On the pathophysiology of severe falciparum malaria with special reference to red cell deformability
Dondorp, A.M.
Preface

The thesis is the result of clinical and laboratory research performed between 1995 and 2000. Main sources and examples include: D. S. Cooper, D. S. Cooper in Transfusion Therapy, 1995. The information contained herein is the original work of the author and has not been published elsewhere. The author has obtained informed consent from all participants, and the research was conducted in accordance with ethical guidelines. Any personal information has been anonymised. The findings described in this thesis represent a novel approach to the understanding of red cell deformability and its implications in transfusion medicine.

As an unexpected physiological abnormality, red cell deformability could not be established in vivo without a clear understanding of its role in blood flow and transport. The importance of red cell deformability in transfusion medicine could be further investigated in vitro through experimental studies on red blood cells from healthy volunteers. The potential implications of these findings in transfusion medicine are significant.

The importance of the findings is further illustrated by the potential implications of these results in transfusion medicine. The findings have been confirmed in vitro and in vivo, and the implications of these findings in transfusion medicine are significant.

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low-resistance to their view, with numerous illustrative examples. Further details in the methodology section.

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E. Hemorrhagic shock: copper and endothelial fluid in patients with severe hemorrhage.

Preface

This thesis is the result of clinical and laboratory studies performed between 1995 and 1999 in Mae Sot and Bangkok (Thailand), Kilifi (Kenya) and Amsterdam (The Netherlands). But the actual start was long before this, when I did an elective in haemorheology in the laboratory of Dr. M.R. Hardeman as a medical student. This elective consisted mainly of staring into a diffracted laser beam, as a step in the development of the Laser-assisted Optical Rotational Cell Analyser (LORCA). The ellipticity of the diffraction pattern as a measure of red cell deformability was measured with a ruler, prone to observer bias. Although blinding of the observer by the laser could lead to more objectivity, the need for a more accurate analysing system became apparent. This led to adaptations with a camera and a computerised curve fitting system, giving the LORCA its great accurateness. After this, still a medical student, I did an elective at the laboratory for haemorheology (Dr. F.J. Nordt) at Sandoz AG in Basel, Switzerland. This led to a temporary job for one year in the laboratory for Rheology and Stroke (Dr. B. Coull) at the Oregon Health Sciences University in Portland, Oregon, U.S.A., studying rheological mechanisms in ischaemic stroke. As in diabetes, a pathophysiological role for disturbances in red cell deformability could not be established in patients with stroke. It seemed that red cell deformability was a well regulated physiological property of erythrocytes, since its normal values were kept within a narrow range. Although these experiences abroad were very enjoyable, they led to no clear scientific path for continuing research. Also internationally, the initial enthusiasm in haemorheology as a clinical science seemed to be waning.

The initiative to measure rheological parameters in malaria came from Johan Vreeken and Piet Kager. It was suggested that altered red cell deformability could be involved in the very quick removal of infected red blood cells from the blood during treatment with artemisinin derivatives and possibly in the neurotoxicity of these new antimalarials. However, in healthy volunteers taking arteether i.m., no reduction in red cell deformability could be observed (also in our later experimental and clinical observations no effect on red cell deformability of artemisinin derivatives in physiological concentrations could be observed). The first important observation came from a pilot study I did together with Karla Peters in Dutch travellers returning home with falciparum malaria. It appeared that these patients did show a reduction in red cell deformability, due to rigidification of the unparasitised red cells. This reduction in red cell deformability predicted the nadir in anaemia that these patients developed. This could offer an explanation for the well known increased clearance of uninfected red cells in falciparum malaria. These preliminary results were presented to Prof. Nick White, who was visiting professor in the AMC hospital in 1994. Because of his invitation to further evaluate this hypothesis in the Wellcome Units in Mae Sot and Bangkok.
in Thailand, this study has become successful. Our Thai studies revealed that in addition to a predictor for anaemia, a severe reduction in red cell deformability was also a strong predictor for a fatal outcome in falciparum malaria. These results were presented at the Oxford Tropical Medicine Meeting in 1997. Prof. K Marsh, who chaired the session of my talk, invited us to visit the Wellcome Unit in Kilifi, Kenya, to expand our observations in children with severe malaria. The Kenyan study confirmed our data in adults and showed that red cell deformability could be restored by blood transfusion. Both in Thailand and Kenya the hospitality I came across was wonderful and the work was a pleasure. Because of the open and sharing atmosphere, I learned a lot more about clinical malaria. In addition to the clinical studies I have spent quite some time in the laboratory with Nok (Kessinee Chotivanich) and Karla Peters, trying to sort out the mechanism behind the rigidification of non-parasitised red cells in falciparum malaria. These mechanisms did not appear to be so straightforward. No conclusive answers yet, and no apotheosis as final chapter for this thesis. But the good thing about it is that it leaves opportunities for future research. And it gives the possibility to stay in contact with a lot of friends that I have met through this research project.

