

HAVE YOU SEEN A “DEATH CRYSTAL?”

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Wisconsin Clinical Laboratory Network Presentation

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NEUTROPHILIC INCLUSIONS...WHY?!

Presentation Goals

Common Neutrophilic Inclusions:

- ✓ Morphology
- ✓ Composition
- ✓ Formation Mechanism

Less Common Neutrophilic Inclusions:

- ✓ Current Literature Findings

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(b). Immature forms include bands, metamyelocytes, myelocytes and promyelocytes. Blasts are rarely seen with infection, with the exception of infants that already display a left shift upon birth.

4. In addition to an increased # of immature neutrophils, other changes to the neutrophils are observed with infection. These changes are called **TOXIC CHANGES**.

B. Neutrophil Toxic Changes

Left Shift

(a). Increased release of immature neutrophils (primarily metamyelocytes and bands) into the blood.

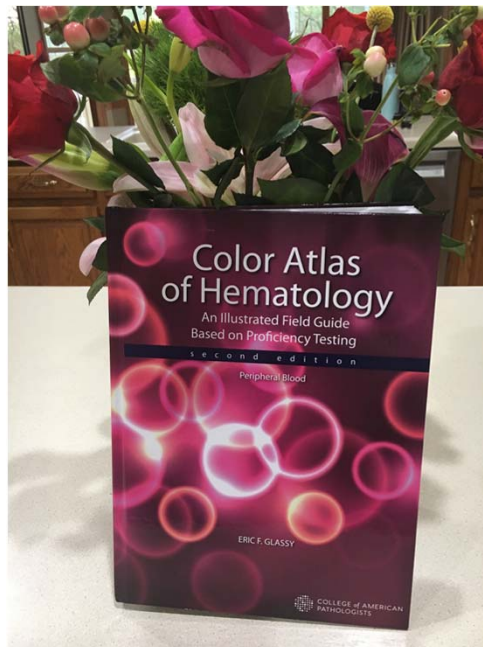
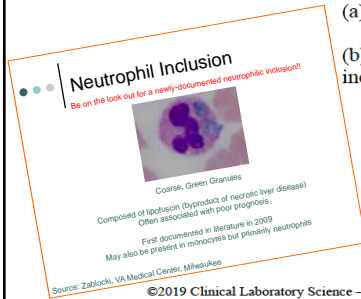
Toxic Granulation

(a). Darkening of neutrophil granules (often purple).

(b). Increased synthesis of granule enzymes; therefore, results in increased stain intensity.

(1). Often due to an increase concentration of **myeloperoxidase** (1^o granule enzyme).

(2). Myeloperoxidase is acidic and binds basic dyes (increased myeloperoxidase results in increased purple staining).

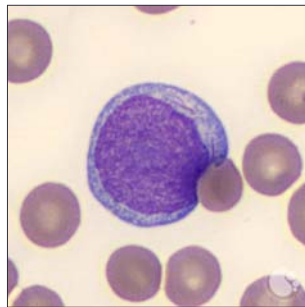


Neutrophilic Inclusions

- Auer Rod ✓
- Toxic Changes ✓
- Ingested Material (microorganisms, crystals, RBC, etc.)

-
-
- Green (Blue-Green) Neutrophilic Inclusion-"Death Crystal" ✓
 - Howell-Jolly Body-Like Neutrophilic Inclusion ✓

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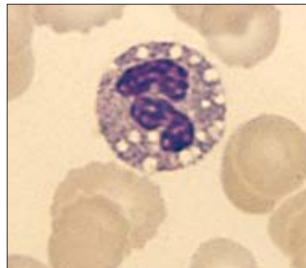
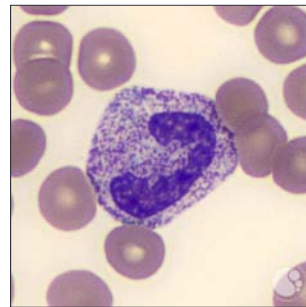
Can you match?

