ISF Awardee Lecture

Malaria predisposes to bacterial sepsis:

Is malaria pigment the key factor?

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Malaria predisposes to bacterial sepsis

Epidemiology

Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital

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Summary

OBJECTIVES To describe the prevalence, aetiology and prognostic implications of coexisting invasive bacterial disease in children admitted with severe malaria in a rural Mozambican hospital.

METHODS Retrospective study of data systematically collected from June 2003 to M[illegible] 2006 in a rural Mozambican hospital, from all children younger than 5 years admitted with severe malaria.

RESULTS Seven thousand and forty-three children were admitted with a diagnosis of malaria. 25.2% fulfilled the criteria for severe malaria. 5.4% of the children with severe malaria had a concomitant bacteraemia. Case fatality rates of severe malaria increase steeply when bacteraemia was also present (from 4.0% to 22.0%, $P < 0.0001$), and bacteraemia was an independent risk factor for death among severe malaria patients (adjusted OR 4.2, 95% CI 2.8–13.7, $P = 0.000$). *Streptococcus pneumoniae*, Gram-negative bacteria, *Staphylococcus aureus* and non-typhoid *Salmonella* (NTS) were the most frequently isolated microorganisms among severe malaria cases. Their frequency and associated case fatality rates (CFR) varied according to age and to syndromic presentation. *Streptococcus pneumoniae* had a relatively low CFR, but was consistently associated with severe malaria syndromes, or anaemia severity groups. No clear-cut relationship between malarial anaemia and NTS bacteraemia was found.

CONCLUSIONS The coexistence of malaria and invasive bacterial infections is a frequent and life-threatening condition in many endemic African settings. In Mozambique, *S. pneumoniae* is the leading pathogen in this interaction, possibly as a consequence of the high HIV prevalence in the area. Measures directed at reducing the burden of both these infections are urgently needed to reduce child mortality in Africa.

Possible Mechanisms

Both Hemolytic Anemia and Malaria Parasite-Specific Factors Increase Susceptibility to Nontyphoidal *Salmonella enterica* Serovar Typhimurium Infection in Mice[▽]

