Measurement of microcirculation in clinical research

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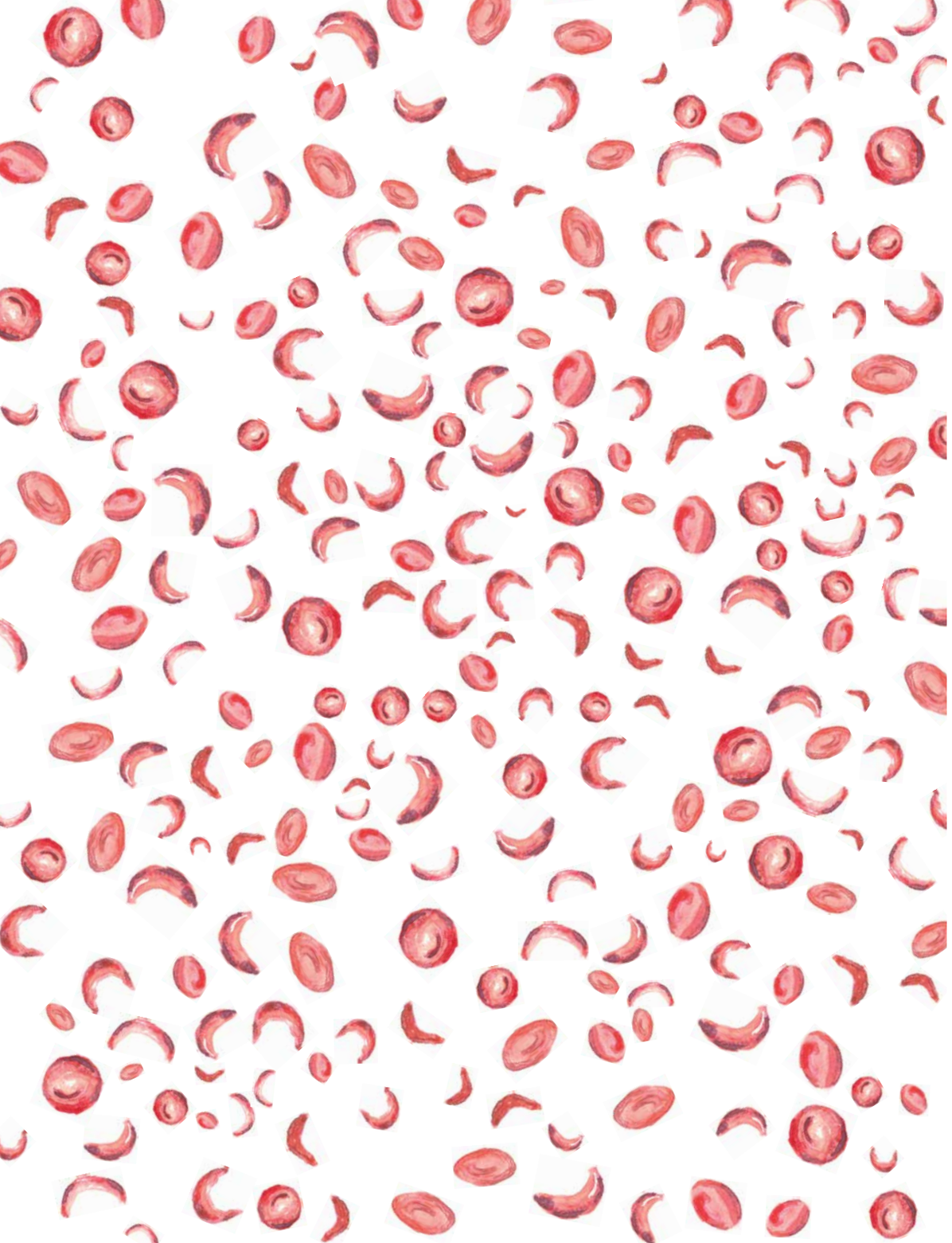

SUMMARY

In this thesis, we have discussed the ability and inability of the microvasculature to be valuable in measuring the pharmacodynamic effects of compounds. This was done in seven chapters, using various angles to evaluate the microvasculature.

Chapter two provides an overview on the application of microcirculation measurements in current practice. Emphasis was put on the clinical utility of the minimal-invasive microvascular imaging techniques that are currently commercially available for skin and retinal microcirculation assessments. Included in this review are the most common used cutaneous and retinal microvascular measurements devices and promising new techniques alongside with challenges and description of minimal invasive biochemical tests to provide guidance when investigating the microcirculation.

