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# Green neutrophilic inclusions and a novel association with gangrenous ischemic colitis

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## ABSTRACT

Blue–green refractile neutrophilic inclusions on peripheral smears are a rare and critical finding. Finding of these inclusions has typically been associated with a poor short-term prognosis causing them to be dubbed, "Green crystals of death." Early reports have suggested a link to acute hepatic failure as the prevailing etiology during which these inclusions appear. Here, we present a case of a 75-year-old male who presented with ischemic colitis in which blue–green neutrophilic inclusions were discovered on peripheral smears. This is the first documented case of ischemic colitis associated with the appearance of green intracytoplasmic inclusions in neutrophils.

**Keywords:** Green crystals, Ischemic colitis, Neutrophilic inclusions

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## INTRODUCTION

Bright blue–green inclusions within neutrophils on peripheral smears are both a rare and critical finding. To date, there have been nine reports encompassing 70 documented cases in the literature, all occurring within the last decade. Their presence on peripheral smears were initially linked to various etiologies of acute liver failure due to significant elevations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). In their retrospective study, Courville et al. (2017) proposed tissue injury as the mechanism leading to inclusion formation due to cases without liver enzyme elevation [1, 2]. Initial reports found that patients often expired within 72 hours after the appearance of aforementioned inclusions. The exact composition of these intra-neutrophilic inclusions is unknown. The leading postulation is that they are composed of lipofuscin, while other authors have speculated that they may be made of biliverdin, or even lysosomal degradation products [2, 3]. We report a case of gangrenous ischemic colitis with associated blue–green neutrophilic inclusions, in which the patient survived and made a complete recovery.

## CASE REPORT

A 75-year-old male with a past medical history of hypothyroidism, osteoporosis, and coronary artery disease presented to the local emergency department with a complaint of acute onset abdominal pain that he graded as 10/10. A computed tomography (CT) scan was performed and showed severe constipation. He was examined by the surgical team and was found to have a rigid abdomen. The patient subsequently became hypotensive and tachycardic and went into shock. He was taken to the operating room on the night of admission for an emergent laparoscopy for his acute abdomen, but suffered cardiac arrest during induction of anesthesia. After three rounds of epinephrine and cardiopulmonary

resuscitation (CPR) the patient returned to spontaneous circulation.

Eventually the patient underwent a total colectomy with partial small bowel resection. He was taken back to the intensive care unit (ICU), intubated, and sedated. He required multiple vasopressors to maintain perfusion. He was found to have an acute kidney injury with an elevated creatinine, lactic acid, and severe metabolic acidosis. The patient was given a bicarbonate drip. However, the patient's abdominal symptoms persisted and was found to have gangrenous ischemic colitis. He had to be taken back to the operative room two more times for further sigmoidectomy and partial small bowel resections on hospital days two and four, respectively. Peripheral smears were ordered by the surgical team throughout the patient's admission. Green inclusions were first visualized on hospital day four, after the patient's third surgery to remove necrotic bowel. His smears were positive for inclusions on subsequent hospital days five and six. After hospital day six, no further inclusions were identified on smears. After 30 days in the hospital the patient made a complete recovery and was discharged to home health.

## DISCUSSION

Rare coarse and bright blue–green intracytoplasmic inclusions have recently been described in the literature. It is likely that they are underreported given that they occur in less than 1.0% of neutrophils or monocytes on positive peripheral smears [2, 3]. The inclusions have an important refractive quality on Giemsa–Wright stains, which allows them to be distinguished from Döhle bodies [2]. In accordance with the literature, the inclusions were found on less than 1.0% of neutrophils on this patient's peripheral smear (Figure 1A–C) [4]. We did not observe any inclusions in monocytes, but did find toxic changes in numerous neutrophils (Figure 2). The patient had a peak white blood cell count of 14,000/mL with a differential of 74.3% neutrophils, 12.9% lymphocytes, 10.2% monocytes, 1.1% eosinophils, and 0.6% basophils, respectively.

The prognosis of these inclusions remains up for debate in the literature. The earliest case reports painted a dismal picture with all three patients expiring within 72 hours of their appearance on peripheral smear. Hodgson et al. documented 20 cases, of which 13 patients died. Of the 13 patients who expired, 12 passed away within 72 hours of identification, further proposing 72 hours as a "critical window" for patient survival [5]. In their retrospective analysis, Courville et al. (2017) showed a lower short-term mortality rate of 31% compared to the previous 68% found in the literature [2]. Elevated blood lactate levels have been shown to ported a poor prognosis with all patients displaying an arterial lactate >5.0 mmol/L expiring in previous reports [3]. Laboratory technicians, pathologists, and hematologists should be made aware of the existence of green neutrophilic inclusions due to the increased risk of mortality that they

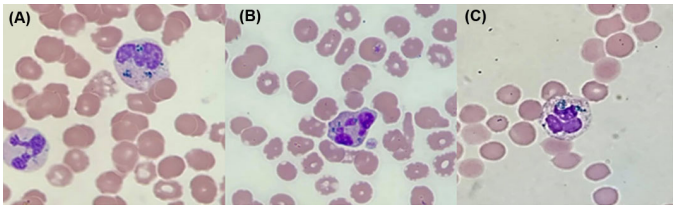


Figure 1: Representative cytoplasmic green inclusions in peripheral blood. Giemsa–Wright 1000× magnification.