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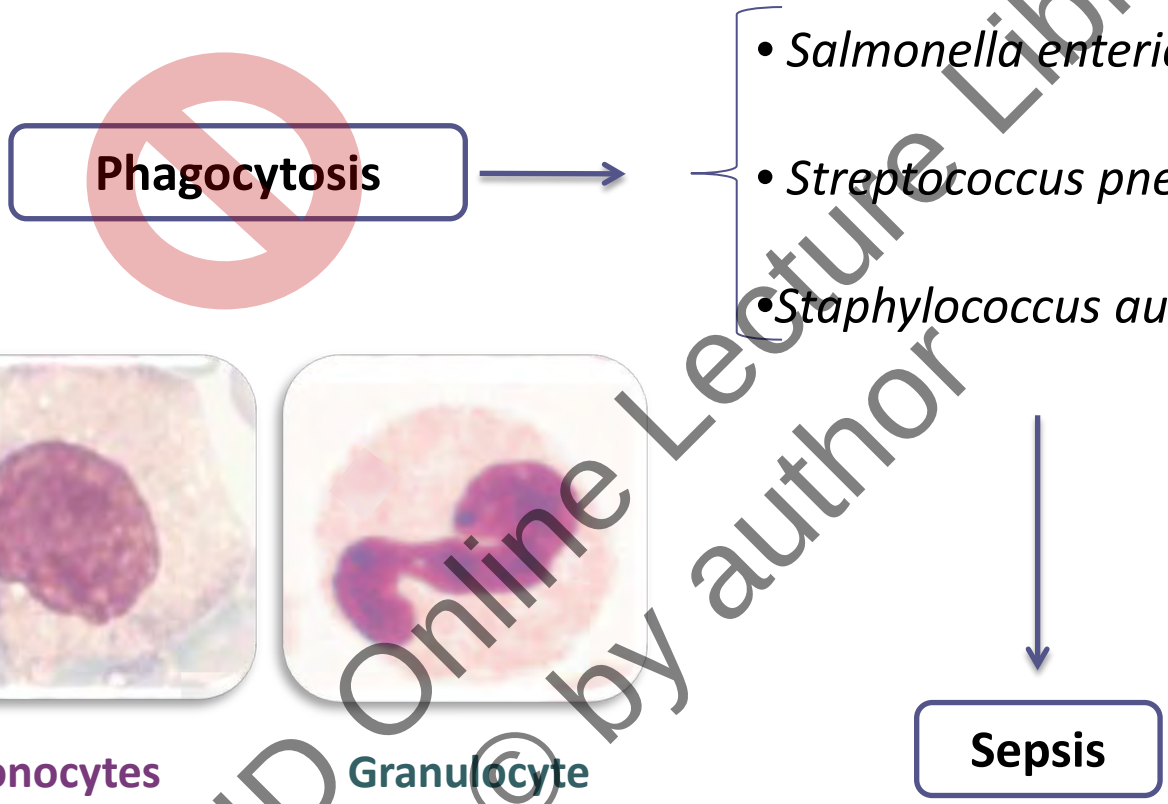
[illegible] malaria is an important risk factor for developing disseminated infections with nontyphoidal [illegible] serotypes (NTS). While recent animal studies on this subject are lacking, early work suggests that [illegible] increased risk for developing systemic NTS infection during malaria is caused by hemolytic anemia, which leads to reduced macrophage microbicidal activity. Here we established a model for oral *Salmonella enterica* serotype Typhimurium challenge in mice infected with *Plasmodium yoelii nigeriensis*. Initial characterization of this model showed that 5 days after coinoculation, *P. yoelii nigeriensis* infection increased the recovery of S. Typhimurium from liver and spleen by approximately 1,000-fold. The increased bacterial burden could be only partially recapitulated by antibody-mediated hemolysis, which increased the recovery of S. Typhimurium from liver and spleen by 10-fold. These data suggested that both hemolysis and *P. yoelii nigeriensis*-specific factors contributed to the increased susceptibility to S. Typhimurium. The mechanism by which hemolysis impaired resistance to S. Typhimurium was further investigated. *In vitro*, S. Typhimurium was recovered 24 h after infection of hemophagocytic macrophages in 2-fold-higher numbers than after infection of mock-treated macrophages, making it unlikely that reduced macrophage microbicidal activity was solely responsible for hemolysis-induced immunosuppression during malaria. Infection with *P. yoelii nigeriensis*, but not antibody-mediated hemolysis, reduced serum levels of interleukin-12p70 (IL-12p70) in response to S. Typhimurium challenge. Collectively, studies establishing a mouse model for this coinfection suggest that multiple distinct malaria-induced immune defects contribute to increased susceptibility to S. Typhimurium.

nigeriensis alone (Fig. 3A). Microscopically, spleens of *P. yoelii nigeriensis*-infected mice exhibited a marked increase of reticuloendothelial cells of the red pulp. Multiple macrophages filled with hemozoin were observed (Fig. 3B). Spleens of *S.* Typhimurium-infected mice exhibited a mild neutrophil infiltrate in the red pulp (Fig. 3B). Both lesions were apparent in spleens

Impairment in phagocytosis can lead to sepsis?



