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BRIEF REPORT

Acute Appendicitis as the Initial Clinical Presentation of Primary HIV-1 Infection

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We report a case of an adolescent who presented at our emergency department with acute abdominal pain. While the initial diagnosis was acute appendicitis, a secondary and coincidental diagnosis of primary HIV-1 infection was made. Concurrent and subsequent clinical and molecular biology findings form the basis of our argument that primary HIV-1 infection was the cause of acute appendicitis in this individual.

Keywords. acute appendicitis; diagnosis; immunohistochemistry; in situ hybridization; primary HIV-1.

Globally, 1.8 million people were newly diagnosed with HIV-1 in 2016 [1]. However, signs and symptoms of primary HIV-1 infection (PHI) are frequently lacking, which creates an obstacle to timely diagnoses of PHI and early treatment initiation [2, 3]. On occasions when clinical presentations of PHI do occur, they are typically flu-like symptoms (eg, fever, rash, aches, and swollen glands) [4]. Other clinical presentations of PHI most frequently originate from the gastrointestinal tract [4]. In 1 case, an atypical clinical presentation of PHI was characterized as "appendicitis-like illness that resulted in explorative laparotomy," but appendicitis was not confirmed [4]. Because gastrointestinal tract–related complaints often are encountered in routine health care settings, this category of patients poses a potential risk of missing an HIV-1 diagnosis.

Although HIV-1-infected individuals have a 4-fold higher prevalence of appendicitis than HIV-1-negative individuals [5], appendicitis has never been reported as the initial clinical presentation of PHI. Appendicitis and HIV-1 have previously been linked in 2 ways. First, in untreated immune-compromised HIV-1-infected individuals, opportunistic infections (eg, cytomegalovirus [CMV], *Streptococcus pneumoniae*, *Mycobacterium avium* complex, and *Cryptosporidium parvum*) have been shown to cause appendicitis [6–9]. Second, at initiation of antiretroviral therapy (ART), HIV-1-infected individuals have a risk of developing immune reconstitution inflammatory syndrome (IRIS), which initiates a pathological inflammatory response to a previous infection resulting in appendicitis [10, 11].

We present a third and novel association of HIV-1 and appendicitis. We describe a case with an adolescent male presenting to our clinic with symptoms of acute appendicitis coincident with his clinical presentation of PHI. Subsequent examinations and findings suggest HIV-1 as the causative agent of the appendicitis.

CASE PRESENTATION

A 17-year-old male of Mediterranean origin was admitted to the emergency department at a regional hospital with a 2-day history of diffuse abdominal pain and diarrhea. One month prior to the admission, the patient had returned to Denmark from a vacation in the western part of Turkey. The patient had a medical history of kidney neuroblastoma at the age of 3. After chemotherapy and radical surgery, he received a bone marrow–derived autologous CD34+ stem cell transplantation. This treatment was successful, and he showed no signs of relapse at scheduled follow-up visits.

At presentation to the emergency department, he was alert, oriented, and febrile. Abdominal palpation elicited both direct and rebound tenderness. Biochemical tests showed an elevated C-reactive protein (CRP) level of 216 mg/L, a leucocytosis (13.2×10^9/L) with lymphocytosis (9.18×10^9/L), and a discretely elevated bilirubin level (32 μmol/L). The patient was closely observed, and the fever resolved spontaneously. The abdominal symptoms abated to the extent that the diagnosis of appendicitis could not be upheld. The next day, his CRP levels had reduced to 145 mg/L. A tentative diagnosis of infectious diarrhea was made, and as the patient had improved, he was discharged to subsequent follow-up in the case that his condition worsened.

On day 14 after the primary contact, the patient returned to the emergency department with complaints of diffuse abdominal pain, multiple diarrheas, pain upon swallowing, headache, a rash primarily on the torso and spreading to the limbs, severe fatigue, a dry cough, severe shortness of breath during physical activity, and an unintended weight loss (6 kg). Upon examination, the patient was febrile and had rebound tenderness in the

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right-lower abdominal quadrant and a maculopapular rash. No lymphadenopathy was detected. Biochemical tests showed an elevated CRP level of 135 mg/L, leucopenia (1.9×10^9/L) with lymphopenia (0.7×10^9/L), thrombocytopenia (72×10^9/L), and a normal hemoglobin level of 8.7 mmol/L. A computed tomography (CT) scan of the thorax and abdomen revealed a thick-walled and distended appendix with surrounding inflammation (Figure 1A). Laparoscopy revealed that a mild fibrin exudate was present at the base of the appendix. Furthermore, prominent and enlarged periappendiceal lymph nodes were observed. Therefore, an appendectomy was performed. Appendicitis was confirmed using histopathological examination. Specifically, tissue inflammation and ulceration of the mucosal membrane were observed in hematoxylin and eosin–stained tissue sections. Standard immunohistochemical stains of the appendix for an immediate early and an early CMV antigen were both negative. The patient was observed for 2 days. At discharge, he was afebrile with a slightly elevated CRP value of 153 mg/L and leucocytes within the normal range.

