Università degli Studi di Brescia
Presentazione *Progetti bando di Ateneo Health & Wealth*
Area tematica "Tecnologie per la Salute"
9 luglio 2021

Sviluppo di nuovi metodi di valutazione della struttura del microcircolo MICROOFT

Damiano Rizzoni, Enrico Agabiti Rosei

Dipartimento di Scienze Cliniche e Sperimentali,
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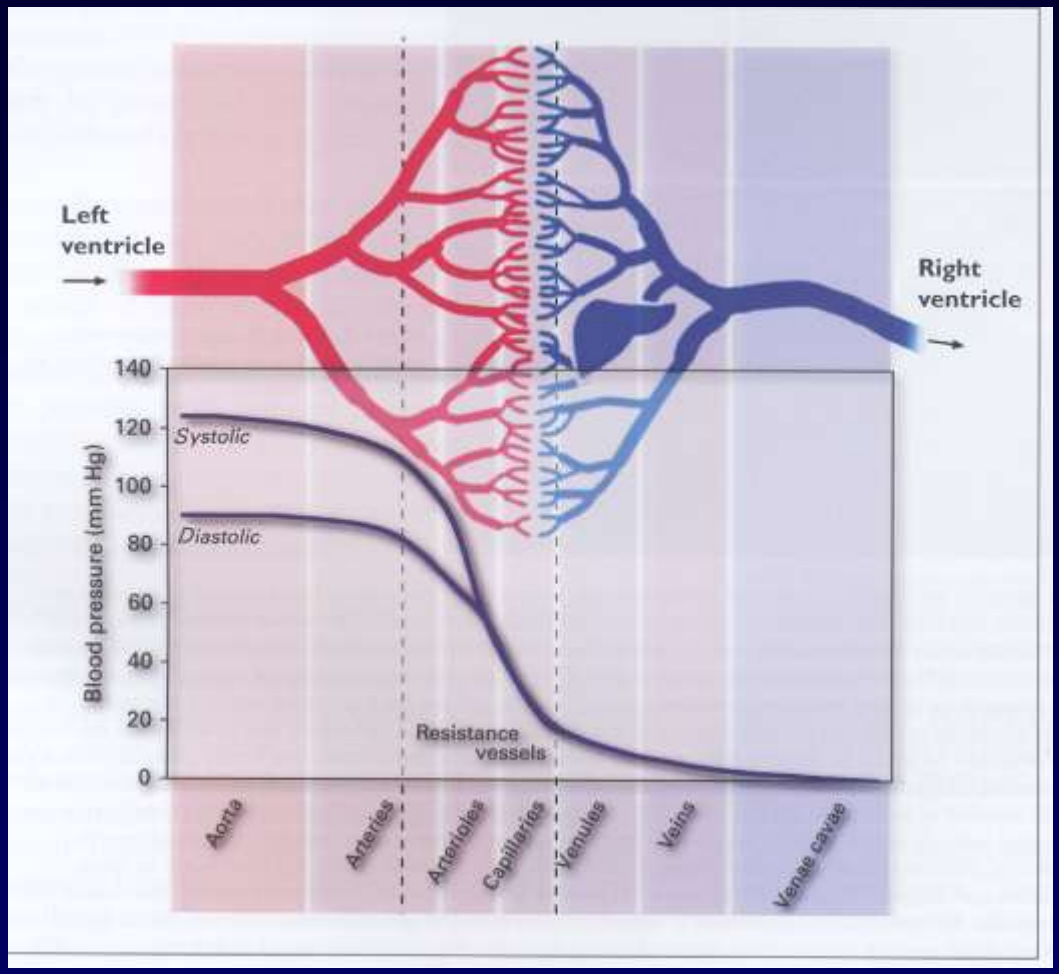
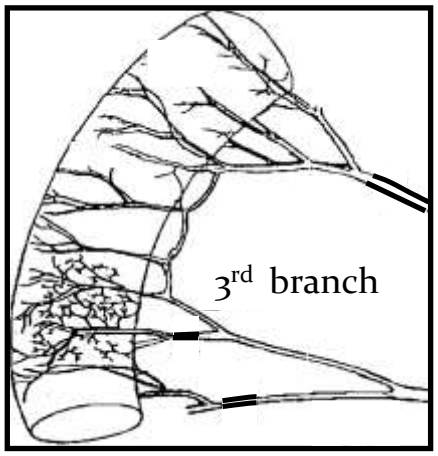
Resistance arteries are key elements in the control of blood pressure



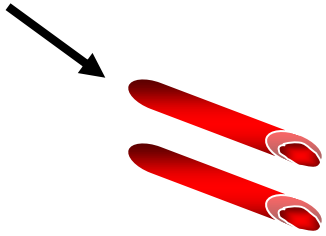
The main drop in hydrostatic pressure occurs al the level of resistance arteries; i.e. the terminal arterioles and the microcirculation. These are vessels with a luminal diameter of less than approximately 400 µm (small arteries/arterioles) and 100 µm (arterioles/capillaries)

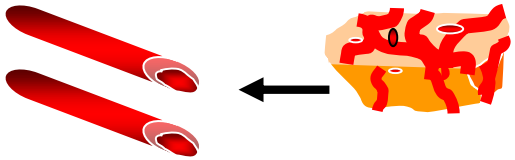
Total peripheral resistance in terminal arteries and arterioles amounts to 45% to 50%, in capillaries to 23% to 30%, in venules to 3% to 4%, and to 3% in veins. Thus, structural changes in small arteries and disappearance (rarefaction) of capillaries strongly affect blood pressure.

Small vessels evaluation
Wire or pressurized micromyograph



Subcutaneous tissue from skin biopsy

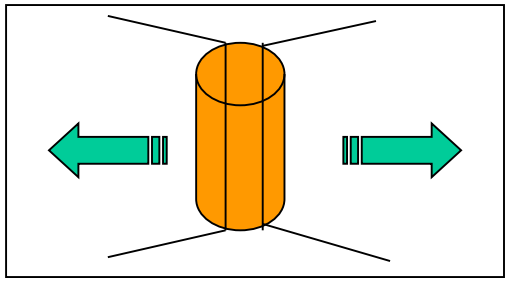




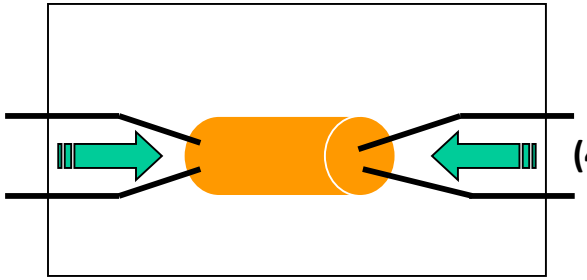
Mesenteric artery
(150 ~350 µm)

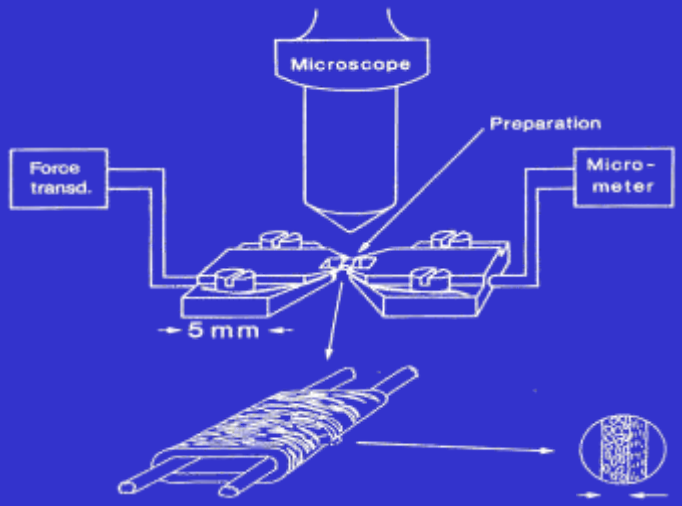
Resistance small artery
(150 ~350 µm)

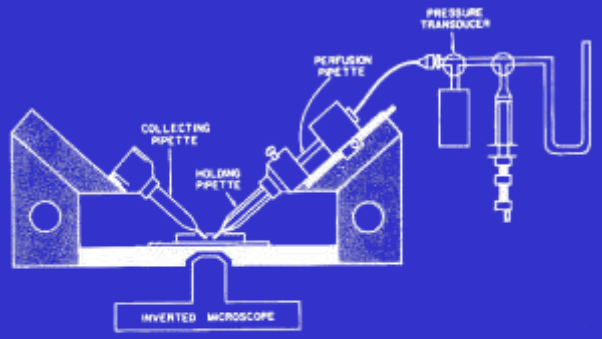
Isometric (wire myograph)