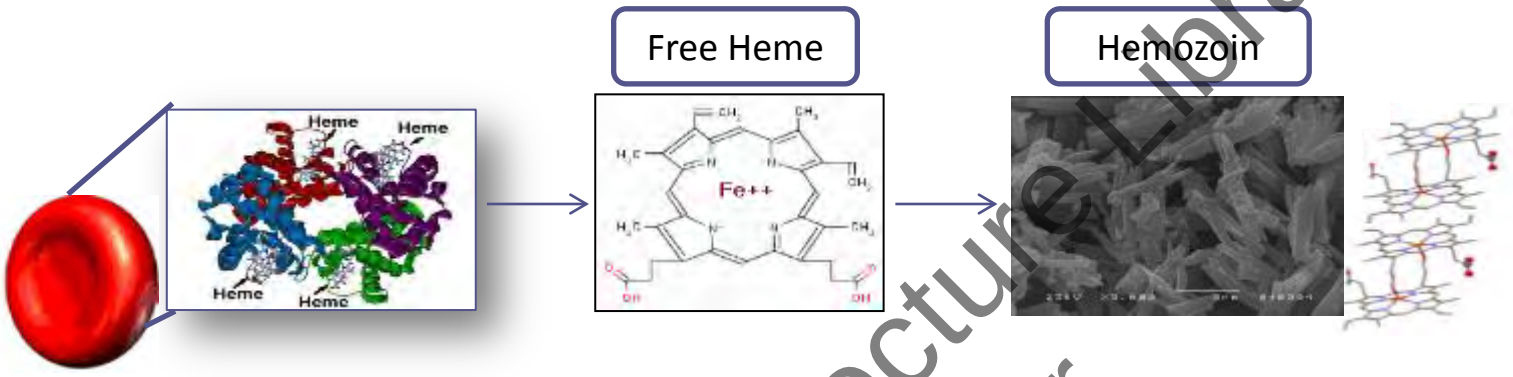
Citation

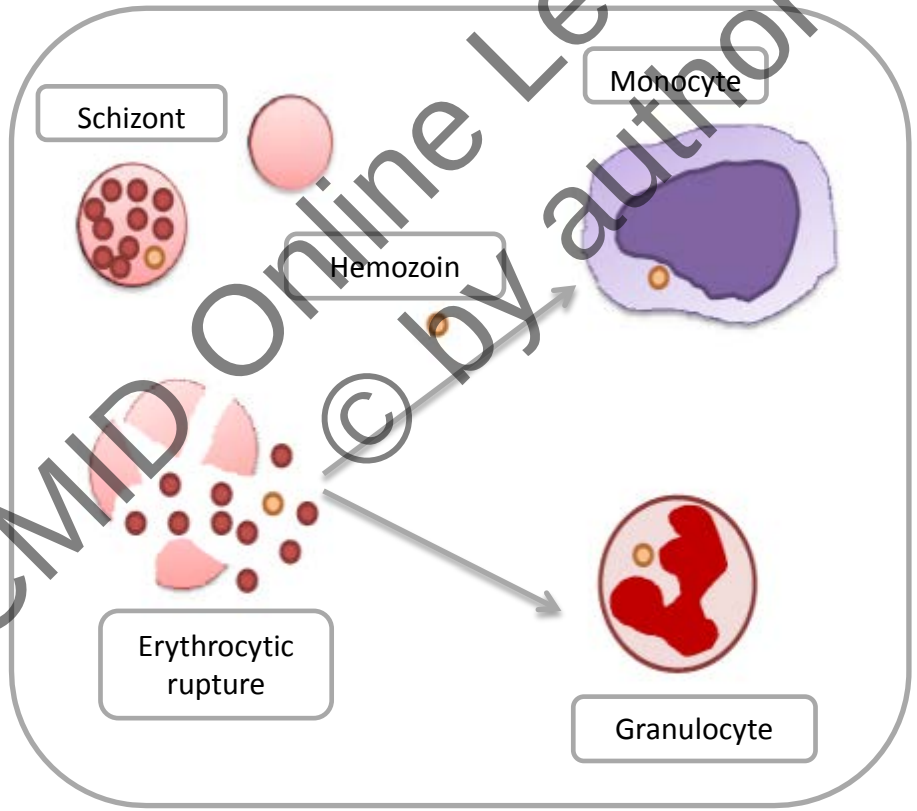
Wenisch C, Fladerer P, Patruta S, Krause R, Hörl W. Assessment of neutrophil function in patients with septic shock: comparison of methods. *Clinical and Diagnostic Laboratory Immunology*. 2001;8(1):8-11.

Taneja R, Sharma AP, Hallett MB, Findlay GP, Morris MR. Immature circulating neutrophils in sepsis have impaired phagocytosis and calcium signaling. *Shock*. 2008;30(6):618-22.

Regel G, Nerlich ML, Dwenger A, et al. Phagocytic function of polymorphonuclear leukocytes and the RES in endotoxemia. *The Journal of Surgical Research*. 1987;42(3):74-84.