On day 18, the patient returned to the emergency department with persistent abdominal pain and a skin rash, as well as fever, headache, and neck stiffness. Biochemical tests showed an elevated CRP level of 81 mg/L, normal leucocytes (4.4×10^9/L) but lymphopenia (0.8×10^9/L), thrombocytopenia (146×10^9/L), and a normal hemoglobin level of 8.3 mmol/L. Meningitis was suspected, and a lumbar puncture was performed. The cerebrospinal fluid (CSF) exhibited 87×10^6/L leucocytes (of which 83×10^6/L were mononuclear), 21×10^6/L erythrocytes, normal protein levels, and normal glucose levels. Penicillin (3 g quater in die [QID, 4 times a day]), ceftriaxone (4 g once daily [OD]), and acyclovir (10 mg/kg/d) were initiated intravenously as empiric treatment. CSF was negative by standard

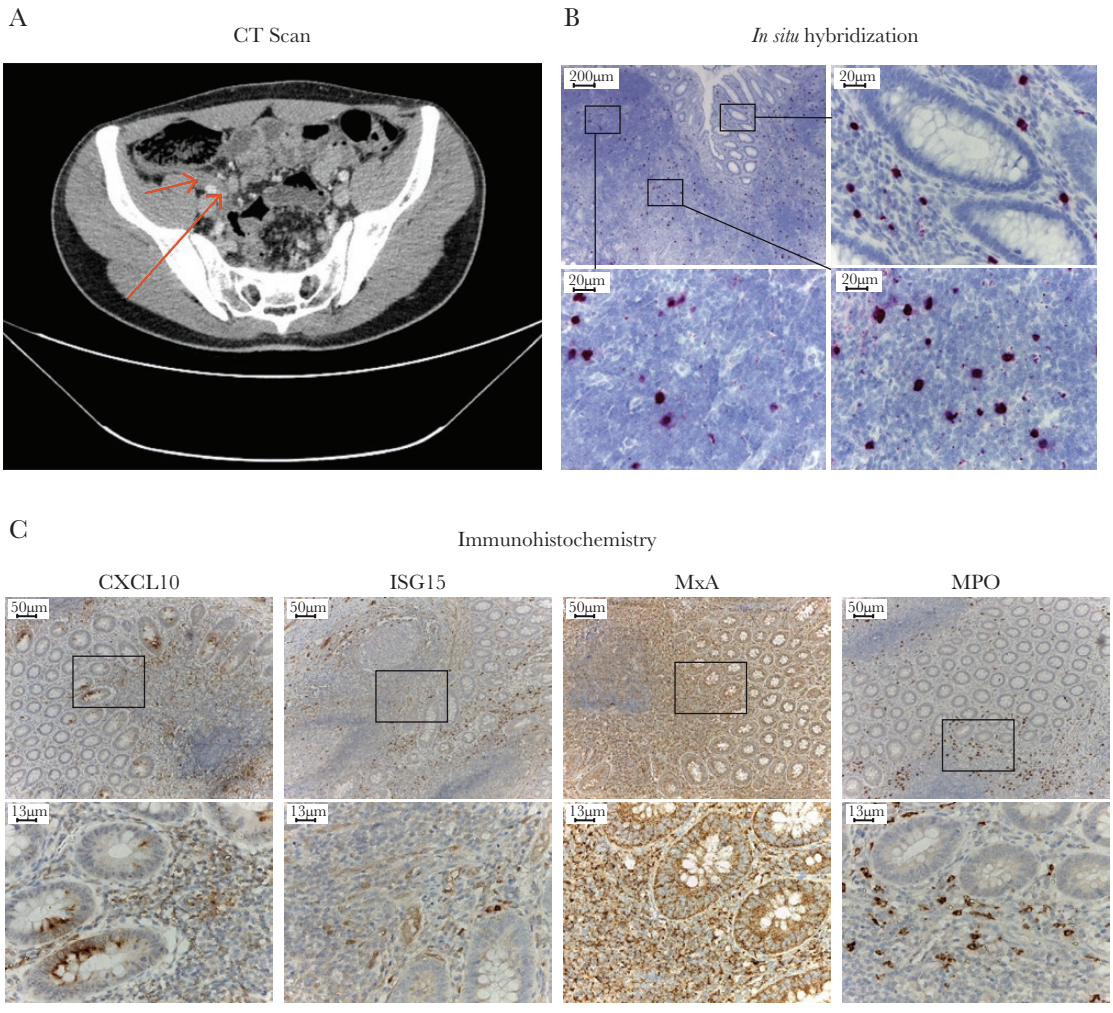

Figure 1. (A) Abdominal computed tomography (CT) scans reveal a dilated appendix with surrounding inflammation. The appendix is indicated by red arrows. (B) In situ hybridization (ISH) of the appendix base. The red ISH signal indicates the presence of HIV-1 RNA transcripts. The upper left image is low magnification. Boxes surround regions of the appendix depicted in higher magnification in the connected images. (C) Immunohistochemistry images of the appendix body. The brown signal indicates the presence of the inflammatory markers, C-X-C motif chemokine 10 (CXCL10), interferon stimulated gene 15 (ISG15), myxovirus resistance protein 1 (MxA), or myeloperoxidase (MPO) indicated for the specific panel. The top column is low magnification. Boxes in the upper column images indicate the region depicted in higher magnification in the bottom column.

clinical polymerase chain reaction (PCR) tests for common pathogens (including *Neisseria meningitidis*, *Streptococcus pneumoniae*, varicella zoster, and herpes simplex virus 1 and 2). Direct microscopic assessment of the CSF was negative for bacteria. Neither CSF nor blood cultures showed evidence of bacteria. Therefore, the patient was treated according to national guidelines for culture-negative meningitis. A new CT scan of the abdomen revealed mild and anticipated postoperative findings and was otherwise considered normal.