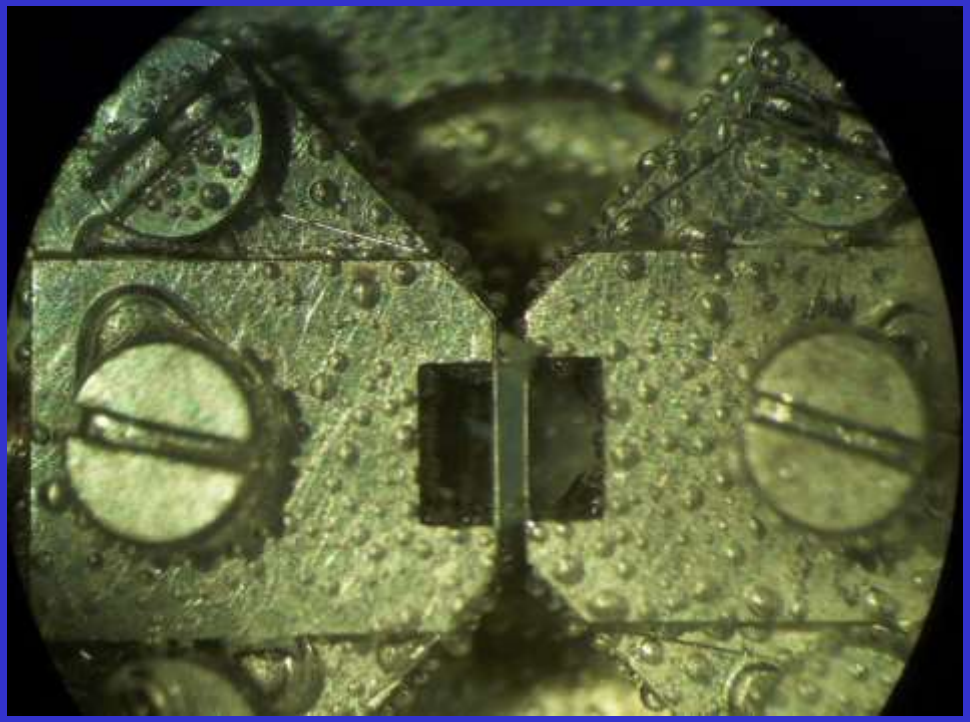


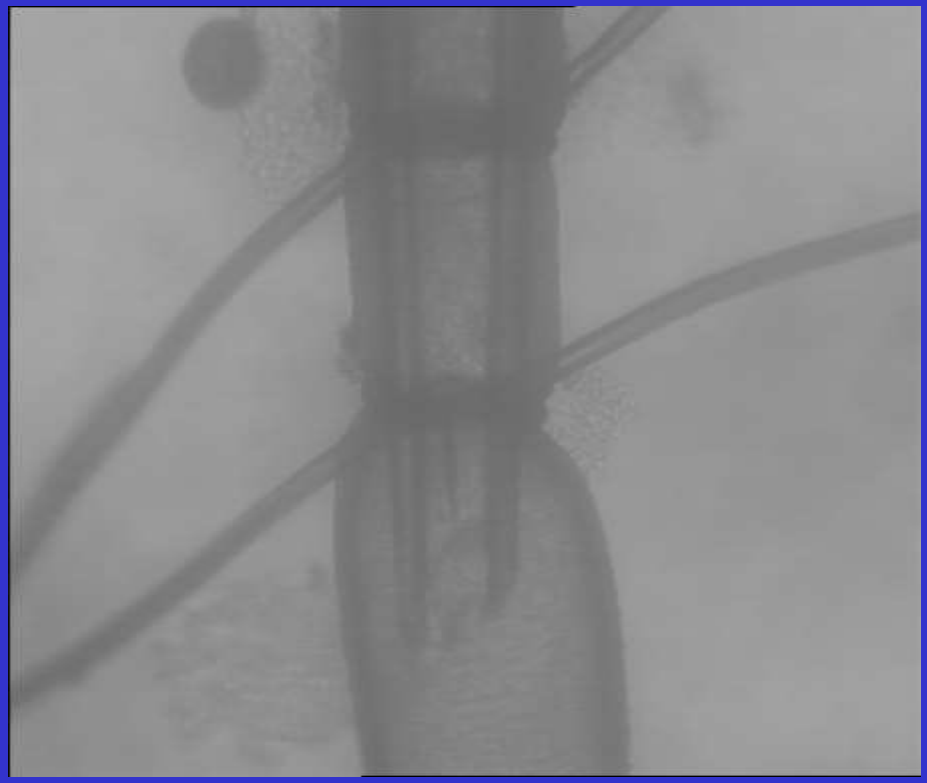
Isobaric (pressure myograph)









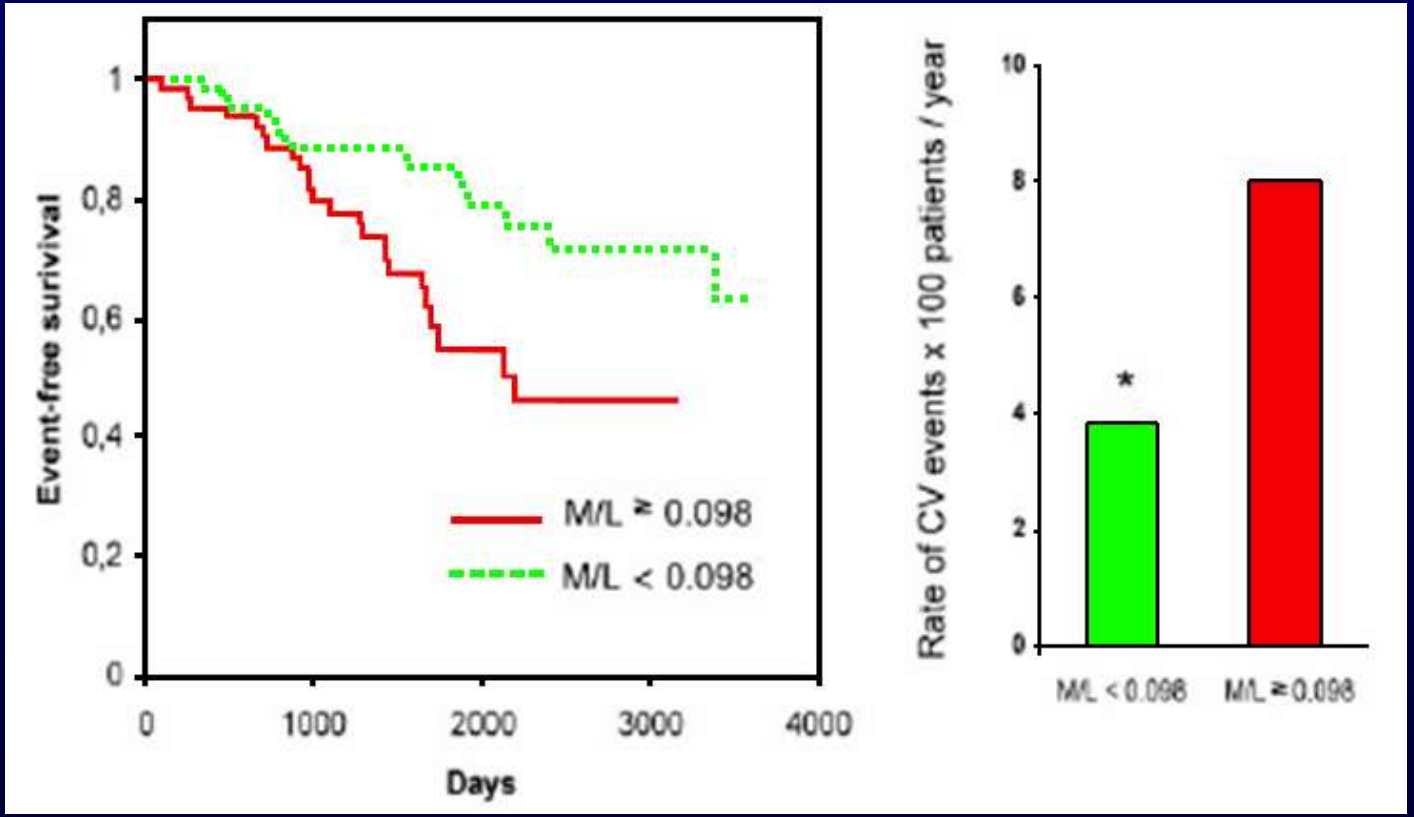


Isometric (wire myograph)

Isobaric (pressure myograph)

Prognostic Significance of Small-Artery Structure in Hypertension

Damiano Rizzoni, Enzo Porteri, Gianluca E.M. Boari, Carolina De Ciuceis, Intissar Sleiman, Maria Lorenza Muiesan, Maurizio Castellano, Marco Miclini, Enrico Agabiti-Rosei.



N = 151

M/L: cutpoint 0.098 (mean and median value)

Mean follow-up 5.6 yrs

Circulation 2003;108:2230-2235.

Microvascular structure as a prognostically relevant endpoint

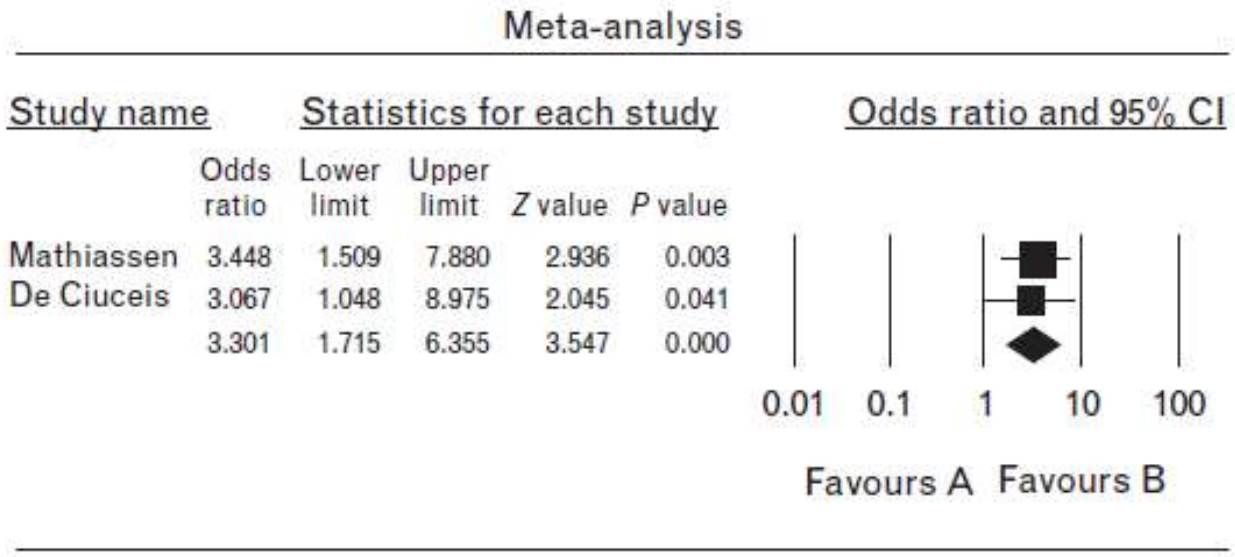


FIGURE 1 Meta-analysis of two studies [49,50]: only patients with essential hypertension are included ($n = 270$). Odds ratio for cardiovascular events in favour of patients with a media-to-lumen ratio of subcutaneous small arteries below 0.098 ($P < 0.0001$). CI, confidence interval.

