Protective or pathogenic effects of vascular endothelial growth factor (VEGF) as potential biomarker in cerebral malaria

Miriam Canavese^{1,2}, Roberta Spaccapelo²

¹Department of Biological Sciences, Imperial College, London, ²Centro di Genomica Funzionale, Università degli Studi di Perugia, Italy

Cerebral malaria (CM) is the major lethal complication of *Plasmodium falciparum* infection. It is characterized by persistent coma along with symmetrical motor signs. Several clinical, histopathological, and laboratory studies have suggested that cytoadherence of parasitized erythrocytes, neural injury by malarial toxin, and excessive inflammatory cytokine production are possible pathogenic mechanisms. Although the detailed pathophysiology of CM remains unsolved, it is thought that the binding of parasitized erythrocytes to the cerebral endothelia of microvessels, leading to their occlusion and the consequent angiogenic dysregulation play a key role in the disease pathogenesis. Recent evidences showed that vascular endothelial growth factor (VEGF) and its receptor-related molecules are over-expressed in the brain tissues of CM patients, as well as increased levels of VEGF are detectable in biologic samples from malaria patients. Whether the modulation of VEGF is causative agent of CM mortality or a specific phenotype of patients with susceptibility to fatal CM needs further evaluation. Currently, there is no biological test available to confirm the diagnosis of CM and its complications. It is hoped that development of biomarkers to identify patients and potential risk for adverse outcomes would greatly enhance better intervention and clinical management to improve the outcomes. We review and discuss here what it is currently known in regard to the role of VEGF in CM as well as VEGF as a potential biomarker.

Keywords: Cerebral malaria, Vascular endothelial growth factor, Biomarker, Cytokines, Angiogenesis, Infected-red blood cells, Blood–brain barrier

Introduction

Despite decades of research, human cerebral malaria (CM) remains one of the most serious complications of *Plasmodium* infection and is a significant burden in Sub-Saharan Africa, where, despite effective antiparasitic treatment, survivors develop long-term neurological sequelae.¹ Until recently, observation regarding human CM has been somewhat limited, because it has relied mainly on examination of post-mortem samples, which are not always accessible.² This limitation has made animal models, despite their imperfections, necessary for longitudinal studies of disease pathogenesis.³

Studies with non-human primate models demonstrated several pathological features similar to those observed in humans, including sequestration of infected red blood cells (iRBCs) and vascular damage. However, these studies are difficult to conduct, due to their ethical and cost constraints and thus are vastly underutilized.^{2,4,5}

Although some differences undoubtedly exist between human and murine experimental cerebral malaria (ECM), several studies highlight the range of similarities, including perturbations in the integrity of the blood–brain barrier (BBB), iRBC impairment, despite successful antimalarial therapy. These similarities support the use of the mouse models to characterize this human disease.^{1,3}

Cerebral microcirculatory disturbances have been implicated as a contributing factor in the pathogenesis of human CM. By a process known as sequestration, iRBCs adhere to the brain endothelium through binding of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a specific cell-surface ligand expressed by iRBCs. Most of the molecules implicated in the adhesion of iRBC on endothelial cells (ECs) are already described; however, the structure of the iRBC/EC junction and the impact of this adhesion on the EC are poorly understood.⁶ To this end, Jambou and colleagues demonstrated the transfer of material from the iRBC to the brain EC plasma membrane in a trogocytosis-like process, followed by a TNF-enhanced iRBC engulfing process. The transfer of iRBC antigens

Correspondence to: Miriam Canavese, Department of Biological Sciences, Imperial College, London, UK/Centro di Genomica, Funzionale, Università degli Studi di Perugia, Italy. Email: m.canavese@imperial.ac.uk

can thus transform EC into a target for the immune response and contribute to the profound EC alterations, including peri-vascular edema, associated with CM.⁶

Moreover, Khaw *et al.*, established a novel tripartite culture system consisting of three major cell types involved in the lesion of CM: human brain endothelial cells (HBECs), iRBC, and peripheral blood mononuclear cell (PBMC) to shed light on the interactions of these three cell types at a critical location, the brain microvasculature. Interestingly, PBMCS, along with HBEC, interact with the iRBCs, leading to up-regulation of the expression of inflammatory genes.⁷

Very recently Moxon *et al.* provided novel insight into the pathogenesis of CM linking loss of the endothelial protein C receptor (EPCR) on brain vessels, caused by cytoadherent iRBCs, with localized coagulation, inflammation, and disruption of endothelial barrier function.^{8,9}

Blood–brain barrier serves as a key interface between the central nervous system and the blood, restricting the free flow of physiological molecules between the bloodstream and parenchyma. The BBB is composed of specialized ECs that line cerebral blood vessels. These ECs are surrounded by a basal lamina and astrocyte end foot processes.¹⁰ Tight junctions are responsible for stitching ECs together for barrier maintenance and integrity, with astrocytes providing additional structural and physical support.¹⁰ Together, these cells serve as the gatekeepers of the BBB and help the barrier to perform its critical function of maintaining proper brain homeostasis. Perturbations to the BBB can lead to the passage of potentially harmful substances into the brain, which may subsequently cause disease. Uncontrolled inflammatory cytokines and infiltration of leukocytes in brain microvessel as well as vascular dysfunction with subsequent BBB damage are the major features of CM.^{11–14}

The relationship among parasite sequestration, signaling events across the BBB, and subsequent neuro-pathological or protective responses within the brain parenchyma in severe malaria is poorly understood.¹⁵ Considering the prominent vascular obstruction it is unclear why there is not more irreversible injury and inflammation. One hypothesis is that parasite sequestration in cerebral microvessels leads to local hypoxia and increased expression of hypoxia-inducible gene products, such as vascular endothelial growth factor (VEGF) and its receptors.¹⁶ However, as shown in Fig. 1, VEGF's functions in CM are controversial and still unknown.