Dohle Body

Toxic Granules

Toxic Vacuoles

Auer Rod

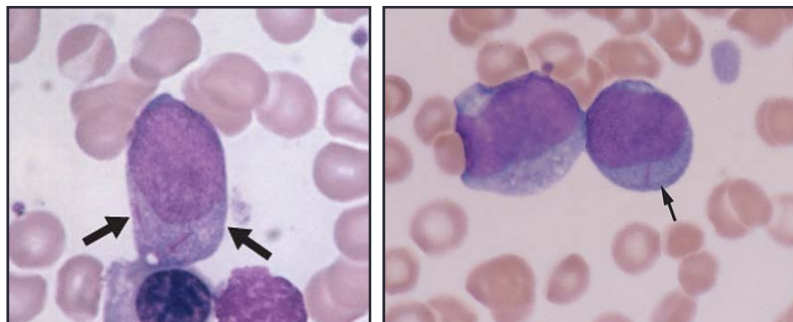


Common Neutrophilic Inclusions

ASH Image Bank

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Auer Rod: Composition/Morphology



Fused 1° (Azurophilic) Granules

- Needle-shaped (comma or diamond)
- 0.2 – 5 um in length
- Highly associated with AML
- May see 1-2 in cell; increased # associated with AML-M3

FA Davis, Harmening, 5th Edition

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John Auer

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AUER: ACUTE LEUKEMIA

SOME HITHERTO UNDESCRIBED STRUCTURES FOUND IN THE LARGE LYMPHOCYTES OF A CASE OF ACUTE LEUKEMIA.

By JOHN AUER, M.D.,

FORMER HOUSE OFFICER OF THE JOHN HOPKINS HOSPITAL; FELLOW OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.

BEFORE entering upon a description of one of the features which marked the blood picture of this case, it will be necessary to give a short clinical history.

T. W., a young man, aged twenty-one years, was admitted April 25, 1903, to Dr. Osler's wards in the Johns Hopkins Hospital, complaining of sore throat and nose-bleed.

Family History. Negative.

Patient's History. Patient had served three years in the U. S. Army in the Philippines, his service ending shortly before October, 1902. While a soldier he had had a severe tonsillitis, for which hospital treatment was found necessary. There was also a history of gonorrhoea. Otherwise negative.

Present History. Began in the middle of March, 1902, with a severe tonsillitis. The throat condition persisted and the patient gradually lost strength. On April 18th he had a severe nose-bleed, which lasted several hours, according to the patient. Since that time there has been nose-bleed at intervals up to admission into the hospital.

On examination the patient looked ill; not emaciated; color sallow; mucous membrane very pale. No jaundice. Teeth in good condition. Gums not spongy. Both tonsils markedly enlarged, almost meeting in the median line; the left showed an area of ulceration. Soft palate showed small areas of hemorrhage.

Neck swollen, due chiefly to enlarged glands. Over trunk, arms, and legs there was an abundant purpuric eruption, most of which was fresh. There was general glandular enlargement.

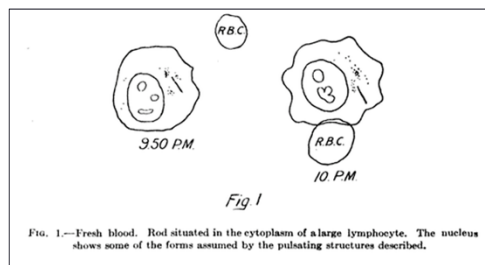


FIG. 1.—Fresh blood. Rod situated in the cytoplasm of a large lymphocyte. The nucleus shows some of the forms assumed by the pulsating structures described.

these cells looked somewhat like myelocytes. In a few of the large lymphocytes the cytoplasm showed large, refractile, rounded bodies; in others a refractile, rod-like body was present. In the nuclei of practically all the large lymphocytes a number of contractile, pulsating, clear areas were noticeable; in one instance pulsating structure.

It was difficult to determine the shape of the large, refractile granules, for it seemed to vary as the granules moved with the cytoplasmic currents (Fig. 2). Some appeared round, others pear-shaped, still others seemed to be composed of two parts linked together in some ways, for they moved as a whole. These large granules changed their position in the cytoplasm, and relatively to each other, due probably to the cytoplasmic currents. In one instance a few of the granules at the same pole of an otherwise normal appearing large lymphocyte moved much more actively than the rest, rotating rapidly on their axis. It was found that there was friction between other granules on their way

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Thomas McCrae

ACUTE LYMPHATIC LEUKAEMIA.

WITH THE REPORT OF FIVE CASES.
By THOMAS McCRAE, M.D., M.R.C.P.,
The Johns Hopkins Hospital, Baltimore.