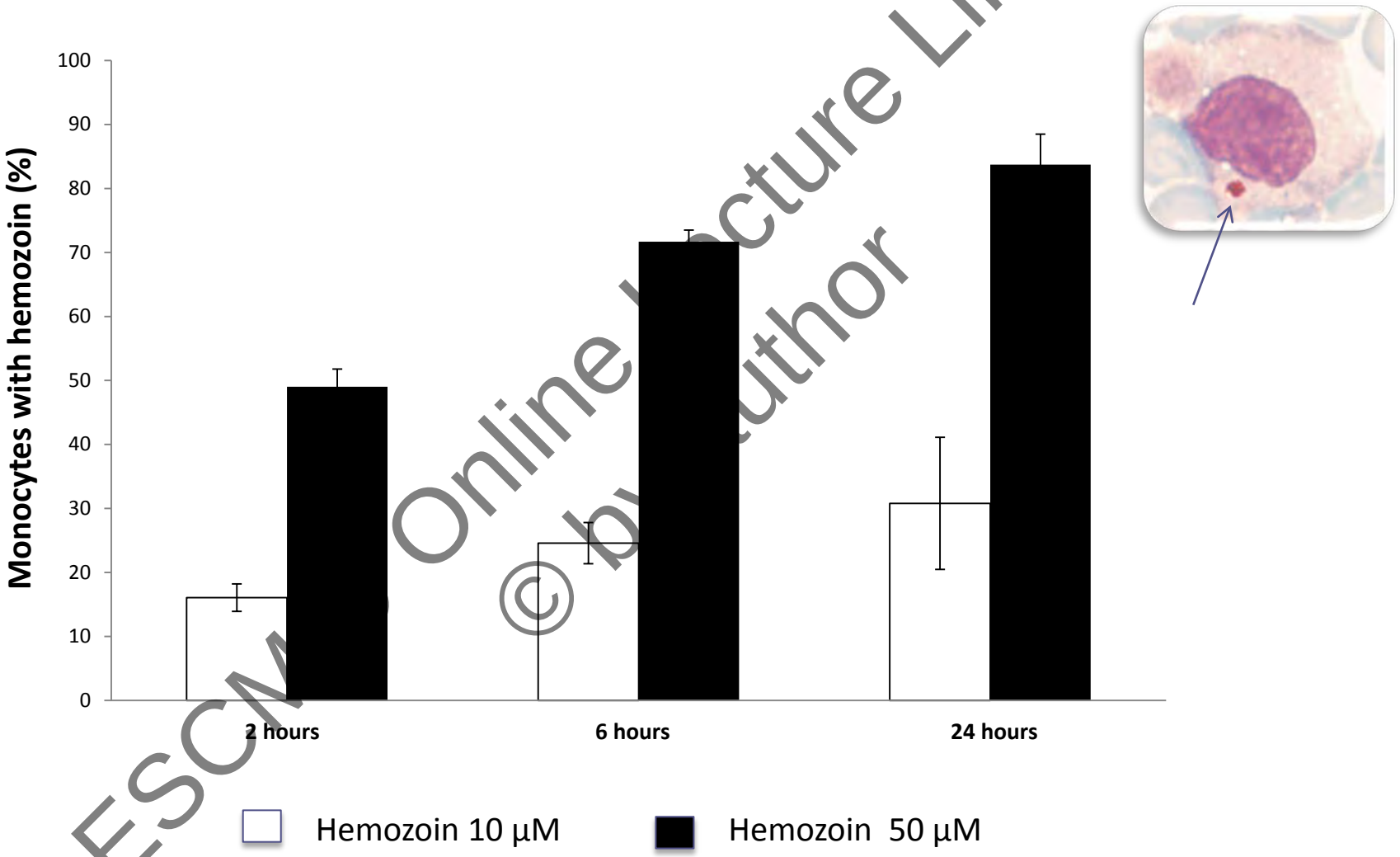
Is malaria pigment the key factor?



