

Pathologic Changes in the Small Bowel in Nine Patients with Diarrhea Associated with a Coccidia-like Body

Bradley A. Connor, MD; David R. Shlim, MD; John V. Scholes, MD; Joseph L. Rayburn, MD; Jason Reidy, MA; and Ramachandran Rajah, ASMLT

■ **Objective:** To confirm a suspected small-bowel injury in patients with a syndrome of protracted diarrhea associated with a coccidia-like body (CLB).

■ **Design:** Investigation of an epidemic including a case-control study.

■ **Setting:** Outpatient clinic in Kathmandu serving primarily the tourist and expatriate community in Nepal.

■ **Patients:** Nine patients with diarrhea with at least one stool specimen that was positive for the presence of a CLB and seven noninfected volunteer controls.

■ **Measurements:** Clinical data, microscopic examination of stool, bacteriologic and viral studies on submitted stool specimens, upper gastrointestinal endoscopy including duodenal aspiration and microscopy, small-bowel biopsy with subsequent light and electron microscopy.

■ **Results:** Endoscopic evidence of inflammation of the distal duodenum was present in five of nine patients with CLB and in none of the seven controls. All nine patients with CLB were noted to have histologic evidence of small-bowel injury, which included acute and chronic inflammation, surface epithelial disarray, and varying degrees of villous atrophy and crypt hyperplasia. One of the seven controls had similar pathologic findings and developed CLB-related diarrhea 5 days later. The other controls had normal distal duodenal histologic results. The organism was found in two of nine duodenal aspirates but was not present in the preserved biopsy specimens as determined by light or electron microscopy.

■ **Conclusions:** The pathologic basis of CLB-associated diarrhea appears to be small-bowel injury whose cause remains to be elucidated.

In recent years, a novel intestinal organism has been noted in the stools of patients with a syndrome characterized by prolonged diarrhea associated with fatigue and anorexia (1-5). The organism resembles either a cyanobacterium or a coccidian and has been called a "cyanobacteria-like or coccidia-like body" (CLB) (6). A recent report suggested that the organism is a coccidian, and the name *Cyclospora cayetanensis* was proposed (7). Until the taxonomic issues are definitively resolved, we refer to the organism as CLB.

The Canadian International Water and Energy Consultants (CIWEC) Clinic in Kathmandu, Nepal, first noted the organism in June 1989. Fifty-five cases were subsequently documented that year in an outbreak that ended 4 months later (4). From May to October 1990, another outbreak was documented, with a total of 86 cases. One hundred four cases were diagnosed in 1991, yielding a total of 245 cases seen at the CIWEC Clinic in three distinct outbreaks (Figure 1). All patients had diarrhea, fatigue, and anorexia. The illness lasted an average of 42 days and was refractory to various antibiotic treatments. All patients recovered completely. The organism has yet to be discovered in the stools of an asymptomatic patient.

The illness is almost invariably associated with weight loss and malabsorption of D-xylose. Many patients also experience nausea, but tenesmus and signs of dysentery are absent. For these reasons, the infection was hypothesized to occur in the upper intestine. We conducted a study to evaluate this possibility by doing upper gastrointestinal endoscopy with duodenal aspiration and small-bowel biopsy in patients infected with CLB.

Methods

Background

The CIWEC Clinic, an outpatient facility that serves the expatriate and tourist populations in Kathmandu, Nepal, treats 5000 patients per year, of whom 30% have a diarrheal complaint. Because endoscopy is not routinely available, we arranged for an American board-certified gastroenterologist to visit the clinic for 1 week during the predicted height of the outbreak in June 1991.

Microbiology

The CLB organism can be observed in plain smears of the stool of infected patients. The organism is spherical and is 9 to 10 microns in width. The organism can be concentrated by flotation in Sheather sucrose solution or by centrifugation of a formalin-ether preparation of stool (8). The organism stains variably red with the modified acid-fast stain used to identify cryptosporidium (Figure 2). All stools submitted for examination to the CIWEC Clinic were screened for the presence of this organism. When CLB was identified, stools were also screened for the presence of ova or parasites, blood, or leu-

Ann Intern Med. 1993;119:377-382.

From the Canadian International Water and Energy Consultants Clinic, Kathmandu, Nepal; the New York Hospital-Cornell University Medical College, New York University School of Medicine; Beth Israel Medical Center and Mount Sinai School of Medicine, New York, New York. For current author addresses, see end of text.