[From the Clinic of Professor Osler.]
In the fifteen years since the opening of the Johns Hopkins Hospital, among 17,100 medical admissions there were 37 cases of leukaemia. Of these, 24 were of the spleno-myelogenous type and all chronic. Among the 13 cases of lymphatic leukaemia 5 were acute. The histories of these cases are as follows:

1. A. H., male, white, tailor, aged 15 years, was admitted on November 25th, 1897, complaining of headache and weakness. The family history was negative. From the age of 4 to 4 years he was sickly, but since then strong and well. He had measles at 5, but no other infectious disease. He had not had tonsillitis, but had been subject to attacks of epistaxis. The present illness began on November 15th, 1897, with headache and general malaise. He kept at work until November 22nd, when, on account of weakness, he had to go to bed. His throat was sore and he had some difficulty in swallowing. He felt feverish. The next day there was nose-bleed, which was severe and lasted for four hours. He

soon passed to stupor, anuria, convulsions, and death; duration about one month. (This case was reported in full in the *Johns Hopkins Bulletin*, No. 110, May, 1900, p. 100. Only a short summary is given here.)

J. L. was admitted on May 11th, 1898. There is nothing of moment in his history, and he did not complain of any serious symptoms. He had been troubled with adenoids, and for a few days had seemed rather easily tired. On examination he was found to be very pale; there was marked swelling of the tonsils and large adenoids. The thorax was clear; liver and spleen were enlarged; there was no glandular enlargement. He had slight irregular fever; the pulse and respirations were rapid. The blood examination showed 35 per cent. of haemoglobin, 1,600,000 red cells and 25,000 white cells per c.mm. The differential count of 1,000 leucocytes showed 13 per cent. of polymorphonuclears, 86.5 per cent. of lymphocytes of which 45 per cent. were large, 41.5 per cent. small. The red cells showed no special changes; no nucleated reds were found. The lymphocytes showed all variations in size, from the smallest to some which were 15 microns in diameter. The general staining was pale. There were, however, a few small lymphocytes with a typical deeply-staining nucleus. Subsequent counts showed an increasing percentage of small lymphocytes. On May 19th the lymphocytes were 99.5 per cent., of which 26.6 per cent. were small, and only 2.6 per cent. large. Death occurred on May 20th, following convulsions. The duration of the attack was probably about four weeks.

4. T. W., male, white, clerk, aged 21 years, was admitted on April 25th, 1903, complaining of sore throat and bleeding from the nose. His family history was negative. There was a

At times, with both the Ehrlich and Wright stains, some of the large mononuclears showed red-staining bodies in the cytoplasm. These were at times rodlike, again broken up into smaller fragments, with rounded ends, and some formed only a red granular mass.

The course was progressively downward. The blood showed on May 13th the small red-staining rods in nearly every large mononuclear and in some of the small mononuclears. The differential count showed much the same percentages as noted before. There were all sizes of mononuclears. On May 14th

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Auer Rod: HISTORY



Dr. John Auer



Dr. Thomas McCrae

Auer Rod: History

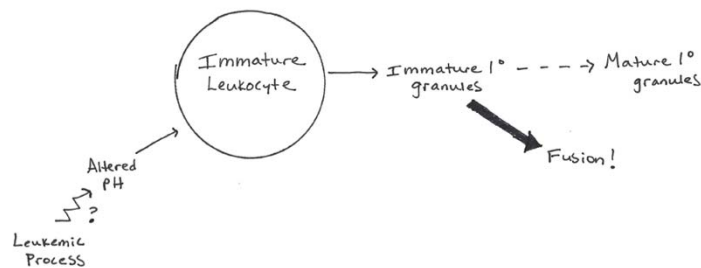
- Fusion of 1° (azurophilic) cytoplasmic granules
- 1st described in 1905-1906
- Hypotheses of composition:
 - “Data are only strong enough to render a comparison justifiable between human intracellular parasites and leukemia granules” (Auer)
 - Infectious Microorganisms
 - Nuclear Fragments
 - Granules ✓
- Hypotheses of formation:
 - Altered cytoplasmic pH ✓?

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Auer Rod: Mechanistic Hypothesis (Ackerman, 1950)

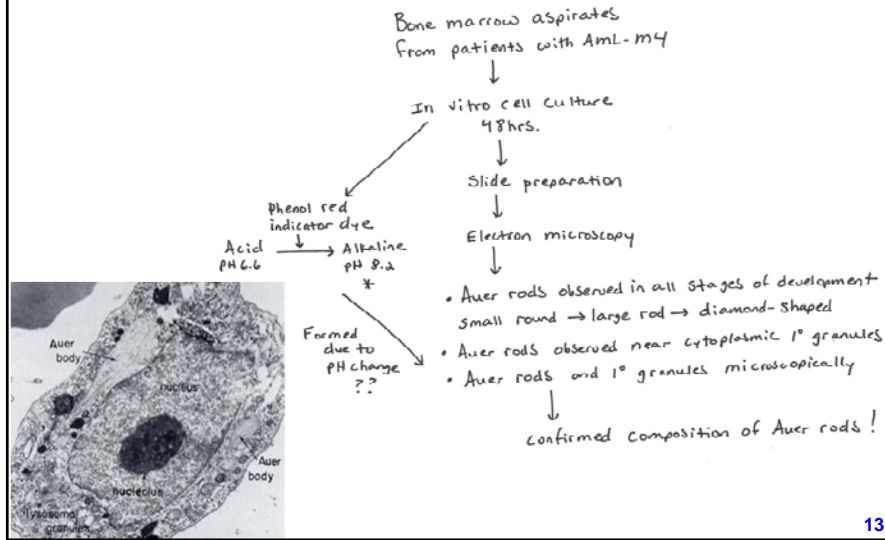
Why do 1° granules fuse in leukemic cells?