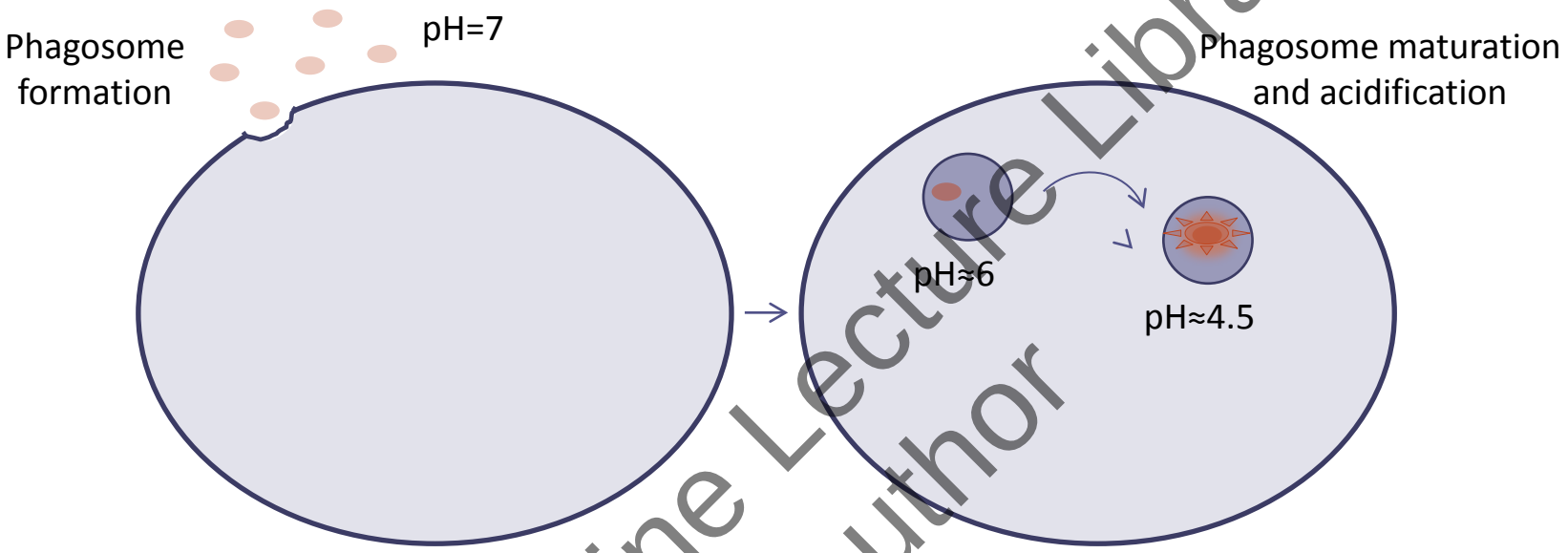


Hemozoin released after erythrocyte rupture is phagocytozed

Flow Cytometric detection of hemozoin inside mocytes (PBMCs)