Therefore, this review will present the current knowledge on the role and functions of VEGF in CM pathogenesis and discuss its future perspectives as adjunctive therapy or biomarker in this disease.

Molecular Mechanisms of VEGF

Vascular endothelial growth factor is a key regulator of physiological angiogenesis during embryogenesis, skeletal growth, and reproductive functions. It has also been implicated in pathological angiogenesis associated with tumors, intraocular neovascular disorders, and other conditions.¹⁷ Vascular endothelial growth factor (also referred as a VEGF A) belongs to a gene family that includes placental growth factor (PLGF), VEGF B, VEGF C, and VEGF D. Vascular endothelial growth factor A is the key regulator of blood vessel growth. Vascular endothelial growth factor C and D regulate lymphatic angiogenesis, emphasizing the unique role of this family in controlling growth and differentiation of multiple anatomic components of the vascular system.¹⁷ A well-documented *in vitro* activity of VEGF is the ability to promote growth of ECs derived from arteries, veins, and lymphatics¹⁸ and it serves as survival factor for ECs, both *in vitro* and *in vivo*.^{19,20} Moreover, VEGF is known as vascular permeability factor, based on its ability to induce vascular leakage.^{21,22}

The VEGF is known to bind to vascular endothelial growth factor receptor 1 (VEGFR-1) (Flt-1) and vascular endothelial growth factor receptor 2 (VEGFR-2) (KDR/Flk-1) on ECs, which results in mitogen-activated protein kinase (MAPK) signaling cascade.²³ As shown in Fig. 2, promotion of neuronal cells proliferation by VEGF is also associated with the increased expression of VEGFR-2 and up-regulation of E2F family transcription factors, cyclin D1, cyclin E, and cdc25.²⁴

The biological effects of VEGF are mediated by the above receptor tyrosine kinases, VEGFR1, and VEGFR2, which differ considerably in signaling properties.¹⁷ Vascular endothelial growth factor receptor 1 expression is up-regulated by hypoxia-inducible factor (HIF-1) dependent mechanism.²⁵ Recent studies have emphasized the role of VEGFR-1 in hematopoiesis and recruitment of endothelial progenitors as well as it has been shown a function of VEGFR-1 signaling in the vascular endothelium is to release tissue-specific growth factors, possibly in a vascular bed-specific fashion.²⁶ Vascular endothelial growth factor receptor 2 is the major mediator of the mitogenic, angiogenic, and permeability-enhancing effects of VEGF.¹⁷ Vascular endothelial growth factor receptor 3 is a member of the same family, but is not a receptor for VEGF, binding instead to VEGF C and VEGF D.¹⁷

In addition to these receptor tyrosine kinases, VEGF interacts with a family of co-receptors, the neuropilins.¹⁷ The role of neuropilin receptor 1 (NRP1) in the development of vascular system has been shown by gene targeting studies documenting embryonic lethality in *Nrp1*-null mice.²⁷ Moreover, Niu and colleagues demonstrated that constitutive

Uncontrolled inflammation and infiltration of lymphocytes in brain microvessels as well as vascular dysfunction with subsequent blood–brain barrier (BBB) damage are the major features of CM. Rapture of infected red blood cells (iRBCs) releases factors that activate brain microvascular endothelial cells (ECs), leading to up-regulation of receptors for iRBC. Sequestration of iRBCs provokes local loss of endothelial protein C receptor (EPCR) and thrombomodulin, which is associated with local inflammation and disturbance of coagulation. Moreover, strong systemic inflammation further amplifies endothelial cell activation in the brain and activates brain-resident perivascular macrophages, microglia, and astrocytes. Activated endothelial and glial cells provide chemotactic signals for lymphocytes and myeloid cells. Sequestered iRBCs and leukocytes interfere with cerebral blood flow and together with cytotoxic factors, damage BBB, leading to edema and hemorrhage. The role of VEGF in this scenario is still unknown. However, at high levels of vessels sequestration there may be increased soluble VEGF production by ECs and perivascular cells as a result of local hypoxia.

Figure 1 Vascular endothelial growth factor's (VEGF) involvement in cerebral malaria (CM) pathogenesis.

In neurons the best characterized signaling pathways are VEGFR-2, PI3K/Akt, and MEK/ERK. Like VEGF A, VEGF B also seems to exert a neuro-protective effect in cerebral ischemia. Accordingly, VEGF B protects cultured cerebral cortical neurons from hypoxia, whereas infarct volume is increased and neurological function is more impaired in VEGF B-knockout than in wild-type mice.

Figure 2 Direct neuronal impact of vascular endothelial growth factor (VEGF) and its mechanisms.