In **chapter three**, microvascular functionality was explored in 8 sickle cell disease patients and matched healthy volunteers with two new devices: the laser speckle contrast imager (LSCI) and the retinal function imager. Both devices showed excellent repeatability (CVC of 8.5% 9.5%, 7.6% and 7.7% respectively) and between measurements on one day (CVC of 7.0%, 7.7%, 7.6% and 4.7% respectively) and were able to measure a difference in microvascular functionality between the patients and healthy volunteers, making these devices feasible for further use in clinical microvascular research. Although the retinal function imager showed great potential, acquisition of the images had a steep learning curve.

Chapter four: The results of the study in chapter three were used to set up a larger study in which microvascular measurements were compared with cerebral hemodynamics using MRI. A total of 17 sickle cell patients and 6 healthy volunteers were included. An increased cerebral and skin blood flow was found in sickle cell patients compared to controls. Furthermore microvascular skin measurements (basal flow) were strongly correlated [ASL vs. LSCI: r = 0.70; PC-MRI vs. LSCI: r = 0.75] with MRI measurements. This suggests that cutaneous microvascular measurements might be a low cost, non-invasive alternative method to obtain details on the hemodynamic state of sickle cell patients.

Chapter five: Retinal microcirculation and retinal oximetry were quantitatively analyzed using fractal analysis in 8 sickle cell patients and matched healthy volunteers. Oximetry pictures and non-invasive capillary perfusion maps (nCPM) were obtained by the retinal function imager. No significant difference was observed for the fractal analysis between the patients and healthy controls, however significantly lower oxygenated Hb levels (Effect size (ES)=850.3; 95% CI 69.16, 1631; p=0.04), and (ES=919.9; 95% CI 171.4, 1668; p=0.02). were detected in sickle cell patients as compared to the healthy controls in the temporal quadrants, suggesting that in sickle cell patients before any structural microvascular changes are present, functional abnormalities could be observed in oximetry measurements.

Chapter six provides an exploration of a new tool the miniaturized Dynamic Light Scattering (MDLS). The MDLS was evaluated in 16 patients with different vascular abnormalities for which they used vitamin K antaganost (amongst them cardiac dysfunction, protein c deficiency pulmonary thrombosis), 8 sickle cell disease patients and 8 unmatched healthy volunteers, by having two measurements on two different days and was benchmarked against LSCI measurements and several biochemical and hematological measurements. There was a large intra-subject variability in MDLS (depending on the type of measurement up to CV> 150%) and none of the endpoints discriminated between the patients and the healthy controls. LSCI and laboratory measurements were stable over time and were able to measure difference between the groups, suggesting that the MDLS device is not suitable for measuring pharmacologically induced changes in microcirculation.

Chapter seven: In this study, we evaluated the effect of recombinant human erythropoietin (RHUEPO) on the cutaneous microvascular function in well-trained cyclists using LSCI. Forty-eight subjects received a weekly dose of either RHUEPO or placebo (1:1 ratio) for 8 weeks, and LSCI was performed

at baseline, after a maximal exercise test in week 6, and before maximal exercise in week 8. Despite an increase in hematocrit levels in the RHUEPO- treated group, we found no statistically significant difference in microvascular function measured between the RHUEPO-treated group and the placebo group. The results of this study suggest that the increased hematocrit levels in RHUEPO-treated well-trained cyclists are not associated with changes in microvascular measurements using LSCI.

Chapter eight: In this study, hyperspectral imaging was evaluated by using a snapshot hyperspectral camera that can measure tissue oxygenation non-invasively using relevant wavelengths in the VIS-NIR region (450-950 nm) and benchmarked the outcomes against LSCI. Several challenges were explored in 16 healthy volunteers including occlusion-reperfusion of the brachial artery and measurements of local changes in skin oxygenation and blood flow after applying a local vasodilator (capsaicin-based cream) and a local vasoconstrictor (brimonidine gel) and outcomes were compared to measurements of an untreated area of the skin. Hyperspectral results (cutaneous StO_2 levels) were correlated to the haemoglobin oxygen saturation measured by an oximeter, furthermore a strong correlation (Pearson R^2: 0.86) of blood flow with StO_2 levels during occlusion-reperfusion phases was observerd. The results of this pilot study showed that the snapshot hyperspectral camera was able to detect different levels of cutaneous StO_2 in human volunteers.

DISCUSSION

Due to technological advances in the last decades the possibilities for microvascular measurements have taken a flight. In this thesis, we have investigated several new devices that have been developed or are that are currently under development and we investigated the applicability of several novel microcirculation measurement devices for their utility in the clinic or in a clinical research setting.