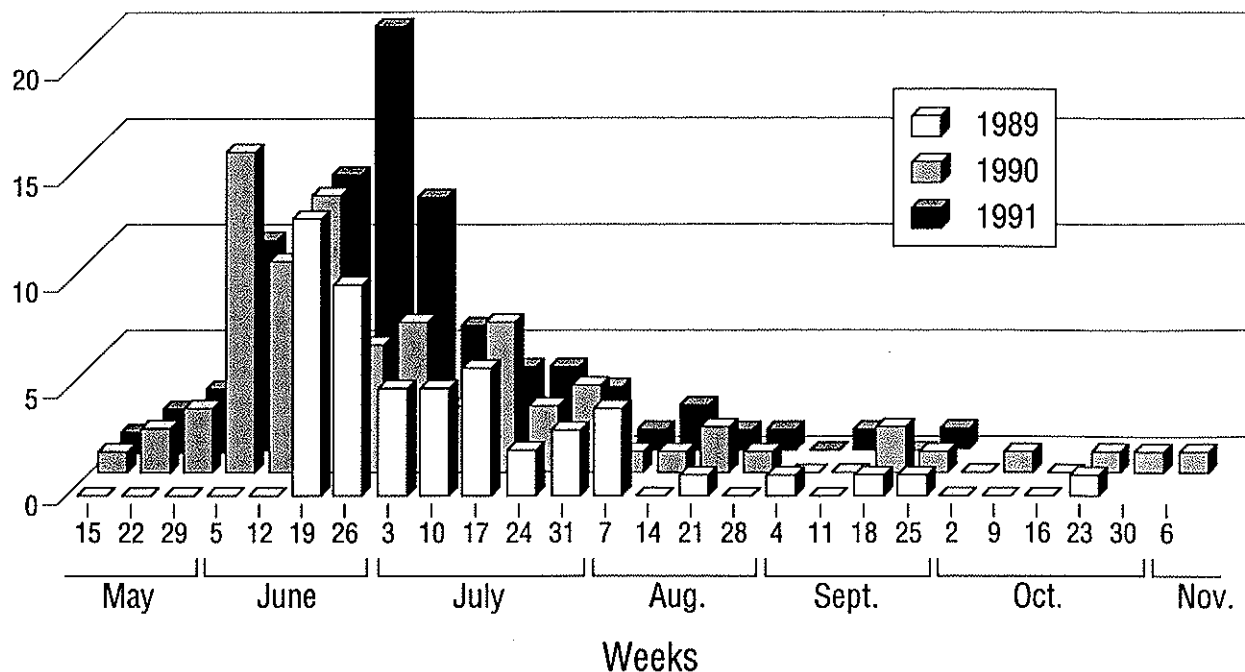


Figure 1. The number of new cases infected with coccidia-like bodies per week, defined as the date of the first positive stool specimen for each patient. Recording of these bodies in the stool began only on 19 June 1989, apparently missing the first part of that year's outbreak. These bodies were not noted in stool examinations during the rest of the year.

kocytes. Bacteriologic and viral studies are not routinely done at our clinic. During the 1991 outbreak, we arranged for a limited number of CLB-positive stools to undergo bacteriologic and virologic studies via the Armed Forces Research Institute of Medical Science in Bangkok, Thailand. Six of the nine patients with CLB who underwent endoscopy had bacteriologic screening for enteric pathogens, using standard culture techniques and DNA probes of prepared stool blots on filter paper (9). The other three patients could not be screened in this fashion because of logistic difficulties. Of 95 other patients with CLB, 33 had similar studies done during the course of the summer. The specimens were screened for rotavirus using a commercially available enzyme-linked immunosorbent assay (ELISA) test (10).

Selection of Patients and Controls

Thirty-four patients had been identified as having diarrhea associated with CLB before the gastroenterologist arrived in Nepal (12 June 1991). Of these 34 patients, 9 were still infected with CLB, were available in Kathmandu, and were willing to have upper endoscopy during the week that the endoscopist was available. Seven persons without diarrhea and without CLB in their stools were recruited as controls. Of these, four were completely asymptomatic and three had requested upper endoscopy because of preexisting upper gastrointestinal symptoms. Informed consent was obtained from all persons having endoscopy.

