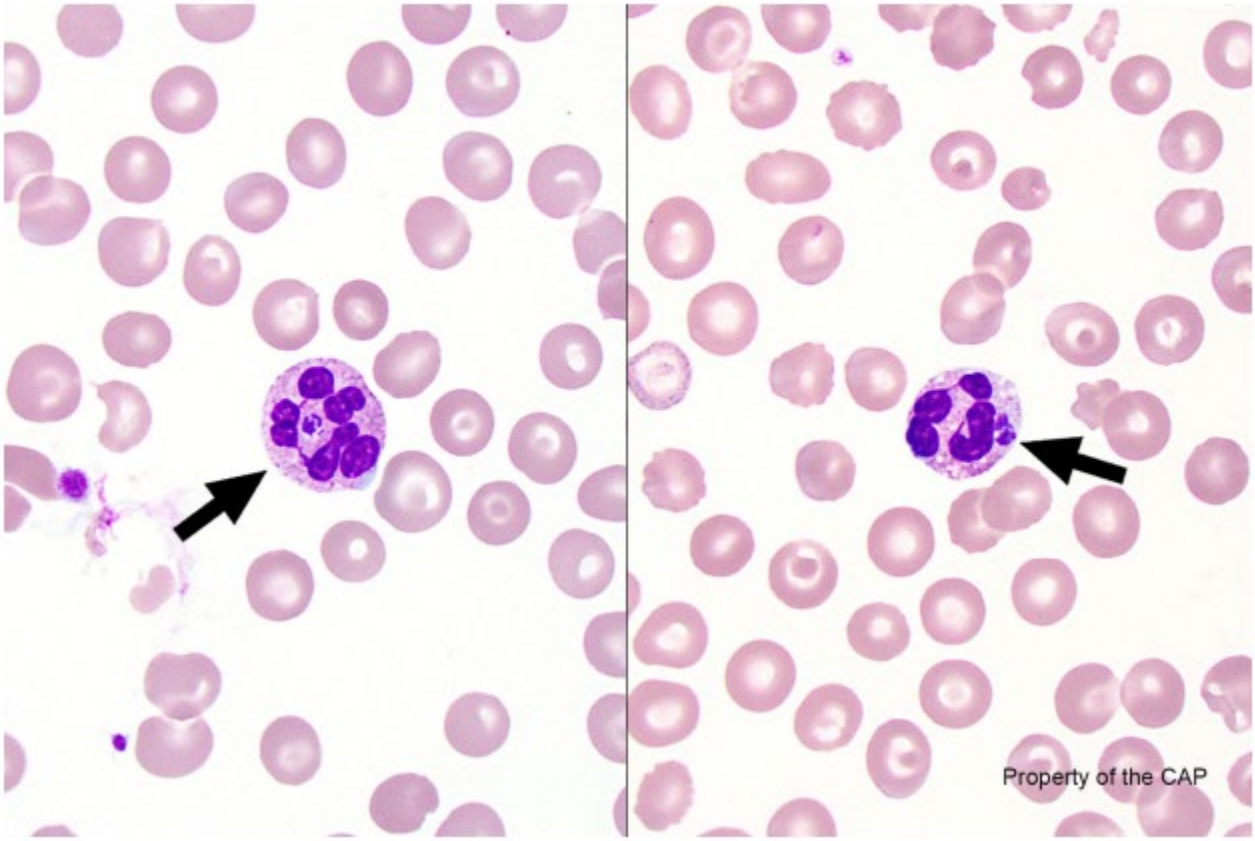
The following is an educational review for a missed image from the hematology survey FH9-B. Please read this review document and complete the very short quiz following.

**Case History**
This peripheral blood smear is from an 80-year-old man from the northeastern US with a tick bite. Laboratory data includes: WBC = 8.1 x 10E9/L; RBC = 4.51 x 10E12/L; HGB = 13.5 g/dL; HCT = 41.1%; MCV = 88 fL; PLT = 50 x 10E9/L; and RDW = 14%.

**BCP-18**




**Identify the cell or cellular element seen in this picture.**

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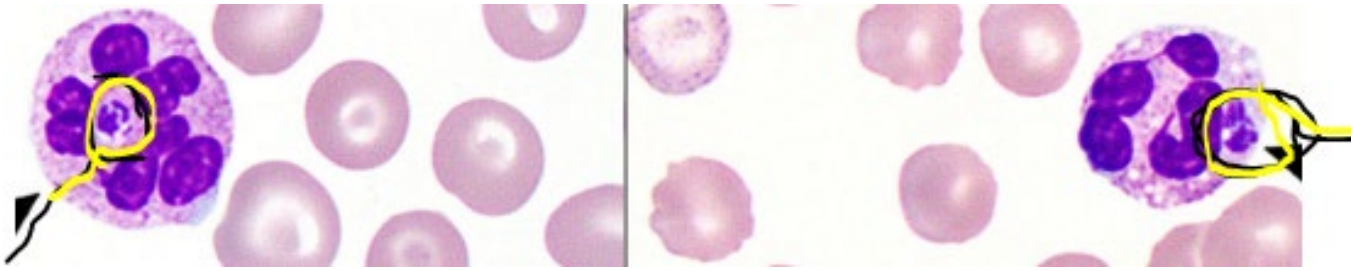
Before you get the answer, look at the clue that CAP hid in the case history: "with a tick bite." Do you have another guess that is NOT a hypersegmented neutrophil? Examine the enlarged images below.



