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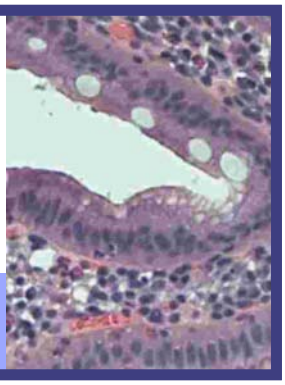
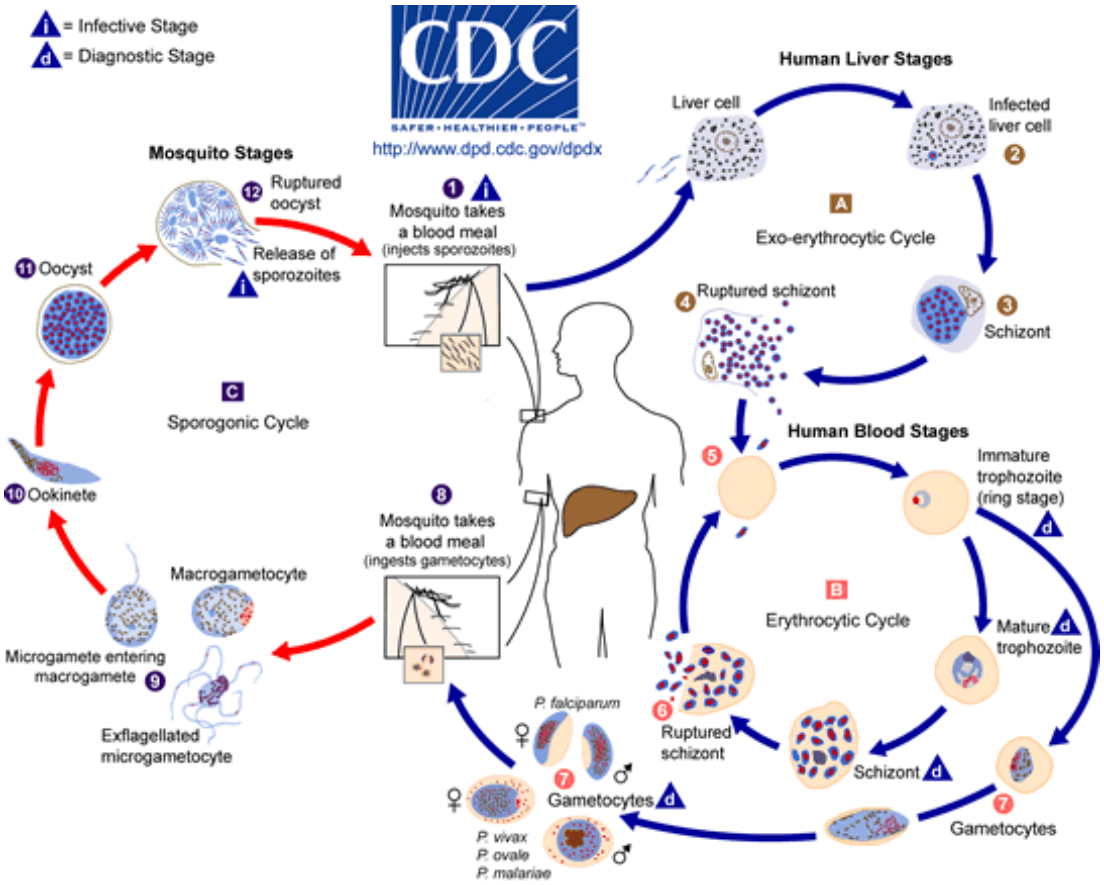


Some Fresh Air Concerning Malaria

Malaria remains as one of the leading causes of morbidity and mortality worldwide. Malaria affects up to 500 million people annually and causes between one and two million deaths, predominantly in sub-Saharan African children.[5] In the United States, endemic malaria was eradicated by 1947 thanks to work done by the Tennessee Valley Authority and the Public Health Service. However, with increasing world travel, the number of cases in the U.S. has risen in recent decades. In 2002, 1,337 cases of malaria were reported to the CDC occurring in 854 U.S. citizens and 483 foreign nationals.[5] All but five cases were imported.

