Applied Cardiopulmonary Pathophysiology 16: 276-282, 2012

Imaging of the sublingual microcirculation in elderly patients – a pilot study

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Abstract

Demographic changes, i. e. worldwide increase in older population, require to direct research efforts to age-relevant topics. The present article focuses on microcirculation in elderly patients. Sidestream dark field (SDF) imaging, a relatively new technology, allows direct visualization of mucosal microcirculation and imaging of surface layers of solid organs using a handheld microscopical camera probe.

Our study aimed at assessing sublingual microcirculation in elderly patients using this new SDF technology in order to identify and compare changes across different age groups. The study results suggest that even in healthy individuals microvascular flow index (MFI) and functional capillary density (FCD) change significantly during the process of aging.

Key words: microcirculation, sublingual, elderly, sidestream dark field imaging (SDF)

Introduction

The last decades witnessed an increase in the world's older population, specially in the developed countries, this being the result of a more advanced health care, better social systems and improved living conditions in developed countries. The U.S. Census Bureau released a report in 2009 that showed that the world's 65-and-older population is projected to triple by mid century, from 516 million in 2009 to 1.53 billion in 2050. In contrast, the population under 15 is expected to increase by only 6 percent during the same period, from 1.83 billion to 1.93 billion. From 2009 to 2050, the world's 85 and older population is projected to increase more than fivefold, from 40 million to 219 million.

These changes in the world's demographics drive focus also of microcirculation research towards elderly patients. An understanding of age-dependent adaptation in the structure and function of microvascular networks is critical to understanding how delivery and distribution of blood flow is controlled across the life span.

Sidestream dark field imaging has risen as a pioneer among different technologies designed to image the human microcirculation (1-3). This relatively new technology allows the direct visualization of mucosal microcirculation and imaging of surface layers of solid organs using a handheld microscopical camera probe. This technology depends on a light guide imaging the microcirculation, which is surrounded by light emitting diodes

at a wavelength of 530nm. The hemoglobin of the erythrocytes absorbs this green light while the rest of the light is scattered to form the white-greyish background. This produces clear images of capillaries with flowing erythrocytes (4). SDF has played a role in minimizing the gap in understanding the microcirculation and helped move microcirculatory research from bench to bedside.

The primary aim of our study was to study the sublingual microcirculation in elderly patients using SDF technology. To identify changes in microcirculatory parameters during the physiological process of aging we compared the microcirculation of different age groups (20-39, 40-69, 75-90 years).

Methods

Subject Selection

Approval for the study was granted by the REB of the University Hospital, Hradec Králové, Czech Republic. Written informed consent was obtained from all participants.

We divided the study subjects in three groups, regardless of their sex. The first group (Group A, n = 10) included healthy subjects (ages 20-39 years), the second group (Group B, n = 10) included 10 healthy subjects (ages 40-69), and the third group (Group C, n = 10) healthy subjects (ages 70-90).

Since the aim was to study the physiological changes during aging, in all groups we included only subjects that were healthy, did not have any chronic diseases and did not take any medications. Furthermore, they were all of good physical condition, with the elderly capable of performing daily activities on their own without the need of assistance. All subjects were of good mental health, with no history of dementia (6), Alzheimers (7), psychosis or behavioral disturbances. All our subjects were non-smokers (8, 9). All subjects had hematocrit values within the normal ranges.

We excluded subjects with any previous history of conditions that could cause pathological changes in the microcirculation. Our subjects did not have any history of hypertension (12), ischemic heart disease, diabetes mellitus, ischemic diseases of the lower extremities, cerebrovascular diseases, critically ill patients, subjects undergoing mechanical ventilation or being treated for all forms of circulatory shock (13-16).

All subjects were instructed to refrain from consuming caffeine-containing substances 2 h prior to the evaluation. No sedation was used during the image recordings and all subjects were cooperative.

Study settings

Image recordings of the microcirculation took place at two locations. Subjects from group A and B where invited to the library of the Department of Anesthesiology and Intensive Care Medicine, University Hospital of Hradec Králové. Subjects from group C where residents of the Senior Home of Hradec Králové, and to ensure their comfort, the image recordings where taken at site at the in-house clinic. Each subject was examined individually in the supine position to allow comfortable measuring for both the subjects and the examiners, the elderly patients were asked to remove any dental prostheses.