As outlined in chapters one and two, new devices must be validated before the can be used in clinical research. This validation process consists of several steps that allows the investigators to determine how precise the measurements are, what the variability of the endpoints is and if the device can pick up diseases or pharmacodynamic effects of compounds. In this thesis, we have attempted to validate several devices, and there are several points that can be discussed. First, the measurements should be fairly easy to perform and operator dependency should be limited. This is applicable for the LSCI, however for other devices such as the RFI or MDLS, there is a steep learning curve and large operator dependency, hampering the possibility of these devices to provide the researchers with quality data during the course of a study. Therefore for experimental devices such as the hyperspectral camera, it is essential that findings on the applicability and validity are taken into account.

The nature of the microvasculature and in particular its accessibility make it a potentially valuable biomarker, although this does require the devices used to evaluate the microvasculature to be as minimally invasive as possible, which is certainly the case for most devices investigated in this thesis. And finally, although most imaging devices require a large initial investment (see chapter 2), the devices have in general low maintenance and low operational costs.

The role of microvascular dysfunction in the pathophysiology of several diseases is well known, and is not only limited to vascular disease but also in ischemic nerve damage, kidney disease and retinal dysfunction. Moreover recent research provides us the insight that microvascular alterations might be an early predictor for onset of certain disease. This has been well studied in ophthalmology with relation to stroke[1], kidney damage and cardiovascular disease.[2,3] Besides the traditional risk factors for cardiovascular diseases such as smoking, hypertension and obesity a substantial proportion of cardiovascular disease is not explained by these risk factors. Therefore more extensive investigation into the mechanisms behind microvascular dysfunction might provide novel insights into the biomarkers that can be used as key endpoints for which devices can then be developed.

In the current global health crisis due to the SARS-CoV-2 virus, evidence is emerging for a key role of the microvasculature in critically ill patients. In COVID-19 severe organ damage has been observed related to a hypercoagulable state.[4] The underlying cause might be microvascular endothelial dysfunction leading to this hypercoagulable state.[5,6] Further research is warranted to evaluate if microvascular assessment can improve risk stratification of patients, or if the microvasculature is a target in the prevention or treatment of the hypercoagulable state.

FUTURE DIRECTIONS

There are plenty novel devices claiming to enable non-invasive measurements of the microcirculation, however for successful wide implementation of microvascular measurements, it is essential that implementation of these devices in clinical studies is regulated in such a way that the outcome of the studies can be relied upon. The new EMA guidelines foresee in this problem and must be adhered to when implementing novel devices in clinical studies.

We foresee a broader implementation of microvascular measurements, not only in the clinic but also in the home situation. For example, PPG measurements are integrated in wearables or mobile devices more and more. As microcirculation might play a dominant role as predictor for certain disease, home monitoring using mobile devices may allow early detection of, for example, myocardial infarction or acute heart failure and provide a time window for cardiologists to take action, preventing hospital admission or worse. Furthermore, microvascular measurements might be more often used as a method to obtain more information about the pharmacodynamics in (early phase) pharmacological research, certainly considering the key role for nitric oxide, also in immune activation and metabolic disease. Currently microvascular measurements are not frequently applied in studies, however due to the relatively low cost, low burden for patients and useful insights this could be valuable add on in pharmacological trials.

Although there have been decades with microvascular measurements it is not only until the last two decades that microvascular research has taken a flight. New imaging techniques, more advanced algorithms for analysis and better computing processing have established microvascular research methods. However before wide implementation these methods should be tested for their validity an integrity. Microvascular research has already brought us interesting new insight in the pathophysiology of diseases and we are only at the beginning of a new era of microvascular research. In this thesis we hope to have contributed with our validation studies of new and existing microcirculation measurement tools to a wider implementation of microvascular measurements in current clinical and research practice.

REFERENCES

1 Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. The Lancet. 2001;358(9288):1134-40.

2 Wong TY, Coresh J, Klein R, Muntner P, Couper DJ, Sharrett AR, et al. Retinal microvascular abnormalities and renal dysfunction: the atherosclerosis risk in communities study. Journal of the American Society of Nephrology. 2004;15(9):2469-76.

3 Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. Survey of ophthalmology. 2001;46(1):59-80.

4 Wadman M, Couzin-Frankel J, Kaiser J, Matacic C. How does coronavirus kill. Clinicians trace a ferocious rampage through the body, from brain to toes. 2020:1502-3.

5 Green SJ. Covid-19 accelerates endothelial dysfunction and nitric oxide deficiency. Microbes Infect. 2020;22(4-5):149-50.

6 Colantuoni A, Martini R, Caprari P, Ballestri M, Capecchi PL, Gnasso A, et al. COVID-19 Sepsis and Microcirculation Dysfunction. Frontiers in Physiology. 2020;11(747).