A change in pH in immature leukemic cells initiate an alternate pathway of granule maturation that leads to fusion.



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Auer Rod: Mechanistic Hypothesis (Freeman, 1966)



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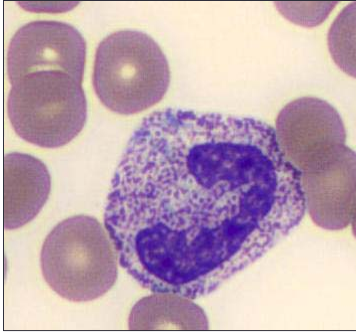
Neutrophilic Inclusions

- Toxic Changes

Dohle Bodies
 Toxic Gran
 Toxic Vac

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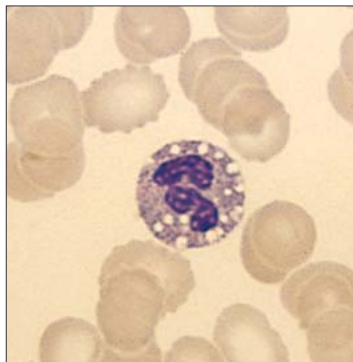
Toxic Granules: Composition/Morphology



1° (Azurophilic) Granules

Larger and more deeply stained 1° granules
Purple or dark blue granules throughout cytoplasm

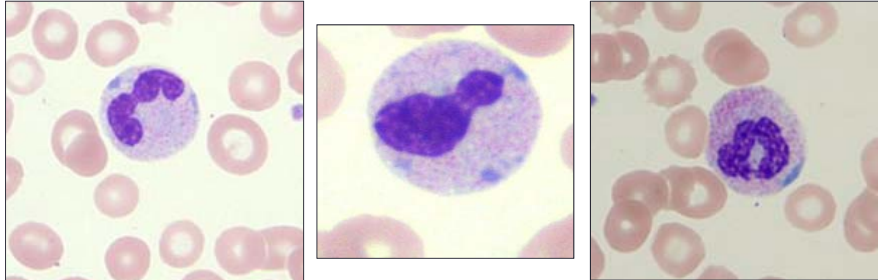
Toxic Vacuoles: Composition/Morphology



Round, clear spaces in cytoplasm

Dr. Karl Döhle

Dohle Bodies: Composition/Morphology



Rough Endoplasmic Reticulum Remnants

Single or multiple blue-gray, irregular inclusion
Often at the periphery

ASH Image Bank; CAP Hematology 2nd Edition; FA Davis, Harmening 5th Edition

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“Toxic” Changes: History

“Toxic Granules” and “Toxic Vacuoles” were
1st described in patients with sepsis and endotoxemia

Endotoxemia = “The presence of endotoxins in the blood”

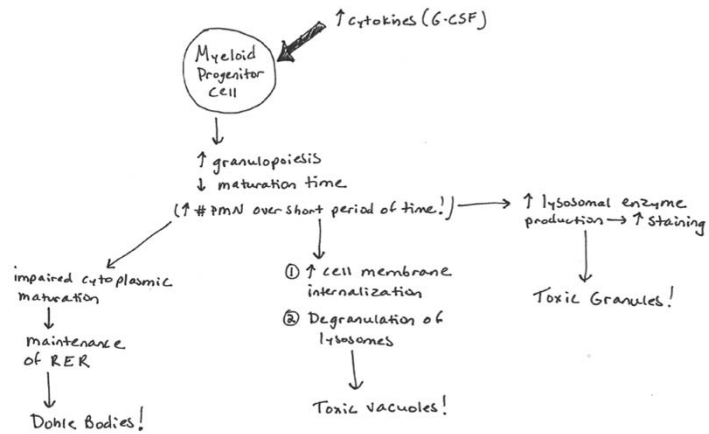
Endotoxins = “A toxic substance present in the outer membrane of gram negative bacteria that is released from the cell upon lysis”