The sublingual microcirculation was visualized using the MicroScan SDF camera (MicrovisionMedical, Amsterdam, The Netherlands). In order to facilitate the procedure and minimize artifacts, all images were obtained by two investigators with long-standing experience in this technique. The SDF probe was covered with a sterile plastic lens and lightly placed on the target sublingual mucosa, the subjects were asked to hold their mouth in a semi-closed position in order to help positioning the camera. Two trained physicians blinded to clinical data performed measurements. Subjects were in supine position, in a temperature controlled room with a temperature of approximately 22°C. The tip of the SDF probe was placed

on sublingual mucosa. To prevent microcirculatory perfusion disturbance due to application of pressure on the imaging area, the probe was first placed on the labial tissue and then retracted to an extent, which minimized contact but enabled visualisation of the capillary bed. Illumination intensity and depth of focus were modulated to fine-tune image quality. Continuous digital image recordings (duration 1 min) were captured in five different locations under the tongue, and digital image recordings were saved on a hard drive as DV-AVI files to enable offline analysis. For high quality image recording we followed the recommendations of the round table consensus on image acquisition and analysis (17).

Off-line analysis

SDF video clips were coded and analysed off line, using the methods described by De Backer et al. (17). Data were analyzed using the AVA3 software (MicrovisionMedical, Amesterdam, The Netherlands). The software enables automatic detection of vessels (7 -100 μm) for the calculation of their diameter, length, density and flow. We used the software to calculate Microvascular Flow Index (MFI), and Functional Capillary Density (FCD).

To calculate the MFI, the image was divided into four quadrants. Characteristic flow scores were assigned in each quadrant for each vessel size category. The flow categories are 0 for no flow, 1 for intermittent flow, 2 for sluggish flow and 3 for continuous flow. The flow category assigned to each vessel category is then summed for the four quadrants and divided by the number of quadrants in which the vessel type is present (MFI range 0–3).

FCD is defined as the length of red blood cell-perfused capillaries per observation area and is given in cm/cm^2. FCD is calculated by applying three equidistant horizontal and three equidistant vertical lines superimposed upon the video sequence. FCD was calculated as the number of capillaries crossing the lines divided by their total length. This gave the number of capillaries per mm. FCD for small vessels was estimated in vessels with less than 25 μm in diameter.

Statistical analysis

Statistical analysis was performed using Prism 4 (GraphPad, La Jolla, CA, USA). All data were analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. A p value < 0.05 was considered significant.

Results

Between groups A (20-39) and B (40-69) we did not observe significant differences in the FCD of all vessels, FCD of small vessels or MFI (Figures 1-3). However, significant decreases in the FCD of all vessels, FCD of small vessels and MFI was found when comparing groups A (20-39) and C (70-90) or B (40-69) and C (70-90), respectively.

Discussion

We found a significant decrease in the microcirculatory parameters FCD and MFI in group C (70-90) when compared to the other two groups A (20-39) and B (40-69). In group C the significant decrease in FCD was found in small capillaries (less than 25 μm in diameter) as well in all vessels. The greatest difference was found in the FCD of small vessels.

The dramatic increase in the number of people reaching age 65 – coupled with their increased life expectancy – has expanded the classification of those of age 65 and older to include three sub-populations commonly referred to as the „young old" (being the age of 65-74), the „old" (74-84), and the „old-old" (being older than 85; (5)). To simplify our study design, we divided the subjects into three sub-groups (group A: 20-39, group B: 40-69 and Group C: 70-90 years).