Stat3 activation directly promotes VEGF expression and stimulates tumor angiogenesis, by down-regulating the expression of angiostatic mediators during malignant progression.²⁸ This progress in the molecular and biological understanding of blood vessel formation and differentiation raises hope that a return to human trials for therapeutic angiogenesis will be more rewarding than the early attempts.

VEGF and Brain: Neuroprotection and Neurodegeneration

In physiological conditions VEGF exerts neuro-protective actions directly through the inhibition of programmed cell death or apoptosis and the stimulation of neurogenesis, as well as it prevents neurons from death under critical conditions such as hypoxia, through binding to specific receptors, which are also expressed on the surface of neuronal cells.²⁹ In addition to its role in physiological angiogenesis, VEGF has been implicated in many disorders.²² Angiogenesis and neurogenesis are prominent features of neurological disease, either as pathophysiological factors or as responses to

injury.¹⁶ One common thread that connects angiogenesis, neurogenesis, and pathogenesis is VEGF, which was identified on the basis of its vascular effects, but has since been recognized as an important signaling molecule in the nervous system as well.¹⁶ Vascular endothelial growth factor is expressed in the brain and it is induced by hypoxia through the transcription factor HIF-1.³⁰ The tyrosine kinase receptors and downstream protein kinases implicated in VEGF signaling in the CNS have been essentially identical to those described previously in non-neural tissues and include VEGFR-2 and to some extent VEGFR-1 receptors as well as MAPKs, phosphatidylinositol 3-kinase (PI3K)/Akt, and Rho/Rac.^{17,31} As described in Fig. 2, at neuronal level, VEGF acts through multiple tyrosine kinase receptors and associated downstream signaling pathways.

The earliest described direct neuronal actions of VEGF were neurotrophic effects.^{16,32} Sondell and colleagues reported that, in cultured superior cervical and dorsal root ganglion neurons, VEGF promotes both axonal outgrowth and cell survival by a VEGFR-2-dependent mechanism,³² and Silverman *et al.* showed that VEGF improves neuronal survival in organotypic midbrain explant cultures.³³ Further studies demonstrated that VEGF caused increase in number of neurites in cultured neurons or cortical explant, which involved signaling through VEGFR-2, MAPK, and Rho/Rac.³⁴ In addition to these trophic, or growth-promoting effects, VEGF can also protect neurons from a range of insults.¹⁶ For example, it reduces death of immortalized hippocampal neurons subjected to serum withdrawal or hypoxia *in vitro*, by activating VEGFR-2, PI3K/Akt, and the transcription factor NF- κ B.^{35,36} Vascular endothelial growth factor also protects cultured cerebral cortical neurons from hypoxia through similar mechanisms,³⁶ as well as by decreasing activation of caspase-3.³⁷ Moreover, VEGF modifies acute and chronic neurodegenerative processes through effects on both blood vessels and neurons and probably also glia^{38,39} and it improves the survival of cultured spinal cord motor neurons, from WT as well as VEGF^{delta/delta} mice. Its protective effect on motor neurons, like most of its other neuronal effects, was mediated by VEGFR-2 and also seemed to involve a neuropilin 1 co-receptor.⁴⁰

Controversially, some other studies have drawn a connection between VEGF and degenerative brain diseases. Kalaria *et al.* reported increased VEGF immunoreactivity in cluster of reactive astrocytes from brains of patients with Alzheimer's disease. Single nucleotide polymorphisms in the promoter regions of the VEGF gene have also been associated with increased risk of Alzheimer's disease.^{41,42}

Therefore, VEGF seems to play a double role in neurodegenerative diseases. On one hand, it offers neuroprotection in neurons via a direct effect or

Vascular endothelial growth factor can induce both beneficial and detrimental effects in the pathogenesis of CM. However, its role is still controversial.

Figure 3 The balance between protective and pathogenic roles of vascular endothelial growth factor (VEGF) in cerebral malaria (CM).

indirectly via maintaining a stable CNS vascular system. On the other hand, excessive VEGF expression, for example by reactive astrocytes, seems to be deleterious, as activated astrocyte-derived VEGF seems to induce BBB breakdown and vascular leakage. So, to design therapies based on VEGF ligands or receptors, a better understanding of the cellular types that participate in the disease, of VEGF expression and its effects, and of the dose needed for a therapeutic window where VEGF is beneficial, are required.⁴³

VEGF in Cerebral Malaria: What is Known?

As described above, VEGF was first identified as a potent stimulator of vascular endothelial permeability and was further reported to promote proliferation, migration, and survival of ECs.^{29,44} The VEGF signaling pathway is known to activate a range of signals that are cytoprotective, angiogenic, and neurotrophic.¹⁵ To this end, there is substantial evidence demonstrating the importance of VEGF in brain tissue repair and in CNS wound healing.^{45–47} Exogenous administration of VEGF to injured brain tissue improved wound repair, functional response, and decreased apoptosis in rats.⁴⁸

Increased levels of VEGF are often detected in tissues and biologic samples from malaria patients.²⁹ However, so far, the role of VEGF in CM has not been clarified yet.

Figure 3 depicts the potential pathogenic versus protective roles that VEGF seems to exert in CM pathology.