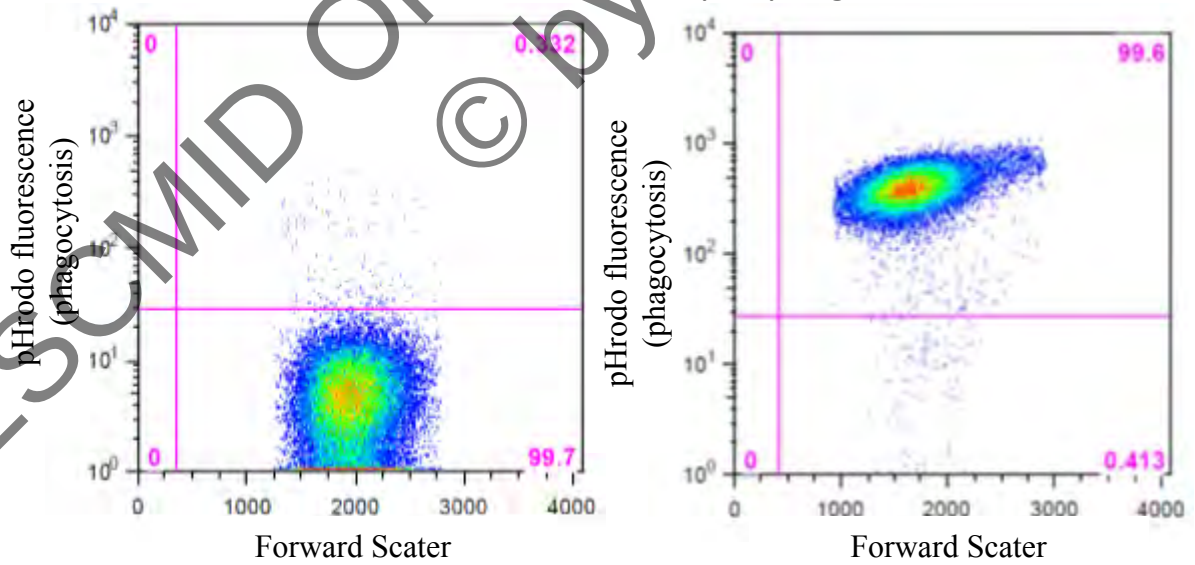


Flow Cytometric measurment of phagocytosis

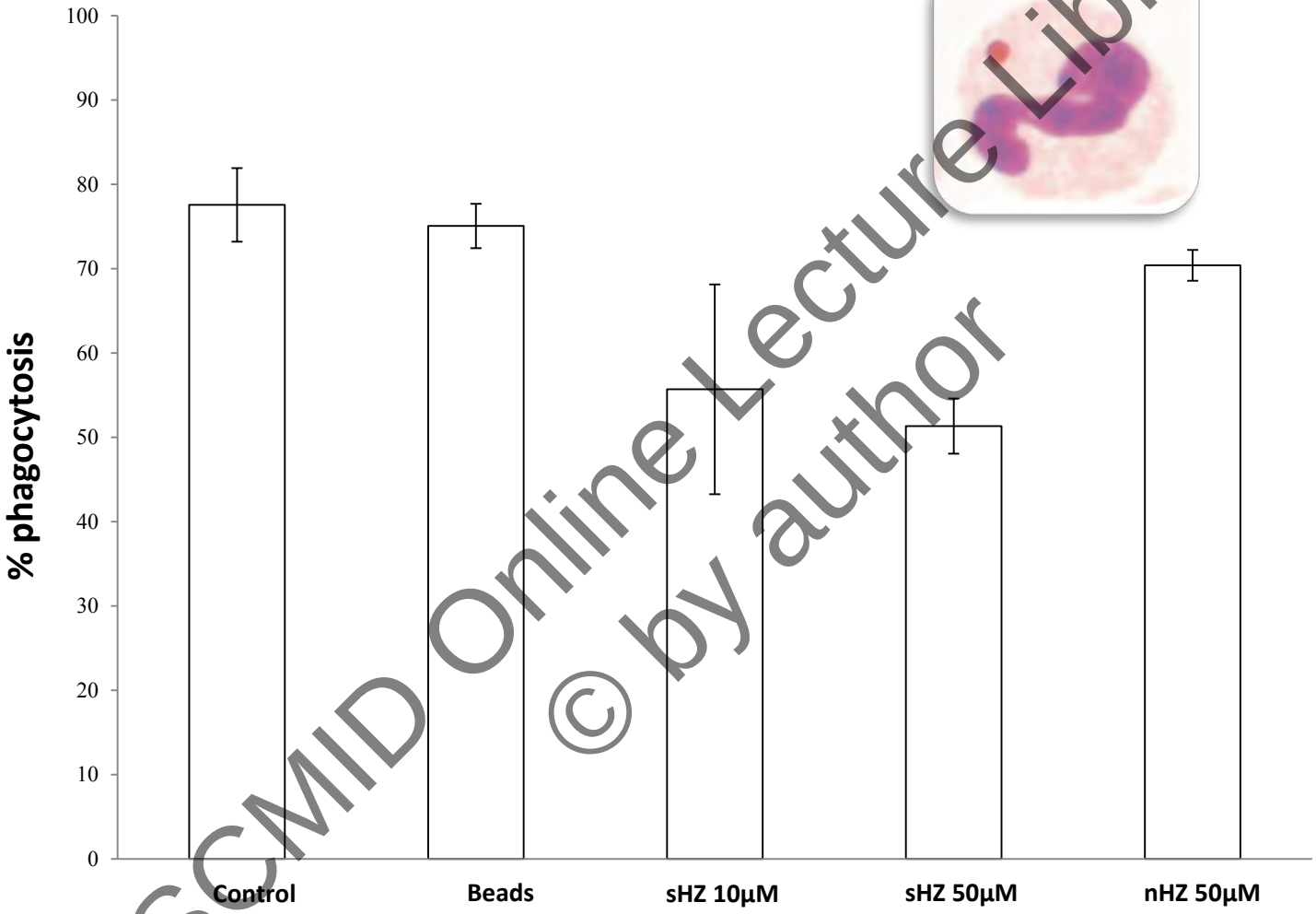