On day 21, the patient was still febrile and exhibiting leucopenia. At this point, the antibiotic treatment was modified considering the travel history to Turkey and exposure to raw unpasteurized milk. Specifically, the antibiotic regimen was switched to ceftriaxone 4 g OD with the addition of ciprofloxacin 400 mg bis in die (BID; 2 times a day), due to clinical suspicion of brucellosis. No known genetic mutations related to Mediterranean fever were identified. The patient was tested for hepatitis A, B, and C virus serology/antigens, CSF 16S PCR analysis, and CMV/Epstein-Barr virus (EBV)/brucellosis serology.

On day 29, the patient remained febrile despite ongoing antibiotic treatment. The patient was tested for *Coxiella burnetii* and parvovirus B19 serology. A blood smear was also performed. Additionally, a fludeoxyglucose (FDG) positron emission tomography (PET) CT revealed pathologic FDG uptake in multiple lymph node sites. To rule out relapse of neuroblastoma, the urine was tested for vanillylmandelic acid and found to be negative. Further investigation into the patient's medical history revealed that the patient had tested negative for HIV-1 88 days earlier at his general practitioner (HIV p24 Ag/Ab Combo, Abbott), and the patient acknowledged having sex with men as an HIV risk factor. Therefore, a new HIV-1 diagnostic test was ordered (HIV p24 Ag/Ab Combo, Abbott).

Of the extensive panel of assays performed to detect an infectious etiology in this case, CMV, EBV and HIV were positive. Regarding CMV, IgM and IgG antibodies against this virus were present in serum, but immunohistochemistry performed immediately after the appendectomy found the appendix to be negative for CMV proteins that would have indicated active viral replication. Regarding EBV, the patient was positive for antibodies against EBV-EBNA, negative for IgM against virus capsid antigen, and negative for EBV DNA in blood. These findings together indicate prior CMV and EBV infections and seroconversions well beyond the time frame of this case. Regarding HIV-1, the day 29 sample was positive for both p24 antigen and anti-HIV antibodies, but negative for p31 antigen (INNO-LIA HIV I/II Score, Fujirebio). These findings place the patient in HIV Fiebig stage V [12] at HIV-1 diagnosis.