The term malaria is from the Italian for "bad air" as the illness was associated with swamps and tropical climate. The disease has been described in historical documents dating back almost 5000 years. In 1880 a French army surgeon, Charles Louis Laveran demonstrated parasites in the blood of patients suffering from malaria. In 1897 the life cycle of malaria was revealed when it was demonstrated that mosquitoes were a necessary stage for parasite development and transmission (figure 1).

Figure 1:



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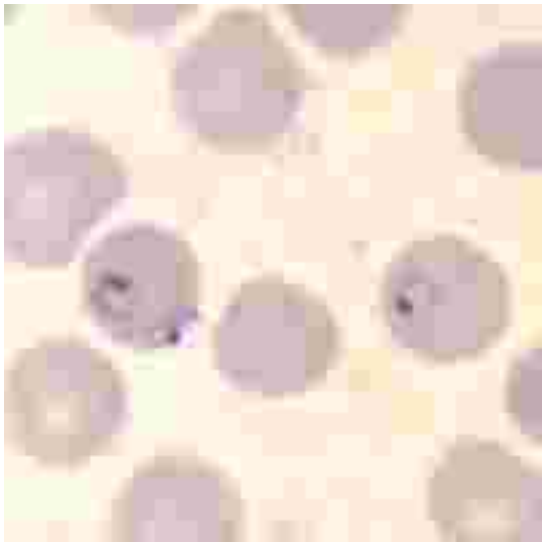
Malaria is caused by four species of the protozoa *Plasmodium*. *Plasmodium falciparum* causes the most severe and life-threatening form of the disease and accounts for nearly all deaths attributed to malaria. This species can propagate to very high densities in the blood stream, and cause marrow suppression, central nervous symptoms, metabolic acidosis, and respiratory distress. *P. vivax* and *P. ovale* cause less severe symptoms than *P. falciparum* because of the lower rates of parasitemia (less than 1% of red cells infected) and lack of associated microvascular sequestration and systemic cytokine effects. Both *P. vivax* and *P. ovale* can have late relapses due to hypnozoites that remain in the liver. This requires additional treatment (discussed later) in addition to blood stage treatment. *P. malariae* is the least commonly isolated species in the U.S. It presents with a three-day periodicity (quartan malaria) and has low levels of parasitemia, often below the level of microscopic detection.