BUT....they are not due to toxic effects of a microorganism on the neutrophil but rather morphological changes due the maturation of the neutrophil



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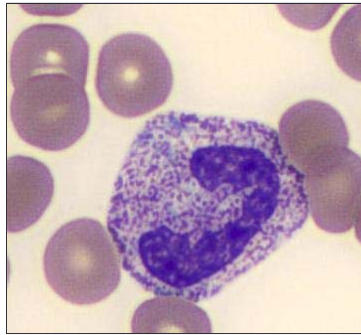
Toxic Changes: Mechanism



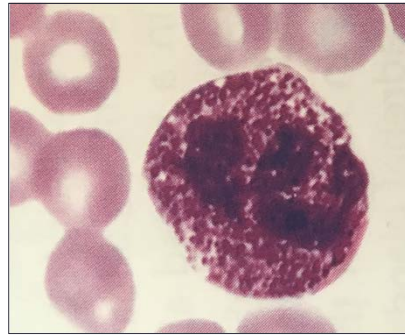
Toxic Changes vs. Anomaly Inclusions

Toxic
Gran
vs.
Alder
Reilly

Toxic Granulation vs. Alder Reilly?



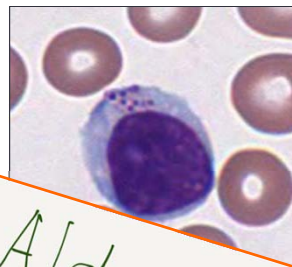
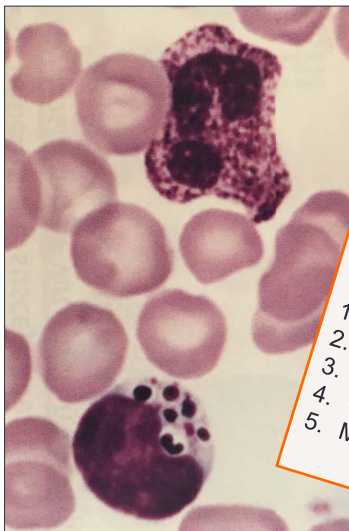
?



ASH Image Bank; CAP Hematology 2nd Edition

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Alder Reilly



- Alder Reilly
1. Granules found in all WBCs
 2. Granule "halos" may be seen
 3. No other toxic changes
 4. Uniform
 5. Mucopolysaccharides

CAP Hematology 2nd Edition

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Toxic Changes vs. Anomaly Inclusions

Dohle
Bodies
vs.
May
Hegglin

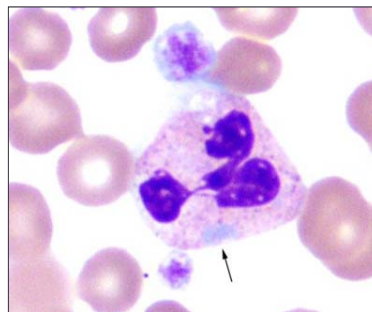
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Dohle Bodies vs. May-Hegglin?



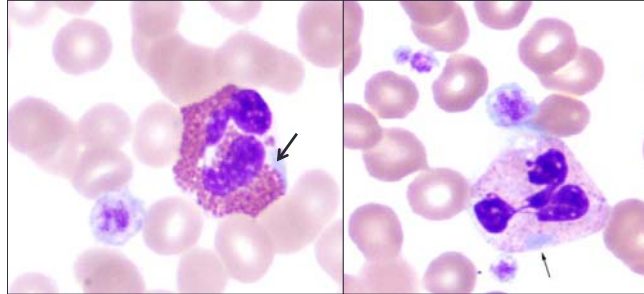
Dohle Body

?



Dohle-Like Body

May-Hegglin



May Hegglin

1. Seen in all granulocytes and some monocytes
2. Platelet abnormalities
3. More basophilic and distinct
4. Ribosomes/endoplasmic reticulum—different arrangement!

CAP Hematology 2nd Edition

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Anomaly Mnemonics

Hereditary conditions that can alter WBC morphologies; “Unexpected or Unusual” WBC morphology; Typically function normally

Mnemonics to remember the anomalies:

P elger Huet	P ince-nez (<u>hypo-segmented neutrophils</u> ; glasses without earpieces)
A lder-Reilly	A ll over 1° <u>granules made of excess mucopolysaccharides</u>
C hediak-Higashi	C hunky granules; C hocolate Chips; <u>fused 1° and 2° granules</u>
M ay Hegglin	I n M ay there are blue skies (<u>dohle-like bodies</u>) and large raindrops (<u>giant platelets</u>)