The patient's CD4 cell count was 680/μL, and his plasma HIV-1 RNA level was 1×10^6 copies/mL. HIV in the CSF fluid was 16×10^6 copies of HIV-1 RNA per mL. Antibiotics were ceased after a total of 14 treatment days. Intensive HIV counseling was immediately begun, and the patient was ready to begin ART 2 weeks later. ART consisted of emtricitabine 200 mg/tenofovir disoproxil fumarate 245 mg (Truvada) in combination with darunavir 800 mg/ritonavir 100 mg once daily. Six months after initiating ART, the CD4 cell count had increased to 850/μL, and plasma HIV-1 RNA was fully suppressed.

To investigate the etiology of this appendicitis and assess whether this case represents a novel clinical presentation of PHI, the appendix was subsequently examined by in situ hybridization and immunohistochemistry. In situ hybridization revealed high levels of HIV-1 RNA-producing cells throughout the appendix base, body, and tip (Figure 1B; Supplementary Figure 1, A and B). Large red nuclei-associated spots represent cell profiles expressing HIV-1 RNA. The levels of productively infected cells observed in this appendix are comparable to those observed in lymph nodes of individuals with untreated HIV-1 infection [13]. Furthermore, immunohistochemical stains against the inflammatory markers CXCL10, ISG15, and MxA reveal a robust production of these proteins throughout the tissue indicative of a stimulation of the IFN-alpha and IFN-gamma immune responses (Figure 1C; Supplementary Figure 1C). Interestingly, no massive neutrophil (MPO) infiltration into the inflamed organ was detected (Figure 1C), which supports that this case was not bacterial-induced appendicitis.

METHODS

Fixation of Appendix

Immediately after the appendectomy, the appendix was fixed overnight in 10% neutral buffered formalin, then cut into 6 pieces (base, body, and tip, alternating longitudinal and cross-sections representing the appendix in full length), and each piece was then embedded in paraffin.

In Situ Hybridization

As we previously described [14], in situ hybridization for HIV-1 RNA was performed using the RNAScope 2.0 RED assay (Advanced Cell Diagnostics) and treated as described in the Supplementary Methods.

Immunohistochemistry

Immunohistochemistry (IHC) to detect C-X-C motif chemokine 10 (CXCL10), interferon stimulated gene 15 (ISG15), myxovirus resistance protein 1 (MxA), or myeloperoxidase (MPO) was performed according to previously published protocols [15, 16] and treated as described in the Supplementary Methods and Supplementary Table 1.

Ethical Statement

Written informed consent was obtained for subsequent analyses and the publication of this report.

DISCUSSION

Here we argue for primary HIV infection as a novel etiology for acute appendicitis. The lack of neutrophil infiltration and the observance of a robust interferon response in the appendix

support a viral etiology for the inflammation. Although we cannot completely rule out a bacterial or other etiology, we did rule out CMV or EBV as the cause of this appendicitis. Further, the patient's CSF was negative for varicella zoster, HSV-1, and HSV-2 replication. Our in situ hybridization analysis of the appendix revealed the presence of HIV-1 RNA-positive cells throughout the entire organ. This observation is consistent with the facts that (i) the appendix, along with Peyer's patches and isolated lymphoid follicles, is a gut-associated lymphoid tissue that functions as an inductive site for mucosal B and T cells [17] and (ii) immune inductive sites are primary sites of HIV-1 replication and pathogenesis [18]. While the primary HIV-1 infection might be purely coincidental and PHI as the cause of the appendicitis cannot be unequivocally proven, the evidence strongly supports PHI as the etiology of the appendicitis in this case.

There is no confirmed precedent for acute appendicitis as PHI, as indicated by Braun et al. in their compilation of rare atypical clinical presentations of PHI [4]. Other atypical clinical presentations of PHI include opportunistic infections (eg, *Candida* stomatitis, *Candida* esophagitis) or damage to the central nervous system (eg, encephalitis, paresis, acute psychiatric disorders). Also, tonsillitis, pneumonia, renal failure, or skin and soft tissue infections have been confirmed as the initial clinical presentation of PHI [4]. Our case supports considering PHI as a differential diagnosis in patients undergoing appendectomy, who subsequently experience low platelets, lymphopenia, prolonged fever, and are refractory to conventional diagnostics and treatment. Overall lessons from this case include a practical reinforcement of the fact that clinical presentations of PHI are often atypical. Our putative finding that the initial clinical presentation of PHI in this case was acute appendicitis underscores the importance of meticulously noting the history of sexual risk behavior and performing HIV tests in high-risk patients presenting with an acute illness not typically associated with PHI.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. The authors declare no conflict of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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