Several studies have investigated VEGF protein expression in the brain of patients with severe or CM pathology.^{49,50} In the first study, immunohistochemical analysis of VEGF was carried out in the brain of six European adults, who had died from CM.⁴⁹ Vascular endothelial growth factor was found to be

overexpressed in astrocytes and VEGFR-1 in Durck's granulomas. The second study showed *in vitro* the unexpected finding of VEGF immunolabeling by sequestered malaria parasites.⁵⁰ A clinical study by Yeo *et al.*²⁴ observed an inverse association between plasma VEGF concentrations and parasite biomass, suggesting potential manipulation of the host response to malaria infection by the parasite.

Taken together these findings suggest that VEGF is of host origin. At high levels of iRBC vessels sequestration there may be increased soluble VEGF production by ECs and perivascular cells as a result of local hypoxia. The sequestered parasites may interrupt autocrine or paracrine signaling in ECs by taking up the VEGF. However, the uptake of circulating VEGF produced at a distance cannot be ruled out. Whether the parasite utilizes VEGF for its benefit, or modulates signaling through uptake or release of VEGF at schizogony is still unknown. There is no *in vitro* evidence that VEGF can be trophic for parasite growth and partially protect them from the effects of drug treatment.^{15,50}

Based on these observations, it seems that VEGF may play a protective role against mortality associated with CM.⁴⁷ Moreover, Lu and colleagues demonstrated that VEGF increases the expression of cell adhesion molecules, such as ICAM-1, on ECs, which would aid sequestration.⁵¹ Taken together, these findings may suggest that VEGF exerts neuro-protective actions directly through the inhibition of programmed cell death or apoptosis and the stimulation of neurogenesis.^{47,52}

Controversially, other studies seem to prove the hypothesis of an adverse role of VEGF in CM.

From the use of mouse models, Epiphany *et al.* demonstrated that VEGF promotes acute lung injury during malaria infection.⁵³ In the context of human malaria, Armah *et al.* showed that decreased levels of serum of VEGF and platelet derived growth factor (PDGFbb) correlated with fatal outcome in CM. Interestingly, a decrease in levels of PDGFbb has been reported in cerebrospinal fluid (CSF) and serum of non-survivors CM.⁵² Whether the decline in levels of VEGF or PDGFbb are causative agents of CM mortality or a specific phenotype of patients with susceptibility to fatal CM needs further evaluation.⁴⁷ Based on this observation, it seems that significant decreases in VEGF may exacerbate neurological damage triggered by inflammatory and or parasite-derived factors, thus leading to death. It has been shown that neutralization of endogenous VEGF in the brains of injured rats results in decreased angiogenic activity and increased vascular and astrocyte degeneration, leading to larger wound cavities.⁴⁵ It is unclear why or how VEGF and PDGFbb production declines in the peripheral blood of CM

patients. Vascular endothelial growth factor expression is negligible in the CNS under normal conditions and it is activated after various types of neurological injuries.⁴⁶ In addition, elevated levels of VEGF-positive astrocytes and deposition of VEGFR-1 in Durck's granulomas were found in non-surviving CM patients.⁵⁴ Thus, it is evident that VEGF is involved in severe manifestations of malaria, although further studies will be required to fully understand its role in fatal outcomes severe malaria.⁴⁷ In a recent study involving Kenyan children, it was demonstrated that erythropoietin, but not VEGF, was associated with neurological sequelae.⁵⁵ It remains to be determined whether this is due to differences in malaria epidemiology, immune status of the patients, or host population genetics.⁴⁷

Moreover, from recent findings, it seems that TNF-alpha, which is activated following the release of malaria antigens after schizont rupture, may activate production of IP-10 in brain capillaries and astrocytes.^{56,57} IP-10 in concert with TNF-alpha can cause vascular injury resulting in breakdown of the BBB, which leads to accumulation of leukocytes that induce local hyper-inflammation. Significant down regulation of VEGF may further inhibit angiogenesis and regeneration of damaged blood capillaries. High systemic levels of IP-10, with its strong angiostatic properties, in CM patients may further inhibit angiogenesis and interfere with VEGF function. IP-10 also blocks VEGF interaction with its natural ligand by interacting with cell-surface glycosaminoglycans, which provide accessory support for VEGF.⁵⁸ Interestingly, another study demonstrated that sTNF-R2/VEGF, IP-10/VEGF, and sFas/VEGF ratios were highest in the non-survivors CM group, and were at least 10-fold higher as compared to the CM-survivor group. These differences supports the hypothesis that the relative balance in inflammatory markers and apoptotic or angiogenic factors, especially VEGF, may predict risk of CM-associated mortality.⁴⁷ Table 1 summarizes the main conclusions of the above published studies, both in human and murine CM.

Potential Serological Biomarkers for Cerebral Malaria: Could VEGF be Considered as a Biomarker to Predict Fatal CM?