There are many people I would like to thank:

First of all, Marja, my wife. You managed the family when I was abroad and gave me the freedom to do this research. My children, Lisa and Marten, who also had to miss their father from time to time. But, it prevented me from getting grumpy, kids.

Johan Vreeken, who was always supportive. A long time ago, when I was a young medical student, you made me enthusiastic for internal medicine. You have played a central role in my specialist training. You initiated this research line and your sharp reasoning often guided into the right direction.

Piet Kager, you have been very supportive and your good relationship with many people involved in tropical medicine throughout the world were crucial for the success of this research. Thank you for the critical and precise reviews of all the protocols and manuscripts.

Nick White, role model, but, unfortunately, unparalleled. Thanks again for your hospitality and the discussions we have had about science and medicine. I reckon that when you are in your late seventies, I will be in my sixties, which gives me some hope to beat you in squash some day.

Kevin Marsh, the Kilifi unit provided a unique place to work. Thank you for the opportunity to share in the open and nice atmosphere. Your amazingly broad scope in science is impressive and I want to thank you for the many learning experiences. Thanks for your essential help in preparing the manuscript of our joint study.

Karla Peters, my mate in good and bad times. Thank you for your dedication to all our
studies. This thesis is also a little yours. Without your dedicated persistence in the follow up of travellers in our pilot study, we would never have discovered the relevance of red cell deformability in falciparum malaria. Your help in the Kilifi study was indispensable.

Brian Angus, my friend. We have spent many hours, day and night, on the wards of Mae Sod hospital keeping patients alive and in the meantime trying to collect all the samples and data (hourly blood sampling, collecting 24-hour urine samples and explaining this in Burmese). Let's keep in touch.

Rachanee Udomsangpetch, thanks for the fruitful discussions on rigid and sticky erythrocytes.

Nok (Kesinee Chotivanich), master of culturing malaria parasites (and of knowing what to do with them afterwards). One day we will unravel the mechanisms behind the rigidification of red cells in falciparum malaria. It was a pleasure to have you as a guest in Amsterdam.

Max Hardeman, without the development of the LORCA, this research project would not have been possible.

Peter Goedhart, who taught me how to use a pipette and was always a standby for technical and computer problems.

Brett Lowe, I think it is a great achievement to manage such a smooth working laboratory. Thanks for your support.

Mike Nyanoti, key player in the carrying out of the Kilifi study. Thanks for your friendship and dedication. Hope to meet you in Amsterdam this September.

Pi Oye (Kamdrat Silamut), Paul Newton, Andy Simpson, Khun Pacharee, Tim Planche and all the other people from the Bangkok unit. Thanks for the good times.

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Director, staff and nurses from Mae Sod provincial hospital, in particular doctor Ronatrai Ruangveerayuth, for their support.

Jane Crawley, Charles Newton, Pauline Lowe, Francis Ndungu, Sam, Peter, Gabriel, technicians and nurses from the Kilifi unit. It was a pleasure to be with you.

Hans Romijn, thanks for your contribution to the chapter on nitric oxides and your support in my clinical career.

Finally, I want to thank my parents. You are the most supportive parents imaginable, as long as my conscious memory spans, irrespective of what I've done or do. And my brother, Jan Jaap, who kept me on the right track through life.