Dr. Everard-Gigot, Marquette University

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NEWLY DOCUMENTED

Green Neutrophilic Inclusion “Death Crystal”

Holly-Jolly Body-Like Neutrophilic Inclusion

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Green Neutrophilic: Composition/Morphology



Lipofuscin-Like Material

- Coarse bright green-blue inclusions
- Variable in size and irregular shape
- “Blocky with ill-defined borders”
- Often refractile
- Often >1 per cell
- Often found in small % of neutrophils
- (May be found in monocytes but primarily in neutrophils)

Permission of Zablocki VA Medical Center, Milwaukee

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Green Neutrophilic: History

Harris, V., et al (2009) Green neutrophilic inclusions in liver disease, *Journal of Clinical Pathology*

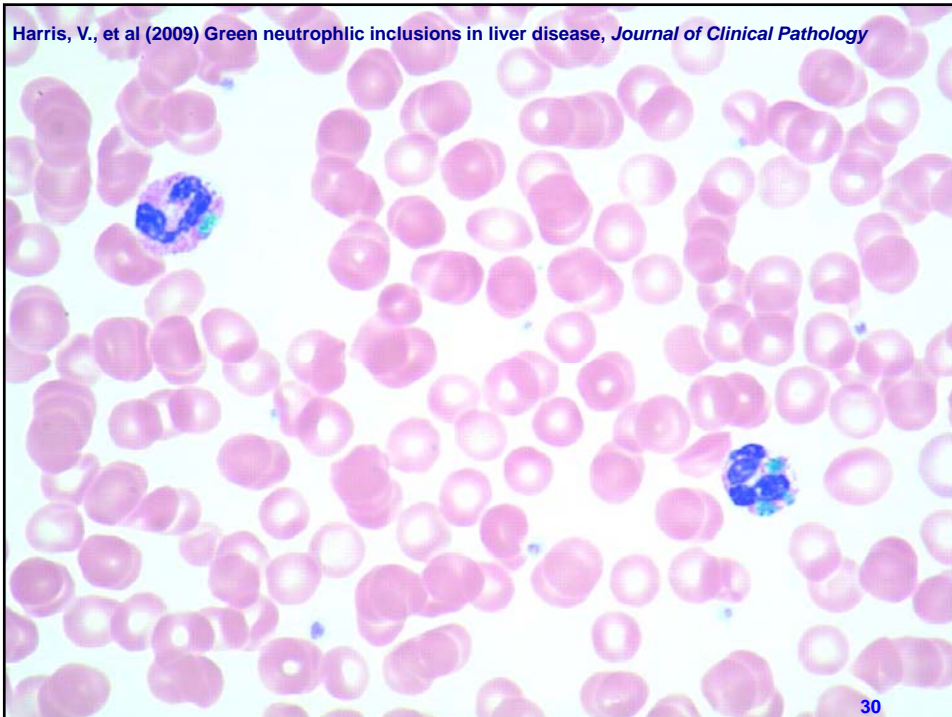
- First documentation of green inclusions + LIVER DISEASE
 - 2 Patients
 - Acute liver failure + inclusions + death within 24 hours

	Patient #1	Patient #2
Diagnosis	Acute Liver Failure 2° to Acetaminophen-Overdose	Trauma-Liver Laceration and Organ Failure
Patient History	Deceased 1 day after admittance	Deceased 1 day after liver enzyme elevation
Significant Smear Findings	Green inclusions-neutrophils (<10%) Toxic granulation/vacuolation	Green inclusions-neutrophils (<10%) Toxic granulation/vacuolation

- Suggested:
 - Inclusions directly related to liver failure
 - "Prognostic indicator of impending death"
 - Composition??
 - "Blood-borne bile product" such as biliverdin
 - Hall's bilirubin stain = negative

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Harris, V., et al (2009) Green neutrophilic inclusions in liver disease, *Journal of Clinical Pathology*



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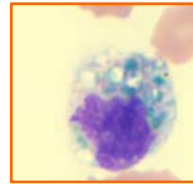
Green Neutrophilic: History

Jazaerly, T., et al (2014) Green neutrophilic inclusions could be a sign of impending death, *Blood*

- Continued evidence of inclusion + liver disease + death within hours

	Patient
Diagnosis	Urinary Tract Infection-Sepsis
Patient History	Elevated liver enzymes Deceased 2 days after admittance
Significant Smear Findings	Green inclusions-neutrophils (<15%) Toxic granulation/vacuolation/dohle bodies