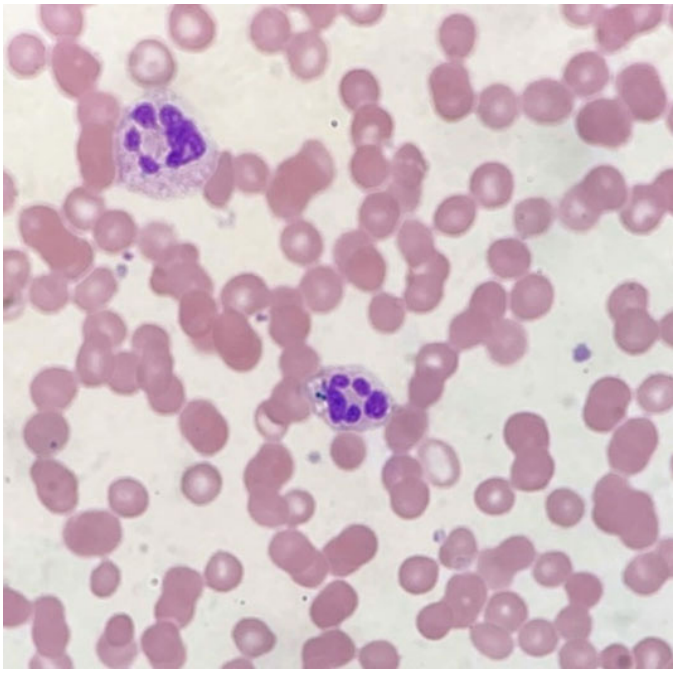

Figure 2: A neutrophil with toxic granulation near another neutrophil with green inclusions. Giemsa–Wright 1000× magnification.

pose. Unlike most patients with the appearance of green inclusions on peripheral smears, our patient has made a complete recovery.

Several authors have suggested etiologies of acute hepatic failure, such as acetaminophen overdose, vascular hypoperfusion, septic shock, Hepatitis D superinfection, Budd–Chiari syndrome, alcoholism, and cirrhosis as responsible for inclusion appearance [3, 6, 7]. Acute liver failure and/or elevation of liver enzymes were present in 24 of the first 25 reported cases [2]. However, Courville et al. (2017) propose tissue injury, with hepatic injury being one class of tissue injury, as the pathogenic mechanism that causes these inclusions to occur given that they found inclusions in four patients without significant elevation of hepatic enzymes [2]. Our case supports this proposition as the patient presented without significant liver damage due to ischemic colitis. This patient had only modest elevation of liver enzymes and ALP thus ruling out acute hepatic failure as an etiology. Several reports have also shown their patients to have comorbid infections at the time of inclusion appearance with associated microorganisms including *Escherichia coli*, *Clostridium difficile*, *Enteroccus*, and *Klebsiella pneumoniae* [2]. Our

patient also likely had an infection at the time of inclusion appearance given the gangrenous necrosis of his colon.

While the exact composition of these intracytoplasmic inclusions remains unknown, it is speculated that they are composed of lipofuscin or a related substance [2, 4, 6]. These inclusions have been noted to stain acid fast Ziehl–Neelson staining. Oil Red O has shown positivity in rare neutrophils, implying high lipid content and transmission electron microscopy studies have confirmed that the inclusions are rich in lipid content consistent with the lipofuscin hypothesis [3]. Inclusions have been demonstrated to have negative iron, myeloperoxidase, Gomori methenamine silver, Warthin–Starry, and Gram staining [3, 5, 8]. Liver autopsies of patients found with inclusions grossly demonstrated yellow–brown pigmentation, which is characteristic of lipofuscin [3, 5]. Giemsa staining of these liver specimens showed the same green pigmentation seen on peripheral smears. It is postulated lipofuscin released by tissue injury is taken up by phagocytes and is thus seen on peripheral smears [3]. As these inclusions are typically associated with serious liver destruction, markers of hepatic damage such as AST and ALT values are often seen in the thousands, with additional liver markers such as ALP and GGT typically elevated [3, 5, 6]. As mentioned previously, blood lactate is usually elevated (93% of patients) and carries a poor prognosis [3, 5]. Lactate dehydrogenase (LDH) elevation, possibly due to tissue necrosis, is often seen as well [3]. As our patient suffered intestinal tissue damage, he had only a modest elevation of liver enzymes with peak AST, ALP, and ALT recorded at 51, 141, and 58 IU/L, respectively. Our patient had an arterial lactate level of 12.2 mmol/L one day after admission. Unfortunately, LDH levels were not drawn in our case. This patient also displayed an elevated neutrophilic-predominate white count.

## CONCLUSION

In conclusion, bright–green intracytoplasmic inclusions within phagocytic leukocytes are a rare phenomenon. While previously associated with acute liver failure, our case demonstrates that they can also be seen in ischemic gastrointestinal disorders. As bright–green inclusions are rare and pose an increased risk of mortality, we advocate for more technician, pathologist, and hematologist awareness of this finding so clinicians can be warned to prevent possible clinical deterioration. Increased awareness will also lead to increased detection of this rare phenomenon and allow us to further elucidate the pathophysiology behind inclusion appearance as well as their biochemical composition.

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### Author Contributions

Mason Marshall – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Isabel Rios – Acquisition of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Written informed consent was obtained from the patient for publication of this article.

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Authors declare no conflict of interest.

## Data Availability
All relevant data are within the paper and its Supporting Information files.