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**Leukocyte with Intracellular *Anaplasma/Ehrlichia***

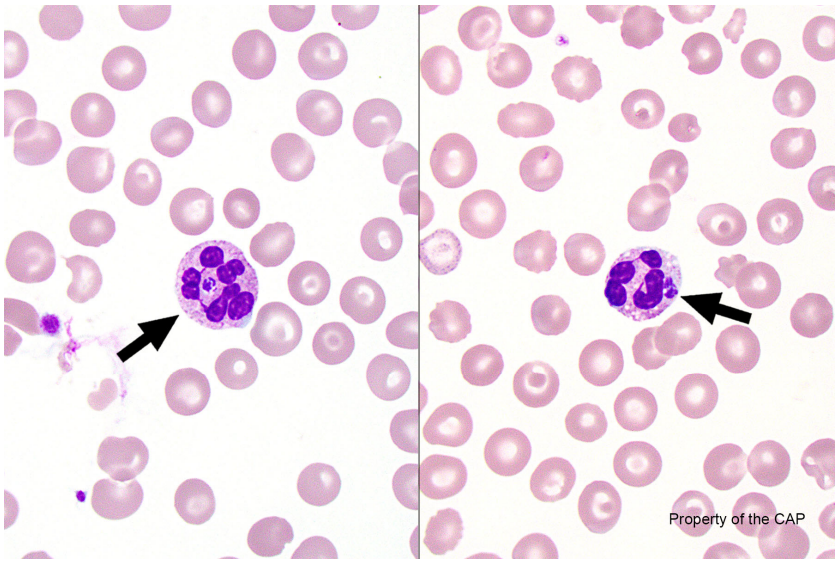
Recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative, obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium)* or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of organisms.

It was a bit unfair of CAP to put a hypersegmented neutrophil in the images when they wanted the answer of intracellular anaplasma/ehrlichia. A majority of survey participants also gave this "wrong" answer, just as we did. The takeaway thought I want us to have is to intake ALL clues offered when deciding an identification.

More importantly, we can put this into practice. If you have a manual differential or slide review on a patient complaining of "tick bite," take an extra minute or two the examine the neutrophils to look for these inclusions. Be wary, also, as they stain purple like the nucleus. Send for path review if suspected.

## Blood Cell Identification – Ungraded

### BCP-18




| Identification | Referees | | Participants | | Evaluation |
|---|---|---|---|---|---|
| | Freq | % | Freq | % | |
| Neutrophil with hypersegmented nucleus | 125 | 68.7 | 3671 | 68.2 | Educational |
| Leukocyte with intracellular *Anaplasma/Ehrlichia* | 42 | 23.1 | 1345 | 25.0 | Educational |
| Neutrophil, segmented or band | 8 | 4.4 | 185 | 3.4 | Educational |
| Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization) | 3 | 1.5 | 69 | 1.3 | Educational |
| Immature or abnormal cell, would refer for identification | 2 | 1.1 | 28 | 0.5 | Educational |
| Eosinophil, any stage | 1 | 0.6 | 4 | 0.1 | Educational |
| Parasite(s) seen, referred for definitive identification | 1 | 0.6 | 31 | 0.6 | Educational |

The arrowed cells are neutrophils with intracellular/intracytoplasmic *Anaplasma/Ehrlichia*, as correctly identified by 23.1% of referees and 25.0% of participants. Recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative, obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium*) or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of organisms.

The arrowed cells were incorrectly identified as neutrophils with hypersegmented nucleus by 68.7% of referees and 68.2% of participants. Although one of these cells depicted shows more than six nuclear lobes, both demonstrate cytoplasmic morulae and thus, the more specific and correct response is “leukocyte with intracellular *Anaplasma/Ehrlichia*.”

A hypersegmented neutrophil necessitates that the neutrophil demonstrates six or more nuclear lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis which occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as

vitamin B12 and folate, and cases in which patients are receiving a nucleotide analog drug (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for the treatment of neoplastic or rheumatologic conditions. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms.

The arrowed cells were incorrectly identified as neutrophils, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization by 1.5% of referees and 1.3% of participants. Despite some toxic changes, there are microcolonies of organisms (morulae) in the cytoplasm of the neutrophils, thus the more specific and intended response is "leukocyte with intracellular *Anaplasma/Ehrlichia*." Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding. Either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization; in this context, only a few, small, punched out appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round or elongated or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF), and they indicate a shortened maturation time and activation of post-mitotic neutrophil precursors.

4.4% of referees and 3.4% of participants correctly identified the cell as a neutrophil (segmented or band) but failed to identify intracellular/intracytoplasmic *Anaplasma* sp.

1.1% of referees and 0.5% of participants identified the cell as an immature/abnormal cell, which is an acceptable answer for laboratories that always refer abnormal cell identification to an outside laboratory.

Here are a few more images of Anaplasma/Ehrlichia inclusions for reference.