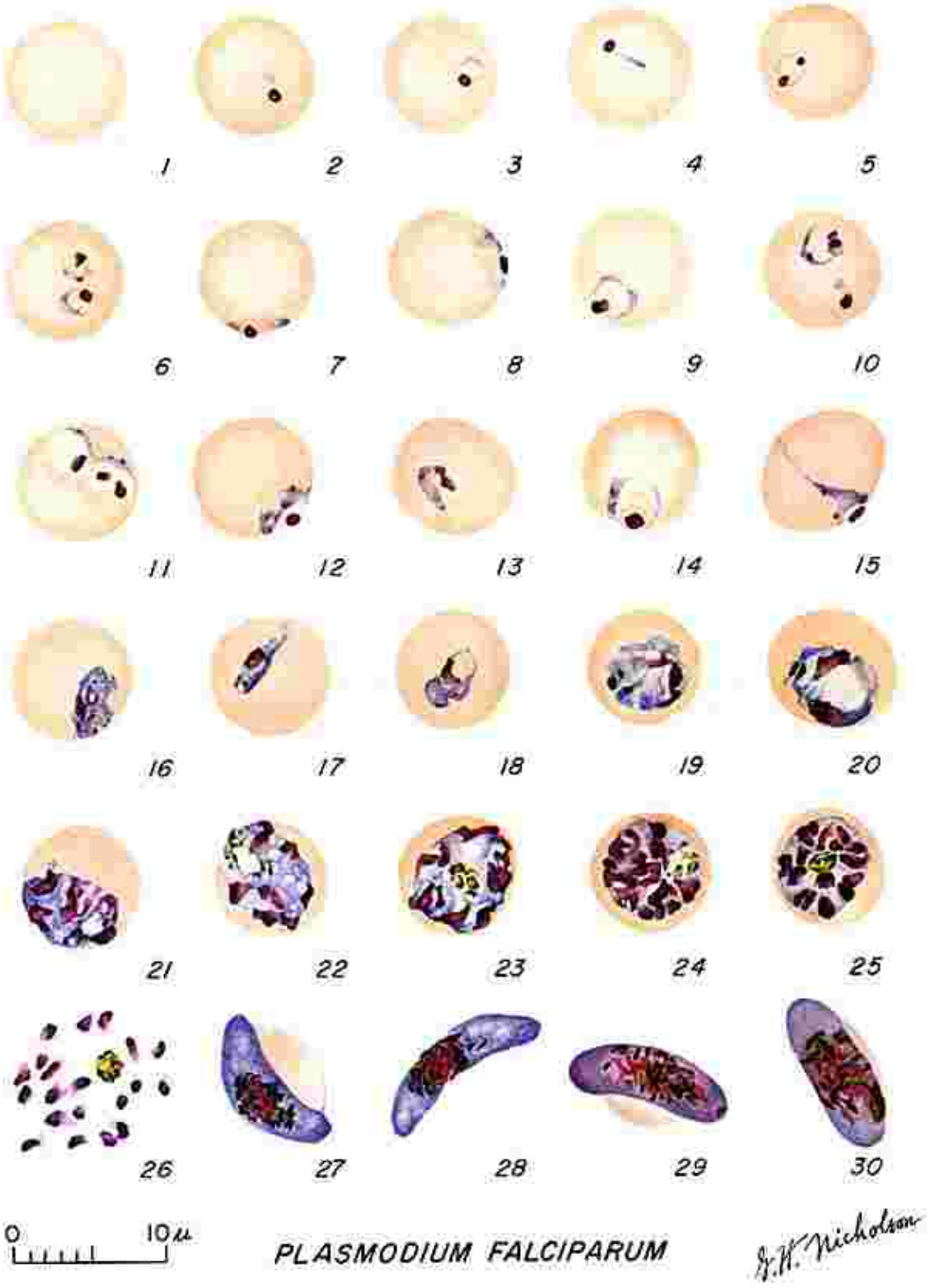
The method of diagnosing malaria has remained unchanged for the last century. The gold standard continues to be thick and thin blood smears examined by a pathologist or other experienced microscopist. A precise diagnosis for treatment is required. *P. falciparum* must be differentiated from other forms of malaria because it can be rapidly fatal and may have different resistance patterns. *P. vivax* and *P. ovale* require primaquine treatment to eliminate a persistent liver stage, and the diagnosis is again, dependent on microscopy. To speciate the parasite, the microscopist relies on infected red cell appearance (normal, enlarged, pigment containing) and the appearance of the parasite by comparing ring forms, trophozoites, schizonts and gametocytes (figures 2 and 3).

Figure 2: Photomicrograph of Plasmodium falciparum within red blood cells.[5]



The importance of accurate diagnosis relates to treatment. Guidelines for treatment are easily accessed on the CDC website at www.cdc.gov/malaria/pdf /treatmenttable.pdf. The website notes that a negative blood smear makes the diagnosis of malaria unlikely. “However, because non-immune individuals may be symptomatic at very low parasite densities that initially may be undetectable by blood smear, blood smears should be repeated every 12-24 hours for a total of 48-72 hours.”[5] Presumptive treatment without laboratory confirmation is discouraged. The type of treatment is chosen based on species, severity of infection (to decide between oral and parenteral therapy), and the presence of resistance. Resistance is determined by the site of origin of the infection and not by susceptibility testing. Chloroquine sensitivity applies to all *P. falciparum* cases from Central America west of the Panama Canal, Haiti, the Dominican Republic, and most of the Middle East. All other *P. falciparum*, and *P. vivax* from Papua New Guinea and Indonesia are considered chloroquine resistant.