Biomarkers have been used to diagnose and prognosticate the progress and outcome of many chronic diseases, such as neoplastic and other conditions.⁵⁹ However, only recently did the field of malaria research move in the direction of actively identifying biomarkers that can accurately discriminate the severe forms of malaria.⁵⁹

A biomarker is a substance that can be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or responses

Table 1 Vascular endothelial growth factor (VEGF) in cerebral malaria (CM): what is known

VEGF levels detected in human malaria samples	Effects of VEGF in murine ECM	Reference	Conclusions	Potential role of VEGF
IHC analysis carried out in brain of 6 European adults. VEGF was found overexpressed in astrocytes and VEGFR1 in Durck's granulomas		Deininger <i>et al.</i> J Neuroimmunol 2003	These data demonstrate the activation of the proangiogenic VEGF signaling cascade in patients with CM, probably reflecting compensatory mechanisms of general and focal brain hypoxia observed in these patients.	Protective
IHC on post mortem brain samples. positive staining for VEGF on iRBC. Confocal microscopy of cultured iRBC revealed accumulation of VEGF within the parasitophorous vacuole, expression of host VEGF-receptor 1 and activated VEGF-receptor 2 on the surface of iRBC		Sachanonta <i>et al.</i> Asian Pac J Allergy Immunol. 2008	<i>P. falciparum</i> iRBC can bind host VEGF-R on the erythrocyte membrane and accumulate host VEGF within the parasitophorous vacuole, which may have a trophic effect on parasite growth.	Protective
Inverse association between plasma VEGF concentrations and parasite biomass were found		Yeo <i>et al.</i> PNAS 2008	VEGF is possibly of host origin. At high levels of iRBC vessels sequestration there may be an increased soluble VEGF production by EC as a result of local hypoxia	Protective
Low levels of VEGF was found in CM patients from India and Ghana who died of CM		Jain <i>et al.</i> Malar J 2008	VEGF was found to be protective against CM associated mortality	Protective
Elevated plasma level of VEGF was found in malarial patients versus febrile illness patients or healthy controls. Increased level of VEGFR2 versus VEGFR1 were observed	Increased level of VEGFR2 versus VEGFR1 were found in mice using ECM	Faruta <i>et al.</i> Am.J.Trop. Med Hyg. 2010	The interaction between VEGF and VEGFR2 is involved in the host immune response to uncomplicated malaria	Protective
High protein expression for VEGF in brain samples of severe malaria patients		Medana <i>et al.</i> Hystopathology 2010	VEGF and its receptor expression levels in severe malaria reflect a non specific response to severe systemic disease	Adverse
	High levels of circulating VEGF were found in mice using ALI mouse model	Epiphanio <i>et al.</i> PLoS Pathogens 2010	VEGF promotes acute lung injury during malaria infection	Adverse
Decreased levels of VEGF and PDGFbb were found in serum and CSF of non survivors CM		Armah <i>et al.</i> Malar J 2007	Decreased serum levels of VEGF and PDGFbb correlate with fatal outcome in CM	Adverse

Table 1 Continued

VEGF levels detected in human malaria samples	Effects of VEGF in murine ECM	Reference	Conclusions	Potential role of VEGF
IP10/VEGF ratio were higher in CM non-survivor group versus survivor		Gengrinovitch <i>et al.</i> Jbiol Chem 1995	The relative balance in inflammatory markers or angiogenic factors, especially VEGF may predict the risk of CM associated mortality	Adverse

to a therapeutic intervention.⁵⁹ While no clear biomarker exists for CM management, to date, recent studies have identified serological factors that have the potential of being biomarkers.⁵⁹ New findings have demonstrated that distinct profiles of cytokine and chemokine are associated with discrete clinical manifestation of *falciparum* malaria.^{47,52,60,61} For example, increased levels of CXCL10, sFas, sTNFR2,^{47,52} IL-8, IL-1ra⁶⁰ and decreased levels of RANTES⁶¹ and VEGF⁴⁷ were all found in CM patients, but not in mild malaria (MM) or healthy control cases.⁵⁹ The endothelial regulators angiopoietin 1 and 2 (ANG-1, ANG-2) were also shown to be differentially expressed in CM compared to MM patients.^{62,63}

Many of the factors discussed above can be viewed as being in the phase 1 of biomarker development, during which, preclinical exploration of potential biomarkers is performed and markers capable of discriminating the severity of the disease are identified. As an example: increase of CXCL10/VEGF, sTNFR2/VEGF, sFas/VEGF,⁴⁷ ANG2/ANG1 ratio,⁶³ increased ANG2 levels,^{24,63} and increased levels of CXCL4 and CXCL10⁶⁴ are considered as prognostic biomarker that can identify patients at risk for CM-associated mortality.⁵⁹ Single biomarkers are rarely used, except in some exceptional cases such as the marker for anemia-hemoglobin level. Most likely for CM, as in many diseases, a composite biomarker panel will be necessary. In recent studies, both in India and Ghana, it has been observed that CM patients, who died, had low VEGF levels,^{47,52} implying that VEGF may be required for tissue recovery. If these findings are confirmed in future studies then one could consider any molecule that can mimic the function of VEGF as a potential candidate for adjunctive therapy. Further studies on the role of these factors for adjunctive therapies are required.⁵⁹

As stated above, in the context of CM, the panel for the prognostic biomarkers could consist of CXCL10, CXCL4, ANG-1, ANG-2, EPO, VEGF, sTNFR2, and sFas.