Agabiti-Rosei E & Rizzoni D, J Hypertens 2017, 35:914–921

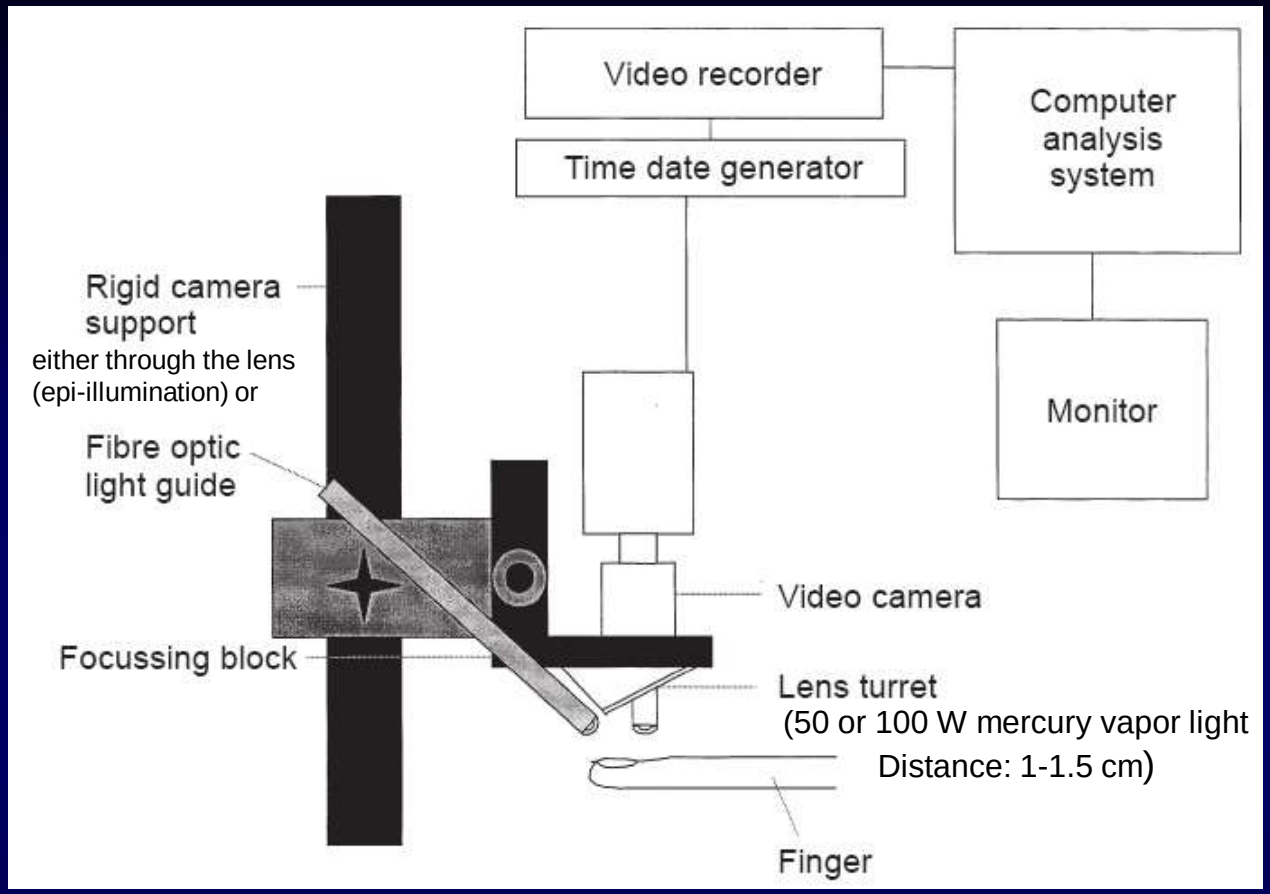
SMALL RESISTANCE ARTERIES

An increase in the wall-lumen ratio of small arteries can be measured in subcutaneous tissues obtained through gluteal biopsies.

These measurements can demonstrate early alterations in diabetes and hypertension and have a predictive value for CV morbidity and mortality, but the invasiveness of the method makes this approach unsuitable for general use.

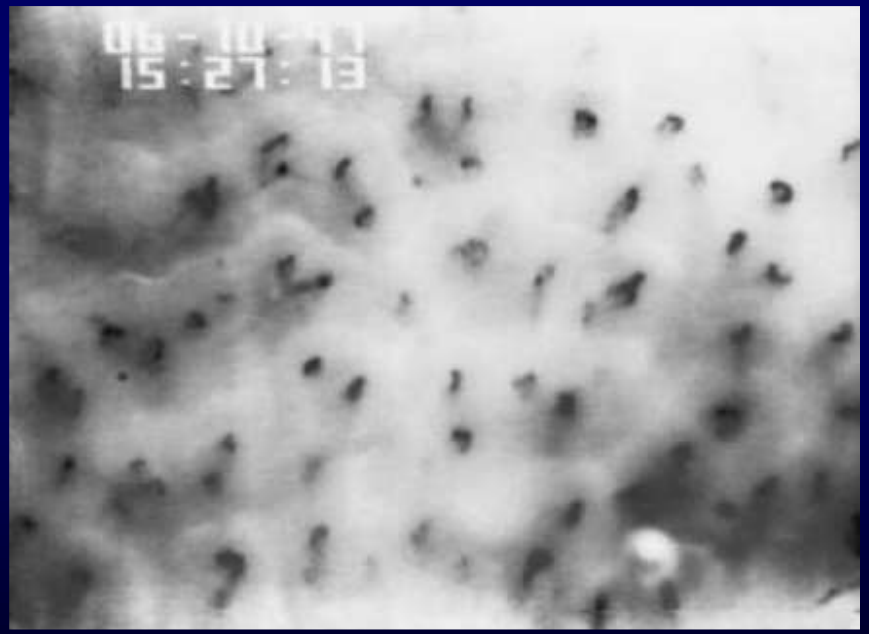
Rizzoni D et al, Circulation 2003; 108:2230–2235.
De Ciuceis C et al, Am J Hypertens 2007; 20:846–852.
Mathiassen et al, J Hypertens 2007; 25:1021–1026.

Videomicroscopy - Capillaroscopy (intra-vital capillaroscopy)

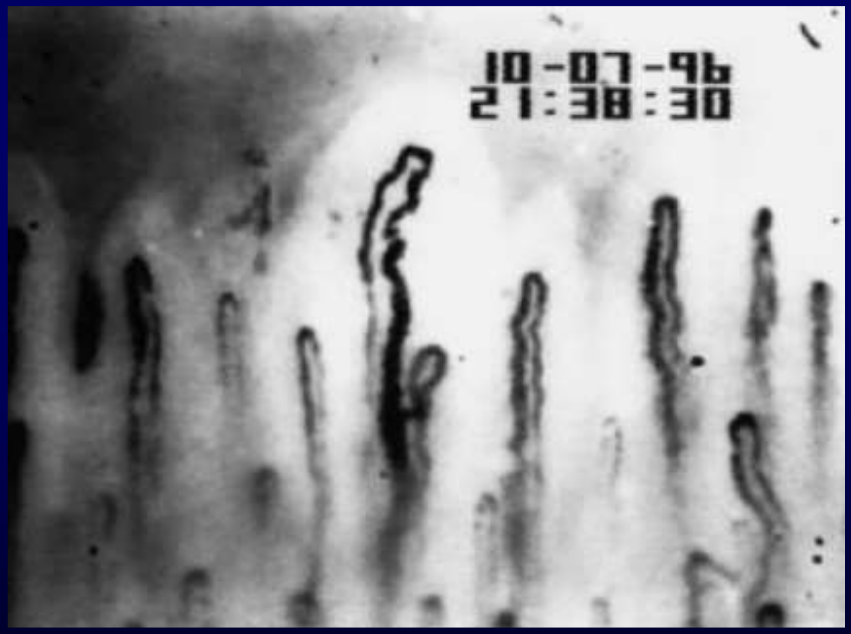


Capillaroscopy or intravital videomicroscopy is a technique providing a 2-D projection of a 3-D capillary network. It generates high-contrast images, videotapes or photographs of skin capillaries by means of television, video and/or informatic systems. It allows the assessment of capillary morphology and capillary density (traditional capillaroscopy), capillary flow velocity (dynamic capillaroscopy), capillary red cell column width.