Figure 3: Drawing of the different stages of Plasmodium falciparum that may be seen in an infected patient's blood smears.[5]



Malaria Testing at Rex

Rex Laboratory offers blood smear review by a pathologist for parasitemia (order Malaria Smear). Wright-Giemsa stained thick and thin blood smears are prepared for detection and identification of malaria. Thick smears are prepared and stained on first shift seven days a week. During the past twenty-one months 67 malaria smears from 58 patients were reviewed. Four positive smears from three patients were detected. There was one case of *P. falciparum* (one patient, two separate specimens), one case of *P. vivax*, and one case indeterminate between *P. vivax* and *P. ovale*. In addition the laboratory participates in the College of American Pathologists clinical microscopy quality assurance program, which requires review of challenge slides of blood parasites.

New Methodology for Malaria Testing

Polymerase Chain Reaction (PCR) has recently become available for detection and speciation of malaria parasites.[1,2,3,4,5] In March of 2006 the CDC published a study comparing PCR to microscopy by parasitologists at the CDC. Microscopy showed a 93.3% sensitivity versus 100% by PCR. Identification to the species level was 100% by PCR and 82.5% by microscopy.[2] In addition, five mixed infections were detected by PCR of which only was one was initially appreciated by microscopy.

In April of 2006 the Mayo Medical Laboratories announced its own molecular test for malaria (Malaria, Molecular Detection by Rapid Polymerase Chain Reaction #87860).[4] This test detects *Plasmodium* spp. and also speciates based on melting curve analysis. The test requires 4.0 ml of EDTA whole blood at ambient temperature along with two thin blood films and two thick blood films (which will be prepared by the Rex Laboratory staff). Because microscopy can be insensitive to low levels of parasitemia and may not allow for definitive speciation, this test should be considered in cases where there is a high suspicion for malaria and the smears are negative. It can also be used when the species identification is not definitive.

The outreach charge for the Rex malaria smear evaluation is $39 (includes both technical and professional charge). The charge for the Mayo malaria PCR test is $218 (includes handling fee). Blood smear examination remains a valuable (albeit over 100 year old) tool to diagnose malaria, but PCR provides a more objective, sensitive and specific test available to help clear the air in problematic cases.

Special thanks to Sheila Smithey (System Support Specialist) for providing Rex malaria data.

Vincent C. Smith M.D.

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3. *Mangold, KA, et al. Real-Time PCR for Detection and Identification of Plasmodium spp. J Clin Microbiol. 2005; 43:2435-40.*
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5. *www.cdc.gov/malaria Centers for Disease Control and Prevention Malaria website.*

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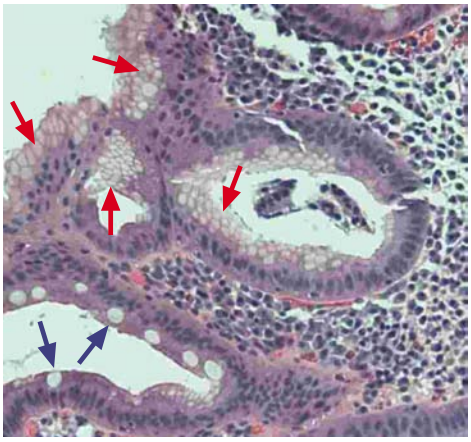
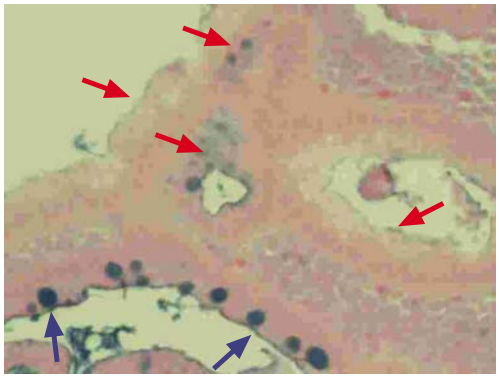
Gut Check #1.1 - Over Diagnosis of Barrett's Esophagus