However, the development of biomarkers is a long journey that will require some technological tools

along the way.⁵⁹ Once validated, the format required for the identified biomarkers will become an issue. Ideally, the panel of biomarkers should be detected in a rapid diagnostic format allowing for a quick and easy-to-use tool in malaria endemic regions. An appropriate format would be a microchip, together with the use of simple and not expensive device for the detection of the biomarkers. Serum obtained from CM patients can be tested for the presence of biomarkers and from the results of either absolute levels and/or ratios of some of the factors, health care workers or clinicians will be able to risk stratify the patients.⁶⁵ However, one of the challenges for advocating the utility of biomarkers in the clinical management of malaria is the cost involved. As most of severe disease occurs in resource limited endemic countries, there is always concern as to whether expensive detection methods can be used as practical tools. While this is a reasonable concern, the recent development of simpler and more field appropriate detection tools^{66,67} indicates that reliable biomarkers can also be measured using appropriate simple methods.⁵⁹ It is hoped that such appropriate technology will be adopted for biomarker detection when these are available. For example, beside biomarkers, ophthalmoscopy is used to improve the diagnosis of CM. Very recently Xiaojin Zhao and colleagues proposed a low cost digital polarimetric ophthalmoscopy technology that enables the capture of retinal abnormalities, which are unique to CM. The proposed technology, that can be integrated into cellphones, offers the basis of a quick and non-invasive screening of CM. It is simple and cost-effective, requiring only a single ultraviolet-exposure of the spin-coated 'guest host' mixture through a 'photoalignment master'.⁶⁸

In summary, to date, there are many different technologies, which could be used to improve the diagnosis of fatal diseases, such as severe malaria. However, the use of biomarkers, as an additional and reliable 'tool' for malaria management would clearly enhance clinical management of severe malaria patients and improve treatment outcomes.

Conclusive Remarks

To date, it has become apparent that alteration of the cerebral microcirculation is an important factor in the pathogenesis of CM.¹

The numerous trials of adjunctive therapy for severe malaria and CM have had generally disappointing results, with no single therapy showing unequivocal benefit in multiple studies, and many therapies yielding conflicting results in different studies. A significant increase in the understanding of malaria pathogenesis has led to several potential new therapies that have shown initial promise, and clinical trials of several of these therapies are currently ongoing.⁶⁹

Although the detailed pathophysiology of CM remains far from resolved, it is thought that the binding of parasitized erythrocytes to the cerebral endothelium and consequent angiogenic dysregulation play a key role in the disease pathogenesis.²⁹ As reviewed above, recent findings showed that VEGF certainly is a key player in the pathophysiology and course of CM disease.

Nevertheless, a better understanding of VEGF expression and function in CM will lead to the development of new strategies to improve treatment outcomes in CM patients and to explore the possibility to use VEGF as biomarker as well as conjunctive therapy.

Disclaimer Statements

Contributors Dr Miriam Canavese wrote and revised the manuscript. Dr Roberta Spaccapelo revised the manuscript.

Funding Dr Canavese is supported by a Senior Postdoctoral Fellowship from I-MOVE Program.

Conflicts of interest The authors have no financial interests to disclose.

Ethics approval N/A.

Acknowledgements

Dr Canavese is supported by a Senior Postdoctoral Fellowship from I-MOVE program.

References

- Shikani HJ, Freeman BD, Lisanti MP, Weiss LM, Tanowitz HB, Desruisseaux MS. Cerebral malaria: we have come a long way. *Am J Pathol*. 2012;181(5):1484–92.
- Newton CR, Krishna S. Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. *Pharmacol Ther*. 1998;79(1):1–53.
- Hunt NH, Grau GE. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol*. 2003;24(9):491–9.
- Martin C. Experimental use of nonhuman primates is not a simple problem. *Nat Med*. 2008;14(10):1011; discussion 1012–3.
- Langhorne J, Buffet P, Galinski M, Good M, Harty J, Leroy D, *et al*. The relevance of non-human primate and rodent malaria models for humans. *Malar J*. 2011;10(1):23.
- Jambou R, Combes V, Jambou MJ, Weksler BB, Couraud PO, Grau GE. *Plasmodium falciparum* adhesion on human brain microvascular endothelial cells involves transmigration-like cup formation and induces opening of intercellular junctions. *PLoS Pathog*. 2010;6(7):e1001021.
- Khaw LT, Ball HJ, Golenser J, Combes V, Grau GE, Wheway J, *et al*. Endothelial cells potentiate interferon-gamma production in a novel tripartite culture model of human cerebral malaria. *PLoS One*. 2013;8(7):e69521.
- Moxon CA, Wassmer SC, Milner DA Jr, Chisala NV, Taylor TE, Seydel KB, *et al*. Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. *Blood*. 2013;122(5):842–51.
- van der Poll T. The endothelial protein C receptor and malaria. *Blood*. 2013;122(5):624–5.
- Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci*. 2006;7(1):41–53.
- Renia L, Howland SW, Claser C, Charlotte Gruner A, Suwanarusk R, Hui Teo T, *et al*. Cerebral malaria: mysteries at the blood–brain barrier. *Virulence*. 2012;3(2):193–201.
- Medana IM, Turner GD. Human cerebral malaria and the blood–brain barrier. *Int J Parasitol*. 2006;36(5):555–68.
- Penet MF, Viola A, Confort-Gouny S, Le Fur Y, Duhamel G, Kober F, *et al*. Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. *J Neurosci*. 2005;25(32):7352–8.
- Liu TP, Fu Y, Xu WY. Immunopathological mechanism of cerebral malaria. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*. 2011;29(1):64–7.
- Medana IM, Day NP, Roberts R, Sachanonta N, Turley H, Pongponratn E, *et al*. Induction of the vascular endothelial growth factor pathway in the brain of adults with fatal falciparum malaria is a non-specific response to severe disease. *Histopathology*. 2010;57(2):282–94.
- Greenberg DA, Jin K. From angiogenesis to neuropathology. *Nature*. 2005;438(7070):954–9.
- Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9(6):669–76.
- Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev*. 1997;18(1):4–25.
- Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem*. 1998;273(21):13313–6.
- Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. *Proc Natl Acad Sci U S A*. 1996;93(25):14765–70.
- Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*. 1983;219(4587):983–5.
- Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol*. 1995;146(5):1029–39.
- Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, *et al*. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature*. 2004;429(6990):413–7.
- Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, Piera K, *et al*. Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. *Proc Natl Acad Sci U S A*. 2008;105(44):17097–102.
- Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem*. 1997;272(38):23659–67.
- LeCouter J, Moritz DR, Li B, Phillips GL, Liang XH, Gerber HP, *et al*. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science*. 2003;299(5608):890–3.
- Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, Yagi T, *et al*. A requirement for neuropilin-1 in embryonic vessel formation. *Development*. 1999;126(21):4895–902.
- Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, *et al*. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene*. 2002;21(13):2000–8.
- Furuta T, Kimura M, Watanabe N. Elevated levels of vascular endothelial growth factor (VEGF) and soluble vascular endothelial growth factor receptor (VEGFR)-2 in human malaria. *Am J Trop Med Hyg*. 2010;82(1):136–9.