- MPO Negative
- Bilirubin stains Negative

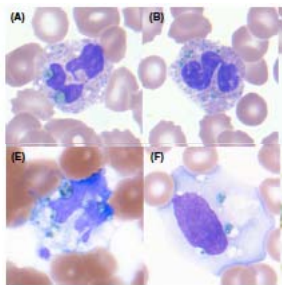


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Green Neutrophilic: History

Hodgson, T., et al (2015) Green neutrophil and monocyte inclusions – time to acknowledge and report, *British Journal of Haematology*

- Evidence that inclusions are not always fatal!
 - 20 patients
 - Green inclusion found in neutrophils AND monocytes
 - Strong association with critical illness = "Critical Green Inclusions"

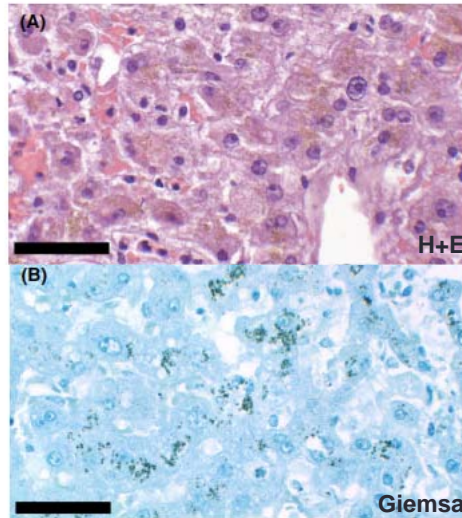


	20 Patients
Patient History	65% deceased within days of detection Detected in 2 patients post-liver transplant (patients lived) 19/20 patients had elevated ALT/AST 24 hours prior to inclusion detection Lactic acidosis correlated with death rates
Significant Smear Findings	90% found in neutrophils 10% found in monocytes Toxic granulation/vacuolation/dohle bodies

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Green Neutrophilic: History

Hodgson, T., et al (2015) Green neutrophil and monocyte inclusions – time to acknowledge and report, *British Journal of Haematology*

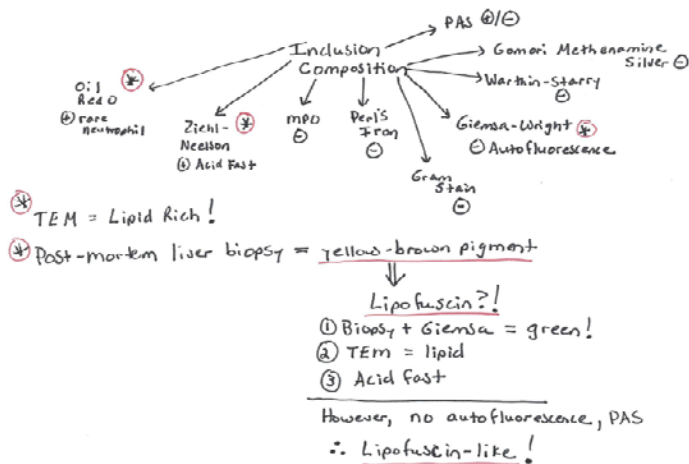


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Green Neutrophilic: History

Hodgson, T., et al (2015) Green neutrophil and monocyte inclusions – time to acknowledge and report, *British Journal of Haematology*

COMPOSITION?!



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Green Neutrophilic: History

Hodgson, T., et al (2015) Green neutrophil and monocyte inclusions – time to acknowledge and report, *British Journal of Haematology*

LIPOFUSCIN

Yellowish brown, auto fluorescent, and lipid-containing insoluble pigment that accumulates in the cytoplasm of aging cells.

Common in aging heart and liver tissue.

2° to lysosome and mitochondria damage.

Oil Red O	Positive
Sudan III	Positive
Acid Fast	Positive
PAS	Positive

Advances in Cellular Neurobiology, 1981
Marani, E. et al, *Encyclopedia of Neuroscience*, 2009

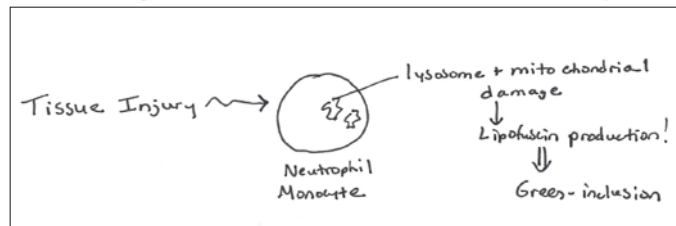
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Green Neutrophilic: History

Courville, E., et al (2016) Green neutrophilic inclusions are frequently associated with liver injury and may portend short-term mortality in critically ill patients, *American Society for Clinical Pathology*

- University of Minnesota Study
 - Analysis of past patient records over a 5 year time span
 - Inclusion associated with high mortality rate BUT also found in:
 - Healthy Individual
 - Individuals with normal liver function

Lipofuscin-like pigment due to “TISSUE INJURY” not solely liver damage



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Who coined "Death Crystal?!"