A diagnosis of Barrett's esophagus has medical, financial, and psychological consequences for the patient. Given the high prevalence of gastroesophageal reflux, many patients undergo upper endoscopy, with esophageal biopsies obtained to "r/o Barrett's". Over diagnosis of Barrett's esophagus may result if the parties involved do not recognize potential pitfalls. The diagnosis of Barrett's esophagus requires collaboration between the gastroenterologist and the pathologist.[1-4] The endoscopist should see columnar (pink) mucosa extending proximally from the gastroesophageal junction (GEJ) into the tubular esophagus. Biopsies should be taken and identified with respect to the distance from the GEJ. The pathologist looks for goblet cell metaplasia (GCM) within the glandular epithelium. If GCM is present in tubular esophageal biopsies taken > 3 cm proximal to the GEJ, the patient has classic ("long segment") Barrett's esophagus. If GCM is present in biopsies taken < 3 cm from the GEJ **AND** the endoscopist notes abnormal mucosa, the patient has "short segment" Barrett's esophagus.

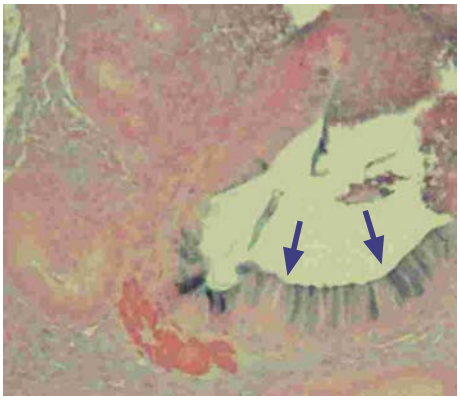
While this sounds straightforward in theory, in practice it can be quite difficult. The anatomic landmarks of the GEJ and squamocolumnar junction ("Z-line") may be obscured in patients with Barrett's. The lower esophageal sphincter (distal 2-3 cm of the tubular esophagus) is often normally lined by gastric mucosa in normal patients or those with reflux esophagitis, and thus may appear abnormal (pink) to the endoscopist. Furthermore, up to a third of patients with reflux esophagitis may have small microscopic foci of GCM at the GEJ or LES, yet they are not believed to have "true" Barrett's esophagus.[3,4] Rather, these changes are attributed to GCM in areas of gastric carditis (perhaps related to *H. pylori*), which have migrated proximally. The minimal histologic criteria for "short segment" Barrett's esophagus have not been defined.[3,4] If the endoscopic picture suggests Barrett's esophagus, separate biopsies should be taken from the GEJ, the Z-line and intervening or proximal pink mucosa.[4] "*Routine sampling of endoscopically normal GEJ should be discouraged, as there is no known clinical significance to the histologic findings in this setting and the practice leads to the over diagnosis of Barrett's esophagus.*"



Black arrows point to "true" goblet cells in areas of goblet cell metoplasia. Note pale blue hue of mucin and isolation of goblet cells. Red arrows point to "pseudogoblet cells". Note pink hue of mucin and cointinuous nature of cells.



Black arrows indicate "true" goblet cells. Note intense Alcian blue positivity. Red arrows indicate pseudogoblet cells, some of which display weak to moderate Alcian blue positivity.



Gastric cardiac mucosa in esophageal biopsy with Alcian blue positivity.

Pathologists can also contribute to over diagnosis by failing to distinguish between "true" goblet cells and "pseudogoblet cells". The latter term refers to foveolar columnar cells, which may be observed in gastric cardiac type mucosa in the esophagus. At times, these cells may become distended, yielding a resemblance to metaplastic intestinal type goblet cells.[4] Alcian blue staining at pH 2.5 has been advocated by some to assist in the recognition of goblet cells. While this stain will decorate true goblet cells, it is certainly not specific. Pseudogoblet cells and even normal gastric cardiac columnar cells may also stain positively with Alcian blue. In most cases, true goblet cells can be distinguished from pseudogoblet cells by careful evaluation of routine H&E stained slides. True goblet cells in Barrett's esophagus have a scattered distribution due to intervening columnar absorptive type or gastric foveolar cells.[4] In addition their cytoplasmic mucin often has a pale blue tint on routine H&E staining. In contrast, pseudogoblet cells are often continuous and have a pink tint on routine H&E staining. *Given the discussion above regarding the lack of consensus regarding the minimal histologic criteria for "short segment" Barrett's esophagus, routine use of Alcian blue stains in this setting probably does more harm than good.*

John D. Benson, MD

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