- 30 Goldberg MA, Schneider TJ. Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. *J Biol Chem.* 1994;269(6):4355–9.
- 31 Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. *Trends Biochem Sci.* 2003;28(9):488–94.
- 32 Sondell M, Lundborg G, Kanje M. Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. *J Neurosci.* 1999;19(14):5731–40.
- 33 Silverman WF, Krum JM, Mani N, Rosenstein JM. Vascular, glial and neuronal effects of vascular endothelial growth factor in mesencephalic explant cultures. *Neuroscience.* 1999;90(4):1529–41.
- 34 Rosenstein JM, Mani N, Khaibullina A, Krum JM. Neurotrophic effects of vascular endothelial growth factor on organotypic cortical explants and primary cortical neurons. *J Neurosci.* 2003;23(35):11036–44.
- 35 Jin KL, Mao XO, Greenberg DA. Vascular endothelial growth factor rescues HN33 neural cells from death induced by serum withdrawal. *J Mol Neurosci.* 2000;14(3):197–203.
- 36 Jin KL, Mao XO, Greenberg DA. Vascular endothelial growth factor: direct neuroprotective effect in *in vitro* ischemia. *Proc Natl Acad Sci U S A.* 2000;97(18):10242–7.
- 37 Jin K, Mao XO, Batteur SP, McEachron E, Leahy A, Greenberg DA. Caspase-3 and the regulation of hypoxic neuronal death by vascular endothelial growth factor. *Neuroscience.* 2001;108(2):351–8.
- 38 Rosenstein JM, Mani N, Silverman WF, Krum JM. Patterns of brain angiogenesis after vascular endothelial growth factor administration *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A.* 1998;95(12):7086–91.
- 39 Krum JM, Mani N, Rosenstein JM. Angiogenic and astroglial responses to vascular endothelial growth factor administration in adult rat brain. *Neuroscience.* 2002;110(4):589–604.
- 40 Oosthuysen B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, *et al.* Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nat Genet.* 2001;28(2):131–8.
- 41 Kalaria RN, Cohen DL, Premkumar DR, Nag S, LaManna JC, Lust WD. Vascular endothelial growth factor in Alzheimer's disease and experimental cerebral ischemia. *Brain Res Mol Brain Res.* 1998;62(1):101–5.
- 42 Del Bo R, Scarlato M, Ghezzi S, Martinelli Boneschi F, Fenoglio C, Galbiati S, *et al.* Vascular endothelial growth factor gene variability is associated with increased risk for AD. *Ann Neurol.* 2005;57(3):373–80.
- 43 Carmeliet P, Ruiz de Almodovar C. VEGF ligands and receptors: implications in neurodevelopment and neurodegeneration. *Cell Mol Life Sci.* 2013;70(10):1763–78.
- 44 Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science.* 1989;246(4935):1306–9.
- 45 Krum JM, Khaibullina A. Inhibition of endogenous VEGF impedes revascularization and astroglial proliferation: roles for VEGF in brain repair. *Exp Neurol.* 2003;181(2):241–57.
- 46 Widenfalk J, Lipson A, Jubran M, Hofstetter C, Ebendal T, Cao Y, *et al.* Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury. *Neuroscience.* 2003;120(4):951–60.
- 47 Jain V, Armah HB, Tongren JE, Ned RM, Wilson NO, Crawford S, *et al.* Plasma IP-10, apoptotic and angiogenic factors associated with fatal cerebral malaria in India. *Malar J.* 2008;7:83.
- 48 Muehlenbachs A, Mutabingwa TK, Edmonds S, Fried M, Duffy PE. Hypertension and maternal-fetal conflict during placental malaria. *PLoS Med.* 2006;3(11):e446.
- 49 Deininger MH, Winkler S, Kreamsner PG, Meyermann R, Schluesener HJ. Angiogenic proteins in brains of patients who died with cerebral malaria. *J Neuroimmunol.* 2003;142(1–2):101–11.
- 50 Sachanonta N, Medana IM, Roberts R, Jones M, Day NP, White NJ, *et al.* Host vascular endothelial growth factor is trophic for *Plasmodium falciparum*-infected red blood cells. *Asian Pac J Allergy Immunol.* 2008;26(1):37–45.