Endoscopic Procedure

Upper gastrointestinal endoscopy was done in all patients by the same gastroenterologist after a fast of at least 6 hours in every case. Topical anesthesia was achieved using dyclonine gargle before each procedure. Using the Olympus XQ20 or Olympus XP20 fiberoptic gastroscope (Olympus Corp., Lake Success, New York), endoscopic examination to the descending duodenum was done in all patients. Endoscopic assessment of the esophagus, stomach, and duodenum was made. Duodenal fluid was aspirated into sterile specimen traps using a sterile saline irrigation of the duodenal bulb and descending duodenum. At least two forceps biopsy specimens of the distal duodenum were obtained in each case. Biopsy specimens were preserved in 10% neutral buffered formalin for light micros-

copy and in 200 mol/L formalin glutaraldehyde for electron microscopy.

Examination of Endoscopically Obtained Material

Duodenal aspirates were examined immediately by light microscopy without stain and after staining with the modified acid-fast stain. The aspirates were preserved at 4 °C for up to 1 week and were then transported at ambient temperature to New York where they were cultured for acid-fast bacteria using Lowenstein-Jensen and American Trudeau Society media.

Duodenal biopsy specimens from cases and controls were examined in a blinded fashion by pathologists from two separate New York institutions. Duodenal biopsy specimens for light and electron microscopy were fixed and prepared as previously described (11).

D-Xylose Absorption Test

Three patients with CLB had D-xylose absorption tests. Twenty-five grams of D-xylose were administered to patients after an overnight fast. Urine was collected for 5 hours. The amount of D-xylose in the urine was calculated using a standard photometric method.

Case Report

The nine patients with CLB had similar clinical histories. All symptoms resolved spontaneously in 2 to 12 weeks. The following case report is representative.

A 40-year-old Canadian woman who had resided in Nepal since October 1990 developed watery diarrhea with urgency on 15 May 1991. She was 7 weeks postpartum at the time and was breast-feeding her infant son. The diarrhea persisted for 3 days with about five loose stools per day. She started taking nalidixic acid on the first day of illness. A stool examination on 16 May showed only a few ascaris eggs, for which she was given mebendazole. She was healthy from 18 May to 22

May, at which time she noted the return of diarrhea and fatigue. The symptoms of anorexia, fatigue, and nausea tended to occur simultaneously, but intermittently, during the ensuing weeks.

On 23 May 1991, a stool specimen submitted to the CIWEC Clinic was positive for the presence of CLBs. Repeated stool examinations on 28 May and 4 June were unchanged. On 10 June, examination showed few CLBs, and on 12 June and 17 June, rare CLBs were reported. A D-xylose absorption test was done on 18 June. A total of 0.9 g of D-xylose was excreted in the 5-hour urine collection (3.6% of the loading dose; normal, >20%).

An upper gastrointestinal endoscopy was done on 17 June 1991. The patient's esophagus and stomach were normal; however mild erythema of the duodenal bulb and marked erythema of the distal duodenum were noted. Symptoms persisted, and a repeated stool examination on 21 June showed rare CLBs. A stool examination was done on 28 June while she was still feeling ill. Results were negative for CLB; her symptoms resolved steadily during the next 7 days, and she has remained well.

Results

Patient Data

We studied six men and three women. Four were tourists, and five were foreign residents of Nepal. Their mean age was 36 ± 6 years (range, 22 to 48 years), and their mean length of time in Nepal was 405 ± 391 days (range, 21 to 1460 days). Symptoms were present in patients with CLB from 4 to 53 days before endoscopy. The mean age of the seven non-CLB controls was 33 ± 6 years (range, 25 to 42 years). The mean length of time in Nepal for controls was 917 ± 727 days (range, 60 to 2920 days).

Clinical Features

The predominant symptoms in the nine patients with CLB were diarrhea and fatigue. Crampy abdominal pain

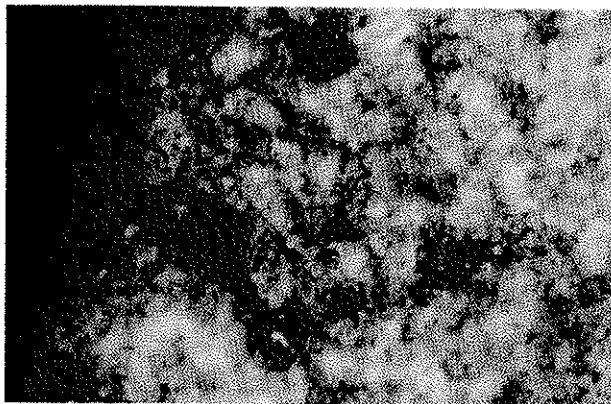


Figure 2. Oil immersion photomicrograph of smear of concentrated stool specimen of patient with chronic diarrhea. Three coccidia-like bodies (red) with central morula containing refractile globules (pale pink to clear) and outer membrane are shown. Organisms measure 9 to 10 μm in width. (Acid-fast stain. Original magnification, $\times 4000$.)