From a structural point of view, the most interesting parameter to be analyzed by capillaroscopy is capillary density. Capillary density is defined as the number of capillaries per unit of skin area. It is measured recording images from the capillary microscope and counting the capillaries in a known skin area. Depending on the underlying investigated area, the capillaries will appear as black dots. (if they are perpendicular to the surface), lines (if the capillaries are lying on an oblique surface) or both. Red blood cells look black in capillaroscopy as the emission spectrum of the mercury lamp is similar to the absorption spectrum of haemoglobin (that is 370-450 nm). Traditional capillaroscopy cannot directly show capillaries that are not perfused at rest. Inducing venous congestion for a few minutes allows the recruitment of previously unperfused vessels, in order to gather information about the total density of vessels of the capillary district, including recruited vessels.



Cutaneous capillaries (dorsum of the finger) (173 x)



Normal capillaries in the nailfoldi (173 x)

Angela C. Shore Br J Clin Pharmacol, 50, 501-513

Structural Skin Capillary Rarefaction in Essential Hypertension

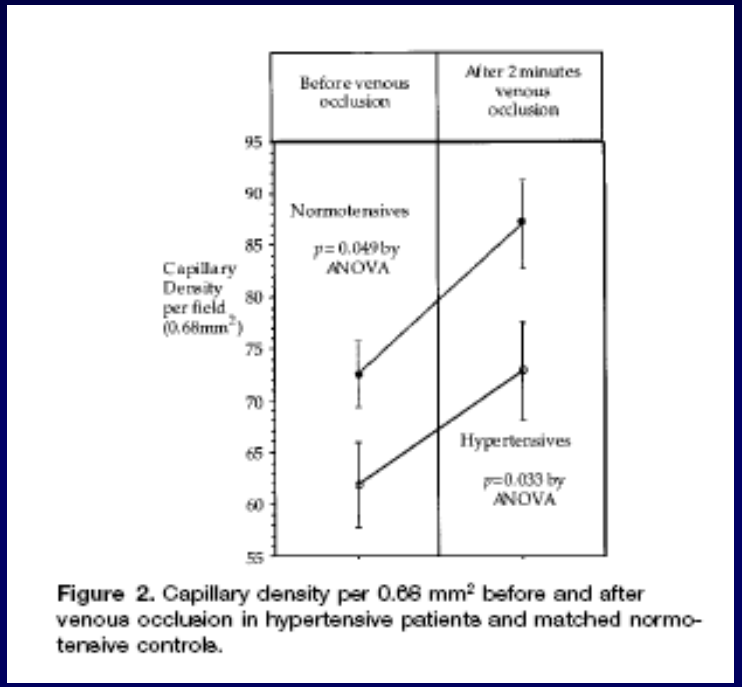


Figure 2. Capillary density per 0.66 mm² before and after venous occlusion in hypertensive patients and matched normotensive controls.

"The aim of this study was to assess the extent of structural versus functional capillary rarefaction in the skin of dorsum of fingers in essential hypertension. The capillary microcirculation was examined with video microscopy before and after maximizing the number of perfused capillaries by venous congestion. The study group comprised 17 patients with essential hypertension (mean supine blood pressure, 155/96 mm Hg) and 17 closely matched normotensive controls (mean blood pressure, 127/77 mm Hg). We used intravital video microscopy with an epi-illuminated microscope to examine the skin of the dorsum of left middle phalanx before and after venous congestion at 60 mm Hg for 2 minutes"

Antonios TF et al, Hypertension 1999;33:998-1001

Scanning Laser Doppler Flowmetry:
A tool to assess structural alterations in the microcirculation

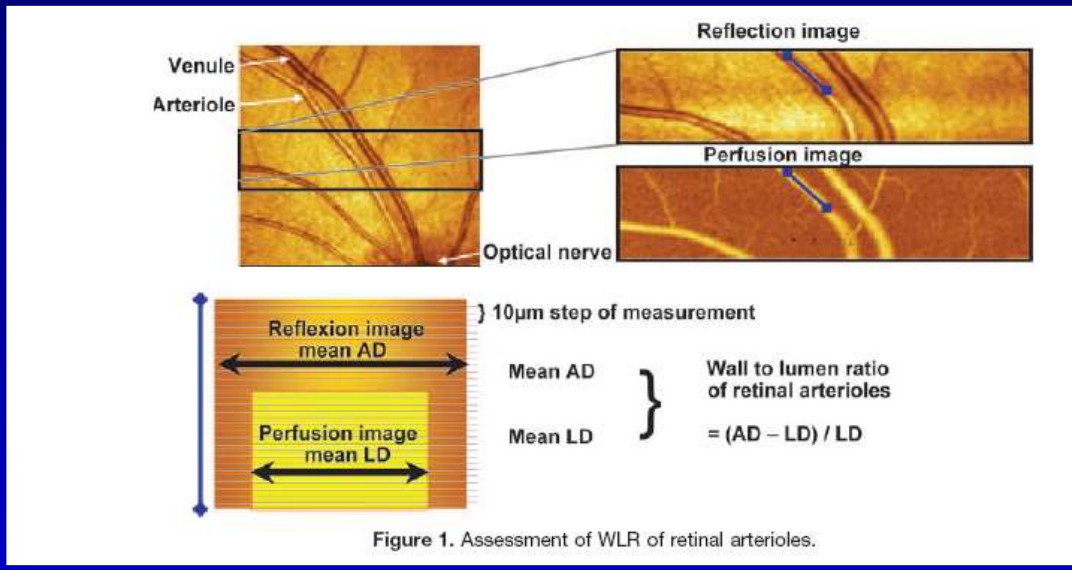
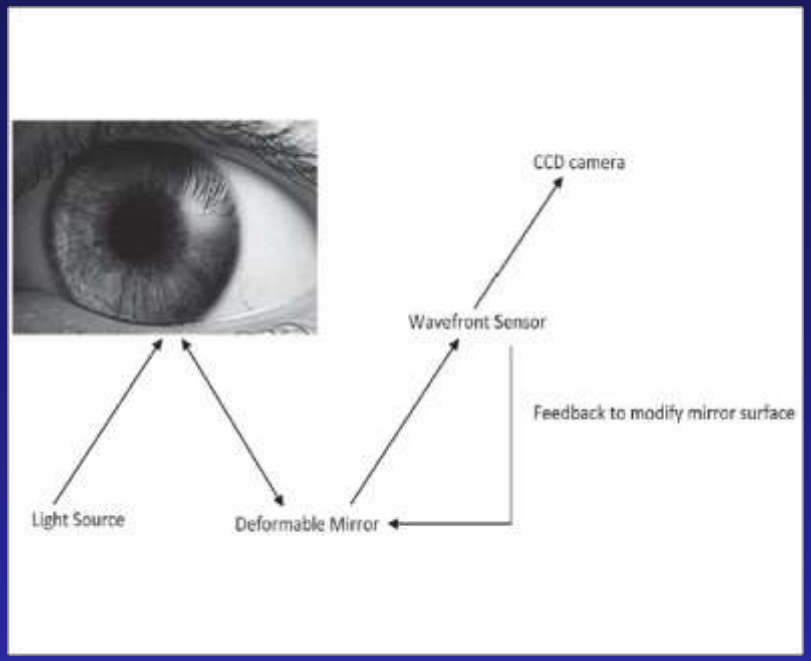


Figure 1. Assessment of WLR of retinal arterioles.

Increased Wall:Lumen Ratio of Retinal Arterioles in Male Patients With a History of a Cerebrovascular Event

Joanna M. Harazny, Martin Ritt, Delia Baleanu, Christian Ott, Josef Heckmann, Markus P. Schlaich, Georg Michelson, Roland E. Schmieder