Phagocytic assay with pHrodo – bio particles

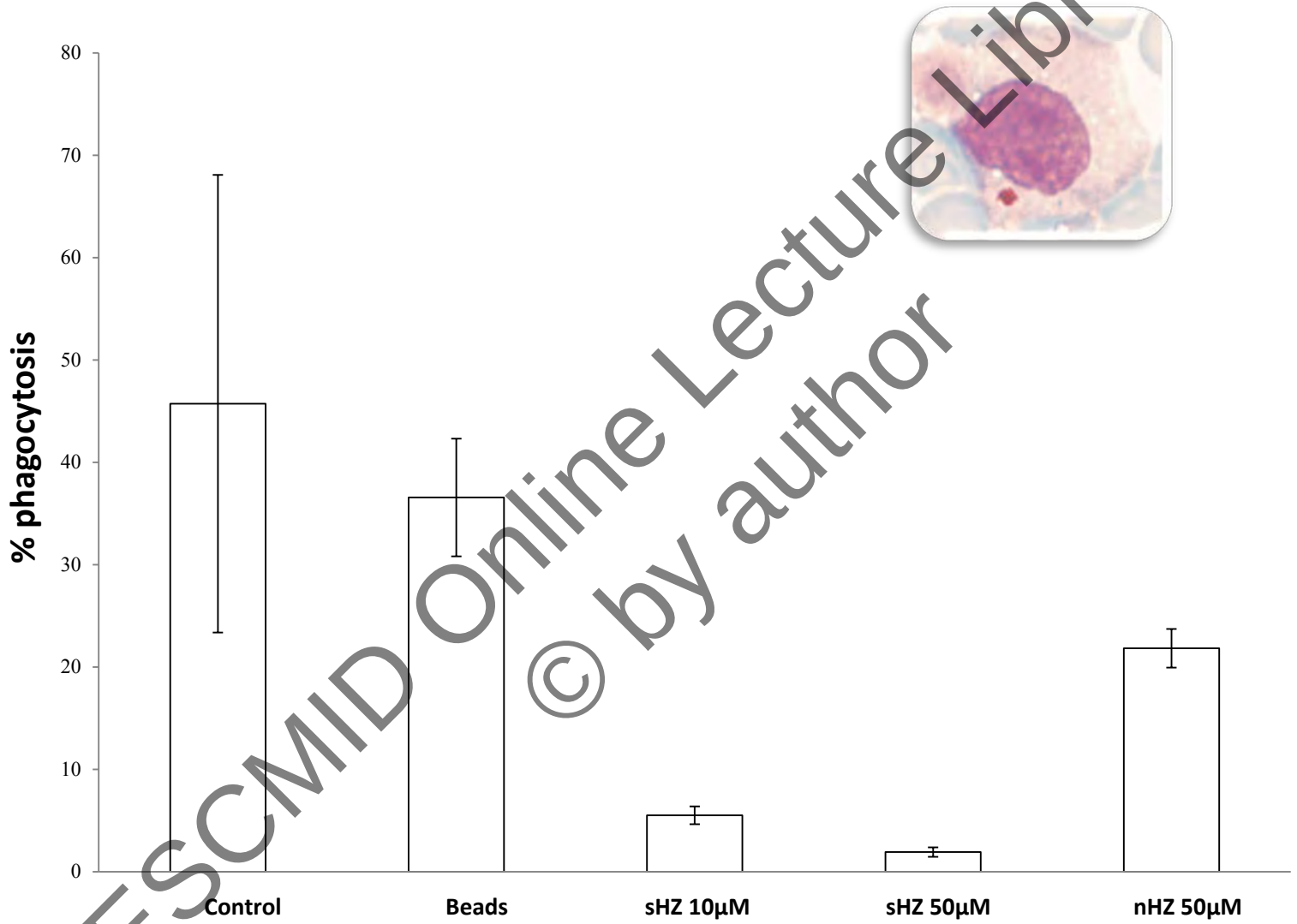
↑ FL3-fluorescence = ↓ pH (phagosome)