ASCLS, May 2018

A case of blue-green neutrophil inclusions (AKA Death Crystals)

S. Renee Hodgkins, PhD, MT(ASCP); Jennifer Jones, MLS(ASCP)CM



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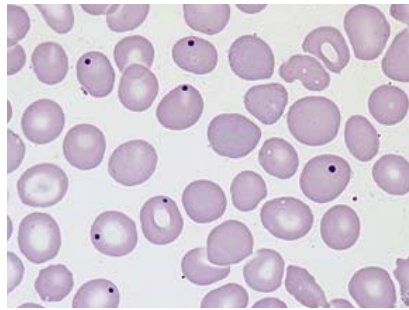
New name?
Have you observed?



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Howell-Jolly Body

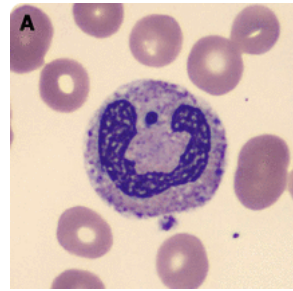
Howell-Jolly Body



Nuclear Remnant

Purple sphere(s) within a RBC

Howell Jolly Body-Like Inclusion



Nuclear Remnant??

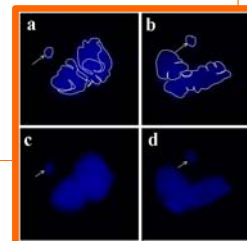
Purple sphere(s) within a neutrophil

ASH Image Bank

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Howell Jolly-Like: History

- First described in 1989
- Associated with:
 - HIV patients
 - Immunosuppressant, anti-viral medication, chemotherapy
- Named based on appearance but composition confirmed
 - Nuclear fragment? ✓
 - DAPI DNA Stain Positive (Omman, et al, 2017)
 - Feulgen Stain Positive (Slagel, et al, 1992)

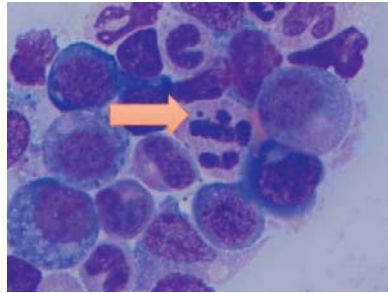
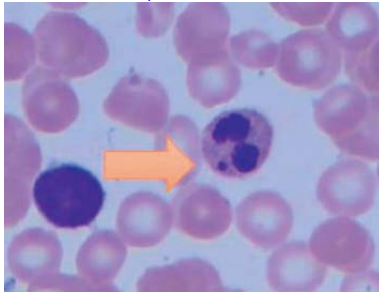


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Howell-Jolly Body-Like: History/Mechanism

Tong, Y., et al, (2019) Howell-Jolly Body-like inclusion in neutrophils in patients with Myelodysplastic Syndrome, *Arch Pathol Lab Med*

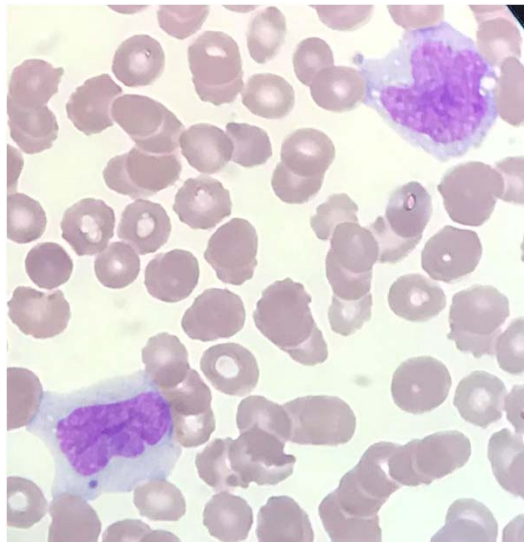
- Most recently, identified in patients with Myelodysplastic Syndrome
 - 11 patients (no history of other underlying inclusion associations)
 - 72% displayed inclusion
 - <5% of neutrophils



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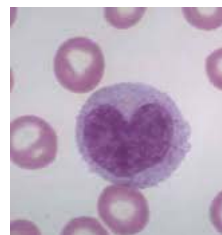


Patient history of CMPD



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Thank You



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