***Hypertension* 2007;50:623-629.**

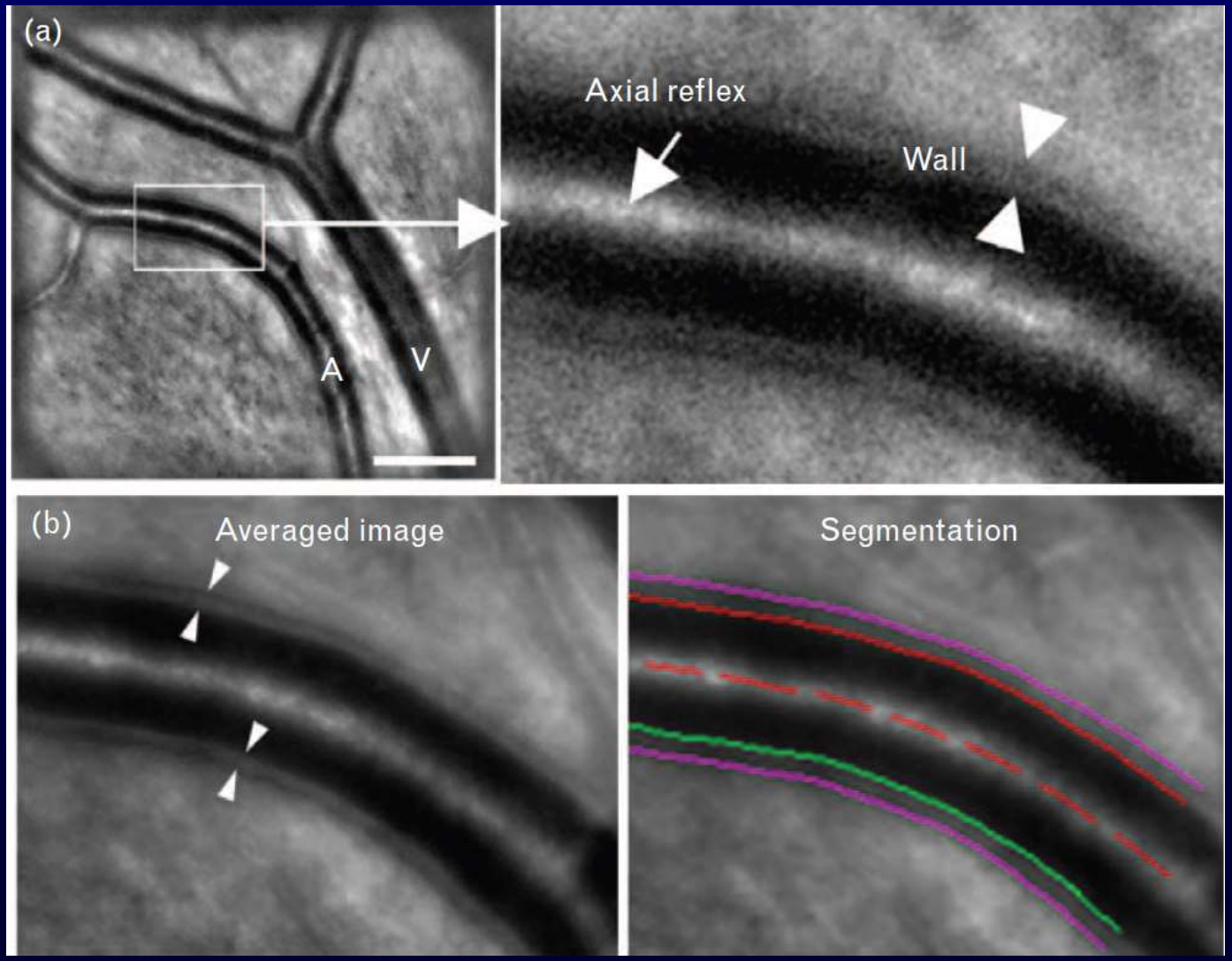


Schematic outline of adaptive optics imaging system. A beam of light enters the eye, and a small amount is reflected back out of the eye and into the optical system. Reflected light is altered by the deformable mirror for optical aberrations based on measurements made by the wave-front sensor. Information about the aberrations of the wave front is processed by the control system that provides feedback to the deformable mirror

Rtx-1 Imagine Eyes, Orsay, FR

Morphometric analysis of small arteries in the human retina using adaptive optics imaging: relationship with blood pressure and focal vascular changes.

Koch E, Rosenbaum D, Brolly A, Sahel JA, Chaumet-Riffaud P, Girerd X, Rossant F, Paques M.



J Hypertens 2014 ; 32(4):890-898

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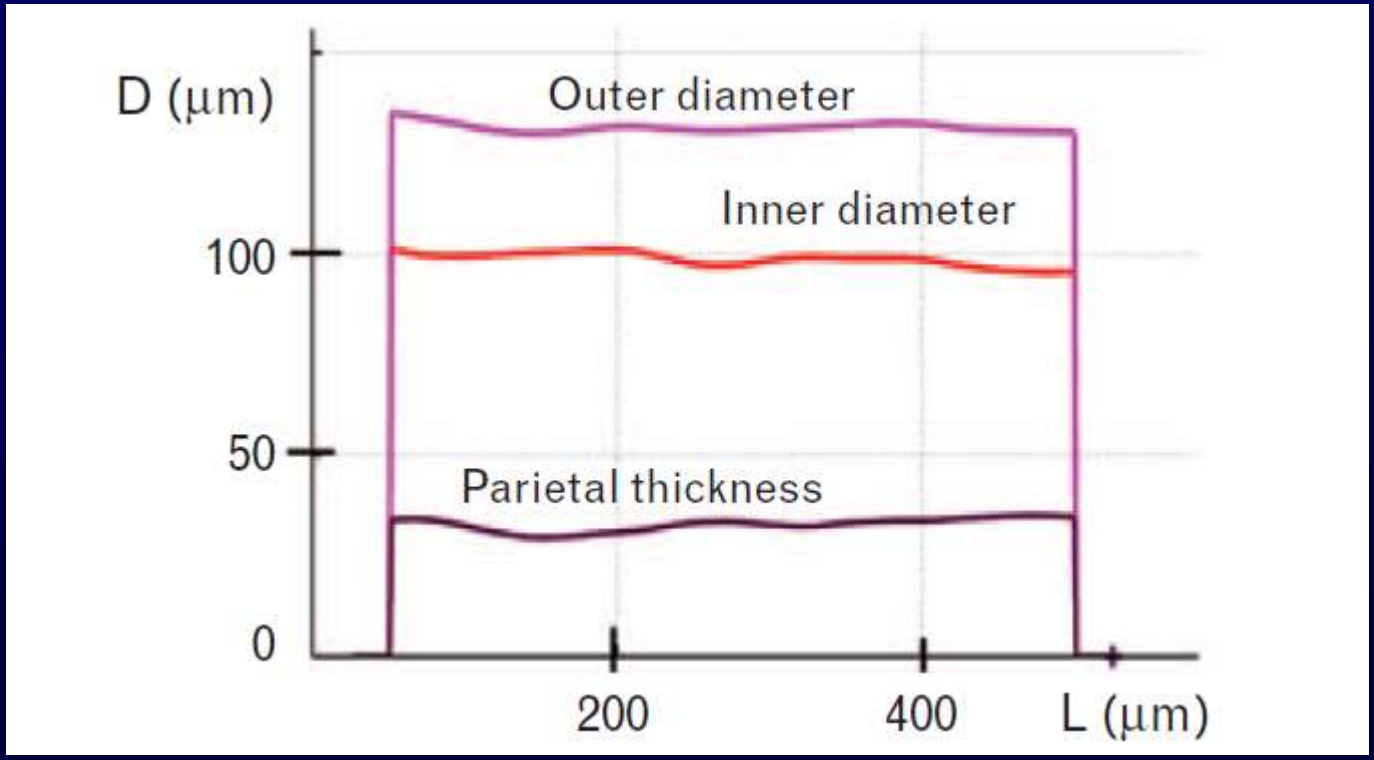