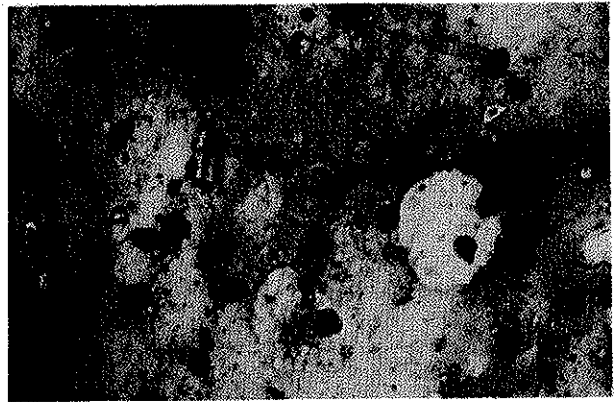


Figure 3. Oil immersion photomicrograph of smear from duodenal aspirate stained with modified acid-fast stain. Three coccidia-like bodies are shown (center). Organisms stain strongly positive (red-pink) but are imperfectly preserved. Acid-fast bacilli are present in the background. (Modified acid-fast stain. Original magnification, $\times 2500$.)

was present in some, but not all, of the patients and varied in intensity. Two patients reported a subjective fever at the onset of their illness. Nausea was variably present in all but two patients studied. Eructation or flatulence was also common. Weight loss, estimated at 5% to 10% of body weight, was reported by all patients and appeared to increase with the duration of symptoms.

Microbiology

Six patients with CLB who had biopsies had bacteriologic cultures done on their stool specimens. Three results were negative, two were positive for enterotoxigenic *Escherichia coli*, and one was positive for enterohemorrhagic *E. coli*. None of these six patients had rotavirus antigen in their stools. Thirty-three of 95 other CLB-positive patients had the same bacteriologic studies done in 1991. Of these 33 patients, 2 tested positive for enterotoxigenic *E. coli*, and 1 each for *Shigella flexnerii* and *Shigella boydii*. The overall rate of bacterial infection in the CLB-positive patients was 7 of 39 (18%).

D-Xylose Testing

Three patients had D-xylose absorption tests. The amount of urinary excretion of D-xylose in a 5-hour urine collection for the three patients was 0.9 g (3.6%), 1.3 g (5.2%), and 2.4 g (9.6%), respectively. Expected values are 5 g or more ($\geq 20\%$).

Endoscopic Findings

Of the nine patients with CLB, five had moderate to marked erythema of the distal duodenum. None of the seven controls had distal duodenal disease. Three controls, all of whom had a history of upper gastrointestinal symptoms, had endoscopic evidence of mild to moderate esophagitis or gastroduodenitis. Of the patients with CLB, one was noted to have a hiatal hernia, antral gastritis, and duodenitis; he reported a premorbid his-

tory of acid peptic disease, for which he was already receiving a histamine H₂-receptor antagonist.

Duodenal Aspirates

The CLB organisms were noted adherent to mucous strands in two of nine patients (Figure 3); however, modified acid-fast staining of the rest of the aspirate could not find any other organisms. Acid-fast bacilli were noted in stains of the aspirates of six of the nine patients with CLB. Similar organisms were seen in one of seven controls. Cultures of the duodenal aspirates for acid-fast bacilli were negative after 6 weeks.