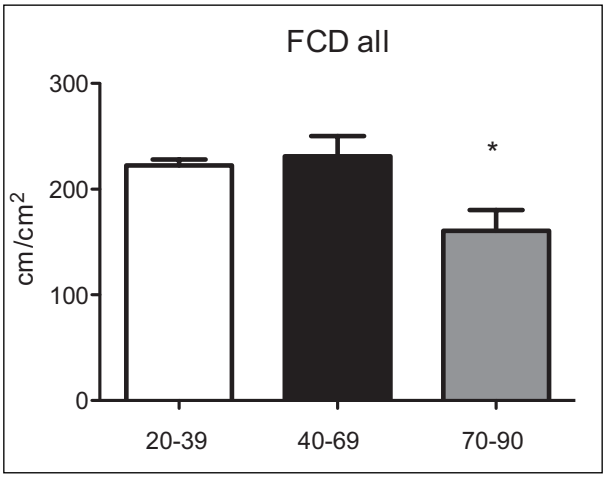

Figure 1: Functional capillary density (FCD) of all microvessels (<100 µm), n=10 per group.
** p<0.05 vs. 20-39 and 40-69 years old*

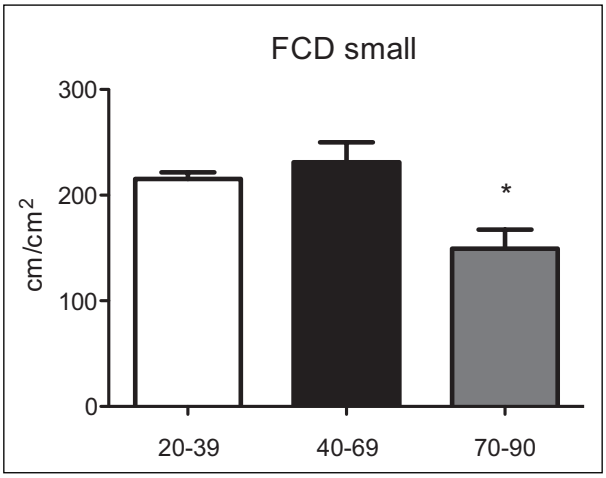

Figure 2: Functional capillary density (FCD) of small microvessels (<25 µm), n=10 per group.
** p<0.05 vs. 20-39 and 40-69 years old*

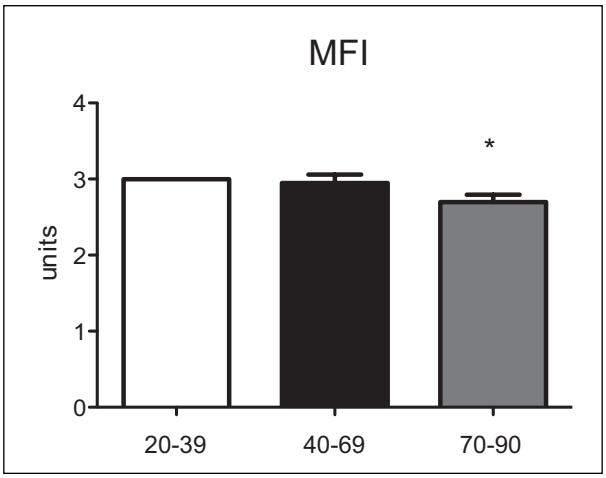

Figure 3: Microvascular Flow Index (MFI) of all microvessels (<100 µm), n=10 per group.
** p<0.05 vs. 20-39 and 40-69 years old*

All subjects had hematocrit values within the normal ranges. The hematocrit in the preferential flow channels is an inverse function of the flow rate for any level of the microcirculatory hematocrit. The increased hematocrit raises the flow resistance in these vessels which reduces flow further and represents a positive feedback condition which may contribute to the intermittent and uneven flow patterns which are present within the microcirculation (10, 11)

Our results concur with previous results from studies that looked at the microcirculatory changes during the process of aging. Experimental data indicate that, independent of the presence of other pathologies, aging alters endothelium-dependent relaxations in both the aorta and small resistance arteries in rats (18-21). Brandes et al. (22) suggested that aging is associated with a reduction in the regenerative capacity of the endothelium and endothelial senescence, which is characterized by an increased rate of endothelial cell apoptosis. Muller et al. (23) showed that aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles.