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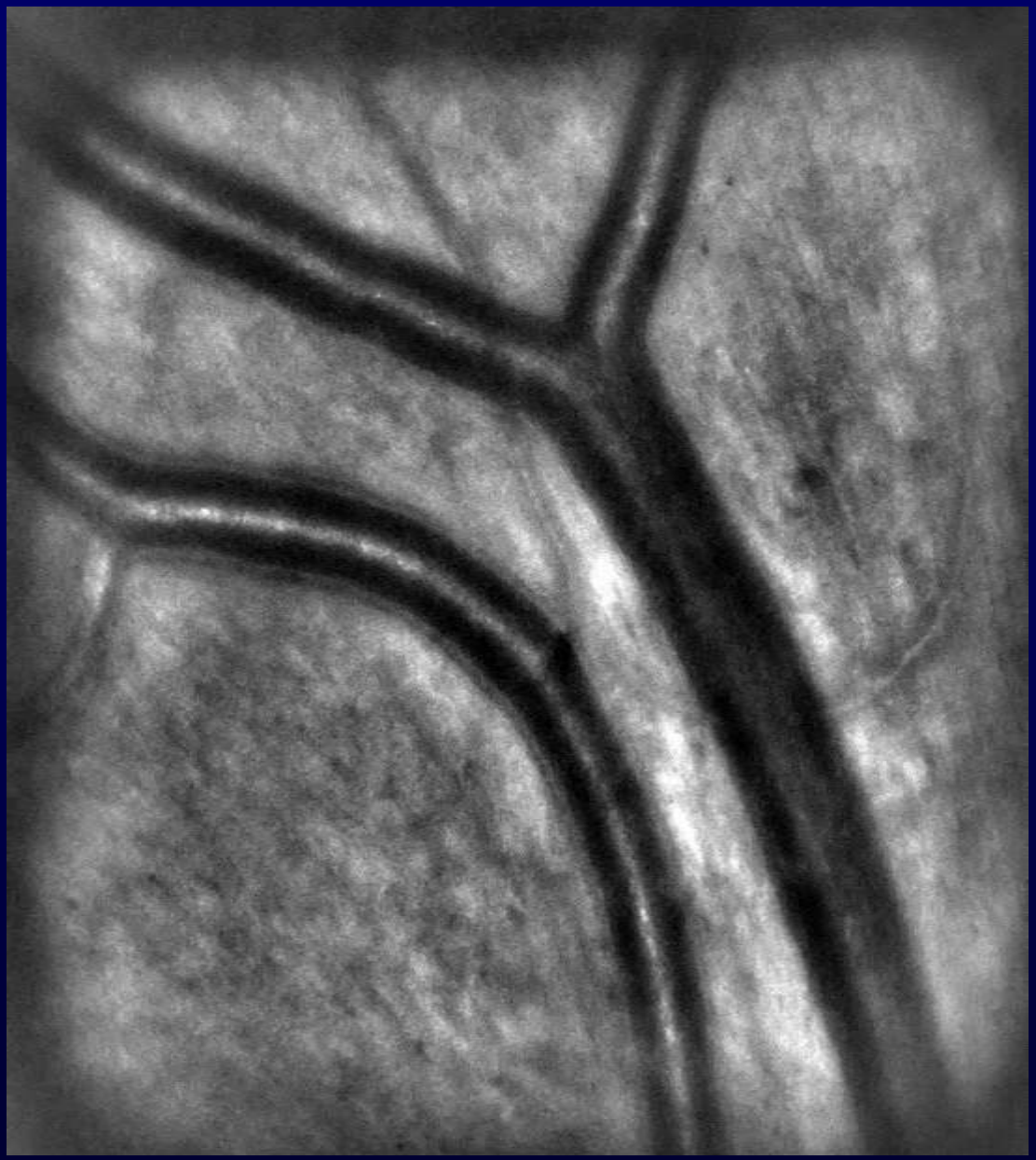
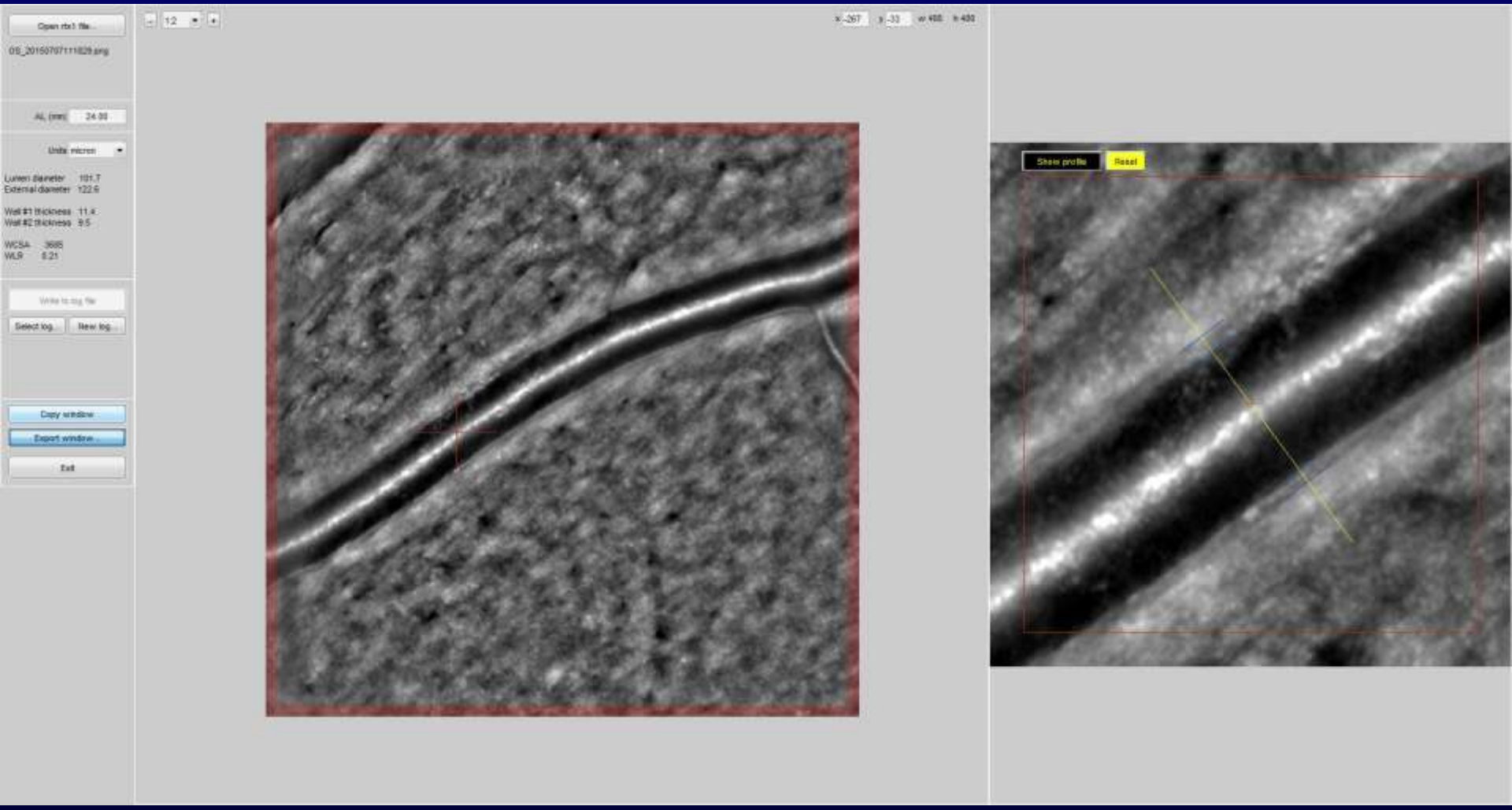


Koch et al, J Hypertens 2014; 32:890-898

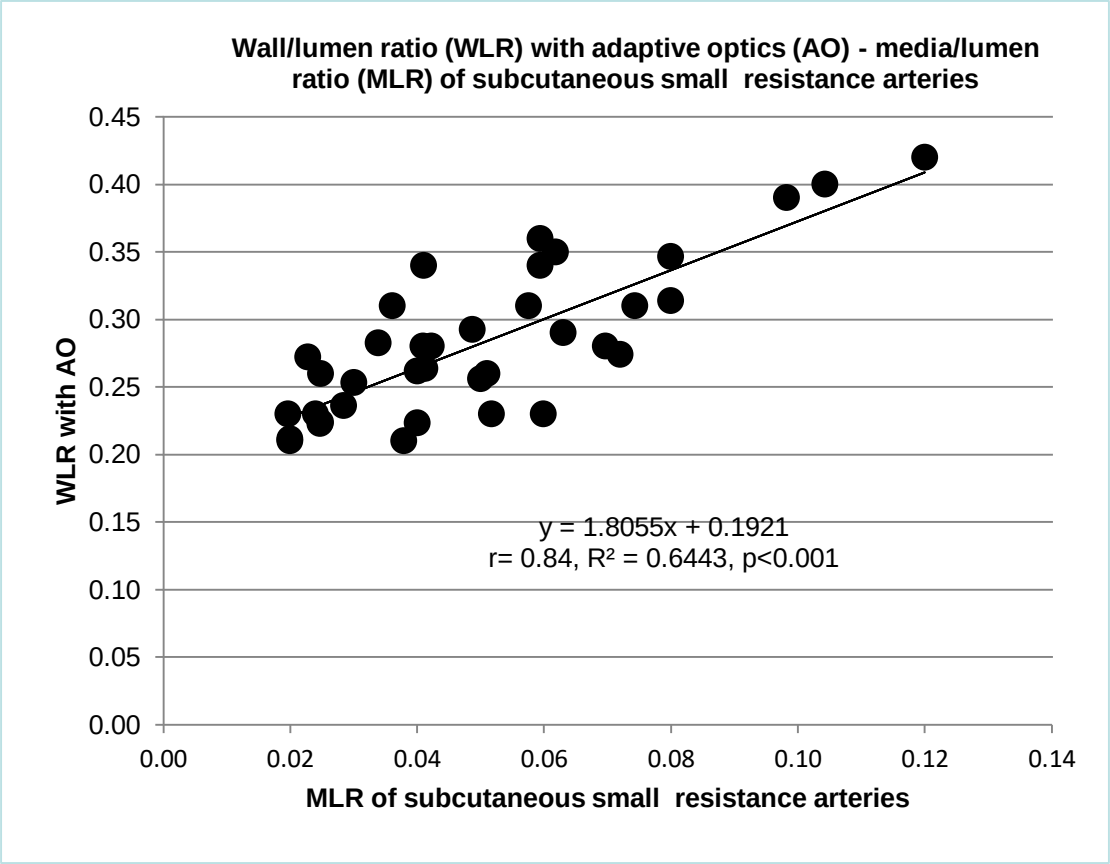


D. Rizzoni Lab of Vascular Biology University of Brescia

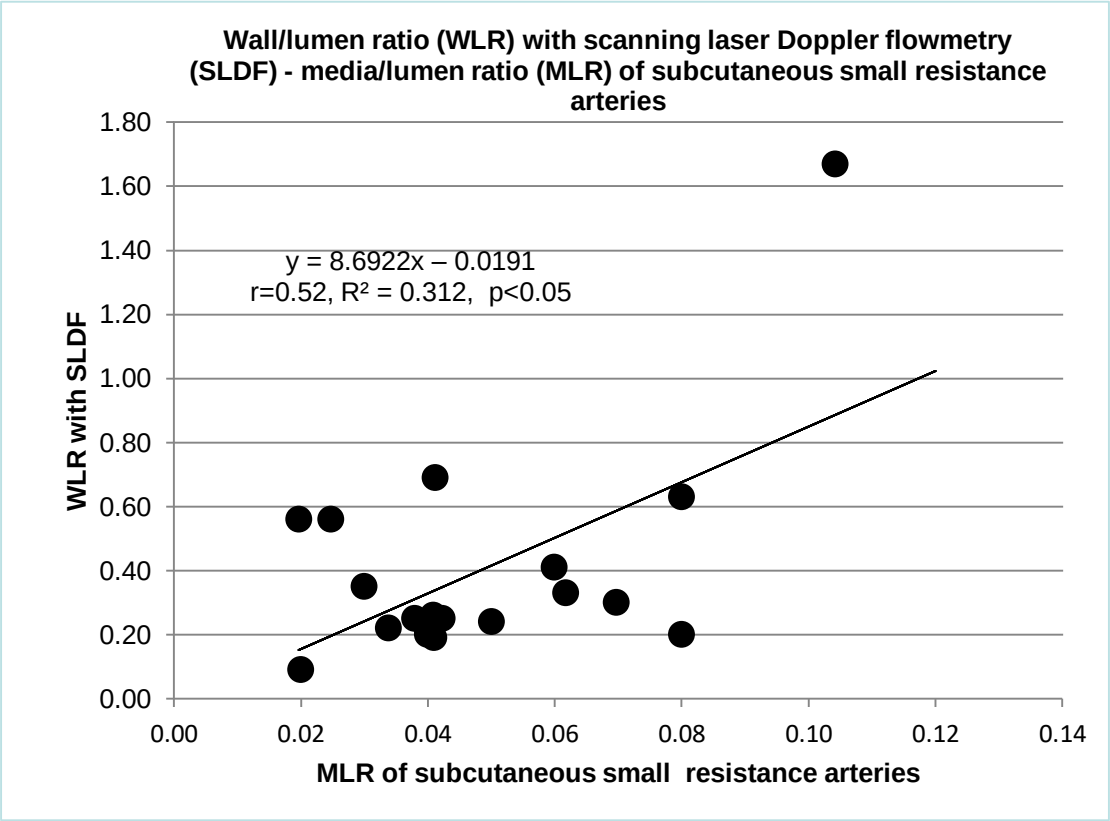
Comparison between invasive and noninvasive techniques of evaluation of microvascular structural alterations