Histologic Findings

Cases with Positive Stool Specimens

Endoscopic duodenal biopsies were randomly oriented during embedding. All cases showed similar histologic abnormalities, although the degree of abnormality varied (Figures 4 and 5). Mild to moderate acute inflammation of the lamina propria was present in all biopsies; neutrophils were also present in the epithelium in five of nine cases. Diffuse chronic inflammation of a mild to moderate degree was present in all specimens, with an increase in plasma cells in the lamina propria being the most prominent feature. Increased intraepithelial lymphocytes were present focally. The surface epithelium, especially near the tips of the villi, showed focal vacuolization, loss of brush border, and an alteration of cells from a columnar to a cuboid shape, suggesting epithelial injury. All cases showed mild to moderately severe partial villous atrophy and crypt hyperplasia characterized by shortened, blunted villi and increased crypt length and mitoses. The villous-to-crypt ratio ranged from 0.6:1 to 1.5:1 (normal, 3:1 to 4:1). No CLBs could be recognized in the biopsy tissue either on routine hematoxylin and eosin or acid-fast stains. No other organisms, including cryptosporidia, microsporidia, and isospora, or viral inclusions were

recognized. No histologic differences could be observed between the patients with CLBs who were concomitantly infected with *E. coli* and those who were not.

A gastric antral biopsy specimen from one case patient showed no histopathologic abnormalities; no organisms, including *Helicobacter pylori*, were identified in the hematoxylin and eosin stain. A colonic biopsy specimen from a second case patient showed no histopathologic abnormalities or organisms.

Cases with Negative Stool Samples (Controls)

Endoscopic duodenal biopsy specimens were randomly oriented during embedding. Five of seven control biopsy specimens showed no histologic abnormalities. One of the remaining two patients showed surface epithelial alteration, inflammation, and architectural changes indistinguishable from those seen in patients with CLBs. This patient developed diarrhea within 5 days of endoscopy, and CLBs were identified in a stool examination done 4 days after the onset of diarrhea. The remaining control patient showed a focal superficial mucosal erosion in one tissue specimen, corresponding to an endoscopically visualized duodenal ulcer.

Discussion

The illness associated with CLB is characterized by the abrupt onset of watery diarrhea, often associated with fever (30%) (4). The initial diarrhea improves within 2 to 4 days but relapses within a few days and is associated with anorexia, fatigue, and nausea. The symptoms wax and wane during the ensuing weeks and are accompanied by weight loss. Treatment with various antimicrobial agents has failed to shorten the illness. The organism is consistently found in stool examinations during this time. Resolution occurs abruptly after 2 to 12 weeks of illness and is associated with the disappearance of the organism from the stool specimens.

We have examined 1500 patients with diarrhea not

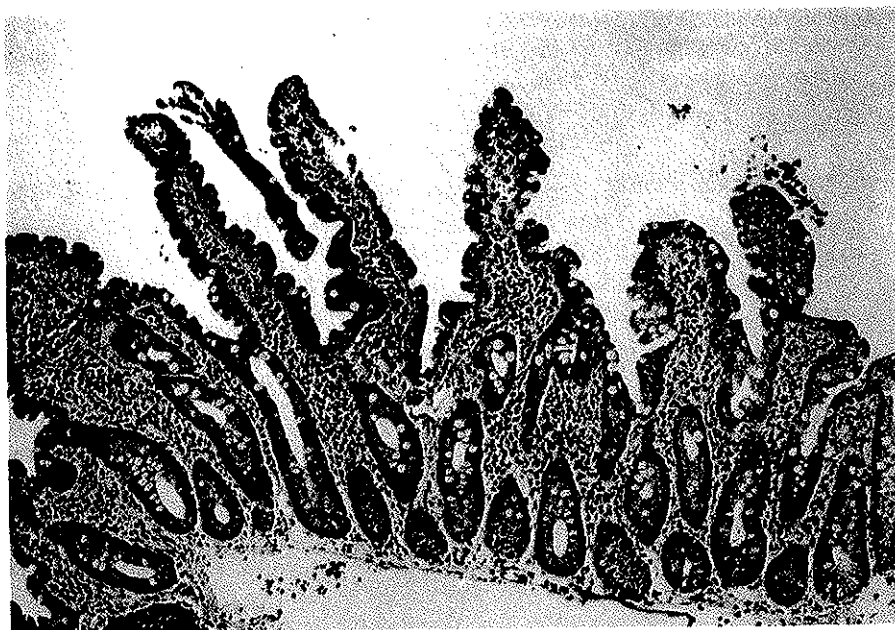


Figure 4. Photomicrograph of section of endoscopic duodenal biopsy showing partial atrophy of villi. Elongated hypertrophic crypts (with a villous-to-crypt ratio of 1:1) and chronic inflammation of lamina propria with increased mononuclear cells are shown. (Hematoxylin and eosin. Original magnification, $\times 250$.)

associated with CLB during the three outbreaks. None manifested prolonged untreatable diarrhea. Forty asymptomatic patients had stool examinations during the outbreak periods, without a finding of CLBs.