Taddei et al. (24) evaluated the role of advancing age as an independent factor that can alter endothelial function. They demonstrated that the vasodilating response to acetylcholine decreased with advancing age in the forearm of both normotensive control subjects and essential hypertensive patients, whereas the vasodilating response to sodium nitroprusside was minimally affected by aging. Taken together, these results are consistent with the finding that endothelial function is progressively impaired with aging (24). In a separate study the same group proposed the age related endothelial dysfunction to be mediated by a progressive reduction of NO availability, since the inhibiting effect of L-NMMA on acetylcholine-induced vasodilation was progressively impaired by advancing age (25). Angula et al. (26) showed that although the aging process by itself, without other concomitant morbidities, causes an impairment of endothelium-dependent vasodilation of human vessels, the presence of cardiovascular risk factors exacerbated such impairment in aged human vessels. Furthermore they suggested that the response of aged human vessels to treatments for improving endothelial function might be different from that of vessels from adult subjects (26). Eskurza et al. (27) confirmed that oxidative stress was the main mediator of the age related endothelial dysfunction.

A group of studies focused on evaluating the blood flow in specific muscle groups during exercise. Wahren et al. (28) showed that the rise in leg blood flow during exercise was decreased in older male subjects (52–59 years) compared to the values measured in young male subjects (25–30 years). Proctor et al. (29) have also shown that leg blood flow and vascular conductance during submaximal cycling exercise at a given level of whole-body oxygen consumption are substantially reduced in older men as compared to their young counterparts. Furthermore, muscle blood flow is lower in older human subjects when a small muscle mass is active and the limits of cardiac output are not approached (30). In conclusion, data obtained in humans indicate that age-induced adaptations of the vasculature contribute to a reduction in muscle blood flow; however, the specific mechanisms that contribute to the age-related diminution of blood flow to muscle have not been discerned in human models (31).

The affect of aging on the density of microvessels has not been studied in detail yet, and there is no striking consensus on the effects of aging on capillary density. Hutchins et al. (32) and Sonntag et al. (33) demonstrated a substantial aging-related rarefaction of the surface arterioles that supply the parenchymal vessels of the cerebral cortex in rats. In normally aging rats, the density of arterioles was almost 40% lower in senescent animals than in young adults (29 months versus 7 months of age).

SDF was not previously used for evaluation of the microcirculation in elderly patients. Previous studies mainly focused on the molecular level to illustrate the age relat-

ed endothelial dysfunction (24-26) or used techniques such as the constant-rate intra-arterial indicator infusion technique to study the changes in blood flow (27, 34). To our knowledge no study has evaluated the capillary density in humans.

Conclusion

We were able to identify in healthy subjects the age group at which the microcirculatory parameters FCD and MFI change significantly. Individuals above the age of 70 years should be excluded from studies evaluating the microcirculation because of the age-related changes found in our study. Even though individuals above the age of 70 years could be free from pathological conditions, standard microcirculatory parameters are altered.

Acknowledgements

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00179906 and by the programme PRVOUK P37/02.