Carolina De Ciuceis[a], Claudia Agabiti Rosei[a], Stefano Caletti[a], Valentina Trapletti[a], Maria A. Coschignano[a], Guido A.M. Tiberio[b], Sarah Duse[c], Franco Docchio[d], Simone Pasinetti[d], Federica Zambonardi[d], Francesco Semeraro[c], Enzo Porteri[a], Leonardo Solaini[b], Giovanna Sansoni[d], Paola Pileri[a], Claudia Rossini[a], Francesco Mittempergher[b], Nazario Portolani[b], Silvia Ministrini[b], Enrico Agabiti-Rosei[a], and Damiano Rizzoni[a,e]

J Hypertens 2018; 36:1154-1163.



De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.



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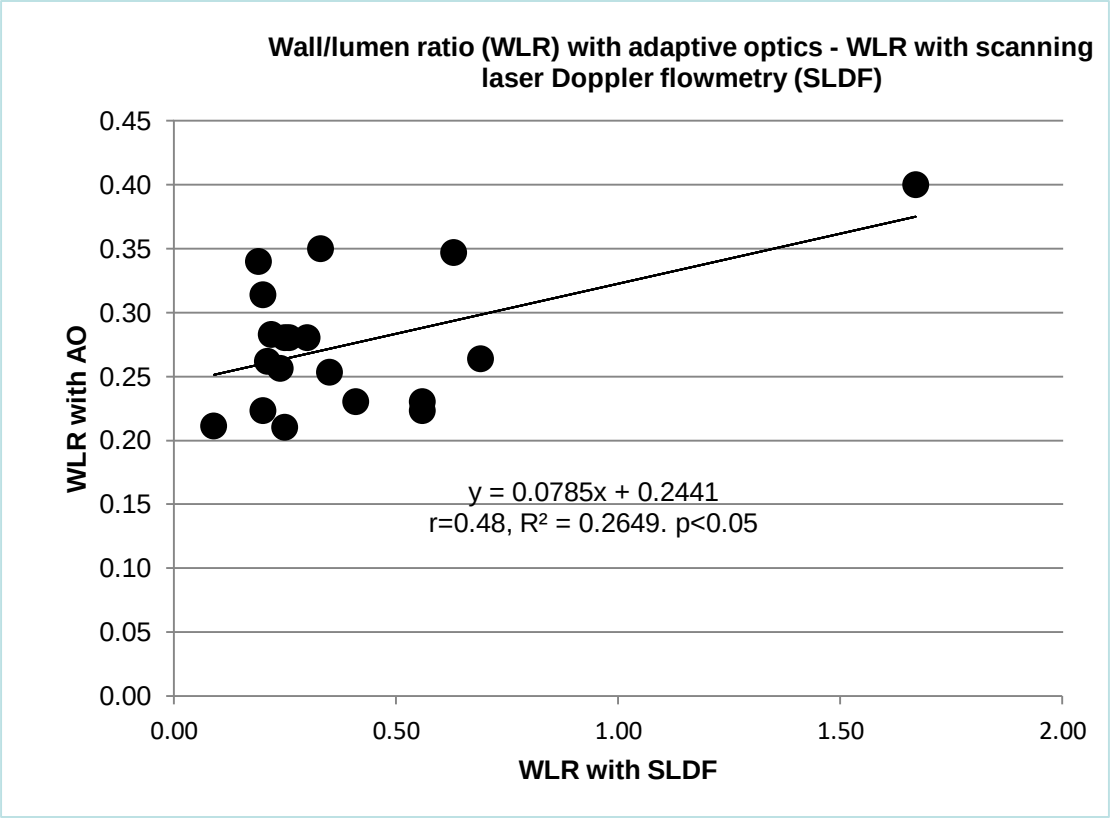


De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.

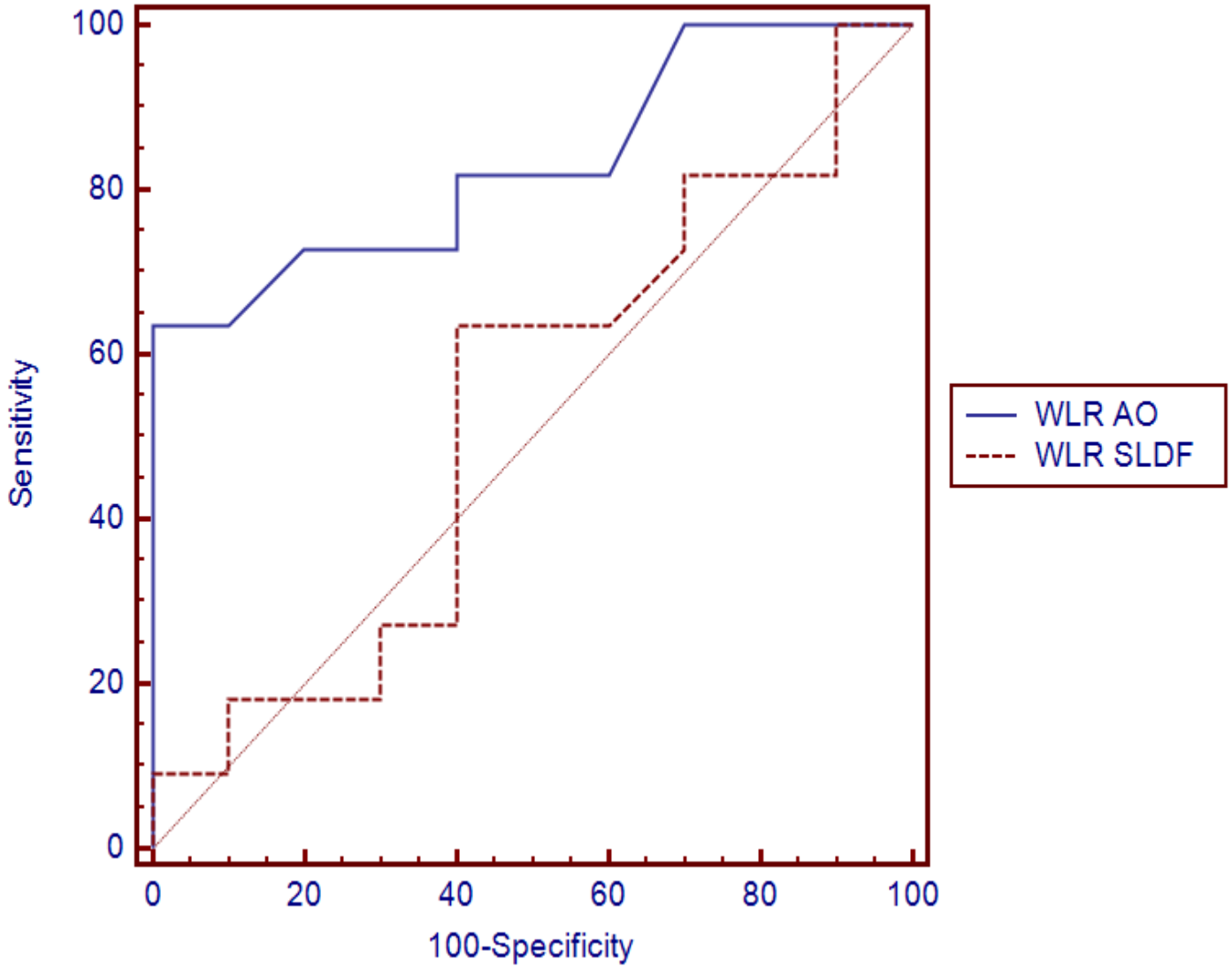
	Basal capillary density in the nailfold / M/L	Total capillary density in the nailfold / ML	Basal capillary density in the dorsum of the finger / M/L	Total capillary density in the dorsum of the finger / ML
Correlation coefficients (n=36)	r=0.49, r^2=0.24, p<0.001	r=0.53, r^2=0.28, p<0.001	r=0.026 r^2=0.0006, P=NS	r=0.050, r^2=0.0025, P=NS
	Basal capillary density in the forearm / M/L	**Total capillary density in the forearm / ML**		
Correlation coefficients (n=36)	r=0.011, r^2=0.001, p=NS	r=0.027, r^2=0.0007, p=NS		

De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.

	W/L retinal arterioles (SLDF) / M/L	W/L retinal arterioles (RTX-1) / M/L	W/L retinal arterioles (SLDF) / W/L retinal arterioles (RTX-1)
Correlation coefficients (n=36)	0.55, r^2=0.30, p<0.001	0.84, r^2=0.81, p<0.001	0.52, r^2=0.27, p<0.001

Slopes of the relations: p<0.01 RTX-1 vs. SLDF.

De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.



De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.