In nine patients infected with CLB, endoscopies and duodenal biopsies were done. All had abnormalities characterized by an inflammatory reaction in small-bowel mucosa, surface epithelial disarray, and evidence of villous atrophy and crypt hyperplasia. One of seven control patients showed similar pathologic findings and developed CLB-related diarrhea 5 days later. In the other controls, the biopsy and endoscopy results were normal. Three of the biopsied patients, selected because of their availability (not for the severity of their symptoms), had D-xylose testing and showed markedly abnormal results.

No CLB organisms were identified on intestinal surface epithelium or intracellularly, either by light or electron microscopy. Electron microscopy also confirmed the absence of other microorganisms such as microsporidia.

The CLBs were found in two of nine duodenal aspirates, in each case clinging to mucous strands. The number of organisms is assumed to have been few because staining of another portion of the aspirate failed to detect any other CLBs. Acid-fast bacilli were noted in the duodenal aspirates of six of the nine patients with CLB and in only one of seven controls. However, efforts to grow the acid-fast bacillus organism failed, and the significance of this finding is unclear. The difference in the presence of acid-fast bacilli in the patients with CLB compared with the controls was not statistically significant.

The mechanism by which CLB causes small-bowel injury is not known. Our study evaluated the duodenum in patients whose duration of illness ranged from 5 days before diagnosis to 53 days after onset. The organism was not found in the biopsy specimens. It does not appear to be invasive or integrated into host enterocytes. It may be loosely bound to surface epithelium or to mucus. The significant amount of architectural disruption and inflammation of the small bowel, without evidence of direct invasion by the organism, suggests that a toxin may be involved. It is worth noting that the mechanism by which a well-studied organism, such as *Giardia lamblia*, causes diarrhea and bowel damage is also not clearly known (12).

Although three of our patients with CLB had positive stool cultures, the pathologic findings in these patients were indistinguishable from those in three other patients with CLBs whose stool specimens were negative. In addition, the pathologic findings that we described have not been noted with the bacterial pathogens that we cultured.

It is unknown whether patients who have CLB infections develop antibodies; however, we have identified six patients who had successive CLB infections in consecutive years (unpublished data), suggesting that their first infection did not confer lasting immunity. The absence of the organism from the environment from November through April each year may allow immunity to fade between the seasonal outbreaks.

The general disruption of small-bowel architecture and the interference with absorption associated with the CLB invites comparison with the syndrome of tropical



Figure 5. Medium-power photomicrograph of section of duodenal biopsy obtained by endoscopy from a patient with coccidia-like bodies in the stool. Partial villous atrophy, crypt hyperplasia with increased crypt length and mitoses (villous-to-crypt ratio, 1:1), and chronic inflammation with increased mononuclear cells in the lamina propria are evident. (Hematoxylin and eosin. Original magnification, $\times 500$.)

sprue, thought to be caused by an unknown pathogen, but the untreated illness is usually of longer duration in patients with sprue. Random treatment of CLB-positive patients with tetracycline and folate has not shortened the course of illness while CLBs are present. We doubt that CLB is the cause of the syndrome of tropical sprue, but the pathologic similarities between the two conditions suggest that, if CLB proves to be toxin-mediated, tropical sprue may also prove to be caused by a toxin-producing organism.

Acknowledgments: The authors thank Peter Echeverria, MD, for his help in performing the bacteriologic and virologic studies.

Requests for Reprints: Bradley A. Connor, MD, 50 East 69th Street, New York, NY 10021.

Current Author Addresses: Dr. Connor: 50 East 69th Street, New York, NY 10021.

Dr. Shlim and Mr. Rajah: CIWEC Clinic, P.O. Box 1340, Kathmandu, Nepal.

Dr. Scholes: Department of Pathology, Tisch Hospital, Room 461, New York University Medical Center, 560 First Avenue, New York, NY 10016.

Dr. Rayburn: Reference Pathology Laboratory, 1721 Patterson Street, Nashville, TN 37203.

Mr. Reidy: Department of Pathology, Beth Israel Medical Center, 1st Avenue and 16th Street, New York, NY 10003.

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