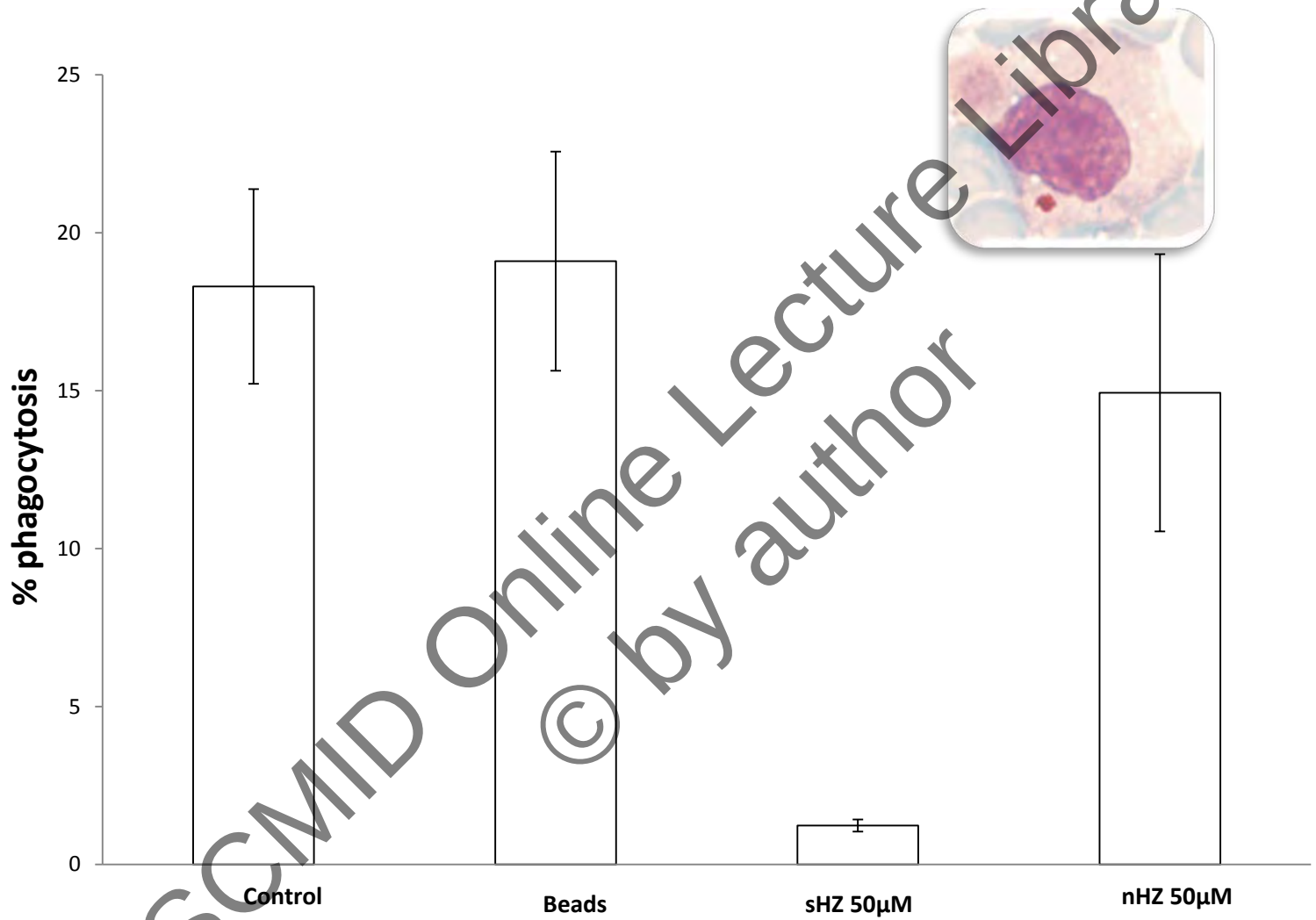
Whole blood pre-incubated with hemozoin for 4 hours - **Granulocytes**



Whole blood pre-incubated with hemozoin for 4 hours - **Monocytes**

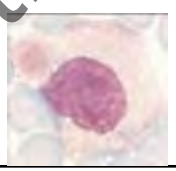
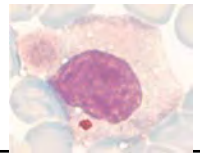


Purified Monocytes (PBMCs) incubated with hemozoin for 6 hours



Whole blood pre-incubated with hemozoin for 4 hours - **Monocytes**

Efect of hemozoin in Hz (+) and Hz (-) monocytes

	Control	Synthetic hemozoin (50µM)	
		Monocytes Hz negative 	**Monocytes Hz positive** 
Healthy donor 1	40.1 %	6.3 %	1.2 %
Healthy donor 2	45.7 %	5.9 %	0.4 %
Healthy donor3	55.1 %	23.2 %	3.9 %

Bactericidal capacity of monocytes pre-incubated with hemozoin

	CFUs/mL (mean) GFP *Salmonella typhimurium* infection 15 minutes	
Monocytes (PBMCs) incubated with hemozoin for 6 hours	Control	473
	Beads	760
	Hemozoin (50 µM)	3410
Monocytes (PBMCs) incubated with hemozoin for 24 hours	Control	230
	Beads	160
	Hemozoin (50 µM)	3780

Bacterial survival increased 7.2 fold

Bacterial survival increased 16.4 fold

control Beads sHZ 50µM

GFP *Salmonella typhimurium* infection - 15 minutes

Conclusions

- Malaria pigment seems to impair the phagocytic capacity of monocytes and granulocytes. This effect is both dose and time dependent.
- Malaria pigment seems to affect the whole monocyte population in the sample, and not only the ones that ingested the pigment.
- Phagocytes ability to kill bacteria seems decreased by malaria pigment.
- These observations may contribute to explain why malaria patients acquire disseminated bacterial infections more easily.

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