Wall lumen ratio of retinal arterioles	**Intraobserver RTX-1**	**Interobserver RTX-1**	**Intraobserver SLDF**	**Interobserver SLDF**
Variation coefficient (%)	3.25±1.40	7.05±4.59	38±22 ***	43±19 ***

***=p<0.001 vs. RTX-1

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 10 March 2021
doi: 10.3389/fcvm.2021.651594

Microvascular Structural Alterations in Cancer Patients Treated With Antiangiogenic Drugs

Maria Antonietta Coschignano[1], Carolina De Ciuceis[1,2], Claudia Agabiti-Rosei[1,2], Valeria Brami[1], Claudia Rossini[1], Giulia Chiarini[1], Paolo Malerba[1], Francesca Famà[1], Deborah Cosentini[3], Maria Lorenza Muiesan[1,2], Massimo Salvetti[1,2], Alina Petelca[1], Sara Capellini[1], Chiara Arnoldi[1], Matteo Nardin[1], Salvatore Grisanti[3], Damiano Rizzoni[1,4], Alfredo Berruti[3] and Anna Paini[2]*

[1] Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy, [2] Spedali Civili di Brescia, Clinica Medica University of Brescia and 2nd Division of Medicine, Brescia, Italy, [3] Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Medical Oncology, Spedali Civili di Brescia, University of Brescia, Brescia, Italy, [4] Division of Medicine, Spedali Civili di Brescia, Brescia, Italy

Objective: Antiangiogenic therapies (tyrosine kinase inhibitors-TKI and direct anti-VEGF monoclonal antibodies) are being increasingly used in the treatment of solid tumors; hypertension represents a common side effect of these agents. Several mechanisms are involved in the development of hypertension, including microvascular rarefaction and other microvascular alterations. Therefore, the aim of our study was to evaluate whether TKI and direct anti-VEGF agents may affect the structure of retinal arterioles or capillary density.

Design and Methods: We investigated 20 patients with a diagnosis of cancer who underwent a treatment with either a TKI or an anti-VEGF antibody. Patients were submitted to ambulatory monitoring blood pressure for blood pressure evaluation. Basal and total capillary density were assessed by capillaroscopy whereas, retinal arteriole morphology was measured by Adaptive Optics. Patients were evaluated before starting the antiangiogenic therapy (T0) and re-evaluated after 3 (T3) and 6 (T6) months after treatment. Fourteen patients completed the study.

Results: Systolic and diastolic blood pressure values were similar in all patients at T3 and T6 compared to T0. However, during the study antihypertensive treatment was optimized (increased dose and/or addition of drugs) in 57% of patients ($n = 8$). No differences were observed in retinal arteriole structural parameters and in large artery stiffness. Basal capillary density was reduced by antiangiogenic drugs after 3 or 6 months.

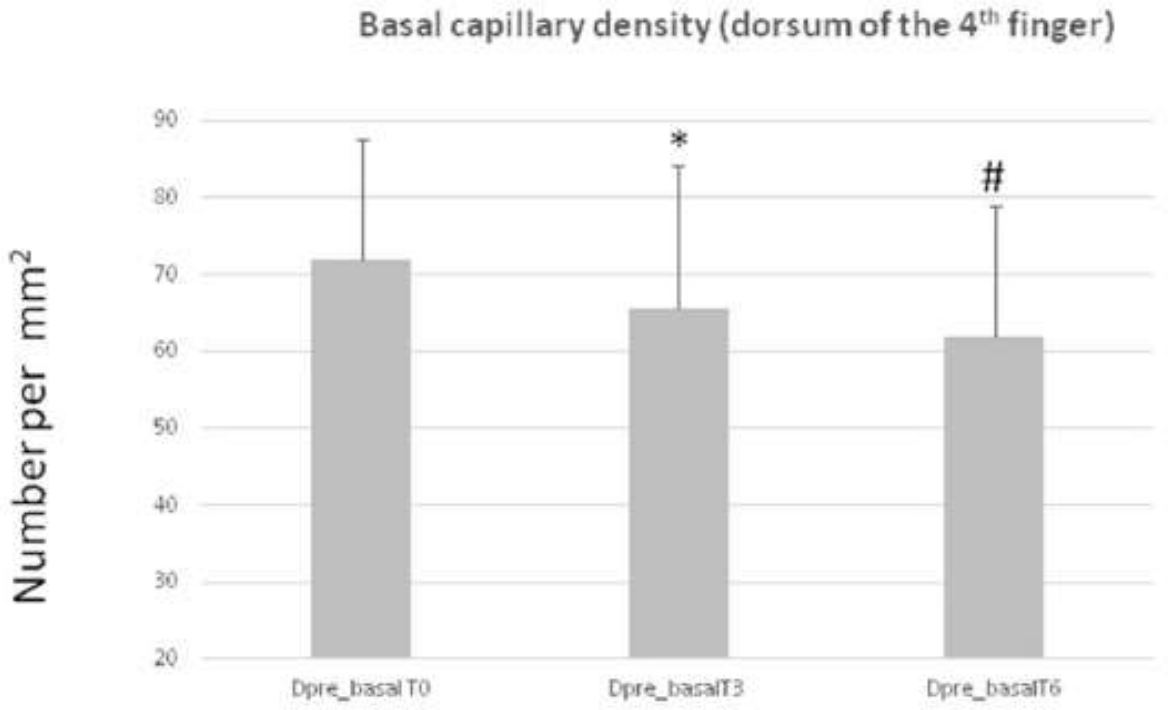


FIGURE 2 | Basal capillary density in the dorsum of the 4th finger (Dpre_basal) at the different time points (T0, T3, T6). *T3 vs. T0 $p = 0.03$; #T6 vs. T0 $p = 0.02$. Data are expressed as mean + standard deviation.

Coschignano ME et al, Front Cardiovasc Med. 2021 Mar 10;8:651594. doi: 10.3389/fcvm.2021.651594. eCollection 2021.

TABLE 3 | Blood pressure values and indices of microvascular structural alterations at the different time points.

Parameter	T0 (Baseline)	T3 (3 months)	T6 (6 months)
ABPM			
24-h systolic blood pressure (mmHg)	122.6 ± 14.7	123.3 ± 12.2	128.3 ± 26.1
24-h diastolic blood pressure (mmHg)	71.7 ± 10.2	70.9 ± 8.0	71.7 ± 9.1
Retinal arterioles			
Internal diameter (μm)	86.4 ±17.0	87.14 ±11.4	89.3 ±11.8
External diameter (μm)	111.5 ±21.6	111.9 ± 15.0	114.4 ± 14.7
Wall thickness (μm)	12.6 ±2.6	12.4 ± 2.0	12.6 ± 1.8
Wall cross sectional area ($\mu m^{2)}$	4,039.6 ± 1,491	3,943.5 ± 1,093.0	3,968.3 ± 1,026
Wall to lumen ratio (WLR)	0.30 ± 0.03	0.28 ± 0.02	0.28 ± 0.03
Capillaries			
Basal capillary density (dorsum), number per area unit	72.0 ± 15.8	65.5 ±18.6*	61.9 ± 17.1#
Total capillary density (dorsum), number per area unit	73.8 ± 14	69.6 ± 20.7	68.5 ± 11.9
Basal capillary density (forearm), number per area unit	56.6± 17.1	47.7 ± 13.2§	49.3 ± 11.6
Total capillary density (forearm), number per area unit	58.4 ± 16.2	54.5 ± 15.8	53.0 ± 8.0

*T-student analysis: *T3 vs. T0 p = 0.03; #T6 vs. T0 p = 0.02; §T3 vs. T0 p = 0.04.*
One-way ANOVA: dorsum basal capillary density p = 0.027, forearm basal capillary density p = 0.17.

Coschignano ME et al, Front Cardiovasc Med. 2021 Mar 10;8:651594. doi: 10.3389/fcvm.2021.651594. eCollection 2021.

Il progetto prevedeva i seguenti obiettivi:
1) Di validare l'apparecchiatura RTX-1 (Imagine Eyes) vs. il gold standard (biopsie tessutali + micromiografia).
2) Di valutare la riproducibilità e il valore clinico della misurazione del rapporto parete/lume delle arteriole retiniche (W/L).
3) Di valutare il possibile effetto di farmaci anti-angiogenetici, ampiamente utilizzati in oncologia, quali i modulatori del VEGF e gli inibitori di tirosina kinasi su indici di struttura del microcircolo valutati non invasivamente (W/L. densità capillare misurata mediante videomicroscopia) e su indici di distensibilità arteriosa.

Results and conclusion: Our data suggest that adaptive optics has a substantial advantage over SLDF in terms of evaluation of microvascular morphology, as WLR measured with adaptive optics is more closely correlated with the M/L of subcutaneous small arteries (r^2¼0.84, $P<0.001$ vs. r^2¼0.52, $P<0.05$, slopes of the relations: $P<0.01$ adaptive optics vs. SLDF). In addition, the reproducibility of the evaluation of the WLR with adaptive optics is far better, as compared with SLDF, as intraobserver and interobserver variation coefficients are clearly smaller. This may be important in terms of clinical evaluation of microvascular morphology in a clinical setting, as micromyography has substantial limitations in its clinical application due to the local invasiveness of the procedure.
De Ciuceis C et al, J Hypertens 2018; 36:1154-1163.

Conclusions: Our data suggest that an increase of antihypertensive treatment is necessary in patients treated with a TKI or a direct VEGF inhibitor, confirming pro-hypertensive effects of these drugs. However, under adequate blood pressure
control, microvascular structure seem to be partially preserved, since a worsening of basal capillary density but no changes in retinal arteriole morphology were observed.
Coschignano ME et al, Front Cardiovasc Med. 2021 Mar 10;8:651594. doi: 10.3389/fcvm.2021.651594. eCollection 2021.

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