- 51 Lu M, Perez VL, Ma N, Miyamoto K, Peng HB, Liao JK, *et al.* VEGF increases retinal vascular ICAM-1 expression *in vivo*. *Invest Ophthalmol Vis Sci.* 1999;40(8):1808–12.
- 52 Armah HB, Wilson NO, Sarfo BY, Powell MD, Bond VC, Anderson W, *et al.* Cerebrospinal fluid and serum biomarkers of cerebral malaria mortality in Ghanaian children. *Malar J.* 2007;6:147.
- 53 Epiphany S, Campos MG, Pamplona A, Carapau D, Pena AC, Ataíde R, *et al.* VEGF promotes malaria-associated acute lung injury in mice. *PLoS Pathog.* 2010;6(5):e1000916.
- 54 Pongponratn E, Turner GD, Day NP, Phu NH, Simpson JA, Stepniewska K, *et al.* An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. *Am J Trop Med Hyg.* 2003;69(4):345–59.
- 55 Casals-Pascual C, Idro R, Gicheru N, Gwer S, Kitsao B, Gitau E, *et al.* High levels of erythropoietin are associated with protection against neurological sequelae in African children with cerebral malaria. *Proc Natl Acad Sci U S A.* 2008;105(7):2634–9.
- 56 Croitoru-Lamourey J, Guillemin GJ, Boussin FD, Moggetti B, Gigout LI, Chéret A, *et al.* Expression of chemokines and their receptors in human and simian astrocytes: evidence for a central role of TNF alpha and IFN gamma in CXCR4 and CCR5 modulation. *Glia.* 2003;41(4):354–70.
- 57 Hanum PS, Hayano M, Kojima S. Cytokine and chemokine responses in a cerebral malaria-susceptible or -resistant strain of mice to *Plasmodium berghei* ANKA infection: early chemokine expression in the brain. *Int Immunol.* 2003;15(5):633–40.
- 58 Gengrinovitch S, Greenberg SM, Cohen T, Gitay-Goren H, Rockwell P, Maione TE, *et al.* Platelet factor-4 inhibits the mitogenic activity of VEGF121 and VEGF165 using several concurrent mechanisms. *J Biol Chem.* 1995;270(25):15059–65.
- 59 Lucchi NW, Jain V, Wilson NO, Singh N, Udhayakumar V, Stiles JK. Potential serological biomarkers of cerebral malaria. *Dis Markers.* 2011;31(6):327–35.
- 60 John CC, Panoskaltis-Mortari A, Opoka RO, Park GS, Orchard PJ, Jurek AM, *et al.* Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. *Am J Trop Med Hyg.* 2008;78(2):198–205.
- 61 John CC, Opoka-Opoka R, Byarugaba J, Idro R, Boivin MJ. Low levels of RANTES are associated with mortality in children with cerebral malaria. *J Infect Dis.* 2006;194(6):837–45.
- 62 Conroy AL, Lafferty EI, Lovegrove FE, Krudsood S, Tangpukdee N, Liles WC, *et al.* Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (non-cerebral) malaria from uncomplicated malaria. *Malar J.* 2009;8:295.
- 63 Lovegrove FE, Tangpukdee N, Opoka RO, Lafferty EI, Rajwans N, Hawkes M, *et al.* Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLoS One.* 2009;4(3):e4912.
- 64 Wilson NO, Jain V, Roberts CE, Lucchi N, Joel PK, Singh MP, *et al.* CXCL4 and CXCL10 predict risk of fatal cerebral malaria. *Dis Markers.* 2011;30(1):39–49.
- 65 Bangirana P, Giordani B, John CC, Page C, Opoka RO, Boivin MJ. Immediate neuropsychological and behavioral benefits of computerized cognitive rehabilitation in Ugandan pediatric cerebral malaria survivors. *J Dev Behav Pediatr.* 2009;30(4):310–8.
- 66 Lucchi NW, Demas A, Narayanan J, Sumari D, Kabanywany A, Kachur SP, *et al.* Real-time fluorescence loop mediated isothermal amplification for the diagnosis of malaria. *PLoS One.* 2010;5(10):e13733.
- 67 Lee WG, Kim YG, Chung BG, Demirci U, Khademhosseini A. Nano/microfluidics for diagnosis of infectious diseases in developing countries. *Adv Drug Deliv Rev.* 2010;62(4–5):449–57.
- 68 Zhao X, Bermak A, Boussaid F. A low cost CMOS polarimetric ophthalmoscope scheme for cerebral malaria diagnostic. *Int Fed Inform Process.* 2012;379:1–9.
- 69 John CC, Kutamba E, Mugarura K, Opoka RO. Adjunctive therapy for cerebral malaria and other severe forms of *Plasmodium falciparum* malaria. *Expert Rev Anti Infect Ther.* 2010;8(9): 997–1008.