, and one patient tested positive for *Ureaplasma Urealitycum* on the rectal swab. These patients were successfully treated with oral doxycycline (100 mg bid for 7 days). Two patients (Pt 2 and Pt 4) were treated with oral tecovirimat (600 mg twice a day for 14 days). The median time to full clinical recovery was 24.5 days (IQR 17.5-30.5). Rectosigmoidoscopy was performed (median time from onset to endoscopy was 15.5 days [IQR 11.25-26.5]). Endoscopic observation showed edematous, erythematous, friable mucosa sprinkled with small erosive ulcerations and occasionally scattered petechial lesions (Fig. 1a). Fibrin and granular areas were observed in Pt 2 and Pt 4. A rectal solitary ulcer covered by fibrin and irregular borders was depicted in Pt 2.

Histological examination of rectal biopsies showed, in all cases, lymphoplasmacytic infiltration with mild crypt distortion (Supplementary Table 2), in absence of basal plasma cells (Supplementary Figure 1). Features of viral cytopathic changes were observed in the glandular epithelium, with irregular nuclei, and nuclear displacement by cytoplasmic vacuoles (Fig. 1b). No intracytoplasmic eosinophilic inclusion bodies, typical for Mpox skin lesions [10,11], were observed. Reactive follicular hyperplasia and lymphoid aggregates were features observed in all cases (Fig. 1c). Reactive follicular hyperplasia was characterized by a distribution of a small number of clusters of differentiation (CD3) T cells within the germinal center, and expanded presence in the mantle zone (Fig. 1d), with strong positivity of CD20 B lymphocytes (Fig. 1e). Follicular microarchitecture disruption, with inward migration of perifollicular small B cells, expressing mantle zone phenotype (BCL2 positive, a protein not expressed in germinal center B cells), was observed (Fig. 1f), associated with the fragmentation of the germinal center, showed by few numbers of cells expressing BCL6 (Fig. 1g). CD23 immunostaining highlighted the follicular dendritic cells' meshwork completely disrupted into clusters (Fig. 1 h,i). This pattern of the follicular lesion is defined progressive transformation of germinal centers (PTGC). Immunohistochemical staining to localize the Mpox virus (MPXV), revealed the presence of infected cells in the epithelium of the crypts and the inflammatory infiltrate around the glands, and, interestingly even in the follicular aggregates (Supplementary Figure 2).

Discussion

Proctitis, commonly caused in MSM by sexually transmitted infections, such as *Neisseria gonorrhoeae*, Lymphogranuloma venereum (LGV), *Treponema pallidum*, and Human Papillomavirus, has been described in recent Mpox outbreaks.

The gastrointestinal tract is a permeable mucosal site, particularly vulnerable to pathogens. Specialized gut-associated lymphoid tissue has evolved to provide protection against invading pathogens, and tolerance to commensal bacteria and self-antigens [12]. It consists of sparse immune cells and organized lymphoid structures, including Peyer patches and isolated lymphoid follicles, which are located in the mucosa and submucosa along the length of the small and large intestines [13].

Typical features of sexually transmitted infection–related proctitis were lymphoplasmacytic infiltration with mild architectural distortion, and lack of basal plasma cells [14]. Acute inflammation of the lamina propria could also be associated with epithelial damage (cryptitis and crypt abscess), up to epithelial necrosis in more severe cases [15]. In addition to these features, we found, in all our cases, reactive follicular hyperplasia with prominent germinal centers assuming the morphological aspect of PTGC. The histological features of PTGC are represented by infiltrating small lymphocytes from the mantle zone, breaking the follicle into separate clusters of B center cells, and disrupting follicular dendritic meshwork.

PTGC usually affects peripheral lymph nodes, while its incidence in extranodal sites appears uncommon. and rarely occurs in the large intestine [16]. Although its etiology remains unclear, a relationship with viral infection has been reported in a subset of cases associated with epstein-barr virus infection [17]. In this study, we showed, for the first time, the presence of PTGC in Mpox proctitis, and the association of this lesion with the detection of the virus by immunohistochemistry. However, we cannot confirm that lymphoid hyperplasia, with PTGC characteristics, is a specific feature of Mpox proctitis, since we have analyzed only a few cases.

Although reactive follicular hyperplasia with PTGC has been occasionally reported adjacent to nodular lymphocytic predominant Hodgkin lymphoma, whether PTGC could represent a prelymphomatous condition is still under debate [18,19]. Due to the small number of cases included, we cannot speculate on the evolution of Mpox-related follicular hyperplasia of the rectum, with PTGC feature, and more studies are needed. To monitor the presence of such histological alteration, it is advisable to manage patients presenting with severe proctitis related to Mpox, with endoscopic biopsy. In our series, MPXV DNA was detected in rectal biopsy in 2/4 patients: this could be related to the interval time between the symptoms' onset and the rectosigmoidoscopy, in fact, the two procedures with both MPXV-negative biological samples were collected on day 19 and 29 since clinical onset. However, fecal or rectal swab samples collected in the same patients before endoscopy were all positive for MPXV, suggesting the presence of the virus in the gastrointestinal tract in the early stages of infection. The cycle threshold values measured in fecal, rectal, or bioptic samples, within two weeks of symptoms onset, showed values compatible with the presence of potentially replication-competent virus [20].

Since the epidemic's beginning, Mpox has been included in the differential diagnosis of the clinical picture of proctitis. Its early clinical recognition is mandatory because of the potential development of severe proctitis, requiring hospitalization and subsequent invasive investigation. Complete histological and microbiological characterization is needed to address both clinical management and specific therapy.

Several limitations exist in this study. This report presents a small number of patients all recruited in one single care center. The lack of a pre-defined timing of sample collection, endoscopy, and antiviral treatment does not allow us to give definitive conclusions apart from this preliminary descriptive report of common histologic features.

Conclusion

In the current Mpox epidemic, proctitis can be considered part of the clinical presentation. Lymphoid hyperplasia with a PTGC pattern can be found as a histopathological peculiarity. Endoscopic procedures, in selected cases with severe presentation, might improve clinical and diagnostic management to identify the degree of tissue damage, the presence of concomitant infection otherwise not diagnosed, and mucosal lesions that require monitoring after the disease resolution.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethics statement

Data were obtained from an observational study approved by the Ethics Committee of the National Infectious Disease Spallanzani. (MonkeyCohort protocol: "Studio di coorte osservazionale monocentrica su soggetti che afferiscono per sospetto clinico o epidemiologico di malattia del vaiolo delle scimmie [Monkeypox])"; approval number 40z, Register of Non-Covid Trials 2022). Written informed consent was obtained from all participants also for the endoscopy procedure and for anonymized publication of images.

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Author contributions

VM, LS, LF, AA, EN, and FDN conceived the study. VM, LS, CA, DK, and LF drafted the first manuscript and revised the final version. AM, CP, GM, FB, and AC followed the patients during the diagnostic and therapeutic path. FCo, FCa, ARG, and FM provided virological assay on samples. RL and MDP performed endoscopies. LF, DC, and FDN provided histopathological analysis. AA, EN, EG, FV, FM, GDO, and FDN reviewed and supervised the manuscript. All authors gave their final approval of the version to be submitted.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.02.021.

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