References

1. Ince C. The microcirculation is the motor of sepsis. Crit Care 2005; 9 (Suppl. 4): 13-19
2. Ince C. Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. Crit Care 2005; 9 (Suppl. 1): 72
3. Turek Z, Cerný V, Pařízková R. Noninvasive in vivo assessment of the skeletal muscle and small intestine serous surface microcirculation in rat: sidestream dark-field (SDF) imaging. Physiol Res 2007; 56
4. Goedhart PT, et al. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Optics express 2007; 15 (23): 15101-15114
5. http://transgenerational.org/aging/demographics.htm#ixzz1zFoGov53
6. Bue L, Hof PR, Delacourte A. Brain microvascular changes in Alzheimer's disease and other dementias. Annals of the New York Academy of Sciences 1997; 826: 7-24
7. De la Torre JC, Stefano GB. Evidence that Alzheimer's disease is a microvascular disorder: the role of constitutive nitric oxide. Brain research reviews 2000; 34 (3): 119-136
8. Leone A, Landini L. Vascular Pathology from Smoking: Look at the Microcirculation! Current vascular pharmacology 2011
9. Henriksson P, Diczfalusy U, Freyschuss A. Microvascular reactivity in response to smoking and oral antioxidants in humans. Microcirculation 2012; 19 (1): 86-93
10. Boyle J. Microcirculatory hematocrit and blood flow. Journal of theoretical biology 1998; 131 (2): 223-229
11. Lowe GD. Blood rheology in vitro and in vivo. Baillière's clinical haematology 1987; 1 (3): 597-636
12. Pretolani E, et al. [The blood rheological and microcirculatory changes in the hypertensive patient]. Cardiologia 1991; 36 (12) Suppl 1: 355-363
13. Szopinski J, Kusza M, Semionow M. Microcirculatory responses to hypovolemic shock. The Journal of trauma 2011; 71: 1779-1788
14. Kanoore E, Vanina S, Dubin A, Ince C. The microcirculation as a therapeutic target in the treatment of sepsis and shock. Seminars in respiratory and critical care medicine 2011; 32: 558-568
15. Ferrari M, et al. [Evaluation of microcirculatory disorders in shock patients]. Deutsche Medizinische Wochenschrift 2011; 136 (19): 1009-1013
16. Jung C, Lauten A, Ferrari M. Microcirculation in cardiogenic shock: from scientific bystander to therapy target. Critical care 2010; 14 (5): 193-193
17. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C. How to evaluate the microcirculation: report of a round table conference. Crit care 2007; 11: R101
18. Soltis EE. Effect of age on blood pressure and membrane-dependent vascular responses in the rat. Circ Res 1987; 61: 889-897

19. Moritoki H, Hosoki E, Ishida Y. Age-related decrease in endothelium-dependent dilator response to histamine in rat mesenteric artery. Eur J Pharmacol 1986; 126: 61-67
20. Hongo K, Nakagomi T, Kassell NF, et al. Effects of aging and hypertension on endothelium-dependent vascular relaxation in rat carotid artery. Stroke 1988; 19: 892-897
21. Mayhan WG, Faraci FM, Baumbach GL, Heistad DD. Effects of aging on responses of cerebral arterioles. Am J Physiol 1990; 258 (Heart Circ Physiol 27): H1138-H1143
22. Brandes RP, Fleming I, Busse R. Endothelial aging. Cardiovascular research 2005; 66 (2): 286-294
23. Muller Delp JM, et al. Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. American journal of physiology 2002; 283: H1662-H1672
24. Taddei S, et al. Aging and endothelial function in normotensive subjects and patients with essential hypertension. Circulation 1995; 91: 1981-1987
25. Taddei S, et al. Age-related reduction of NO availability and oxidative stress in humans. Hypertension 2001; 38: 274-279
26. Angulo J, et al. Age-related differences in the effects of α and γ peroxisome proliferator-activated receptor subtype agonists on endothelial vasodilation in human microvessels." Experimental gerontology 2012
27. Eskurza I, Kahn ZD, Seals DR. Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. Journal of physiology 2006; 571: 661-668
28. Wahren J, Saltin B, Jorfeldt L, Pernow B. Influence of age on the local circulatory adaptation to leg exercise. Scand J Clin Lab Invest 1974; 33: 79-8
29. Proctor DN, Shen PH, Dietz NM, Eickhoff TJ, Lawler LA, Ebersold EJ, Loeffler DL, Joyner MJ. Reduced leg blood flow during dynamic exercise in older endurance-trained men. J Appl Physiol 1998; 85: 68-75
30. Lawrenson L, Poole JG, Kim J, Brown C, Patel P, Richardson RS. Vascular and metabolic response to isolated small muscle mass exercise: effect of age. Am J Physiol Heart Circ Physiol 2003; 285: H1023-H1024
31. Muller D, Judy M. Aging-induced adaptations of microvascular reactivity. Microcirculation 2006; 13 (4): 301-314
32. Jorfeldt L, Wahren I. Leg blood flow during exercise in man. Clin Sci 1971; 41: 459
33. Hutchins PM, Lynch CD, Cooney PT, Curseen KA. The microcirculation in experimental hypertension and aging. Cardiovasc Res 1996; 32: 772-780
34. Sonntag WE, Lynch CD, Cooney PT, Hutchins PM. Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor. Endocrinology 1997; 138: 3515-3520

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